THE INVOLVEMENT OF *FUSARIUM*, AUTOTOXINS AND HERBICIDE RESIDUES IN THE ASPARAGUS (*Asparagus officinalis* L.) REPLANT PROBLEM.

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Science at Massey University.

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Abstract.

In temperate climates, asparagus reaches peak production five to eight years after planting and thereafter yield declines until production is no longer economically viable, normally between years 12 to 15. In many of the asparagus producing areas of the world the availability of land suitable for asparagus production is limited, therefore, replanting of old asparagus beds is undertaken. Replanted asparagus often has poor establishment and a short commercial life compared with planting on sites that have no history of asparagus production.

In this research, field trials indicated that replanted stands will yield 20% to 30% less marketable asparagus than those on similar sites with no previous asparagus cropping history. Pre-planting treatments with the fungicides thiabendazole and/or metalaxyl did not alleviate the problem but may improve establishment in replant sites. Treatment of plants or field soils with *Trichoderma viride* did not improve establishment or plant performance in old asparagus soils. The replant problem was common to all asparagus cultivars evaluated with the most vigorous varieties in a replant site also performing best in virgin soils.

Plants that died out in replant soil field trials exhibited symptoms typical of *Fusarium* spp. infections and isolations confirmed the involvement of both *F. oxysporum* and *F. moniliforme* in the early decline of replanted asparagus stands.

Greenhouse studies confirmed the importance of *Fusarium* inoculum level in inciting disease in asparagus plants. As inoculum levels increased the disease levels on roots and crowns of developing seedlings also increased and the plant vigour decreased. A Root Necrosis Potential bioassay which measured the infectivity of *Fusarium* propagules in field soils proved to be useful in separating soils with a previous history of asparagus production from virgin soils.

Residual herbicides commonly used in asparagus production significantly reduced asparagus seedling growth at levels likely to be found after several years of asparagus cropping demonstrating the importance of planning for the removal of an old asparagus planting some years before the crop is terminated.

Evaluation of soil with and without asparagus cropping history showed that an abiotic cause to the replant problem may also be important.

The presence of autotoxic material in asparagus storage roots was confirmed in laboratory experiments and the toxic material reduced growth of asparagus. Bioassays using pre-germinated asparagus seed on blotting paper demonstrated that the toxin was water soluble and heat stable. The toxins were present in roots of all ages and all asparagus cultivars tested. All asparagus cultivars tested were inhibited by the toxin. A range of other plant species were shown to be suppressed by asparagus storage root extract and some species were unaffected.

The level of toxicity in replant soils at two sites was monitored over a twelve month period using a lettuce seed, paper bioassay procedure. The toxin levels found in asparagus soils after the termination of the asparagus crop by cultivation was probably only high enough to directly inhibit replanted asparagus for a short time (up to five or six months) after terminating the crop. Autotoxins are likely to be present in old asparagus soils for many years following the termination of the asparagus crop and their importance in the replant problem is most likely to be as a result of an interaction with the pathogenic *Fusarium* spp. present.

Fusarium appeared to be the main factor involved in the replant problem and inoculum levels of pathogenic *Fusarium* spp. in soils are likely to be high for many years after asparagus cropping has ceased. In most cases the asparagus replant problem is therefore a replant disease that is likely to persist for many years.

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Section One

1 The Asparagus Plant.

1.1 Introduction

Asparagus *(Asparagus officinalis* L. subspecies *officinalis)* is an ancient crop first cultivated over 2000 years ago (Douglas 1990). It is a native of the eastern Mediterranean and Asia where it is found on the coastal sand dunes above the high tide mark (Hughes 1992). This natural habitat indicates the plants ability to thrive in well drained low fertility sites and also its ability to withstand strong winds, salinity, drought and temperature variations. The cultivated varieties today are strains of *A. officinalis* which differ little in general appearance or growth habit although, through local adaptation, they vary greatly in yield, size, shape and colour of spears, earliness, disease resistance and longevity (Hughes 1992).

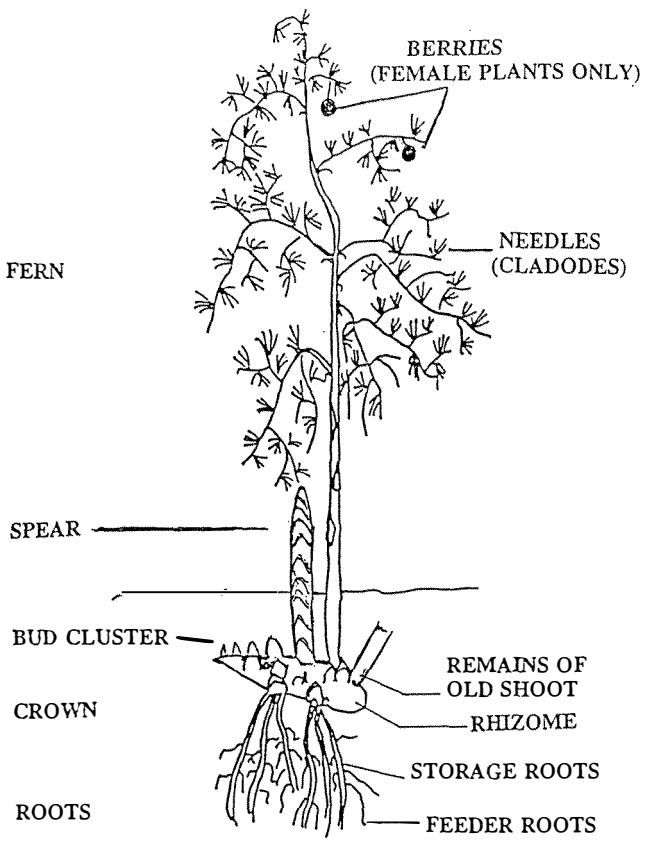
Approximately 215,000 ha of asparagus are grown world wide and world demand, particularly for fresh, green asparagus is increasing (Nichols 1990). Unlike most vegetables, asparagus is a monocotyledon and the main storage carbohydrates are fructans rather than starch. The genus Asparagus belongs to the family Liliaceae and there are approximately 300 species of which *A. officinalis* is the predominant one grown for food (Sudjatmiko 1993) although stems, ferns and berries of several species are eaten in their natural habitats and cultivated for floral displays. A tetraploid purple cultivar from Northern Italy, *A. officinalis* cv violetto d'albenga, is grown for spear production on a small scale.

1.2 Botany

Asparagus is a herbaceous perennial with erect foliage (fern) growing from an underground stem with many long fleshy storage roots. The underground parts are known as a crown. The stalks are smooth with many branches carrying small needle like cladophylls (the main photosynthetic organs), in whorls at the nodes. The leaves on the cladophylls are no more than very small scales (Mullendore, 1935).

Figure 1.1

Diagram of *A. officinalis* above and below ground parts.



The crown consists of many fleshy storage roots attached to an underground stem (rhizome) which consists of the closely spaced basal internodes of old stems, with clusters of buds at the growing tips. These buds become the aerial shoots which elongate and emerge in a definite order with the bud nearest the last formed spear being the next to elongate (Tiedjens, 1926).

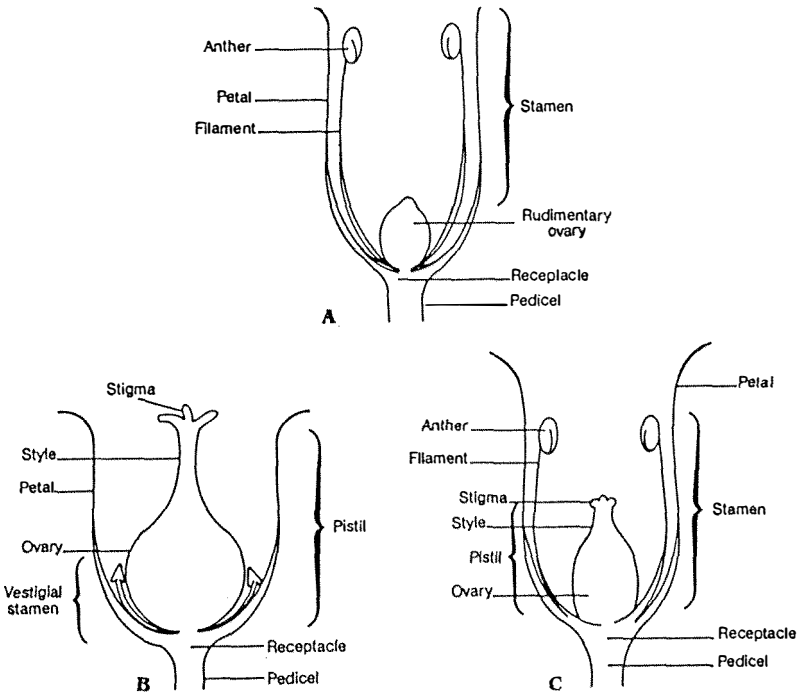
The fleshy roots are unbranched, grow up to 2 m in length, and are the plant's storage organs (Blasberg 1932, Yaeger and Scott 1938). The crowns of older plants exhibit branching as some lateral buds develop into clusters of buds that extend the crown in new directions. Each bud cluster, termed a crownlet, tends to grow independently of the rest of the plant, with the older (non-functional) parts of the stem decaying with time. New ferns and new roots are produced by the plant each year. The ferns die at the end of the growing season (at the onset of winter in temperate climates) and the roots remain to provide carbohydrate for growth the following spring. Older roots do eventually decay. Younger, recently developed storage roots are white and readily distinguished from the darker brown, rusty coloured older storage roots. Feeder roots are small hairlike structures that grow from the storage roots. They are formed during the spear and fern growth stage and are associated with younger newly developed storage roots.

A. officinalis is normally dioecious, ie. with male and female flowers borne on different plants. There are no obvious differences in the growth or vegetative morphology of male and female plants however male plants consistently produce a higher marketable yield and live longer than females in a commercial situation (Ellison et al 1960, Hughes 1992). Flower primordia contain both male and female structures and all the flowers are potentially hermaphroditic. Usually the stamens (females) or pistils (males) abort but in some cases a few hermaphroditic flowers form on andromonoecious plants which self pollinate and can thus give rise to homozygous andromonoecious plants or supermales (Ellison 1986).
A. officinalis has 10 chromosomes and is diploid (2n = 20). Normally triploid or tetraploid lines have not been cultivated commercially except for *A. officinalis* cv violetto d'albenga which is tetraploid and has the typical larger cladophylls, flowers and stems of polyploid plants (Ammal et al. 1966).

Sex expression in asparagus is controlled by a single gene factor dominant for maleness with female plants being homozygous (xx) and male plants heterozygous (xy) (Benson 1982).

The time before individual plants flower varies considerably but most plants produce flowers in their first or second growing season. In temperate climates female plants generally start flowering later than males (Ellison et al. 1960).

Figure 1.2 Diagram of male, female and hermaphroditic flowers (Ellison 1986).



(A) Normal flower of male asparagus. Note rudimentary sterile ovary. Anthers are yellow and produce much pollen. (B) Normal flower of female asparagus. Note vestigial stamens. Anthers are white and do not produce pollen. (C) Androgenous flower of andromonoecious asparagus. Such flowers are rare, are self-pollinated, and can set one or more viable seeds. Functional androgenous flowers are limited to 1–10 per andromonoecious plant.

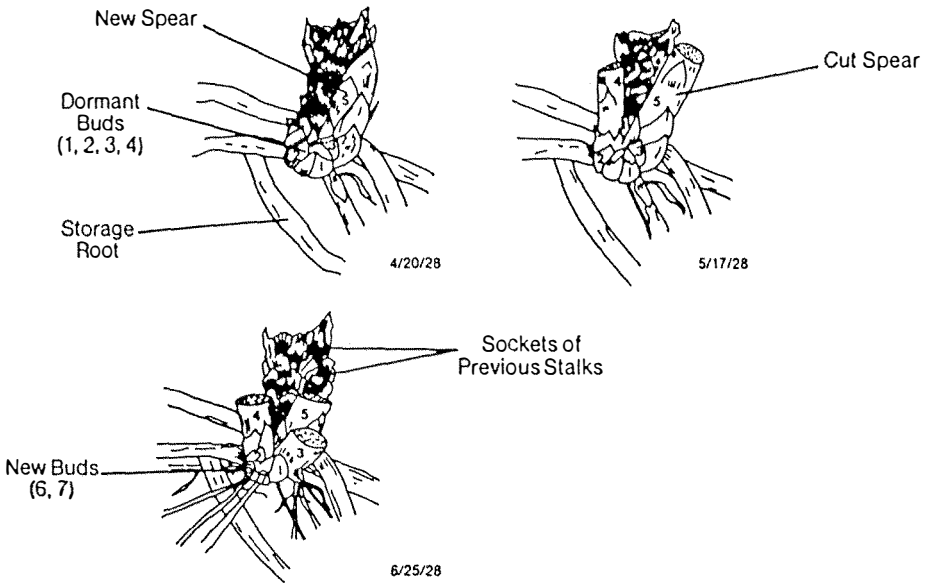
Drawing by J. J. Kinelski.

1.2.1 Seedling Structure and Development

Female plants normally flower and produce berries in their second growing season although some females will flower in their first season - at about six months of age. When mature berries are red and contain one to nine seeds. Seeds have a hard black testa and are 3-4 mm in diameter.

At the optimum temperature for seed germination (30°C) the hypocotyl will emerge within 10 days. In the field, in spring, germination and emergence usually takes 20-25 days. The hypocotyl develops into a 15-20 cm tall primary shoot with a diameter of 1-2 mm. The second bud arises from the axil of the basal scale leaf (bract) of the primary shoot. An axillary bud, to the side of the second spear, will then elongate to form the third spear. Another lateral axillary bud may be formed at the base of the second spear lying on another plane, although normally in the first season, growth is along one axis with the lateral buds remaining dormant. Successive buds and spears originate from alternate sides of the main growth axis so that two rows of spear or fern sockets are apparent on the crown or rootstock.

Figure 1.3 Diagram of bud cluster and pattern of bud development (Ellison 1986).



Three views of a bud cluster of a mature crown of asparagus showing the sequence of spear production and new bud development during the cutting season. Drawing by G. T. Nightingale.

1.2.2 Root Structure and Development

New storage roots are formed at the base of young actively growing buds, spears or ferns. These roots may grow up to 2m long depending on environmental conditions. The epidermal cells of older roots become suberised and as old roots decay only these epidermal layers and the lignified inner stele remain. The cortex is the main part of the root with the outer 6 to 8 layers of cells having walls thickened with suberin. The inner cortical cells have large inter-cellular spaces and these cells are the principle storage region of the plant (Blasberg 1932, Yaeger and Scott 1938). Approximately 2 storage roots are formed for every shoot that forms (Yen, 1993).

The secondary fibrous roots or feeder roots arise from the pericycle region just under the endodermis. The xylem occupies the greatest part of the vascular area or stele. The number of protoxylem strands varies from 7 to 18. Each xylem strand has at least one trachea. The xylem parenchyma cell walls are thickened with lignin so that old roots decay leaving a cylinder of lignified tissue. Each phloem strand has a few large sieve tubes and a large number of companion cells (Blasberg 1932).

The fibrous feeder roots are small in diameter and usually have a suberised outer layer. As their function is uptake of moisture and nutrients the cortex is much reduced compared with the storage organs.

1.2.3 Stem Structure and Development

In young plants as each bud develops to form an aerial shoot an axillary bud is differentiated and it will form the next spear either to the left or right of the axis of growth (Hughes 1992). As the plant gets older, buds differentiate more rapidly so that a cluster of successive buds forms at the growth terminal of the crown. On older plants occasionally one of the dormant lateral buds develops to commence growth in a new direction. This gives rise to crowns with several clusters of buds or crownlets developing independently and in different directions.

The bud size immediately prior to elongation indicates the potential size of the spear which will develop. Most of the differentiation occurs prior to elongation as evidenced by sections cut through young buds. Elongation first begins in the lower portion of the stem and continues upward so that the tip is still embryonic when the base is almost mature (Blasberg 1932).

Both the epidermis and the outer cortical layers of the stem contain chloroplasts but the epidermis contains few stomata. A cylinder of lignified fibres lies within the cortex and contributes to the support of the stem. Vascular bundles are numerous and scattered through the parenchyma or ground tissue (Blasberg 1932).

1.3 Commercial Production.

1.3.1 Establishment

Commercial beds of asparagus are established in three ways.

1) One year old crowns
2) Seedling transplants
3) Direct seeding.

1.3.1.1 Crown transplants.

Crowns are commonly grown in nursery ground at densities of 200,000 to 250,000 per ha. Seed is spring sown and crowns are dug when dormant in the winter or early spring. Crowns are transplanted to commercial beds in 150 mm deep trenches 1 to 2 m apart with in-row spacings of 250 to 400 mm to give a population of 20,000 to 30,000 plants per ha.

1.3.1.2 Seedling transplants.

Seedlings are produced in the greenhouse in cells and planted in the field eight to twelve weeks from sowing as an actively growing seedling with four or more shoots and a small crown of eight or more storage roots.

1.3.1.3 Direct seeding.

Seed is sown directly into the production area normally at a rate higher than the population required for the commercial planting. Sowing is normally done in furrows so that the crown will be positioned below the soil surface after cultivation. Direct seeding is not commonly undertaken in New Zealand.

The main advantages of crowns over both seedlings and direct seeding are that the management of irrigation, weed control and insect control are less critical as the grower is dealing with a more robust plant. The positioning of the plant well under the soil surface is also easier with crowns than with seedlings or seed.

Seedlings have the advantage of being easier, cheaper and faster to produce. Garrison (1977) has suggested that seedlings might be less suitable in an asparagus replant situation since the younger plants have a greater susceptibility to *Fusarium* spp. Conversely Falloon (1990) suggests that seedlings are less susceptible to *Phytophthora* spp. mainly as they are normally planted later in the season and shallower in the soil.

1.3.2 Replanting Asparagus Soils

1.3.2.1 Introduction

In many traditional asparagus producing areas of the world the availability of land suitable for asparagus production is limited (Grogan & Kimble 1959; Yang 1982; Huiskamp & Kanters 1989; Blok & Bollen 1993, 1996(a)). In New Zealand there are about 3000 ha of asparagus in production. New Zealand's production is only 2% of the world total but compared to other vegetables produced here this level is high. Annual production is about 8000 tonnes of which approximately 4000 tonnes are processed for export and 1500 tonnes are exported fresh. Domestic fresh consumption is about 2000 tonnes and the domestic market consumes about 500 tonnes of processed asparagus.

Much of the area that is in production in this country is planted with old cultivars and is nearing the end of its commercial life (Falloon & Fraser 1990).

Many New Zealand growers see replanting of existing stands as the best means of changing to new cultivars of superior quality and capable of two to three times the yield of those currently being grown. As many growers do not have fresh land available, replanting, if it can be done successfully, may provide the most attractive means of staying in asparagus production and changing from old declining stands to new higher yielding stands. However further areas of free draining soils suitable for asparagus production do exist.

1.3.2.2 Asparagus Decline.

There are numerous reports of declining productivity from old asparagus plantings (Grogan & Kimble 1959; Yang 1982,1986; Gordon-Lennox & Gindrat 1987; Peirce & Colby 1987). It is generally accepted that, in temperate climates, asparagus stands reach peak production after five to eight years and slowly weaken thereafter so that the commercial life is ten to twenty years (Johnson et al. 1979; Falloon & Tate 1986; Bussell & Ellison 1987).

Grogan and Kimble (1959) have defined asparagus decline as a gradual decrease in productivity of the stand to a point where it is no longer profitable to maintain. They described typical symptoms as; a reduced number of fern stalks and thinner stems; spears shrivel or wilt at any stage of growth; ferns are usually yellowed and stunted with reddish brown vascular discolouration and rusty flecks or lesions on the external layers of the lower portion of fern stalks; reddish discolouration may extend into the crowns from fern sockets or from discoloured fleshy roots; feeder roots may be absent or shrivelled and where present, show discoloured, reddish areas at the junction with fleshy storage roots; some of the fleshy roots show vascular discolouration and many collapse completely with soft rot bacteria or secondary organisms usually associated with such collapse.

In all situations where asparagus decline is reported, isolations have confirmed that *Fusarium* infections are associated with the disorder (Grogan & Kimble 1959; Conroy 1975; Johnson et al. 1979; Fantino 1990).

Of the species of *Fusarium* that cause infections in asparagus, *F. oxysporum* (Schlecht) emend Snyd. and Hans. *f. sp. asparagi* Cohen, is considered to be the most important pathogen associated with asparagus decline. *F. moniliforme* (Sheldon) emend Snyd. and Hans. is also commonly isolated and, although not as prevalent as *F. oxysporum*, it is generally considered to be part of the complex (Cohen & Heald 1941; Endo & Burkholder 1971; Hartung & Putnam 1986).

F. oxysporum is a vascular wilt pathogen and the symptoms associated with infections of asparagus are as described for the symptoms of asparagus decline. It is the most frequently isolated *Fusarium* spp. found in vascular root tissue, cortical root lesions, crown and stem lesions, and stem vascular tissue. *F. moniliforme* is less commonly isolated from roots but is frequently isolated from stem and crown tissue where it causes a more invasive, dry, brown rot than *F. oxysporum.*

Most researchers agree that the asparagus crown/root rot complex associated with declining stands is caused by either or both of these pathogens acting in combination. (Grogan & Kimble 1959; Falloon & Tate 1986).

More recent research on the effect of Asparagus Virus 2 (AV2) on asparagus in New Zealand indicates that the symptoms of AV2 infection of asparagus plants are the same as those described for asparagus decline (Jaspers & Falloon 1997).

It is possible that AV2 infection directly causes asparagus decline and the *Fusarium* spp. are opportunists that infect the plants under stress from AV2 infection. Alternatively AV2 infection may predispose asparagus to *Fusarium* infection and thus give rise to decline symptoms. In either case the result of AV2 and *Fusarium* infection is a reduction in plant vigour, reduced spear size and a reduction in yield through a decrease in spear number and size of spears and a reduced plant population as plants with severe decline symptoms die out.

Johnson et al. (1979) studied the incidence of *F. oxysporum* and *F. moniliforme* in asparagus beds of differing ages and found that *F. oxysporum* tended to predominate in young stands whereas *F. moniliforme* was the predominant species in older stands. Endo & Burkholder (1971) also suggested that *F. moniliforme* tends to be more prevalent in older stands. In the Netherlands *F. oxysporum* was more commonly isolated from asparagus roots crowns and stem bases than *F. moniliforme* (Blok & Bollen 1995) and the two species were the main pathogenic fungi present. Blok and Bollen (1996(b)) found *F. oxysporum* was the most common pathogen isolated from replanted asparagus and it incited a disease they termed asparagus replant-bound early decline (ARED).

The means of spread and survival of these two *Fusarium* species explains their different incidence in asparagus stands of differing ages and histories. Both *F. oxysporum* and *F. moniliforme* are seed borne as conidia in cracks or fissures in the asparagus seed testa. *F. oxysporum* is the more common of the two however and young plants infected from seed contamination are frequently infected with *F. oxysporum* (Inglis 1980; Manning et al. 1980). *F. oxysporum* is ubiquitous and survives in the soil as a saprophyte on decaying organic matter, and also as chlamydospores which remain viable for many years.

In a newly established block of asparagus the inoculum level increases over time as the amount of host material (asparagus roots, crowns and stems) increases. In seed blocks the number of spores increases with time and hence the level of seed contamination tends to be higher from older seed blocks than from younger ones (Gilbertson & Manning 1983). *F. oxysporum* spreads slowly in soil: for example Grogan and Kimble (1959) demonstrated that 20-25 mm movement towards host material occurred after one month. *F. moniliforme* on the other hand, produces airborne conidia in sporodochia on the stems of infected plants and can thus spread rapidly in a field of asparagus. *F. monilforme* also survives in the soil as resting mycelium that has thickened and darkened walls (Booth 1971). Strains of *F. moniliforme* that are pathogenic on asparagus can also survive on maize and sweetcorn and thus cause severe disease in subsequent asparagus plantings (Damicone & Manning 1980). Some research suggests that the strains of *F. moniliforme* that show cross pathogenicity between corn and asparagus are not

highly virulent on asparagus in the laboratory and may not cause disease in the field (Elmer pers. comm.).

The virulence of isolates of *F. oxysporum* and *F. moniliforme* varies between strains but invariably isolates of *F. moniliforme* produce the more aggressive or invasive rot of asparagus root and crown tissues. It is reasonable to assume then that *F. moniliforme* is of major importance in the asparagus decline syndrome and is also implicated in re-establishment failures (Endo & Burkholder 1971; Johnson et al. 1979).

Fusarium proliferatum and *F. subglutinans* were taxonomically synonymous with *F. moniliforme* using Snyder and Hansen (1940) taxonomy but have been separated from *F. moniliforme* on the basis of their conidiophore morphology (Nelson et al. 1983). *F. proliferatum* produces microconidia in chains and false heads on polyphialides and monophialides whereas *F. moniliforme* has only monophialides. *F. subglutinans* produces microconidia only in false heads on both monophialides and polyphialides. The three species have all been implicated as incitants of *Fusarium* crown and root rot in asparagus (Elmer & Ferrandino 1992).

In South Africa *F proliferatum*, *F. oxysporum*, and to a lesser extent *F. solani* were the fungi found to be most commonly associated with asparagus decline (Schreuder et al 1995).

1.4. The Replant Problem

1.4.1 Introduction

Where asparagus plants have declined, it is difficult if not impossible to re-establish a commercially profitable stand. Reports from Asia, Australia, North America and Europe all indicate that replanting land to asparagus leads to establishment failure and reduced plant vigour. Where re-establishment is achieved, the economic life of the stand is shorter than with fields established on land that has not previously been in asparagus (Grogan & Kimble 1959; Conroy 1975; Young 1984; Huiskamp & Kanters 1989; Blok & Bollen 1993).

Earlier reports on the asparagus replant problem have attributed the failure of new plants to establish and thrive to the carry over of *Fusarium* spp. The symptoms on plants that fail in a replant situation have often been identical to symptoms associated with the asparagus decline and *Fusarium* root and crown rot complex. It appeared reasonable to assume then that *Fusarium* infections in a replanting situation are one of the major causes of the replant problem, particularly where stands with severe decline symptoms were replanted. Grogan and Kimble (1959) report trials where direct replant seeding of asparagus gave low seedling emergence, and wilting and death of all seedlings within 3 months, with typical *Fusarium* wilt and root rot symptoms.

More recent work has shown that, while chemical fumigation or soil sterilisation improves establishment in a replant situation (presumably by eliminating or reducing the *Fusarium* inoculum) the effect is only short lived and the replant problem still persists to some extent (Yang 1982; Poll pers. comm.; Blok & Bollen 1993). Many studies have shown that asparagus tissue and asparagus plant residues contain a toxic substance that is inhibitory to growth of asparagus plants (Hartung & Putnam 1986; Hartung et al. 1989; Young & Chou 1985).

Studies by Proebsting and Gilmore (1941) and Chandler and Daniell (1974) indicate that a replant problem with stonefruit was most likely due to a water soluble toxin released from the roots and bark that was taken up by the newly planted trees, reducing the vigour of the new trees and predisposing them to attack by microorganisms.

1.4.2 Potential major causes of replant problems.

1.4.2.1 Autotoxins

Laufer and Garrison (1977) reported toxicity of asparagus tissue to the growth of asparagus and other vegetable seedlings. Yang (1982) showed that extracts from asparagus tissue that had been dead for at least eight months in the field still contained substances that retarded development of asparagus seedlings, and that the autotoxin was heat stable and water soluble.

More recent reports describe how crude extracts from asparagus roots have a negligible effect on radicle emergence but dramatically reduce radicle elongation of asparagus and several other plant species (Hartung & Stephens 1983; Hazebroek et al. 1989; Shafer & Garrison 1986).

An interaction of root autotoxin with the two common *Fusarium* spp. affecting asparagus was first reported by Hartung and Stephens (1983). They found that neither *Fusarium* nor root fragments alone reduced asparagus seedling dry weights appreciably, but when combined they significantly decreased dry weight and increased seedling root rot. Subsequent trials by Peirce and Colby (1987) demonstrated that the autotoxin in asparagus residues predisposed young roots and shoots of germinating asparagus seedlings to *Fusarium* infection and that at low rates the toxins and *Fusarium* had a synergistic effect. One could conclude that this synergism would be accentuated in the field as the pathogenic effect would result in more root deaths with subsequent increases in autotoxin release. Hazebroek et al. (1989) used various extraction techniques in an attempt to characterise the autotoxin. Their work suggested that more than one compound was involved and that the compounds were phenolic in character. Lake et al. (1993) showed that the phenolic and saponin components of asparagus roots were inhibitory but the most inhibitory material was located in the amino acid / carbohydrate fraction. Work by Miller et al. (1991) and Hartung et al. (1990) has identified ferulic, isoferulic, malic, citric, fumaric and caffeic acids as phytoinhibitory components of asparagus root extracts.

The importance of the toxins in the replant problem appears to be related to the effect they have in predisposing asparagus roots and stems to attack by pathogens (Peirce & Miller 1990; Miller et al. 1991). Hartung and Stephens (1983) postulated that either more sites on the asparagus roots were colonised by *Fusarium* in the presence of the toxins or that the *Fusarium* can spread more rapidly once fungal penetration has taken place when the roots are exposed to toxins. Either explanation implies a breakdown of the plant's natural defence mechanisms in the presence of both the autotoxic compounds and *Fusarium* inoculum.

The work of Hazebroek et al. (1989) also indicated that the autotoxic components of asparagus tissue are biologically active against a wide range of organisms. Extracts of asparagus tissue have reduced the growth of bacteria, fungi and nematodes.

This wide range of biological activity indicates that synergism between autotoxins and *Fusarium* in asparagus tissue may not be a simple relationship, but could involve the inhibition of other antagonistic microorganisms in the rhizosphere as well as breaking down the plant's natural defence system. Ferulic acid has been shown to inhibit hyphal elongation *in vitro* and fungal root colonisation *in vivo* of the vesicular-arbuscular mycorrizhae coloniser of asparagus, *Glomus fasciculatum* (Wacker et al. 1990). This suggests that one of the autotoxic products of asparagus roots could reduce the symbiotic effectiveness of mycorrizhal fungi and reduce plant growth in this way.

There is ample evidence that asparagus roots produce a water soluble phytotoxin. In the laboratory it is possible to wash the toxin out of a soil with sufficient water. However in the field, a large reservoir of roots and crown material is present that breaks down slowly. It is reasonable to assume that in the field, unless the old plants are physically removed entirely (an uneconomic operation), autotoxins may be present for some years as the old root and crown material slowly breaks down (Blok and Bollen 1993,1996(a)).

Work described in section 4 investigates effect of the toxin on other plant species and measures the toxicity of field soil over a 12 month period.

1.4.2.2 Herbicide residues

The situations where herbicide residues might affect replant behaviour are when relatively insoluble or strongly adsorbed residual herbicides have been used on soils high in clay or organic matter. As asparagus is typically grown on free draining soils, herbicides are not likely to persist at phytotoxic levels beyond 12 months, so provided adequate planning precedes replanting, herbicide residues are not likely to pose a threat to establishing plants.

Where herbicide residues are likely to be present eg. where long lasting residuals have been recently applied, a 12 month wait and thorough, deep cultivation should be sufficient to allow breakdown of the residues (Holly & Roberts 1963; Anderson 1977). The pot trials (section 3) in this work show inhibitory effect of low levels of residual herbicides on young asparagus.

1.4.2.3 *Fusarium*

1.4.2.3.1 Chemical Control.

Economic methods have yet to be developed for chemical control of *Fusarium* in established stands (Yang 1982; Gordon-Lennox & Grindrat 1987; Elmer et al. 1996). This is not surprising as control of a soil-borne fungus by chemical means requires treatment of the entire rhizosphere soil volume or maintenance of an effective level of fungicide within the plant roots. The increasing consumer demand for reduced pesticide use in food production is placing more emphasis on research efforts to control disease by alternative means.

Work by Falloon & Fraser (1990) has shown it is possible to achieve some control of *Fusarium* on establishing asparagus crowns using an acid formulation of thiabendazole. In the presence of high levels of inoculum however, this effect may only be short lived as the developing roots grow out of the protected area around the treated crown.
Field trials described in section 2 show the effect of fungicides on seedlings and crowns planted in a replant site. Some of the work in section 3 shows the effect of fungicides and NaCl on the level of *Fusarium* infectivity in old asparagus soil.

1.4.2.3.2 Disinfestation

Soil sterilisation using chemicals, steam or solarisation could eliminate many of the pathogenic microorganisms. Greenhouse Trials have shown growth of replants to be better in sterilised soil but still not as good as in sterilised virgin soil (Poll & Huiskamp 1990; Blok & Bollen 1993), the superiority of the virgin soil performance being attributed to the lack of autotoxins. As it is difficult to eliminate all *Fusarium* from planting material and as large amounts of host material are present in a replant situation, sterilisation is not expected to achieve long-term control of *Fusarium*. Other problems with sterilisation as a cure for the replant problem are expense on a field scale and the likelihood of escapes reducing the effectiveness of the treatment. Soil sterilisation also creates a biological vacuum which can readily be invaded by a new population of plant pathogens. Any sterilisation treatment should be followed by the reinoculation of the young plants with the mycorrhizal fungi required by asparagus for enhanced nutrient uptake particularly in soils with low levels of plant available phosphate (McCormick & Thomsen 1989).
The use of thiabendazole to control *Fusarium* in established stands of asparagus has shown limited success (Falloon & Fraser 1990) however as a means of control of the

decline and replant problem it is not favoured, as the cost of treatment is excessive and soil drenches have shown little control of the disease.

F. oxysporum and *F. moniliforme* are seed-borne. Seed treatments have been used with varying degrees of success. Damicone et al. (1981) showed that soaking seeds for 24 hours in an agitated mixture of 25g/litre benomyl in acetone gave complete control, however the same treatment used by Tate (pers. comm.) did not give complete control. The standard seed treatment in New Zealand at present is dusting with benomyl and thiram however this does not give complete control either. Falloon & Fraser (1990) suggest that *Fusarium* free seedlings can be grown using an acid formulation of thiabendazole as a seed treatment. The seeds must then be grown in sterile soil or potting media to avoid infection by the ubiquitous *F. oxysporum* following germination.

Pot trials described in section 3 show the effect of soil sterilisation on growth of plants in replant soil.

1.4.2.3.3 Resistance/Tolerance to *Fusarium*

Asparagus cultivars resistant to *Fusarium* spp. offer a possible means of avoiding this aspect of the replant problem. However, despite many years of breeding for resistance to *F. oxysporum* and *F. moniliforme*, resistant cultivars have not been developed (Bussell & Ellison 1987). Field screening in New Zealand of 175 cultivars and advanced breeding lines, 45 of which were the result of crosses between plants selected for resistance to *F. oxysporum* and *F. moniliforme*, showed that progeny from parents selected for *Fusarium* resistance were amongst the most susceptible lines tested (Bussell & Ellison 1987). The greater tolerance to crown and root rot of several parents selected for *Fusarium* resistance in the field was probably due to increased vigour rather than any genetic resistance or specific defence mechanism. Elmer et al. (1990) attributed failure to breed resistant lines to the existence of genetically-distinct strains of *Fusarium* found infecting *Asparagus*. Certain *Asparagus* spp. have been shown to be immune to *Fusarium*. *A. densiflorus* cv. Sprengeri is highly resistant to *F. oxysporum* (Lewis & Shoemaker 1964), however *A. officinalis* (2n=20) and *A densiflorus* (2n=60) are not closely related and hybridisation to improve resistance in *A. officinalis* has proved unsuccessful to date. The use of gene transfer vectors such as *Agrobacterium tumefaciens*, has been explored to transfer genes into *A. officinalis* but transformation efficiency has been low. The use of particle gun techniques is thought to hold some promise in successfully transferring genes into *A. officinalis* (Eberhardt et al. 1997).

In vitro cell selection processes that identify novel mutations may also provide a means of selecting variants with desirable resistance traits (Ganeshan et al. 1997).

An important aspect of *Fusarium* tolerance in asparagus is the vigour of the plant. This has implications in the replant situation as any aspect of establishment that can enhance the vigour of the establishing plants will help limit the effect of *Fusarium* infections (Nigh 1990; Elmer et al. 1996). Such things as weed control, adequate moisture and fertility, insect control and control of other diseases such as *Stemphylium* and *Phytophthora* might be expected to minimise the effect of *Fusarium*, but these alone cannot control the disease in the presence of autotoxins and a high level of inoculum in a replant soil.

1.4.2.3.4 Rock Salt

The use of sodium chloride (rock salt) to improve the vigour of declining asparagus and reduce the level of *Fusarium* infections is being investigated in New Zealand and the U.S.A. Elmer (1989; 1992; 1990; 1997) found that increased yields were achieved in declining asparagus stands using 560 to 1120 kg/ha of rock salt. The form of nitrogen fertiliser applied with the rock salt also had an effect on disease levels. A soil pH of 6 or higher was also necessary before a response to rock salt was evident (Elmer 1997).

Falloon and Fraser (1990) have also shown that slight yield increases are achieved using rock salt at 560 kg/ha at pre-harvest on established asparagus.

1.4.2.3.5 Biological Control.

Biological control measures (such as inoculation with a non-pathogenic strain of *F. oxysporum*) have been shown to protect plants against crown rot development in the field for up to eight weeks (Damicone & Manning 1982).

Armstrong and Armstrong (1969) identified several races of *Fusarium* that showed cross-pathogenicity for various crops but were non-pathogenic on asparagus, and showed potential for the protection techniques demonstrated by Damicone and Manning (1982). Research in Taiwan with soil amendments has shown that a mixture consisting of rice husks, bagasse, oyster shell, urea, potassium, nitrogen, calcium, phosphate and mineral ash suppressed natural populations of *Fusarium oxysporum* and reduced both the incidence and severity of the disease (Sun & Huang 1985; Tu et al. 1990). The mechanisms involved are not well understood but these workers found that the soil amendment inhibited conidial germination and enhanced germtube lysis of both *F. moniliforme* and *F. oxysporum*. This result could be a direct effect of the mineral components of the amendment or an indirect effect through modification of the microbial population. Scher and Baker (1980) found a species of *Pseudomonas* that suppressed *Fusarium* infections in soils with high

inoculum levels. Other research into biological control by exposure of the pathogen to other fungal communities has shown some promise (Marrois et al. 1981; Marrois & Mitchell 1981). It appears that biological control may offer a potential means of controlling *Fusarium* infections in asparagus for prolonged periods, if an antagonist or non pathogenic stable strain can be found.

Use of *Trichoderma viride* mycelium as a crown dip to enhance establishment in a replant situation has been trialed as described in section 2.

1.4.3 Potential minor causes of replant problems.

1.4.3.1 Phytophthora

A survey of asparagus fields in New Zealand in 1986 showed that 68% of established beds of asparagus were affected to various degrees by Phytophthora rot (Falloon & Fraser 1991) caused by *Phytophthora megasperma* var. *sojae* which has been shown to cause establishment failures particularly in wet conditions and in a replant situation (Gordon-Lennox & Grindrat 1987; Falloon & Fraser 1991). Presumably where asparagus is being replanted into old asparagus soils large numbers of oospores of *Phytophthora* are present and germinate in the presence of fresh host material if conditions are wet enough. Although Phytophthora root rot is not widely reported as being important in asparagus decline, losses through this disease in a replant situation can be dramatic and readily differentiated from *Fusarium*.

Falloon & Fraser (1991) showed that during establishment almost complete control of Phytophthora rot can be achieved. Control measures include;

- avoidance of wet conditions at or soon after planting crowns.
- use of seedling transplants to avoid cool wet conditions often encountered by crown transplants.
- crown dip and seedling drench with metalaxyl.

As a result of this research it is possible to control Phytophthora rot during replanting so that this possible cause of the replant problem can be eliminated.

1.4.3.2 Asparagus Virus 2

AV2 predisposes plants to *Fusarium* infection (Falloon & Tate 1986). The elimination of this virus from seed and from cloned propagating material should improve longevity of stands by reducing susceptibility to *Fusarium* spp. An understanding of the mechanisms involved in the AV2/ *Fusarium* interaction in asparagus plants may help in avoiding *Fusarium* infections. Jaspers & Falloon (1997) demonstrated the effect of AV2 on the vigour of field grown asparagus in New Zealand. The symptoms of AV2 are identical to those of asparagus decline reported to be caused by *Fusarium* spp. and it is possible that infection by the virus incites decline symptoms in asparagus plants and the stress induced by the virus allows *Fusarium* spp. infections to take place.

1.4.3.3 Soil structure

Soil structure may be involved in the asparagus replant problem. Work on the specific replant disease of stone fruit by Hein (1980) indicated that the problem could be overcome by improving the soil structure by deep cultivation. This work also implicated a build up of noxious soil microorganisms in the stone fruit replant problem, and sterilising replant soils tended to improve growth of replanted root stocks.

Soil structural problems in an asparagus replant situation are unlikely to be a major factor as was found with stonefruit by Chandler & Daniell (1974). Where soil structural problems are encountered in an old asparagus stand or at the time of replanting it would be possible to correct these by appropriate cultivation techniques such as subsoiling to break up impermeable layers and allow water infiltration.

1.5 Replant problems in other crops.

Replant problems in agriculture and horticulture are not uncommon and arise in the main through a close rotation or monoculture although such replant situations will not always lead to replant problems.

Replant problems are soil borne and are often very complex. Replant diseases are often the causes of replant problems and can be specific and very persistent such as the potato cyst nematode which can survive in the soil for several years as cysts and for which the potato is practically the only host (Hoestra, 1994). The persistence of replant diseases is often a result of specific and persistent resting stages of the pathogen such as cysts or chlamydospores but also arises when the parasite enters a passive waiting stage such as the saprophytic behaviour of pathogenic fungi surviving on plants other than the host without causing undue damage. Another

example of the replant disease persistence is the nematode *Pratylenchus penetrans* which is polyphagous, feeding on many crops without causing much damage but apples and other rosaceous species are very susceptible and the nematode significantly reduces their growth.

The causes of replant problems are often divided into biotic causes which are called replant diseases, and may be incited by fungi, actinomycetes, bacteria, nematodes or interactions of these, or abiotic causes of which the most common are phytotoxins, nutrient imbalance, soil pH problems, herbicide residues or soil structure and drainage problems (Utkhede & Smith 1994(a)). Replant diseases are generally controlled by soil fumigation, soil sterilisation or soil pasteurisation thus supporting the 'biotic cause' theory.

1.5.1 Replant problems of apples.

Replant problems causing reduced vigour and uneconomic yield in newly planted apple trees have been known for many years.

A specific apple replant disease (SARD) has been distinguished but a single causal organism has not been identified. Causes of SARD that have been identified in literature include *Pythium* spp., *Peniophore sacrata*, *Cylindrocarpon* spp., Actinomycetes, Bacteria and massive invasions by mycorrhizae (Hoestra 1994; Utkhede & Smith 1994(a)).

Replant problems of apple trees with abiotic causes have been attributed to deficiency of certain soil nutrients, toxicity caused by excess Mn or Al, high soil pH and hard pans at the sub-soil level (Utkhede & Smith, 1994(a)).

In most apple growing areas apple replant diseases must be controlled in order to establish high yielding, profitable new plantings of modern varieties. The most common successful control methods involve removing the old trees, deep ripping, soil fumigation with methyl bromide or metham sodium and supply of adequate moisture and nutrients to the new planting to avoid stress during establishment (Peterson and Hinman 1994; Smith 1994).

It is clear that the apple replant problem is a very complex phenomenon and the factors involved in specific growing areas must be studied before lasting control methods are developed. Current research into possible biological control measures is encouraging and may provide the best long term means of control of the apple replant problem (Utkhede & Smith 1994(b); Catska & Taube-Baab 1994).

1.5.2 Replant problems of stonefruit.

Stonefruit replant problems have been attributed to biotic and abiotic factors as with apples. Actinomycetes, bacteria, fungi and nematodes have all been implicated in replant diseases in peaches (Gur & Cohen 1988).

Soil structural problems have also been involved in the replant problem of stonefruit in some instances (Hein 1980) There has been much research on the production of allelochemicals by peaches and the autotoxicity problems of old peach orchard soils (Tagliavini & Marangoni 1994) Peach roots contain a cyanogenic glucoside, prunasin, which when hydrolysed during decomposition of peach roots yields HCN (hydrocyanic acid) and benzaldehyde. These products of the decomposition of peach roots are toxic to the growth of new peach plantings and the toxicity is apparently specific to members of *Prunacea* so apple trees can be planted in old peach soils without encountering the same replant problem (Cohen & Gur 1988).

The replant problem in stonefruit appears to be as complex as any other replant problem with many soil factors involved and possible interactions.

1.6 Summary of the asparagus replant problem

Asparagus is a perennial crop which can remain in production for more than 15 years. Reports from throughout the world indicate that replanting old asparagus stands often leads to establishment failure and reduced plant vigour and where re-establishment is achieved the economic life of the stand is often shorter than on land not previously used for asparagus. This phenomenon is widely known as the 'replant problem'.

Early reports (Grogan & Kimble 1959) on the asparagus replant problem attributed the failure of new plants to establish and thrive to the carry-over of *Fusarium* spp. The symptoms on plants that fail in a replant situation have often been identical to symptoms associated with asparagus decline and the *Fusarium* root and crown rot complex. It has often been assumed that *Fusarium* infection in a replant situation is one of the major causes of the replant problem, particularly where stands with severe decline symptoms were replanted. The symptoms of decline of asparagus in a site with no history of asparagus production are similar to the symptoms of early decline of asparagus in a replant site (Blok & Bollen 1996 (b)).

More recent work (Blok & Bollen 1993) has shown that although chemical fumigation or soil sterilisation can improve replant establishment (presumably by eliminating or reducing the *Fusarium* inoculum) the effect is short lived and the replant problem persists to some extent.

Many studies (Hartung & Stephens 1983; Hazebroek et al. 1989; Shafer & Garrison 1986) have shown that asparagus tissue, asparagus plant residues, and soils from old asparagus beds contain substances which are inhibitory to asparagus plants. There is strong evidence that the toxic material, often called an autotoxin, is located in the amino acid/carbohydrate fraction of the asparagus roots, but the phenolic and saponin components of asparagus roots have also been shown to be phytotoxic.

Studies have shown that the importance of toxins in the replant problem is related to the effect they have in predisposing asparagus roots and stems to attack by pathogens (Peirce & Miller 1990; Miller et al. 1991).

Residual herbicide effects are also likely to be involved in replant failures or suppression in some cases. Quite strong and long lasting herbicides are used with asparagus and there is ample evidence to show that some herbicide residues can suppress asparagus plant growth as well as predisposing them to attack from fungal pathogens particularly *Fusarium*.

1.7 Objectives

The objectives of this research are to:

a) Measure the magnitude of the replant suppression in two old asparagus fields.

b) Assess the importance of herbicide residue, *Fusarium* and autotoxins as potential major causes of asparagus replant problems.

c) Develop bioassays which will predict the likelihood of replant problems in specific field situations.

Section Two
Field Trials

2.1 Introduction.

It is widely reported that asparagus planted in a site that has previously grown asparagus has poor survival and poor yield compared with a virgin site (Grogan & Kimble 1959; Conroy 1975; Young 1984; Huiskamp & Kanters 1989; Blok & Bollen 1993, 1996(a)).

These observations are anecdotal and no reports of studies which quantify differences between replanted fields and virgin sites have been reported in the literature.

The replant problem has been attributed mainly to a carryover of soil-borne inoculum of *Fusarium* spp. and to the toxic material associated with the residue of previous asparagus plants (Grogan & Kimble 1959; Young 1984; Young & Chou 1985; Huiskamp & Kanters 1989; Hartung et al. 1989). Herbicide residues carried over from previous plantings have also been suggested as a cause of the replant problems (Davison & Clay 1972).

Five field trials are described in this chapter:

1) A comparison of growth and yield of crowns and seedlings planted after various fungicide treatments on a replant and adjacent virgin site. Different soil preparation treatments were also compared to minimise the release of toxic material from the previous crop.

2) Growth and yield comparison from crowns following different fungicide treatments on a second replant soil and adjacent virgin soil.

3) An investigation of the effectiveness of a biological control agent in a replant situation.

4) An assessment of the performance of different cultivars in a replant site.

5) An evaluation of the effectiveness of rock salt and fungicide applications to a declining asparagus planting.

2.2 Field Trial No. 1

2.2.1 Objectives.

To determine:

1. The difference in survival and yield of asparagus planted on a site with no history of asparagus production compared with that on an adjacent site with a long history of asparagus production.

2. The effect of crown transplants and seedling transplants in a replant or virgin site.

3. The effect on the replant problem of cultivating the old crop into the soil or of leaving the old plants intact and planting between the rows.

4. The effect of thiabendazole as a crown dip or seedling drench on *Fusarium* crown and root rot in a replant site.

2.2.2 Materials and Methods.

The trial site was an alluvial-based deep (> 2 m) silt loam soil in Twyford, Hawkes Bay. The replant site had been used for the production of asparagus (cv NZ Beacon) for eight years. The most recent residual herbicide applications to the site was 3200 g a.i./ha of diuron on 15 December 1988 and 8 l/ha Caragard (2000 g a.i./ha each of terbuthylazine and terbumeton) on 18 December 1987.

The virgin soil area was separated from the old asparagus area by an open drain and was the same soil type but with no history of asparagus production or residual herbicide use.

2.2.2.1 Ground preparation.

The old asparagus plants on the replant site were destroyed by chopping the plants with three passes of a rotary hoe, first at 50 mm depth then 50 mm deeper on each pass. The area was then deep ripped to a depth of 500 mm and finally rotary hoed to a depth of 200 mm.

The virgin soil areas were deep ripped to 500 mm and rotary hoed to a depth of 200 mm.

Where the old asparagus plants were left intact, a 1 m wide strip between the rows of old plants was cultivated to a depth of 300 mm using rippers followed by a final pass with a rotary hoe set 150 mm deep.

2.2.2.2 Planting Material.

Both crowns and seedling transplants were produced from seed that had been surface disinfested by dusting with thiram (Thiram 80W) and benomyl (Benlate).

Crowns were produced in the field by direct seeding four rows of seed into beds at 1800 mm centres 300 mm between rows and 60 mm within the rows to give a plant population of 150,000/ha. The seed was sown 30 mm deep into a stale seedbed that had 250 kg/ha Cropmaster 12:10:10 N:P:K fertiliser incorporated when the beds were formed, three weeks before sowing. Following sowing on 3 October 1988, 35 mm of water was applied using a travelling irrigator and two further irrigations (40 mm each) were applied on 15 December 1988 and 12 January 1989. Weed control was with metribuzin (400 g a.i./ha) and diuron (1600 g a.i./ha) applied 18 days after sowing but prior to seedling emergence. Some hand weeding was also required. During the growing season two applications of iprodione (1000 g a.i./ha) were made (2 February and 11 March 1989) to control *Stemphylium.*

The fern on the crowns died back in June 1989 and the crowns were harvested in August 1989 using a potato harvester to lift them from the soil. The crowns were separated by hand, the soil shaken from the roots, and they were then stored in wooden bins in a coolroom at +4°C until planting.

Seedlings were grown in peat in 75 mm deep root trainers in a greenhouse. Seed was hand sown, 10 mm deep, one per cell on 1 August 1989. The seed trays were watered daily and a liquid feed supplying 100 ppm N, 135 ppm P and 100 ppm K was applied three times each week.

2.2.2.3 Experimental Design.

The experiment was a randomised split plot design with four replicate blocks.

Main plots were the ground preparation method, old plants cultivated in or left intact.

Sub plots were the plant material treatments, crowns or seedlings with or without fungicide.

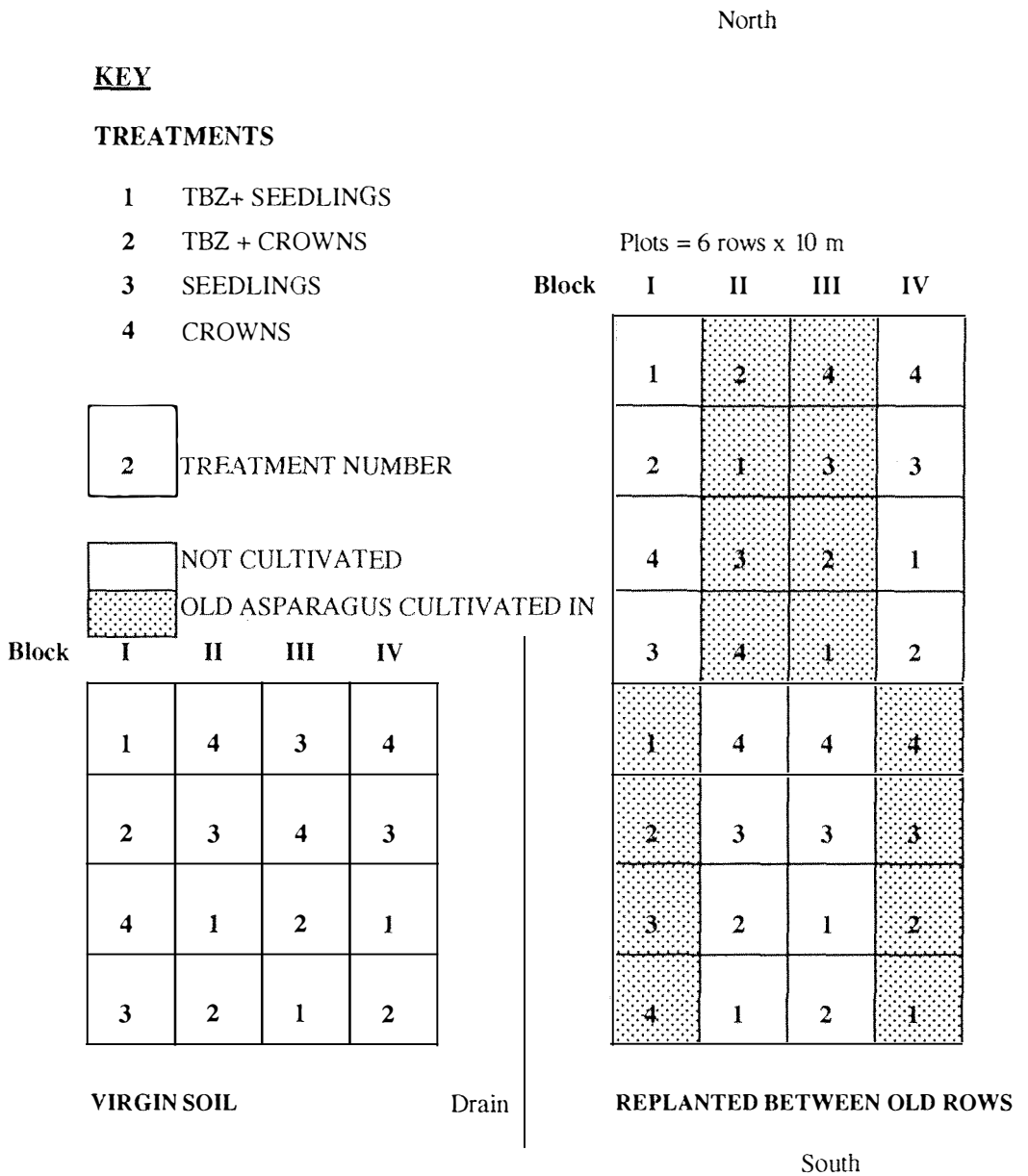
Treatments:

1. Virgin and replant site.
2. Crowns and seedlings.
3. With and without thiabendazole drench.

and on the replant site only

4. Old plants left intact and old plants cultivated in.

Figure 2.1: Field Trial No. 1 plot layout and treatment randomisation.



2.2.2.4 Trial Planting.

Crowns were planted on 28 September 1989 and seedlings on 20 November 1989. Planting was done in rows 1800 mm apart between the existing old rows or the sites of old rows. Plants were spaced 250 mm apart in the rows. A trench 120 mm deep and 100 mm wide at the bottom was prepared and crowns were laid in the bottom of the trench, buds upwards, before it was backfilled. The seedlings were planted with the cell 20 mm below the soil in the bottom of the trench. Each plot was 6 rows wide by 10 m long (240 plants). The trial was established in a commercial planting and was thus surrounded by guard plants.

2.2.2.5 Fungicide treatments.

Crowns and seedlings in the thiabendazole treatments were immersed in a trough of thiabendazole at 1.15 g a.i. per litre of water (4.33 ml Storite/l water) before leaving them to drain prior to planting. Thiabendazole is hereafter referred to as TBZ. The untreated plants were similarly immersed, but in water.

The entire trial area and surrounding commercial plantings were treated with metalaxyl at 1000 g a.i./ha (10 kg/ha Ridomil 10g prills) immediately after the crowns were planted, to protect against *Phytophthora.*

2.2.2.6. Trial site management.

Ground preparation was carried out 21 days before the crowns were planted. The entire area was irrigated (35 mm) when the seedlings were planted on 20 November 1989 and one further irrigation of 35 mm was applied on 12 January 1990.

Methabenzthiazuron at 700 g a.i./ha (1 kg/ha Tribunil) was applied after the first irrigation for residual weed control. Spears were harvested until 15 January 1990 from the plots where old plants were left intact. All plots were cultivated between the rows on 29 January 1990 and 25 February 1990 to control weeds and fern growth on the old intact plants. Seedling treatments were sprayed with demeton-S-methyl (Metasystox) at 50 g a.i./ha to control thrips on 19 December 1989 and 20 January 1990.

During the five years of the trial cultural practices were carried out in accordance with normal commercial practice. Each winter, the fern was mowed when it was dry and brittle (June), and the soil was mounded up over the rows after mowing. Prior to spear emergence in September the area was cultivated flat and residual herbicides (linuron 500 g a.i./ha and diuron 1000 g a.i./ha) and the fungicide metalaxyl (1000 g a.i./ha) were applied.

Two hundred and fifty kilograms per hectare of Cropmaster 15:10:10 (N:P:K) fertiliser was applied before planting (20 September 1989) and then annually on the final harvest day each year.

The trial area was irrigated (35 mm each irrigation) on 20 December 1990 and 25 January 1991. Three cultivations between the rows were made on 12 January, 15 February and 18 March 1991 using a shallow rotary hoe mainly to control fern growth from old plants which were still alive.

Residual and knockdown herbicides, terbumeton 800 g a.i./ha, terbuthylazine 800 g a.i./ha and glyphosate 1440 g a.i./ha, were applied after the last harvest each spring (31 October 1991, 25 November 1992 and 3 December 1993).

The trial area was not irrigated after January 1991.

2.2.2.7 Harvesting.

The harvesting strategy was similar to that used on commercial stands. All spears were picked once they were greater than 21 cm in height or shorter if the head was opening. Any spears that were non-saleable were cut off, left on the ground and not recorded. All spears greater than 21 cm in length were trimmed back to 21 cm and the total weight was recorded. Spears were then graded as export quality or non export on the basis of length, diameter, straightness, head tightness and freedom from physical defects.

Export quality spears were straight, had no markings or punctures on the stems and were in the diameter range of 10 mm to 20 mm at the base. Four records were made for each plot at each harvest. (total spear no.; total marketable weight; export or 1st grade spear no.; export or 1st grade weight)

Only the two centre rows of each plot were used for obtaining data and the other four rows (guards) were harvested as normal production beds.

2.2.2.8 Assessments and analysis.

Plots were evaluated on 17 April 1990 for plant survival, number of ferns per plant, average height and diameter of the ferns. Plant survival and average fern height per plot was again determined on 20 March 1991. In 1991 a daily spear harvest was taken starting on 1 October and ending 31 October. In 1992 it was eight weeks starting on 1 October and ending on 25 November. In 1993 the daily spear harvest was nine weeks starting on 1 October and ending on 3 December.

The trial data was initially analysed to test for differences between the soil preparation methods in the replant site. Data from the 32 replant plots were analysed by analysis of variance (Anova) as a split plot with randomised blocks using the general linear models procedure in the SAS statistical package (SAS 1989). However as the two soil preparation methods in the replant trial area had no significant effect on survival or yield, data from these treatments were pooled for further analysis of the experiment and comparison of the replant and virgin sites.

2.2.2.10 Isolations

During the 1989/1990 summer several plants that showed signs of stress or dieback were excavated from Field Trial No. 1 and inspected. (Plates 1, 2, 3 & 4, p28-29)

Several *Fusarium* spp. isolates were obtained from these plants using the following procedures.

Plate 1. Plants from Field Trial No. 1 showing severe chlorosis and necrosis.

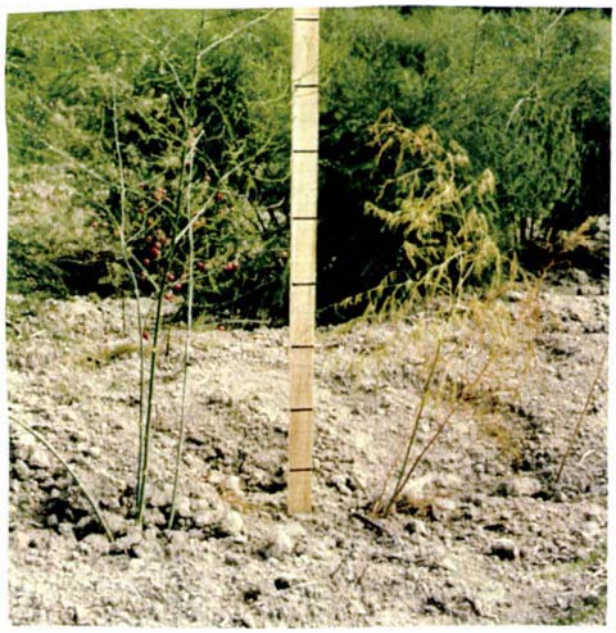


Plate 2 Cross section of healthy crown 3 months after planting.

Plate 3 Cross section of crown showing symptoms of *Fusarium* infection 3 months after planting.

Plate 4 Cross section of fern stalk showing symptoms of *Fusarium* infection.

2.2.2.11 Culture Media.

Preparation procedures for the three media used for isolating and identifying *Fusarium* spp. from diseased plants in Field Trial No. 1 are given in Appendix 1.

2.2.2.12 Isolating procedure

After washing all soil from diseased or dying plants, the roots, crowns and stems were separated and dissected. Reddish brown discolouration typical of *Fusarium* spp. infections were evident in the vascular and connective tissues. Infected parts of the roots, crowns and stems were surface sterilised with 1000 ppm sodium hypochlorite and pieces from the margin between diseased and healthy tissue were plated on PDA and FSM.

Isolates were identified from the conidial stage. *Fusarium* spp. isolates were subcultured onto fresh PDA and grown for five days until sporulation was evident. Plates were flooded with sterile distilled water and the resultant spore suspensions were streaked onto fresh PDA. After 24 hours colonies growing from single spores were able to be seen under the dissecting microscope and transferred to fresh media to be grown on as single spore isolates.

2.2.2.13 Identification and Storage of Isolates.

Single spore isolates of the cultures obtained were grown on PDA and CLA for three to four weeks for identification and then stored on sterilised soil in McCartney bottles for later use as inoculum for pot trials.

Colony characteristics on PDA and conidia and conidiophore morphology on CLA were used to identify the isolates. Isolates of *F. moniliforme* were readily separated from *F. oxysporum* by the presence of single celled micro conidia in chains when grown on CLA (Booth 1971).

A total of 65 isolates were collected from the field trials. Using the taxonomy of Snyder and Hansen (1940) 61 of the isolates were identified as *F. oxysporum* and 4 as *F. moniliforme*. The *F. moniliforme* isolates were not separated further into *F. proliferatum*, *F. moniliforme* and *F. subglutinans* as described by Nelson et al (1983). Of the isolates collected fourteen were placed in long term storage on soil, 13 of which were F. *oxysporum* and 1 was *F. moniliforme*.

Rate of growth on PDA was graded at 25°C under (50 cm) 2 x 1 m Sylvania Gro-Lux (F30w/GRO) fluorescent tubes with 12 hours light and 12 hours dark:

Fast	50 mm diameter colony in < 5 days.
Medium	50 mm diameter colony in 5 - 7 days.
Slow	50 mm diameter colony in > 7 days.

Table 2.1 Source, cultural characteristics and identification of fourteen *Fusarium* isolates grown on Difco PDA at 25°C with 12 hours light and 12 hours dark.

Source	Code	PDA colour/growth rate/rings	Species.
crown	1	dark red/fast/growth rings	*F. oxysporum*
crown	11	red / medium/growth rings	*F. oxysporum*
crown	12	pink/medium/no growth rings	*F. oxysporum*
crown	15	pink/medium/growth rings	*F. oxysporum*
crown	16	red/medium/no growth rings	*F. oxysporum*
crown	17	pink/slow/no growth rings	*F. oxysporum*
root	3	red/slow/growth rings	*F. oxysporum*
root	4	red/medium/no growth rings	*F. oxysporum*
bud	9	pale red/fast/growth rings	*F. oxysporum*
bud	10	pink/medium/growth rings	*F. oxysporum*
bud	19	pink/medium/no growth rings	*F. oxysporum*
stem	13	pink/medium/no growth rings	*F. moniliforme*
stem	14	pink/fast/growth rings	*F. oxysporum*
stem	18	red/medium/no growth rings	*F. oxysporum*

2.2.2.14 Pathogenicity Test Method

A modified version of the tests described by Gordon-Lennox & Gindrat (1987) and Stephens and Elmer (1988) was used.

Asparagus (cv UC157) seed was surface sterilised for one minute using 1000 ppm sodium hypochlorite with three drops of Tween 20 (wetting agent) per 100 ml. After rinsing, the seed was sown in autoclaved sand at 20 mm depth and germinated in an incubator at 25°C.

When the seed had germinated and shoots appeared above the sand germlings were planted into nutrient water agar in Petri plates by inserting the radicle into the agar with forceps, care being taken to avoid crushing the radicle. Ten seedlings were planted in each Petri plate of nutrient water agar. Nutrient water agar was prepared using nutrient solution that contained 100 ppm N, 135 ppm P and 100 ppm K in the form of ammonium nitrate, mono ammonium phosphate and potassium nitrate.

Spore suspensions of each of the isolates to be tested were obtained from 5 day old cultures on PDA. Thirty millilitres of sterile water was swirled around the Petri plate and poured off through a cheese cloth. The resultant spore suspensions were standardised to 10,000 total conidia (macroconidia and microconidia) per ml by dilution after using a haemocytometer to count the conidial concentration.

Five millilitres of each suspension (50,000 conidia) was placed on six replicate plates for each isolate. The test plates were grown in an incubator for two weeks at

20°C with 12 h light and 12 h dark. After assessment of the seedlings Kochs postulates were completed by making isolations from the pathogenicity test seedlings and comparing the cultural characteristics of the resulting isolates, on PDA, to the original cultures used for inoculation.

2.2.2.15 Pathogenicity test assessment and analysis.

The test was measured by scoring the plates on a 1 to 5 scale based on root disease level and discolouration.

The scoring system was as follows:

1 Healthy white roots.

2 Few brown/red flecks or lesions on up to 25% of roots.

3 Several lesions evident on most roots. 25% to 50% of root system infected.

4 75% to 50% of roots infected, showing red/brown lesions

5 More than 75% of roots infected with often dead, water soaked roots.

Test scores for the six replicate plates of each isolate were analysed by analysis of variance and isolate mean scores were ranked using Duncan's multiple range test. The SAS statistical package was used for the analysis.

2.2.3 Results

2.2.3.1 Field trial results

The survival and growth rates of plants during the first two growing seasons in the replanted area was not affected by cultivating the old asparagus crowns compared with leaving them intact in the soil. (Table 2.2)

The survival rate after the first growing season was 95% but by the end of the second growing season the survival rate had fallen to 62%. The plant deaths in the replant area that led to poor survival occurred in random patches in the trial area.

Table 2.2 Mean fern height (cm) and survival (%) during the first two growing seasons of a replant site where old plants were left intact or cultivated into the soil.

		Cultivation	No Cultivation	SE 18 df
Mean fern	**1990**	116(ns)	130(ns)	7.7
Height	**1991**	122(ns)	118(ns)	2.1
Survival %	**1990**	95(ns)	95(ns)	1
	1991	62(ns)	63(ns)	2

(ns)= analysis of variance not significant at P=0.05

The survival and plant vigour in the replant site after the first two growing seasons was very patchy with some areas (up to 6 m of row length) where no plants survived while in nearby areas (adjacent rows) all plants survived and growth was excellent. (plate 5) Plant deaths in the replant area continued throughout the first two growing seasons and plants that died exhibited typical symptoms of *Fusarium* infection. Ferns were usually yellowed and stunted with reddish brown vascular discolouration and rusty flecks or lesions on the external layers of the lower portion of fern stalks. Reddish discolouration extended into the crowns from fern sockets or from discoloured fleshy roots. *Fusarium* spp. were readily isolated from the disease margin of tissue taken from dead or dying plants in the replant site.

Plate 5. Patchy survival and growth in replant area of Field Trial No. 1.

Table 2.3 Mean spear numbers (,000s/ha) and weight (t/ha) over three harvest seasons from a replant site where old plants were left intact or cultivated into the soil.

		Cultivation	No Cultivation	SE 18df
Total	**1991**	100(ns)	128(ns)	6.5
Spear	**1992**	133(ns)	157(ns)	9.7
Number	**1993**	84(ns)	117(ns)	8.7
Total	**1991**	1.69(ns)	2.01(ns)	.09
Yield	**1992**	2.03(ns)	2.33(ns)	.16
	1993	0.88(ns)	1.23(ns)	.08

(ns) = analysis of variance not significant at P=0.05

Although there was consistent trend for more spears and a higher total weight to be produced from the plots where the old plants were left intact the differences were not statistically significant. (Table 2.3)

The patchy nature of survival and growth in the trial area led to a large experimental error that was not controlled by the placing of treatments in blocks.

Table 2.4 Fern height (cm) and survival (%) for all treatments after the first and second growing seasons in Field Trial No. 1. (SE 37df in brackets)

	Fern Height (cm)		Survival %	
	1990	**1991**	**1990**	**1991**
Virgin	123 ns	130 A	99 A	73 A
	(9.6)	(2.2)	(0.8)	(3.0)
Replant	107 ns	120 B	95 B	63 B
	(6.8)	(3.1)	(0.6)	(2.1)
Crowns	145 A	134 A	97 ns	67 ns
	(8.3)	(2.7)	(0.7)	(2.6)
Seedlings	85 B	117 B	97 ns	68 ns
	(8.3)	(2.7)	(0.7)	(2.6)
With TBZ	107 ns	124 ns	97 ns	65 ns
	(8.3)	(2.7)	(0.7)	(2.6)
Without TBZ	122 ns	127 ns	97 ns	70 ns
	(8.3)	(2.7)	(0.7)	(2.6)

Means followed by different letters are significantly different (P=0.05).

ns = Anova not significant (P=0.05)

The mean height of ferns was greater in the virgin soil than in the replant soil in both growing seasons but was only significant in 1991. (Table 2.4) Survival was significantly higher in the virgin than in the replant soil. Crowns produced taller ferns than seedling transplants in the first two growing seasons but there was no difference in the survival rates from crowns or seedlings after two growing seasons. Treatment of the plants with TBZ had no effect on the height of fern produced or on the survival rate of the plants.

There were no significant interactions between any of the treatments.

The survival rate in both the replant and virgin areas by the end of the second growing season was low. In the replant area patches of dead and dying plants were scattered throughout the trial site. In the virgin soil however the death of plants followed a distinct pattern. There was a low lying area on the roadway adjacent to the south eastern corner of the virgin soil plots. During the winter of 1990 and early spring of 1991 water pooled in this area and asparagus plants progressively died out. The deaths continued to progress across the trial area from this corner throughout the life of the trial (Figure 2.2). Deaths were a result of *Phytophthora* (*Phytophthora*

megasperma var *sojae*) infection which was characterised by the soft, wet, mushy rot of crowns and roots and the distinctive smell of rotten potatoes.

Phytophthora megasperma var *sojae* was baited out of the soil with equal frequency from all plots in the virgin soil area indicating the presence of the pathogen which caused severe infections in the wettest plots where water tended to pond in heavy rain.

Figure 2.2 Pattern of progression of *Phytophthora* infection across virgin soil plots in Field Trial No. 1.

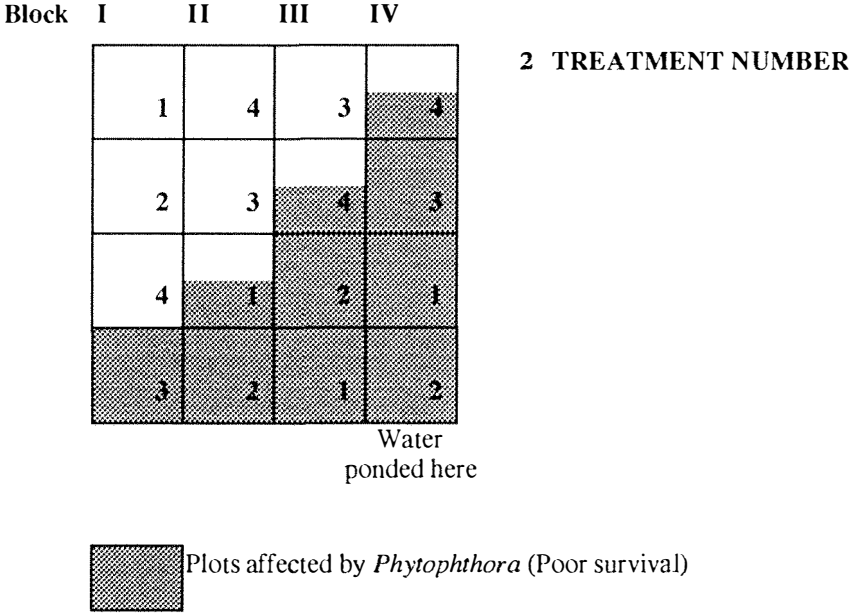


Table 2.5 Total spear numbers (,000s/ha) and total yield (t/ha) from 1991, 1992, 1993 harvests of Field Trial No. 1 (SE 37df in brackets).

	Total Spear No.			Total Yield		
	1991	**1992**	**1993**	**1991**	**1992**	**1993**
Virgin	91	153	133 A	1.60	2.68	1.29
	(13)	(15)	(7)	(0.24)	(0.27)	(0.14)
Replant	114	145	100 B	1.85	2.18	1.06
	(9)	(11)	(9)	(0.17)	(0.19)	(0.10)
Crowns	99	163	139 A	1.68	2.16	1.32
	(11)	(13)	(8)	(0.21)	(0.24)	(0.13)
Seedlings	106	135	94 B	1.76	2.09	1.02
	(11)	(13)	(8)	(0.21)	(0.24)	(0.13)
With TBZ	95	142	109	1.57	2.09	1.1
	(11)	(13)	(8)	(0.21)	(0.24)	(0.13)
Without TBZ	111	155	124	1.88	2.16	1.24
	(11)	(13)	(8)	(0.21)	(0.24)	(0.13)

Means followed by different letters are significantly different at P=0.05.

There were no significant differences in the numbers of spears or of yield between any of the treatments in this experiment during the 1991 or 1992 harvests. (Table 2.5) In 1993, the virgin soil produced more spears than the replant soil. The total yield was also 20% higher on the virgin soil although the difference was not significant (P=0.05).

Crowns produced more spears than seedlings in the 1993 harvest but the higher total yield from crowns compared with seedlings in 1993 was not significant.

The TBZ treatment resulted in no differences in spear numbers or yields in any of the three harvests seasons. There were no significant interactions between the treatments.

When the problem of Phytophthora rot progressing through part of the virgin soil area of the trial site became evident in 1991 it was closely monitored and all plots were scored (1 to 5) in May according to the fern height, numbers of ferns and diameter of ferns. This score was called a vigour score and was recorded for each plot in May 1991 and again May 1992. The vigour scores for each plot for the two years were pooled and along with the survival figures taken in 1991 were used as concomitant variables in linear regression analysis (Steel and Torrie 1980) of the accumulated yields from the three harvests using the SAS statistical package.

Table 2.6 Accumulated total spear numbers and 1st grade spears (,000s/ha), Total yield and 1st grade yield (t/ha), from three harvests (1991-1993) of Field Trial No. 1, analysed using concomitant variables. (SE 37df in brackets).

	Total spear No.	No. 1st Grade	Total Yield	1st Grade Yield
Virgin	370	131 A	5.36 A	2.23 A
	(16)	(6)	(0.25)	(0.1)
Replant	356	103 B	4.41 B	1.67 B
	(24)	(9)	(0.37)	(0.15)
Crowns	384	118	4.86	1.87
	(19)	(7)	(0.29)	(0.12)
Seedlings	342	117	4.91	2.03
	(20)	(7)	(0.3)	(0.13)
With TBZ	376	121	5.27	2.09
	(19)	(7)	(0.30)	(0.12)
Without TBZ	350	113	4.5	1.8
	(20)	(7)	(0.3)	(0.13)

Means followed by different letters are significantly different at P=0.05.

Analysis using the concomitant variables helped demonstrate the differences between the accumulated yields for the three harvests on replant and virgin soils. (table 2.6) Total spear number and 1st grade spear number were greater on virgin than on replant soil but only the latter was significant at P=0.05.

The total yield and the first grade yield was significantly higher on virgin soil than on replant soil. Total yield was 21% higher on virgin soil than on replant and 1st grade yield was 33% higher on virgin soil compared with replant.

There were no significant differences between spear numbers or yield for the crowns or seedlings or for the TBZ treatments when the accumulated data from the three harvests was analysed. There were no significant interactions between the treatments.

2.2.3.2 Pathogenicity Test Results.

Although the pathogenicity test was not highly sensitive it did show that the isolates tested were all pathogenic in the test conditions (Table 2.7). The test has provided a ranking of the isolates on the basis of the strength of their pathogenicity under the test conditions.

Table 2.7 Pathogenicity Test score (1=healthy to 5=more than 75% of roots affected) and ranking for thirteen *F. oxysporum* isolates and one *F. moniliforme* isolate from Field Trial No.1.

Isolate Code	Test score
16	3.5 A
15	3.3 AB
1	3.3 AB
18	3.3 AB
17	3.2 ABC
12	**3.2 ABC**
11	2.8 ABC
13	**2.8 ABC**
14	2.8 ABC
9	2.5 ABC
4	2.5 ABC
19	2.3 BC
10	2.3 BC
3	2.2 C
WATER	1.0 D

SE = 0.30 (76 df)

Means separation in the table by Duncan's Multiple Range Test (P=0.05)
Means followed by the same letter are not significantly different.
Isolates 12 and 13 (Bold) were used in preparation of inoculum in later experiments.

All of the isolates obtained from seedlings in the test were identical in culture to their parent isolates indicating that no cross contamination occurred in preparation of the conidial suspensions or during the incubation period of the test.

2.2.4 Discussion

2.2.4.1 Cultivating the old plants or leaving them intact.

Plant vigour and the survival rate measured in the first two growing seasons were not significantly affected by chopping up the old plants compared to leaving them intact.

Blok and Bollen (1993) measured 4180 and 11060 kg (dw)/ha of asparagus root residues in two former asparagus fields. Assessments done in this trial indicated that approximately 15000 kg (dw)/ha of asparagus residue was incorporated into the top 500 mm of soil during the chopping up of the old plants with the rotary hoe whereas 1500 kg (dw)/ha of root residues were incorporated where the old plants were left intact and only the strip between the rows was cultivated. This did not affect initial plant growth or survival although the spear numbers produced and total weight of spears produced over three successive harvest seasons was slightly (but not significantly) higher where the old plants were left intact. (P=0.0735 for total spear numbers and P=0.1014 for total weight of spears) However this difference could be important in a commercial situation as the total accumulated yield over the three harvests was 20% higher where the old plants were left intact. As well as the yield advantage from the new crop by leaving the old planting undisturbed there would be a significant saving in cultivation costs.

The cultivation process described for destruction of the previous asparagus planting (2.2.2.1) involved at least four passes with a powerful tractor and a total cost of $300.00/ha. Cultivation for replanting between old rows of intact plants would cost approximately $70.00/ha thus saving $230.00/ha. Another advantage of this method of replanting is the production achieved from the old planting in the first growing season of the new planting which could have a significant impact on cash flow in the first year of the new development. Production would not be high as presumably the planting was being replaced because of low yields but harvesting could continue as long as the return for spears harvested is equal or greater than the cost of harvesting them. The main disadvantage of leaving the old plants intact when replanting is the control of the fern growth on the old plants once harvesting spears from them is uneconomic. In this and other similar trials this was done by rotary hoeing over the top of the old plants monthly to ensure no ferns developed fully and after two seasons of such treatment the carbohydrate reserves of the old planting were completely exhausted and the plants died out.

Chopping all the old asparagus plant material into the soil prior to planting a new crop would be expected to release higher levels of autotoxin into the soil compared to leaving the old plants relatively intact. The presence of higher levels of autotoxic material may have led to the reduced yields from plots where the old plants were

chopped in either through a direct inhibition (Huiskamp & Kanters 1989; Blok & Bollen 1993, 1996(a)) or through an interaction with *Fusarium* spp. causing greater disease levels (Young & Chou 1985; Huiskamp & Kanters 1989; Hartung et al. 1989; Blok & Bollen 1993)

The large amounts of asparagus plant residues may have depleted plant available nitrogen levels as the soil microbial population broke down the organic matter. This might also explain the difference in performance between the two cultivation treatments with yield being reduced where the old plants were chopped into the soil because of a reduction in available nitrogen compared with where the old plants were left intact.

2.2.4.2 Survival and Vigour comparisons.

Fern height and survival measurements during the first eighteen months after planting showed that crowns produced taller plants than seedlings. This is to be expected as the crowns are effectively bigger plants at planting time, with bigger buds, as they are twelve months old at planting compared to seedlings at ten weeks old. Crowns were also planted six weeks earlier than the seedlings so commenced growing sooner. Sudjatmiko (1993) found that earlier plantings of seedlings resulted in larger plants at the end of the first and second growing seasons.

Although survival after the first season of growth was 95% by the end of the second growing season there was only 62% survival in the replant site which would not normally be considered enough to sustain commercial levels of production. Similar research on the replant problem with apple trees has shown that unless steps are taken during development, such as soil sterilisation, to minimise replant deaths commercial yields from new apple developments on old apple orchard sites will not be economically viable (Smith 1994).

The virgin soil site had a higher survival rate and produced taller and stronger plants than the replant site. This supports findings in other reports that the replant problem may be due to the presence of some or all of the three main factors implicated in the problem, namely - autotoxins, herbicide residues and inoculum of pathogenic *Fusarium* spp. (Grogan and Kimble 1959; Young and Chou 1984; Blok and Bollen 1993). The TBZ treatment had no affect on the survival rate or fern height during the first eighteen months of the trial and indicates that either *Fusarium* infection is not involved in survival or vigour or that the TBZ treatment was not effective for control of *Fusarium* infections in this trial. This is contrary to the results achieved by Falloon et al. (1989) who reported improved survival and vigour by treating crown transplants with TBZ in a replant site.

The pathogenicity test was an adaptation of the tests used by other workers (Gordon-Lennox & Gindrat 1987; Stephens and Elmer 1988) and was a satisfactory

means of demonstrating the ability of numerous isolates to cause infection. A total of 65 isolates were collected from diseased plants in the field trials and all of them caused infection in the test described above. The test results for 14 of the isolates are given as these isolates were ones that were put into long term storage on soil to be used as an inoculum source in later experiments.

Of the 65 *Fusarium* isolates obtained from the trial site 61 were *F. oxysporum* and 4 were *F. moniliforme* indicating that both species were present and causing disease, reduced survival and death of new plants in the replant site. This supports the findings of Endo & Burkholder (1971) and Johnson et al (1979) who found that both pathogens were involved in the crown and root rot complex.

Blok and Bollen (1993, 1996(b)) also found that *F. oxysporum* was by far the most commonly isolated pathogen in old or declining asparagus fields and concluded that *F. oxysporum* f.sp. *asparagi* (*FOA*) was the main cause of asparagus replant-bound early decline (ARED) in the Netherlands. The evidence from isolations taken from roots crowns and stem bases of the new plants in Field Trial No. 1 suggests that although *FOA* may be the most commonly isolated pathogen *F. moniliforme* is likely to also be involved.

In the virgin site the pattern of deaths was different than in the replant site. In the spring of the second growing season after planting (September/October 1990) it was noted that the plants were slow emerging in the south eastern corner of the virgin trial site or in the adjacent commercial stand. This area was near a slightly lower lying area where water ponded occasionally during the winter. Excavation of asparagus plants in this area revealed typical symptoms of *Phytophthora* infection of the storage roots and even of entire crowns. As the spring in 1990 was very wet the invasion of *Phytophthora* continued throughout the trial period despite treatment with metalaxyl. The likely cause of this problem was the introduction of the disease to the virgin site on vehicle wheels or soil associated with cultivation equipment as the virgin site was immediately adjacent to older asparagus plantings. Conditions were ideal for *Phytophthora* infection for three months in spring 1990 and the metalaxyl application would not control the disease for this long (Falloon & Fraser 1991).

In summary the replant area suffered poor survival (63% after eighteen months) due mainly to sporadic plant deaths throughout the trial area usually associated with *Fusarium* infections. The virgin area suffered poor survival (73% after eighteen months) but the deaths were mainly confined to one area where survival was as low as 25% and the deaths were associated with the unintentional introduction of *Phytophthora* to the trial area 8 to 10 months after planting.

2.2.4.3 Spear numbers and Yield comparisons.

Analysis of variance of the data collected during the three harvests shows that only in the 1993 harvest season were there any significant differences (P=0.05) between the treatments.

In 1993 the virgin soil site produced 33% more spears than the replant site (P=0.0083) although the difference in total weight of spears produced (21% more from the virgin site) was not significant (P=0.2087).

To increase the sensitivity of the analysis, particularly the comparison of virgin and replant sites, concomitant variables were assigned to each plot in 1991 and 1992 to reflect both survival and vigour of the surviving plants. Regression analysis using the concomitant variables reduced the variance in the experiment caused by the poor survival and growth in part of the virgin soil site.

The analysis of the accumulated total yields from the three harvests showed that the virgin soil produced 30% more 1st grade spears, 21% higher total yield and 33% higher first grade yield than the replant site. As the total number of spears produced on the replant and virgin sites was not significantly different but the total yield was higher on the virgin site it is evident that the virgin site produced slightly larger spears on average than the replant site. This also explains why the 1st grade yield was relatively higher on the virgin site.

There was no difference in accumulated spear numbers or yield from crowns or seedlings over the three harvests. As none of the treatment interactions show any differences, neither type of planting material performed better than the other in a replant site compared with a virgin site, and both crowns and seedlings performed poorly in the replant site but well in the unaffected areas of the virgin site.

The TBZ treatment caused slight increase in spear numbers and yields but the differences (7% more spears and 16% higher yield) were not significant at P=0.05. There was no interaction between the fungicide treatment and site or planting material.

The main result from this experiment has been to show a higher number of spears and a higher yield on the virgin site compared to the replant site. This result confirms reports from other researchers that replanted asparagus has poorer performance than when the crop is grown on new ground (Blok and Bollen 1993, 1996(c); Gordon Lennox & Gindrat 1987; Young 1985; Conroy 1975). It also goes some way towards quantifying the yield reduction to be expected if old asparagus ground is replanted.

2.3 Field Trial No. 2

This trial was established on a peat and pumice based sandy loam soil in Hawkes Bay. An area that had been used for asparagus production (cv Rutgers Beacon) for fourteen years was replanted and the virgin soil treatments were planted on an adjacent area with no history of asparagus production.
The most recent residual herbicide applications to both virgin and replant areas were diuron 1600 g a.i./ha of and terbuthylazine 1500 g a.i./ha on 3 December 1988 and 1500g a.i./ha each of terbuthylazine and terbumeton on 10 December 1987.

2.3.1 Objectives.

To determine the effect of:

1. Using a replant site or virgin site on survival rate, vigour and yield of newly planted asparagus.

2. Two fungicides (thiabendazole and metalaxyl) either alone or in combination as crown dips to control *Fusarium* crown and root rot and Phytophthora rot in a replant site.

2.3.2 Materials and Methods.

2.3.2.1 Ground preparation.

Ground was rotary hoed in August five weeks before the crowns were planted, as described in 2.2.2.1. The virgin soil area had the same preparation as the replant soil.

2.3.2.2 Planting.

Crowns were planted on 28 September 1989 in a 150 mm deep trench, 250 mm apart in rows with 1500 mm between rows. After planting the crowns were covered by 150 mm of soil. Plots were 3 rows wide by 10 m long. (120 plants)
The experiment was planted in the middle of a commercial asparagus planting and was surrounded with guard plants.

2.3.2.3 Plant Material

One year old crown transplants (cv Syn 4-56) were produced as described in 2.2.2.2.

2.3.2.4 Treatments.

Crowns were treated with thiabendazole, metalaxyl, a combination of both fungicides or water only and allowed to drain for 24 h before planting.

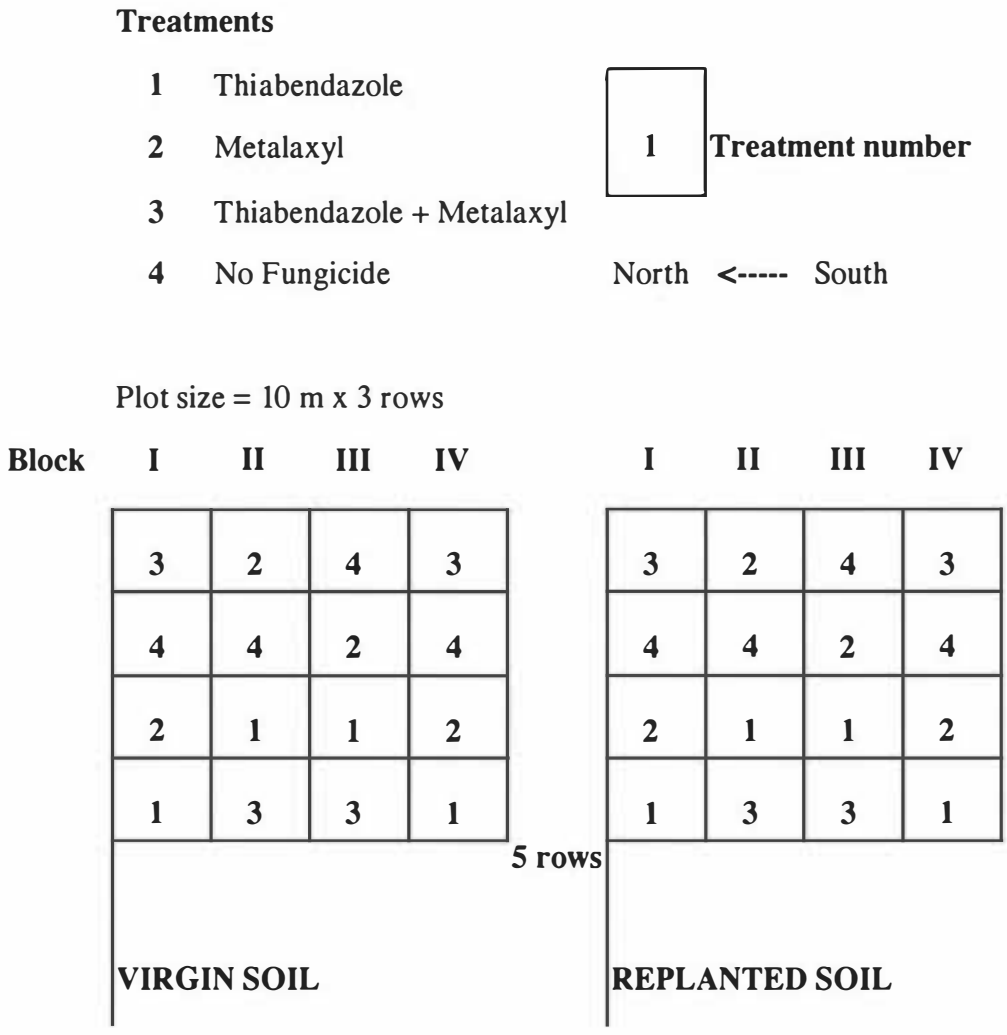
Site treatments were:

1. Replant site.
2. Virgin site.

Fungicide treatments applied at each site were:

1. Thiabendazole crown dip, 1.15 g a.i. per litre of water (4.3 ml Storite/l).
2. Metalaxyl crown dip, 250 mg a.i. per litre of water (1 g Ridomil 25WP/l).
3. Thiabendazole plus metalaxyl crown dip (4.3 ml Storite plus 1 g Ridomil 25WP/l).
4. No fungicide (water crown dip).

Figure 2.5 Plot layout and treatment randomisation of Field Trial No. 2



2.3.2.5 Experimental design.

The experiment was a factorial design with four randomised complete blocks on each of the adjacent sites.

2.3.2.6 Trial site management.

Methabenzthiazuron at 700 g a.i./ha (1 kg/ha Tribunil) was applied after planting for residual weed control. Haloxyfop at 400 g a.i./ha of (4 l/ha Gallant) was applied 12 December 1989 to control grass weeds. Because of strong weed growth a mower was used between the rows in January 1990 and further herbicide application of linuron at 500 g a.i./ha was applied in January 1990. There was no irrigation applied to the trial.

Cultural practices were carried out in accordance with the normal practice on commercial plantings as described in 2.2.2.5. Each winter fern was mowed once it was dry and brittle. The area was cultivated twice over the winter and early spring with the final cultivation bringing the ground flat prior to spear emergence in September. Residual herbicide was applied prior to spear emergence (linuron 500 g a.i./ha and diuron 1000 g a.i./ha) together with 1000 g a.i./ha metalaxyl.

At the end of the harvest period a knockdown herbicide (1800 g a.i./ha glyphosate) and residual herbicide (3200 g a.i./ha simazine or 1800 g a.i./ha terbuthylazine and 1800 g a.i./ha terbumeton) were applied each year. An annual application of 250 kg/ha 12:10:10 NPK fertiliser was made on the day of the final harvest each year.

2.3.2.7 Harvesting

The first harvest was taken in spring 1991 and harvests were carried out as described in 2.2.2.7. The two southern rows of each plot were used for yield assessment at each harvest with the other rows harvested with the commercial production beds that surrounded the trial.

2.3.2.8 Assessments and analysis.

Plots were evaluated for plant survival on 17 April 1990. Number of ferns per plant and mean height of the fern were recorded on the same date. Plant survival and the average fern height per plot was determined after two growing seasons on 20 March 1991.

A daily spear harvest was taken in October 1991 starting on 1 October and ending 31 October. In 1992 the daily spear harvest started on 1 October and ended on 25 November. A daily spear harvest starting on 1 October and ending on 3 December was taken in 1993.

Survival and growth records and the harvest data for three harvest seasons were analysed by analysis of variance using the general linear models procedure and the SAS statistical package.

2.3.3 Results

2.3.3.1 Survival and vigour comparisons.

The fern numbers per plant were not significantly different between sites or between fungicide treatments and these data are not presented, however there were large differences in fern height between the sites in both years and the survival rate differed significantly between the various fungicide treatments by the end of the second growing season.(Table 2.8)

Table 2.8 Mean fern height (cm) and survival (%) of replant or virgin site crowns or crowns with four possible fungicide treatments after the first and second growing seasons in Field Trial No. 2.

	Survival (%)		Height (cm)	
	1990	**1991**	**1990**	**1991**
Replant	98.3	85.3	155.6 B	107.5 B
Virgin	98.9	85.1	168.8 A	126.9 A
SE 18df	0.45	1.04	3.36	1.26
Metalaxyl	98.1	84.5 AB	153.8 B	115.6
TBZ	98.5	89.4 A	174.3 A	118.8
Both fungicides	98.5	85.8 AB	160.3 B	116.9
No fungicide	99.3	81.3 B	160.5 B	117.5
SE 18df	0.63	1.48	4.75	1.78

Means followed by a different letter are significantly different at P=0.05

There was no difference in survival between the replant or virgin site after one or two growing seasons, however the virgin site grew significantly taller ferns in both growing seasons compared with the replant site.

The fungicide treatments had no effect on the fern height in either growing season although all three fungicide treatments improved the plant survival slightly compared with the no fungicide by the end of the second growing season.

2.3.3.2 Spear yield comparisons

The total numbers of spears and total weight of spears produced was consistently higher from the virgin soil site in all three harvests (Table 2.9)

The TBZ fungicide treatment produced slightly more spears and a greater total yield than the other fungicide treatments in all three harvests although only the differences between the total yield were significant at P=0.05.

Table 2.9 **Total spear numbers (,000s/ha), total yield (t/ha) and mean spear weight (g) from three spear harvests of Field Trial No. 2.**

	Total spear no.			Total Yield			Mean Spear Weight		
	1991	**1992**	**1993**	**1991**	**1992**	**1993**	**1991**	**1992**	**1993**
Replant	141	281 B	194 B	2.29 B	5.62 B	3.46 B	16.2	20.0	17.8
Virgin	151	358 A	250 A	3.08 A	8.32 A	5.13 A	20.4	23.2	20.5
SE 18df	6	8	7	0.15	0.23	0.15	0.79	0.83	1.12
Metalaxyl	145	319 A	219 AB	2.41 B	6.82 B	4.08 B	16.6	21.3	18.6
TBZ	159	331 A	237 A	3.15 A	7.62 A	4.82 A	19.8	23.0	20.3
Both	141	327 A	221 AB	2.62 B	6.93 B	4.25 B	18.6	21.2	19.1
None	140	299 B	211 B	2.56 B	6.50 B	4.03 B	18.3	21.7	19.1
SE 18df	9	11	10	0.21	0.32	0.21	1.24	1.18	0.97

Means followed by a different letter are significantly different at P<0.05

Total yield from the virgin site was significantly (34%, 48% and 48%) higher and spear numbers were 7%, 27%, and 30% higher than the replant site in the 1991, 1992 and 1993 seasons respectively. The yield difference was in part due to a significantly greater total number of spears produced in 1992 and 1993 in the virgin site and partly due to significantly greater mean size of spears in each of the harvests from the virgin site.

2.3.4 Discussion

Establishment on the virgin and replant sites in this trial was the same with very similar survival and numbers of ferns produced. However ferns were taller during the establishment phase on the virgin site and this difference was reinforced by the greater spear numbers and higher total yields during the harvests. In the first harvest season the greater total yield from the virgin site was mainly due to the larger spears produced (7% more spears but 34% higher yield on the virgin site). As the size of a spear is governed by (a) the size of the bud it grows from and (b) the amount of soil it grows through (Liao 1997), it can be concluded that the virgin soil plants had larger buds than those in the replant site because the soil cover over the crowns was similar on both sites.

The difference in spear size between the virgin soil and replant soil diminished during the course of the experiment (4.2 g, 3.2 g, and 2.7 g, in 1991, 1992 and 1993 respectively) although the difference in yield increased over the same period through an increasingly higher number of spears being produced on the virgin site compared with the replant site.

As the virgin and replant areas had a similar history of herbicide applications residual herbicide carryover is unlikely to have caused the difference between their performance.

The differences between the fungicide treatments were much smaller than the differences between the two sites and there was no interaction between the fungicide treatments and the sites. The TBZ treatment resulted in the highest spear numbers and greatest total yield. In the first harvest season the metalaxyl treatment produced the fewest spears but in the subsequent harvest season the no-fungicide treatment had the lowest spear numbers and yield. It appears that the metalaxyl treatment on its own had little beneficial effect either during establishment or in the yield from the first three harvests whereas the TBZ treatment was beneficial but the combined treatment was not as effective as TBZ alone.

Other researchers have reported increases in yield and improved establishment with the use of thiabendazole as a hypophosphite salt formulation and generally no effect from other formulations (Falloon et al. 1989). Thiabendazole presumably reduces *Fusarium* infection in some way and the biggest responses have been reported in situations where the *Fusarium* inoculum level would be expected to be high such as old declining asparagus stands or replant sites.

The metalaxyl treatment is normally used on asparagus crown transplants to protect against *Phytophthora* during establishment (Falloon & Fraser 1991). That the metalaxyl treatment had little effect in this experiment is an indication that during

the establishment period conditions did not favour *Phytophthora* and this conclusion is substantiated by the fact that the soil type in the trial area is a very free draining sandy loam and there were no periods of heavy rain during the three months following planting of the trial.

The combination of the two fungicides appears in some way to have reduced the activity of the thiabendazole. Only the acid formulation (Storite clear or Tecto 20S) of TBZ improves the performance of asparagus in established beds or in new plantings which indicates that the fungicidal activity is very dependant on the form of the thiabendazole on the plants or in the soil (Falloon pers. comm.). It is possible that the combination with metalaxyl changed the form of thiabendazole in some way thus reducing its activity.

The effect of the thiabendazole treatment is likely to have resulted from reduced *Fusarium* infection during the months following planting thus allowing thiabendazole treated plants to establish better. Thiabendazole is unlikely to have had any affect on the plants after the first few months as the plants would have grown beyond the treatment provided by the initial crown drench applied prior to planting. As asparagus has a vigour related tolerance to *Fusarium* it is likely that the initial control of this disease by the thiabendazole treatment prior to planting could continue on for several years through improved growth and vigour in the treated plants (Nigh 1990).

Although the thiabendazole treatment was no more effective on the replant site than on the virgin site it is still likely that *Fusarium* was a major factor in the difference in performance between the two sites. Inoculum of *Fusarium* spp. pathogenic to asparagus was present in both sites as evidenced by the presence of *Fusarium* lesions on roots of plants dug from both sites in the winter following planting of the trial. In the replant soil a much higher inoculum level would have been present due to the large amount of asparagus crown and root material distributed through the soil during the destruction of the previous asparagus crop (Blok & Bollen 1993, 1996(b)). Increased *Fusarium* infections through the increased disease pressure from the high inoculum level is likely to have contributed to the difference between the replant and virgin sites. The autotoxic material from the old asparagus crop residues is also likely to have reduced the vigour of the plants in the replant area. This could have a combined effect in reducing the vigour related tolerance of the replant site plants to *Fusarium* attack and also in some way predispose the plants to *Fusarium* infection or enhance the ability of the *Fusarium* inoculum to cause disease (Hartung & Stephens 1983; Peirce & Colby 1987; Wacker et al. 1990).

As with Field Trial No. 1 the main result from this experiment has been to show an increased number of spears and a higher yield on the virgin site compared to the replant site. This result confirms reports from other researchers that replanted asparagus has poorer performance than when the crop is grown on new ground (Blok and Bollen 1993, 1996(c); Gordon-Lennox & Gindrat 1987; Young 1985; Conroy 1975).

Along with Field Trial No. 1 this trial helps to quantify the difference in yield between developing a new asparagus crop on new ground compared with putting the same development on an old asparagus soil. Over the three years of harvest the accumulated total yield was 18% and 32% lower on the replant sites than on the virgin sites in Field Trials No. 1 and 2 respectively. If the average of these figures is used as a guideline then growers should expect the yield from a new asparagus planting in an old asparagus soil to be approximately 25% lower than if the same development was in ground that had not previously grown asparagus.

2.4 Field Trial No. 3

The use of fungicides as plant dips or drenches for long term control of soil borne pathogens is often not successful as the plant roots quickly develop away from the zone protected by the original treatment. Initial indications from field trials were that the fungicide treatments under evaluation did not provide adequate disease control in replant sites. There is also an increasing trend towards reduced use of pesticides and alternatives to fungicides are being sought for many applications.
Biological control agents (BCAs) have the advantage that they may grow with the developing plants and protect them from pathogenic attack. *Trichoderma viride* has proved to be a useful BCA for a range of fungal diseases and it was therefore appropriate to evaluate its use in an asparagus replant site.

2.4.1 Objective

(a commercial strain).

To evaluate the potential use of a mycelial suspension of *Trichoderma viride* as an aid to establishment and growth of asparagus crowns planted into an asparagus replant site.

2.4.2 Materials and Methods

The trial was established in September 1991 on a deep, alluvial, silt loam soil adjacent to Field Trial No. 1. The site had been in asparagus production (cv NZ Beacon) for ten years. The most recent residual herbicide applications to the soil were 3200 g a.i./ha diuron on 20 December 1990 and 3000 g a.i./ha simazine applied on 15 December 1989.

2.4.2.1 Ground preparation and planting.

Cultivation was carried out on 12 August six weeks before the crowns were planted on 24 September 1991 as described in 2.2.2.1.
Planting was in rows 1800 mm apart with plants spaced 250 mm apart in the rows as described in 2.2.2.4.

2.4.2.2 Planting Material

The trial was planted with one year old crowns (cv Syn4-56) produced as described in 2.2.2.2.

2.4.2.3 Treatments

Crown treatments were applied immediately before planting.

There were three treatments.

1. - No Treatment. (Water crown dip)
2. - Metalaxyl and thiabendazole crown dip.
3. - *Trichoderma viride* crown dip.

The fungicide treatments were applied by dipping the crowns in a solution containing both fungicides as described in 2.3.2.4.

Trichoderma viride was applied as a suspension of mycelium and spores mixed with water and the crowns were immersed in the suspension for thirty seconds prior to draining and planting out. No-treatment crowns were immersed for thirty seconds in water prior to planting. The plants were covered with soil within 30 min of placing them buds upwards in the trench.

2.4.2.4 Trial Design

The trial was planted in a randomised complete block with six blocks. Each plot contained 80 plants and was 2 rows wide by 10 m long. The trial was completely surrounded by two rows of guard plants.

2.4.2.5 Trial site management:

Linuron (800 g a.i./ha) and diuron (1800g a.i./ha) applied on 25 October 1991 for pre-emergent weed control and hand weeding carried out in January and February 1992 to control weeds that were not controlled by the herbicide. Haloxyfop 400 g a.i./ ha was applied in February 1992 to control grass weeds. No irrigation was applied. Cultural practices were carried out in accordance with the normal practice on commercial plantings as described in 2.2.2.6. In winter fern was mowed when it was dry and brittle. The area was cultivated twice over the winter and early spring with the final cultivation bringing the ground flat prior to spear emergence in September. Residual herbicide was applied prior to spear emergence (linuron 500 g a.i./ha and diuron 1000 g a.i./ha) plus metalaxyl at 1000g a.i./ha for *Phytophthora* control. Two applications of 250 kg/ha 12:10:10 NPK fertiliser were made, one prior to planting out the trial and another twelve months later before spear emergence. No spear harvest was taken during the second growing season.

2.4.2.6 Assessments and analysis.

On 2 March 1992 fern number per plant, average fern height per plot and survival rate for each plot were recorded. On 12 March 1993 the survival rate for each plot was recorded.

A daily spear harvest was taken in 1993 starting on 1 October and finishing on 2 November. Harvesting and recording was carried out as described in 2.2.2.7.

As the yields from the trial area were very low and there appeared to be no differences between the treatments the trial was discontinued after the 1993 harvest season.

Survival, growth and harvest data were analysed by analysis of variance using the SAS statistical package.

2.4.3 Results

The mean survival rate at the end of the first growing season was 85%. By the end of the second growing season the plant mean population had fallen to 63% of that planted.

At the end of the first summer there were no differences in the survival rate, number of ferns per plant or mean height of ferns between the three treatments. The survival rate was the same for all three treatments after the second year of growth also. (Table 2.10)

There were no significant differences between the treatments for the total numbers of spears produced, the total weight of spears, numbers of first grade spears and weight of first grade spears from the 1993 harvest. As the pattern of total yield and 1st grade yield was similar, only total spear numbers and total yield are presented in Table 2.10.

Table 2.10 No. of ferns per plant, mean fern height (cm), survival (%) in 1992 and 1993, spear number (,000s/ha) and total yield (kg/ha) from a four week harvest of crowns, with three pre-planting treatments, grown in an old asparagus soil .

	Crown Treatment			
	No Treatment	**TBZ+Metalaxyl**	***Trichoderma***	SE 10df
Fern Number/Plant	4.3	4.6	4.1	0.27
Fern Height	90	99	94	3.3
Survival 1992	88	80	86	3.2
Survival 1993	65	61	60	3.9
Spear Number	54	48	55	4.1
Total Yield	662	567	774	90

During the first growing season there were plant deaths throughout the trial area. Many plants that survived the first season of growth did not emerge in the second spring and plant deaths continued throughout the trial area in the second growing season. Plants that died exhibited typical symptoms of *Fusarium* infection. Ferns were usually yellowed and stunted with reddish brown vascular discolouration and rusty coloured lesions on the external layers of the lower portion of fern stalks. Reddish discolouration extended into the crowns from fern sockets or from discoloured fleshy roots. *Fusarium* spp. were readily isolated from the disease margin of tissue taken from dead or dying plants in the trial site.

There was no response by way of survival, growth rate or yield from the TBZ plus metalaxyl treatment or from the Trichoderma treatment compared with untreated crowns.

2.4.4 Discussion.

The trial area exhibited problems typically associated with the asparagus replant problem and the none of the treatments improved either the initial establishment or the longer term vigour and yield from the crop.

In Field Trial No.1 the use of TBZ as a crown dip improved establishment and vigour in the replant site and in Field Trial No.2 the TBZ treatment increased spear yield. In this trial the TBZ was applied along with metalaxyl and as in Trial No. 2 this seems have reduced the effectiveness of the thiabendazole treatment. Research reported by Falloon et al. (1989) demonstrated that TBZ alone or with metalaxyl at the rates used in this work improved establishment and plant growth rate in an asparagus replant site. Possible reasons for the difference in results in this trial compared with the work of Falloon et al. (1989) are, in this trial;

a) Soil conditions were more favourable for *Fusarium* infection with hot dry conditions during February and March 1991 and in January and February 1992.

b) Ideal conditions for *Phytophthora* during early spring 1992 leading to plant deaths in early spring and also leading to increased susceptibility to *Fusarium* infection later in the season due to the stress induced by *Phytophthora* infection.

c) Possibly differing *Fusarium* inoculum levels compared to the other trials.

McCormick and Geddes (1992) evaluated the use of thiabendazole plus metalaxyl mixture and *Trichoderma* as crown treatments in replant and virgin soil sites in two successive plantings (1990 and 1991). Their results were similar to those reported here with generally no differences detected between the crown treatments in either replant or virgin soil but substantially higher survival and yield from all treatments in the virgin soil compared with replant. In the 1990 planting which was followed by very wet ground conditions the survival rate was substantially lower in the *Trichoderma* treatment.

2.5 Field Trial No. 4

Experience in New Zealand and overseas suggests that some cultivars of *Asparagus officinalis* L. are better suited to a replant situation than others. It is not clear whether differences are due to differing physiological ability to grow in the presence of asparagus toxins and/or high levels of *Fusarium* inoculum. Differing performance in a replant situation could simply be a reflection of the growth capability of differing genotypes at any one site.

2.5.1 Objective

To compare the establishment and yield of twenty *Asparagus officinalis* L. cultivars in old asparagus soil.

2.5.2 Materials and Methods

The trial was established on a deep, alluvial, silt loam soil adjacent to Field Trial No. 1. The site had been in production of asparagus cv NZ Beacon for ten years. The most recent residual herbicide applications to the site were diuron (3200 g a.i./ha) on 20 December 1990 and simazine (3000 g a.i./ha) on 15 December 1989.

2.5.2.1 Plant Material

Seedlings of the twenty cultivars were produced either from seed or in the case of the clones, rooted minicrowns. The seedlings were grown as described in 2.2.2.2. Clones were grown in rootrainers in the same manner as the seed lines but using rooted minicrowns which were acclimatised to the peat media by being held in a high humidity tent in the greenhouse for ten days after exflasking as described by Conner et al. (1992).

2.5.2.2 Ground preparation

The site was prepared on 28 August twelve weeks before the seedlings were planted. The preparation of the site was as described in 2.2.2.1. The experiment was planted on 20 November 1991 in rows 1800 mm apart and plants were spaced 250 mm apart in the rows. A trench 100 mm deep was prepared and the seedlings were planted into the bottom of the trench with the top of the seedling cell approximately 20 mm below the soil surface.

2.5.2.3 Experimental Design and Treatments.

The experiment was planted as a randomised complete block design with twenty treatments and four blocks. Each plot was 1 row x 5m and contained 19 plants.

Table 2.11 Treatment numbers and Cultivars evaluated in Field Trial No. 4

Treatment No.	Cultivar	
1	JWC1	
2	UC157	
3	CP3 * CP 4	
4	CP3 * CP 6	
5	Syn 4 - 56	
6	Syn 4 - MD10	
7	Syn 4 - 362M	
8	Syn 4 - 51	
9	Syn 4 - 53	
10	Franklim	
11	Astora	
12	Pacifica	Clone
13	CP 1	Clone
14	S 8	Clone
15	RT 8	Clone
16	CP 3	Clone
17	CP 6	Clone
18	Pole Tom	
19	CPA 6b	
20	Tainan 3	

2.5.2.4 Trial site management.

The trial area received 35 mm of irrigation at planting by overhead sprinklers. Methabenzthiazuron (700 g a.i./ha) and haloxyfop (400 g a.i./ha) were applied during December 1991 for weed control and hand weeding was used during January and February 1992. Applications of demeton-S-methyl (100 g a.i./ha) were made in December 1991, January 1992 and February 1992 to control thrips on the establishing seedlings. Cultural practices were carried out in accordance with the normal practice on commercial plantings as described in 2.2.2.6. In winter fern was mowed when it was dry and brittle and the area was cultivated twice over the winter and early spring with the final cultivation bringing the ground flat prior to spear emergence in September. Residual herbicide was applied prior to spear emergence (linuron 500 g a.i./ha and diuron 1000 g a.i./ha) as was metalaxyl (1000 g a.i./ha) for *Phytophthora* control. Two applications of 250 kg/ha 12:10:10 NPK fertiliser were made, one prior to planting out the trial and another twelve months later before spear emergence. No spear harvest was taken during the second growing season.

2.5.2.5 Assessments and data analysis.

The number of plants surviving and the height and numbers of ferns per plant were recorded in February 1993. Spears were harvested daily from 1 October 1993 until 2 November following the method described in 2.2.2.7

The trial was discontinued after the 1993 spring harvest as the survival rate was poor and the yield was correspondingly low.

Data from the 1993 harvest and survival rates recorded in February 1993 were subjected to analysis of variance and ranked using Duncan's Multiple Range test for each of the variables total yield, total spear number and survival using the SAS statistical software.

2.5.4 Results

The cultivars with the highest survival rates tended to have greater spear numbers and high total yields reflecting the higher population contributing to the yield. (Table 2.12)

The best performing varieties were Franklim, Syn 4-56, Syn 4-53, CP3xCP4, JWC1 and Astora. Intermediate in performance were Syn4-51, CPA6B, Tainan 3, UC157 and Syn 4-362M. Poor performing seed grown cultivars were CP3xCP6, Syn4-MD10 and Pole Tom. The six clones all had low survival and low yields.

Table 2.12 Total yield (kg/ha), Total spear number (,000s/ha) and Survival (%) from a four week harvest of twenty asparagus cultivars grown in a replant site.

	Total Yield	Total Spear no.	Survival
Franklim	1618 A	18.0 ABC	30.5 ABC
Syn4-56	1558 A	19.3 AB	35.3 ABC
Syn4-53	1413 AB	13.3 ABCD	33.0 ABC
CP3xCP4	1313 ABC	16.3 ABCD	35.5 ABC
JWC1	1175 ABCD	22.0 A	43.5 A
Astora	1080 ABCD	19.5 AB	38.3 AB
Syn4-51	1015 ABCDE	16.3 ABCD	31.8 ABC
CPA 6B	933 ABCDE	14.3 ABCD	31.5 ABC
Tainan 3	888 ABCDE	16.0 ABCD	34.5 ABC
UC157	888 ABCDE	14.0 ABCD	23.8 ABCD
S8	878 ABCDE	5.8 D	15.8 CD
Syn4-362M	720 BCDE	13.5 ABCD	35.5 ABC
CP3xCP6	647 BCDE	13.5 ABCD	36.8 ABC
Syn4-MD10	637 BCDE	13.3 ABCD	35.8 ABC
RT8	513 CDE	10.5 BCD	33.0 ABC
CP3	438 DE	12.0 ABCD	26.3 ABCD
CP1	393 DE	5.0 D	8.0 D
Pole Tom	393 DE	10.2 BCD	20.0 BCD
Pacifica	358 DE	9.3 BCD	21.0 BCD
CP6	208 E	15.0 ABCD	23.8 ABCD
SE 57df	240	3.3	6.4

Means separation in the table by Duncan's Multiple Range Test (P=0.05) Means followed by the same letter are not significantly different.

2.5.5 Discussion.

There was a wide range of survival and yield from the twenty cultivars evaluated. The poorest performing varieties were predominantly the clones and this was mainly attributable to inferior planting material at the commencement of the experiment. The clonal plants, grown from recently ex-flasked rooted minicrowns, were not as large at planting as the seed grown cultivars and had poor root development at planting time compared with the seed grown cultivars.

Although poorly developed seedlings, as used in planting the clonal material in this experiment, can establish and develop successfully in soils with no history of asparagus production it is apparent from this experiment that only strong and vigorous seedling transplants will establish successfully in a replant situation. The

clones that were used in this experiment were taken from larger production lines of those clones that were planted out in commercial plantings on two virgin sites in Hawkes Bay. After two years the survival rates of all the clones was 75% at one virgin site and 81% at the other indicating that the poor performance of the clones in this experiment was at least partly due to the replant site.

The best performing cultivars in this experiment had a 35% to 40% survival rate eighteen months after planting the trial. In a normal commercial planting on virgin soil one would expect a survival rate in excess of 80%. Similarly the highest total yield of 1618 kg/ha two years after planting the trial was less than half the yield one would expect at this stage from a new planting on a virgin site.

The ranking of the cultivars in this experiment is the same as the ranking of these cultivars in other trials on virgin sites. The best performing varieties Franklim, Syn4-56, Syn 4-53, JWC1 and Astora are commonly the best performing in other trials in Hawkes Bay and Manawatu on virgin soil. (Schofield et al. 1994; Nichols 1992, 1994) Similarly the varieties that performed poorly in this experiment have also done poorly in other North Island cultivar trials that were conducted on virgin soil.

None of the cultivars evaluated in this experiment were suited to establishment in a replant situation and the differences between the cultivars in a replant site appear to be the same or possibly more accentuated than in a site with no history of asparagus production. It appears that establishment failures are very much more likely in a replant site. These findings support observations from other workers regarding establishment failures and reduced vigour in replanted asparagus although comparisons of cultivars in a replant field trial have not been previously reported (Grogan & Kimble 1959; Young 1984; Blok & Bollen 1993).

2.6 Field Trial No. 5

Asparagus is very tolerant of saline soils (Francois 1987) and applications of rock salt (NaCl) were once recommended in U.S.A. because they increased yields (Walker 1905; Rudolph 1927). Elmer's (1990, 1992) work suggests that NaCl and other forms of Chloride (Cl) suppress soil-borne diseases in various crops including asparagus, cereals, corn, celery, and fodder beet.

As Elmer's research (1990, 1992) suggested that the main effect of rock salt on declining asparagus stands was a reduction in *Fusarium* infection levels and increased spear yield a field trial was established to evaluate the use of rock salt on a declining asparagus stand under New Zealand conditions.

Thiabendazole also controls *Fusarium* in asparagus. English work (Giltrap pers. comm.) showed that applications of thiabendazole to established asparagus resulted in yield increases of between 30% and 35%. This was confirmed at Lincoln (Falloon & Fraser 1990) where thiabendazole applications increased yields of marketable spears of between 3% and 30% in three cultivars.

The bio-control agent *Trichoderma viride* was also evaluated in the field trial as a potential soil treatment to control decline in old asparagus beds.

2.6.1 Objective

To evaluate the ability of rock salt, thiabendazole drench and *Trichoderma viride* to improve the spear yield in a declining asparagus stand.

2.6.2 Materials and Methods

The trial was established in September 1990 on a deep, alluvial, silt loam soil adjacent to Field Trial No. 1. The site had been in asparagus production (cv NZ Beacon) for nine years and the yield had declined in each of the three previous harvest seasons.

The trial plots were 3 rows wide and 10 m long and were established in a uniform area of the old stand. Row spacing in the old stand was 1.8 m and soil was mounded over the rows to give 150 mm of soil over the crown buds.

2.6.2.1 Treatments.

The treatments were first applied on 15 September 1990 after the pre-harvest cultivation of the beds had been completed. Treatments were re-applied on 12 September 1991.

Irrigation was applied (20 mm) by overhead sprinklers immediately following the application of the treatments in both years.

The treatments were:

1. Rock salt applied at 1120 kg/ha by hand broadcasting.
2. Thiabendazole applied at 3200 g a.i./ha as a soil drench at 1000 l/ha.
3. *Trichoderma viride* as a mycelial and conidial suspension at 1000 l/ha
4. No treatment (control)

2.6.2.2 Trial design and management.

The treatments were arranged in a completely randomised block design with six replicates. Each plot was 3 rows (5.4 m) wide and 10 m long. Following the treatment applications in 1990 a spear harvest was taken finishing on 15 December 1990. After the final harvest in 1990 knockdown and residual herbicides were applied (2000 g a.i./ha each of terbuthylazine and terbumeton and glyphosate 1800 g a.i./ha). Following summer fern growth the fern was mowed when it was dry and brittle before the beds were cultivated flat. Prior to the second application of the treatments in September 1991 the beds were cultivated to give a mound over the crowns with 150 mm soil over the crown buds. The trial was discontinued at the end of the second harvest season in December 1991.

2.6.2.3 Assessments and Analysis

Daily spear harvests were carried out and recorded as described in 2.2.2.7. The 1990 harvest commenced on 29 September and finished on 15 December. In 1991 the daily spear harvest commenced on 1 October and finished on 18 December.

Daily harvest records were summed for each plot to give total plot figures for each of the two harvest seasons. The data were analysed by analysis of variance using the SAS statistical package.

2.6.3 Results

As the total spear numbers, total yield, first grade spear numbers and first grade yield all followed the same pattern only the total yield for each of the treatments is presented. (Table 2.13)

In 1990 the rock salt and *Trichoderma* treatments had the same yield as the untreated control whereas TBZ application resulted in significantly lower yield than the other treatments. In 1991 there were no significant differences between the treatments however the TBZ treatment resulted in the lowest yield as in the previous year.

The 1991 yields were approximately 25% lower than the 1990 yields in this trial indicating that the asparagus bed was continuing to decline in yield.

Table 2.13 Total spear yield (T/ha) from an old asparagus bed following pre-harvest applications of rock salt, fungicide, BCA or nothing for two seasons.

	Total yield 1990	Total yield 1991
Rock Salt	5.61 A	3.95 A
Thiabendazole	4.54 B	3.28 A
Trichoderma viride	5.23 A	3.47 A
Control	5.35 A	3.62 A
SE 14df	0.38	0.33

Means followed by a different letter are significantly different at P=0.05

2.6.4 Discussion.

In this trial two years of rock salt and *Trichoderma viride* applications had no effect on yield on a declining asparagus bed. The thiabendazole treatment resulted in a lower yield in both years however this was significant only in the first season of the trial. This result is contrary to those obtained by Falloon & Fraser (1990) and Elmer (1990).

The slight reduction in yield caused by the TBZ application in this experiment may be a result of TBZ reducing the activity of beneficial micro-organisms such as mycorrhizal fungi. In other trials where TBZ has been used on established asparagus beds (Falloon & Fraser 1990; Giltrap pers. comm.) the resultant increase in yield has been statistically significant but generally uneconomic with the cost of the TBZ application being higher than the value of the increased marketable crop.

The levels of Trichoderma *viride* in the soil were not monitored during the experiment. Treatment with this BCA did not result in an increase in yield in this experiment and as with other work with this organism (McCormick & Geddes 1992), *Trichoderma viride* does not appear to show promise as a useful BCA for *Fusarium* on asparagus.

The lack of response from rock salt applications in this experiment could be explained by the soil pH. In recent work Elmer (pers. comm.) has found that rock salt does not have a beneficial effect on declining asparagus beds unless the soil pH is over 6. Where the soil pH is over 6, Elmer (1997) found rock salt applications resulted in, reduced fern water potential, reduced organic acids in root exudates, changed micro-flora populations in the rhizosphere, increased Mn availability. These changes may enhance host resistance. In this trial the soil pH was 5.6 and probably too low to allow the rock salt to affect the rhizosphere enough to reduce *Fusarium* infections.

Section Three
Greenhouse experiments.

3.1 Introduction.

Reports on the asparagus replant problem have attributed the failure of new plants to establish and thrive to the carry over of *Fusarium* spp. in the old asparagus soil (Blok & Bollen 1996(c)). The symptoms on plants that fail in replant situations have often been identical to symptoms associated with asparagus decline and the *Fusarium* root and crown rot complex. It has often been assumed that *Fusarium* infection in a replant situation is one of the major causes of the replant problem, particularly where replanting land where stands with severe decline symptoms were previously reported (Grogan & Kimble, 1959). Recent work has shown that chemical fumigation or soil sterilisation can improve establishment in a replant situation, presumably by eliminating or reducing the *Fusarium* inoculum, but the effect may be only short lived and the replant problem still persists to some extent (Yang 1982 ; Blok & Bollen, 1993).

Long lasting residual herbicides are generally used on asparagus beds to control weeds during the harvest period and the fern growing period. It is thought that after several years levels of these low solubility compounds may build up to cause establishment problems and reduced vigour when asparagus beds are replanted.

Many studies have shown that asparagus tissue, asparagus plant residues and soil from old asparagus beds contain substances that are inhibitory to asparagus plant growth (Young & Chou, 1985; Hartung & Putnam, 1986; Hartung et al, 1989).
Some studies have shown that the importance of toxins in the replant problem is related to the effect they have in predisposing asparagus roots and stems to attack by pathogens (Hartung & Stephens, 1983; Hazebroek et al., 1989; Peirce & Miller, 1990).

3.2 Greenhouse Trial No. 1

3.2.1 Objectives

To determine the effect of asparagus root residues, *Fusarium* spore suspensions and herbicide residues on the growth of asparagus seedlings in soil.

3.2. 2 Materials and Methods.

3.2.2.1 Soils

Two soils were used in the experiment neither of which had a history of asparagus production. Soil #1 was collected from an area adjacent to Field Trial No. 1 and is described as Pakowhai silt-loam, a fertile alluvial silt-loam soil. Soil #2 was a lighter soil collected from an area adjacent to Field Trial No. 2 and is a Paki Paki sandy-loam based on peat and pumice. Both soils were mixed with an equal volume of sand and fumigated for seven days with Chloropicrin then ventilated for ten days before use.

3.2.2.2 Seedlings

Asparagus cv Syn 4-56 seed was surface sterilised by soaking for 60 seconds in a 500 ppm sodium hypochlorite/0.01% Tween 20 solution, then rinsed three times in sterile distilled water. It was sown in 5 ml cells of peat, one seed in each cell, and held in the greenhouse for eight weeks. The greenhouse was heated when the temperature fell below 15°C and vented above 25°C. The seedlings had three to four shoots and six to eight storage roots at the time they were transplanted, one per pot into plastic pots (100 mm square and 150 mm high) that held 500 g of dry soil, containing the desired treatment combination (Table 3.1).

3.2.2.3 Asparagus storage root additions.

Root additions were prepared from 1 year old asparagus cv Syn 4-56 crowns that were field grown in a sandy loam soil as described in 2.2.2.2. Storage roots were cut from the rhizomes and washed thoroughly in tap water before being closely inspected for *Fusarium* lesions. Any roots with symptoms or signs of *Fusarium* infection were discarded and the "clean" roots were surface sterilised in 500 ppm sodium hypochlorite solution before slicing in a food processor to an average 2 mm length or 0.1 g piece.

The fresh root pieces were stored in the refrigerator at 4°C for 24 h before mixing with soil in a concrete mixer at a rate of 20 g of roots per 1000 g of dry soil.

3.2.2.4 Herbicide

The herbicide 'Caragard' which contains equal proportions of terbumeton and terbuthylazine was diluted with the soil in a concrete mixer to give 1 ppm total active ingredient in the treated soil. This rate was used as indications from trials done by Ciba, (the manufacturer of 'Caragard') in Switzerland, indicate that between 0.5 ppm and 1 ppm total active ingredient may be present in soil twelve months after applying this product at the highest of the recommended rates.

3.2.2.5 *Fusarium* Inoculum.

The isolate used was *F. oxysporum* strain No. 12, originally isolated from a necrotic crown in Field Trial No.1. (Table 2.1)

Fusarium inoculum was applied in the form of a spore suspension prepared by flooding seven day old cultures on PDA with sterile distilled water and stirring the surface with a glass rod. Conidial numbers in the resultant spore suspension were counted using a haemocytometer and the suspension adjusted to give 4000 conidia (both macro conidia and micro conidia) per ml. Five millilitres of the suspension (20,000 conidia) was applied to the top of the pots after the seedlings had been planted and the experiment set out on the greenhouse bench.

3.2.2.6 Experimental design and Treatments.

One seedling was transplanted into each 100 ml square pot containing 500 g of dry soil. There were four pots of each treatment in each block.

The experiment was a factorial design with two soils and three additions (roots, herbicide, *Fusarium*) giving 16 possible treatments arranged in a randomised complete block design with five blocks. (Table 3.1)

Table 3.1 Treatment combinations of soil, roots, herbicide and *Fusarium* in Greenhouse Trial No. 1.

Treatment Number	Soil	Roots	Herbicide	*Fusarium*
1	1	-	-	-
2	1	-	-	+
3	1	-	+	-
4	1	+	-	-
5	1	+	-	+
6	1	+	+	-
7	1	-	+	+
8	1	+	+	+
9	2	-	-	-
10	2	-	-	+
11	2	-	+	-
12	2	+	-	-
13	2	+	-	+
14	2	+	+	-
15	2	-	+	+
16	2	+	+	+

3.2.2.7 Management.

Plants were grown in the greenhouse for 14 to 16 weeks heated below 15°C and vented above 25°C. During the growing period a liquid feed containing 150 ppm N, 100 ppm P and 100 ppm K was applied twice a week and the experiment was watered three times per week.

The five replicates were assessed on the following dates:

Rep 1	2-Oct
Rep 2	9-Oct
Rep 3	9-Oct
Rep 4	16-Oct
Rep 5	16-Oct

3.2.2.8 Assessment Procedure and analysis.

Pots were soaked in water for several min. and the seedlings removed from the soil with the roots intact. The soil was washed from the roots and the following measurements taken for each seedling:

-Number of live shoots and dead shoots.

-Number of live roots and dead roots.

-Fresh weight of shoots and roots

-Dry weight of shoots and roots

Level of *Fusarium* infection on roots:

Fusarium infection level was recorded as a lesion score from 1 to 5 based on the numbers of *Fusarium* lesions on both feeder and storage roots:

1 = no lesions evident and a healthy white root system. (0%)*

2 = a few lesions could be seen on a few roots. (< 25%)

3 = several lesions on most of the storage roots and feeder roots. (26% to 50%)

4 = lesions evident on all storage roots even if only tiny red flecks and the feeder roots all infected. (51% to 75%)

5 = mainly dead root system. (> 76%)

* - Estimate of % of root system affected.

Level of *Fusarium* infection on crowns:

Each crown was cut in half and given a crown rot score:

1= healthy clean white crown. (0%)*

2= slight brown discolouration. (<25%)

3= red brown discolouration and stunted crown development.(26% to 50%)

4= entire crown discoloured stunted but still alive. (51% to 75%)

5= mainly dead crown. (>76%)

* - Estimate of % of crown affected.

The records for individual plants were summed to give total plot figures and this data was analysed by analysis of variance using the general linear models procedure in the SAS statistical package.

3.2.2.9 Isolations.

Samples of root and crown material showing evidence of *Fusarium* infection were taken from 12 pots in replicate 1 for isolation. Fresh weights of these samples were taken and the dry weight calculated on the basis of the fresh to dry weight ratio of the rest of the root or crown material in the pots from which they were taken. The dry weight of roots or crowns could then be corrected for this missing material before analysis.

Diseased material was surface sterilised by immersion in 1000 ppm sodium hypochlorite solution for sixty seconds then pieces of the disease margin were removed and plated onto PDA

Single spore isolates were prepared from all *Fusarium* cultures obtained as described in 2.2.2.12 and grown on PDA and CLA to identify the *Fusarium* isolate.

3.2.3 Results.

The fresh weight of roots and shoots and the dry weight of roots and shoots followed the same pattern as the total plant dry weight, therefore only the mean total dry weights per plant for treatments are presented.

There was a significant difference in dry weight of plants between the five replicate harvesting dates with later harvested replicates producing more dry matter.

As significant (P=0.05) two way treatment interactions were found between soil x herbicide and roots x herbicide the main effects are not presented. There were no significant differences between the means of the three way or four way interactions.

The major factor to affect dry matter, shoot number, root number and crown rot score was the herbicide treatment. (Table 3.2)

The addition of *Fusarium* inoculum as a spore suspension had no significant effect on shoot numbers, root numbers, or total dry weight of asparagus seedlings in this experiment although it was the only factor in the experiment to significantly (P=0.05) increase the lesion score.

Herbicide addition either alone or in combination with any of the other treatments significantly restricted the plant growth. The effect of the herbicide was greater on the lighter soil than on the heavier soil. The root residues added in combination with the herbicide considerably reduced the suppression caused by the herbicide.

Table 3.2 Mean shoot number, root number, crown rot score and total dry weight (g) for the soil x herbicide and root x herbicide interactions in Greenhouse Trial No. 1.

		Shoot No.	Root No.	Crown Rot Score	Total Dry Weight
Soil #1	**+ Herbicide**	7.1a	13.8b	1.8b	2.4b
	- Herbicide	6.0b	17.2a	1.0b	3.8a
Soil #2	**+ Herbicide**	5.0c	10.1c	4.5a	1.4c
	- Herbicide	6.7ab	17.6a	1.0b	3.7a
Plus roots	**+ Herbicide**	6.8a	14.9b	1.8b	2.3b
	- Herbicide	6.5ab	17.7a	1.3b	3.7a
Minus roots	**+ Herbicide**	5.3c	9.1c	4.4a	1.5c
	- Herbicide	6.2ab	17.1a	0.7b	3.7a
SE (20 df)		0.32	0.86	0.42	0.18

Means followed by different letters are significantly different (P=0.05) using Duncan's Multiple range test.

Fusarium oxysporum was isolated from all disease lesions tested.

Of the eleven *F. oxysporum* isolates obtained from material recovered from the experiment seven appeared to be the strain originally used as inoculum. New strains were distinguished from the original isolate used for inoculum in the experiment on the basis of colony morphology and media pigmentation when single spore isolates were grown on PDA. The presence of four new strains of *F. oxysporum* indicated that *Fusarium* may have been present on or in the fresh root material, in the soil, or may have been introduced with the seedlings. (Table 3.3)

Disease lesions were evident on most of the plants at the end of the experiment with most disease present on treatments that resulted in the most plant suppression. It appeared that the *Fusarium* used as inoculum did not remain confined to the treated pots and that other sources of *Fusarium* also caused infection during the experiment.

Table 3.3 Treatment, type of material, presence of inoculum or roots and identity of *Fusarium* isolate for eleven isolates obtained from disease lesions in Greenhouse Trial No. 1

Treatment no.	Type of material	Inoculum added	Roots added	*Fusarium* Isolate
4	crown	no	yes	12
4	root	no	yes	12
5	root	yes	yes	new strain
5	crown	yes	yes	12
8	root	yes	yes	new strain
10	crown	yes	no	12
10	root	yes	no	12
11	root	no	no	12
11	crown	no	no	12
15	crown	yes	no	new strain
16	crown	yes	yes	new strain

3.2.4 Discussion.

3.2.4.1 Herbicide.

Of the replant factors that were evaluated in this experiment the residual herbicide had the greatest effect.

There was no difference in plant performance or disease levels between the two soils in the absence of the herbicide treatment however with herbicide present the suppression was much greater in Soil #2 the lighter textured pumice based soil. As the plants were supplied with liquid fertiliser twice each week these differences were not likely due to differing nutrient status of the soils but most likely due to differing plant availability of the herbicide molecules in the two soils. The difference between the suppression caused by the herbicide on the two soils is probably due to the presence, in the heavier soil, of more colloidal particles which provide adsorption sites for the herbicide molecules. The herbicide concentration in soil solution would then be lower in the heavier soil than in the lighter textured soil where it would be more readily available for uptake by the plants. A similar effect may have arisen through the differing water holding capacity of the two soils with the lighter textured soil having a lower water holding capacity and therefore a greater likelihood of herbicide being available to plants in the soil solution following watering than the heavier soil which would be effectively 'drier' when given the same watering regime. Studies of the persistence and plant-availability of triazine herbicides in soils with differing textures and water holding capacities indicate that the triazine herbicides are more strongly adsorbed in soils with a higher colloidal content (either mineral clays or organic matter) and that dry

conditions will increase persistence of herbicides in the soil (Holly & Roberts, 1963; Hance, 1976).

The organic matter contents of the two soils were 5.0% w/w (Soil #1) and 10.4% w/w (Soil #2). It could be expected that herbicide adsorption in Soil #2 would be greater than in Soil #1 based on the higher organic matter content however the suppression caused by herbicide was greater in Soil #2 than Soil #1. This could be explained by a higher total colloidal content in Soil #1 despite the difference on organic matter due to the presence of more clay colloids in the alluvial based soil.

In the field the effectiveness of the residual herbicide used in the experiment follows a similar pattern to that in the pot trial with good initial weed control on both soils but control is achieved for a longer period on the heavier alluvial silt loam soil where it persists longer than in the lighter textured pumice based soil.

The herbicide had a different effect on the numbers of shoots produced from the two soils. In Soil #1 addition of herbicide resulted in production of more shoots per plant whereas in Soil #2 fewer shoots were produced in the presence of herbicide than when no herbicide was added. The triazine group of herbicides act in plants by blocking electron transport during photosynthesis and are known as photosynthesis inhibitors. It is apparent that in Soil #2 uptake of the herbicide resulted in reduced plant growth and therefore a reduced shoot number. In Soil #1 the herbicide uptake and therefore the inhibition of photosynthesis was not as great as in Soil #2 but did cause a change in the growth pattern of the plants resulting in the production of more shoots. The increased shoot number could be due to reduced apical dominance in herbicide affected shoots thus allowing new shoot initiation earlier than when the herbicide was not present.

The addition of asparagus root material enhanced asparagus seedling growth in the presence of residual herbicide. This effect could be explained by the increased organic matter added in the form of asparagus roots providing binding sites for the herbicide molecules and thus reducing the amount in the soil solution in a plant-available form. Alternatively an increase in microbial activity in pots where root material was added could have caused a more rapid breakdown of the herbicide molecules by soil micro-organisms.

The level of herbicide added was 1 ppm of both active ingredients in combination, so that 0.5 ppm of terbumeton and 0.5 ppm of terbuthylazine were added on a dry weight of soil basis. This is equivalent to about 10% of the level present in soil if a recommended rate of Caragard is applied in the field and then mixed with the top 300 mm of soil.

The rates of degradation of these chemicals in the soil have not been studied in New Zealand but studies in Switzerland (data provided by Ciba) indicate that levels as high as 0.5 ppm of these two chemicals could be expected in an asparagus

field after several years of Caragard use at recommended rates even if no herbicide had been applied in the preceding twelve months.

It is apparent from this experiment that where an asparagus crop is in decline and termination of the crop is planned the likely carryover of herbicides in the soil should be taken into account when using herbicides in the two years before terminating the crop.

3.2.4.2 *Fusarium.*

Addition of *Fusarium* inoculum of 20,000 conidia per pot had no effect on asparagus seedling growth rate or crown rot score but did increase the lesion score. The inoculum caused more infection of the asparagus seedling roots as evidenced by the increased lesion score but the increased disease level did not reduce the seedling growth over the course of the experiment. There was no interaction between the *Fusarium* and the storage root treatments in this experiment. This contrasts with other research where levels as low as 3,000 conidia of *Fusarium oxysporum* f.sp. *asparagi* added to similar sized pots significantly reduced emergence of asparagus seedlings in the presence of water extract from asparagus storage roots (Peirce & Colby 1987). Hartung et al. (1989) also demonstrated that inoculum of both *Fusarium oxysporum* f.sp. *asparagi* and *Fusarium moniliforme* when added alone or in combination with asparagus root tissue increased root rot scores and decreased total seedling dry weight of asparagus seedlings grown in pots in the greenhouse. Peirce and Colby's (1987) experiment examined the emergence of asparagus seedlings after exposure to various treatments. The effect of the treatments is likely to be different during the germination and early shoot and root growth of seedlings in their study than might be expected with much larger eight week old asparagus seedlings. Studies that show asparagus seedling suppression by *Fusarium* inoculum on its own or in combination with other factors have generally used higher levels of inoculum eg. 8 g millet seed inoculum per 500 g pot of soil (Hartung & Stephens 1983; Hartung et al. 1989). The inoculum level added in this study relates to approximately 40 colony forming units added per gram of soil (cfu/g) whereas levels of 10^3 to 10^6 cfu/g may be found in natural soils (Nash and Snyder 1962; Komada 1975). Addition of 16 g millet seed inoculum per kg of soil as in Hartung's experiments would provide a much greater fungal biomass than the spore suspension used in this study. Blok & Bollen (1996a) also found no interaction between *Fusarium* and asparagus root additions in pot trials conducted in non sterilised soil and attributed the difference in those results to their earlier work and that of Hartung & Stephens (1983), Hartung et al. (1989) and Peirce & Colby (1987) to the fact that the experiments that showed interactions between *Fusarium* and autotoxins used sterile growing media or very high levels of

root residues. In this experiment however the soil had been sterilised and the rate of inoculum was similar to that used in experiments where an interaction between the two was reported (Peirce & Colby 1987). It is likely that the complex nature of the interaction between asparagus autotoxic material and *Fusarium* has led to the variable results reported with many factors involved that may change the strength of the interaction such as the presence or absence of other soil micro-flora, the tendency of different *Fusarium* isolates to interact with the phytotoxic material and the availability or adsorption of the toxins in the soil.

Inoculum isolate (12) was isolated from non-inoculated pots so some pot to pot contamination must have occurred during the experiment. It is likely that the contamination was by means of conidia transferred from pot to pot by insects walking on the soil surface or by irrigation water splash. Insects were noted in the greenhouse during the experiment and insecticide was applied to the experiment to try and minimise this form of cross-contamination.

Four new isolates (morphologically different from isolate 12 used for inoculum) were identified. All of these were isolated from plants that had originally been inoculated with isolate 12. The most likely source of the new isolates of *F. oxysporum* was the root material that was added to the soil. Three of the new isolates were found in pots where root material had been added. Although the obviously infected roots were left out in preparation of the fresh storage root additions and the clean roots were surface sterilised it is likely that some *Fusarium* would be present in the roots unless they were sterilised by autoclaving. Autoclaving was avoided as the effect of high temperatures on the autotoxic compounds or on other material in the roots was not known.

It is possible that the seedlings were a source of *Fusarium* contamination although the seed was surface sterilised. Damicone et al. (1981) demonstrated that some conidia on asparagus seed may not be eliminated by surface sterilisation with sodium hypochlorite but found that treatment with benomyl in acetone was effective in disinfesting asparagus seed. This disinfestation process was evaluated but resulted in many abnormal seedlings and was therefore not used in the production of seedlings for pot trials or bioassays. Another possible source of *Fusarium* contamination in the experiment was the soil. Sterilisation of the soil with chloropicrin should have eliminated all fungi however some chlamydospores or thick walled mycelium could have survived the fumigation particularly if embedded in dense soil particles or organic material in the soil.

3.2.4.3 Storage Roots

The storage root additions in this experiment had no effect on plant growth or disease levels on their own or in the presence of *Fusarium* inoculum but did enhance growth when the herbicide was added. An inhibition of growth and an increased disease level, through the activity of autotoxic material in storage roots, could have been expected in this study as found by others (Hartung et al. 1989; Blok & Bollen 1993). Application of a crude water extract from storage roots at rates equivalent to a water extract from 2.5 g dw/kg of soil was found to inhibit emergence of asparagus seed (Peirce & Colby 1987). Similarly Shafer & Garrison (1986) found rates of 20 to 60 g dw/kg of asparagus roots incorporated in soil inhibited emergence of lettuce, tomato and asparagus seedlings. However the level of autotoxin required to reduce emergence is likely to be different from that required to have an effect on the growth of eight week old asparagus seedlings. Hartung et al. (1989) added 0 - 40 g dried, ground storage root material per kilo of soil in their study and found rates of 20 g dw/kg and higher inhibited growth of asparagus seedlings in pots while lower rates did not. Blok & Bollen (1993) also found that storage root additions of 20 g dw/kg inhibited the growth of asparagus seedlings whereas 2 g dw/kg did not if the roots had been sterilised by gamma irradiation but this lower rate did cause inhibition to seedlings in pots if the roots had not been sterilised.

Estimates of the weight of asparagus crop residues incorporated into the soil during the destruction of an old crop are in the range of 12 to 34 tonnes fresh weight per hectare (2.5 g to 7 g dw/kg). These estimates are based on excavating plants to a depth of 500 mm in the trial sites of the two field trials described in section 2. Blok and Bollen (1993) calculated that 2 g dw/kg was approximately the level of storage root material likely to be found after taking an old asparagus block out of production by cultivating in the old plants. The rate of root addition in this study was 20 g of fresh root per kg of soil (20 g fw/kg). This is equivalent to 4 g dw/kg and is lower than the rates that inhibited growth in the studies referred to and approximates the rate that could be expected in a replant asparagus soil.

It is likely that the lack of an inhibitory response to the root additions either alone or in combination with the *Fusarium* inoculum in this experiment was because the rate of storage roots added was too low. This implies that at levels likely to be encountered in the field the autotoxic material from storage roots of the previous asparagus crop may not directly cause suppressed growth in a subsequent asparagus crop. As the levels of *Fusarium* inoculum added in this study were much lower than those likely to be encountered in an old asparagus field the possibility of an interaction of the autotoxic material with *Fusarium* in a replant site cannot be discounted.

3.3 Greenhouse Trial No. 2

3.3.1 Objectives

The objectives of this experiment were to confirm that:

1. The soil used in pot and field trials has a replant problem.
2. *Fusarium* is an important factor in the replant problem.

3.3.2 Materials and Methods

3.3.2.1 Soil

The soil was Pakowhai silt loam as in Field Trial No. 1.

A sample of this soil was collected from an area that had been in production of asparagus cv NZ Beacon for the past eleven years and where the old asparagus plants had been destroyed by cultivation two months before the soil was collected. A further sample was collected from an adjacent area that had no history of asparagus production.

Both soils were mixed with an equal volume of sand and half the volume of each soil was fumigated with chloropicrin at 0.5 g/kg for 14 days in sealed 20 l containers before air drying and venting for 10 days.

3.3.2.2 Seedlings

Seedlings were grown as described in 3.2.2.2.

3.3.2.3 *Fusarium* inoculum

Single spore isolates of *F. moniliforme* (isolate 13) and *F. oxysporum* (isolate 12) that were isolated from necrotic crown and root material in Field Trial No. 1. were grown on PDA for seven days at 20 °C with a twelve hour photoperiod and spore suspensions were prepared by flooding the plates with sterile distilled water and agitating with a sterile glass rod. Conidial numbers were counted with a haemocytometer and the suspensions adjusted and mixed to give 50:50 mixtures with a total of 4,000 and 16,000 macro and micro conidia per ml. Five millilitres of the appropriate suspension was added to each pot to give inoculum loadings of 20,000 or 80,000 conidia per pot.

Oatseed inoculum was prepared by autoclaving 2 l flasks containing 800 g of oatseed and 100 ml water. After cooling the oatseed was inoculated with 5 mm diameter agar plugs of the *Fusarium* isolates and incubated at 20°C for seven days

by which time mycelial development was evident throughout the flasks of seed. Oatseed inoculum of each of the two isolates (12 and 13) was mixed in equal quantities with each soil before potting up to give added rates of 5 g or 25 g of inoculum per pot.

An autoclaved oatseed treatment with no inoculum was added to control pots.

3.3.2.4 Experiment design and management

The experiment was a factorial design with four soils and six inoculum treatments to give a total of 24 treatments. (Table 3.4)

One seedling was transplanted into each 100 ml square pot containing 500 g of dry soil. There were four pots per plot of each treatment in four blocks. The experiment was laid out in the greenhouse in a randomised complete block design.

Plants were grown in the greenhouse heated below 15°C and vented above 25°C for 14 weeks. During the growing period a liquid feed containing 150 ppm N, 100 ppm P and 100 ppm K was applied twice a week and the experiment was watered to field capacity three times per week.

3.3.2.5 Assessment procedure.

The assessment procedure is described in section 3.2.2.8.

Records for individual plants were summed to give total plot figures and these data were analysed by analysis of variance using the general linear models procedure in the SAS statistical package.

Table 3.4 Treatment numbers, soil treatments and inoculum combinations for Greenhouse Trial No. 2.

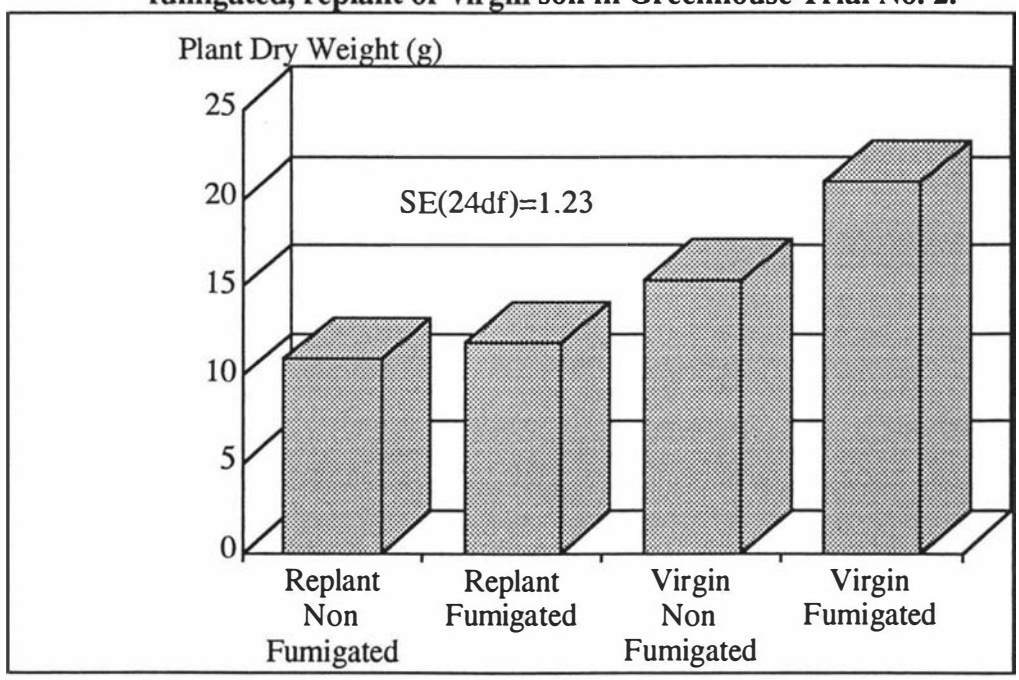
Treatment no.	Soil	Inoculum
R1	replant soil	Nil
S1	sterilised rep.	Nil
V1	virgin soil	Nil
SV1	sterilised virg.	Nil
R2	replant soil	20,000 conidia per pot
S2	sterilised rep.	20,000 conidia per pot
V2	virgin soil	20,000 conidia per pot
SV2	sterilised virg.	20,000 conidia per pot
R3	replant soil	80,000 conidia per pot
S3	sterilised rep.	80,000 conidia per pot
V3	virgin soil	80,000 conidia per pot
SV3	sterilised virg.	80,000 conidia per pot
R4	replant soil	25 g ster. oats per pot
S4	sterilised rep.	25 g ster. oats per pot
V4	virgin soil	25 g ster. oats per pot
SV4	sterilised virg.	25 g ster. oats per pot
R5	replant soil	5 g oat inoc. & 20 g oats
S5	sterilised rep.	5 g oat inoc. & 20 g oats
V5	virgin soil	5 g oat inoc. & 20 g oats
SV5	sterilised virg.	5 g oat inoc. & 20 g oats
R6	replant soil	25 g oat inoc. per pot
S6	sterilised rep.	25 g oat inoc. per pot
V6	virgin soil	25 g oat inoc. per pot
SV6	sterilised virg.	25 g oat inoc. per pot

3.3.3 Results.

The patterns of root and shoot numbers and the fresh weight and dry weight of shoots and roots were similar to those of total plant dry weight, consequently only the mean total dry weight per plant and the two disease scores for the treatment means are presented here.

There was no interaction between the treatments for the mean total dry weight or crown rot scores. There was an interaction (P=0.05) between the effect of inoculum and soil treatments on root lesion scores

Figure 3.1 Mean total dry weight/plant (g) grown in fumigated or non-fumigated, replant or virgin soil in Greenhouse Trial No. 2.



Plants grown in the virgin soil were significantly bigger than those in the replant soil as indicated by the dry matter accumulated. (Figure 3.1) Soil fumigation with chloropicrin increased total plant dry weight in both soils although this increase was significant (P=0.05) only in the virgin soil.

Both rates of oatseed inoculum reduced total plant dry weight on fumigated or non fumigated replant soil and on fumigated virgin soil by up to 300% but on non fumigated virgin soil these treatments reduced dry weight by only 130%. Oatseed on its own also reduced total plant dry weight in the virgin soil and fumigated replant soil but, except in the non fumigated virgin soil, the reduction was less than either rate of oatseed inoculum. (Figure 3.2)

Figure 3.2 Mean total dry weight/plant (g) grown in presence of two rates of conidia or oatseed inoculum or no inoculum or oatseed in Greenhouse Trial No. 2.

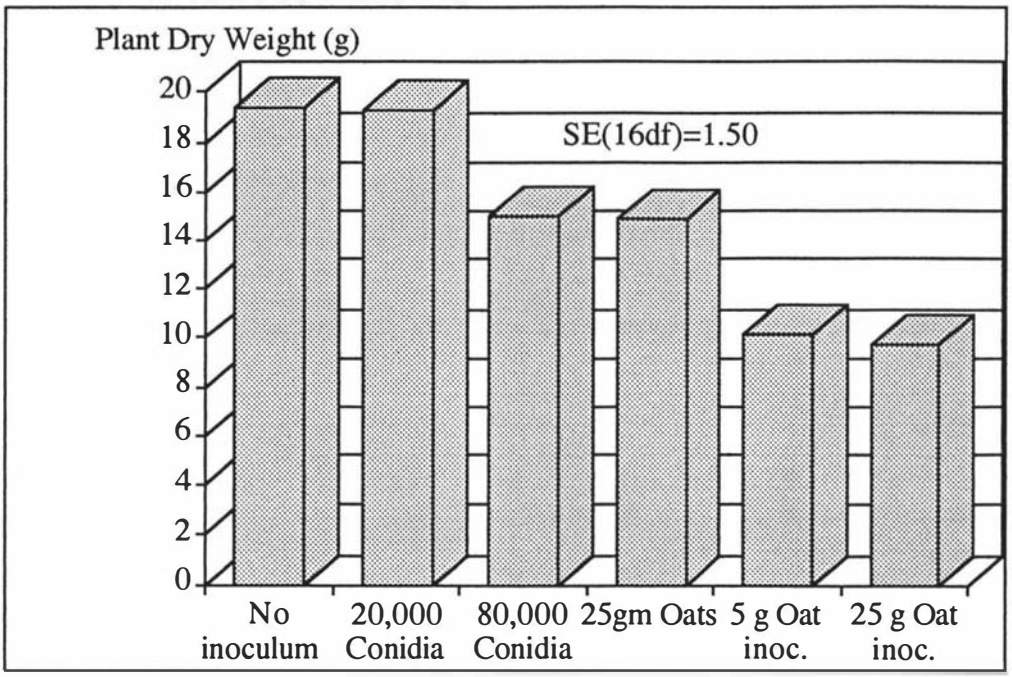
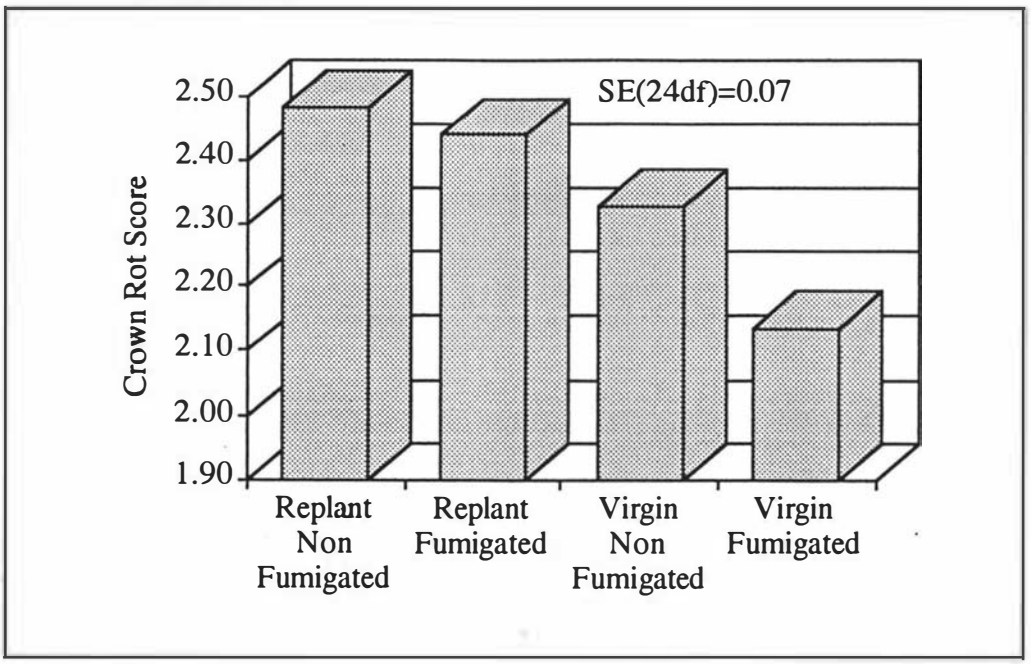


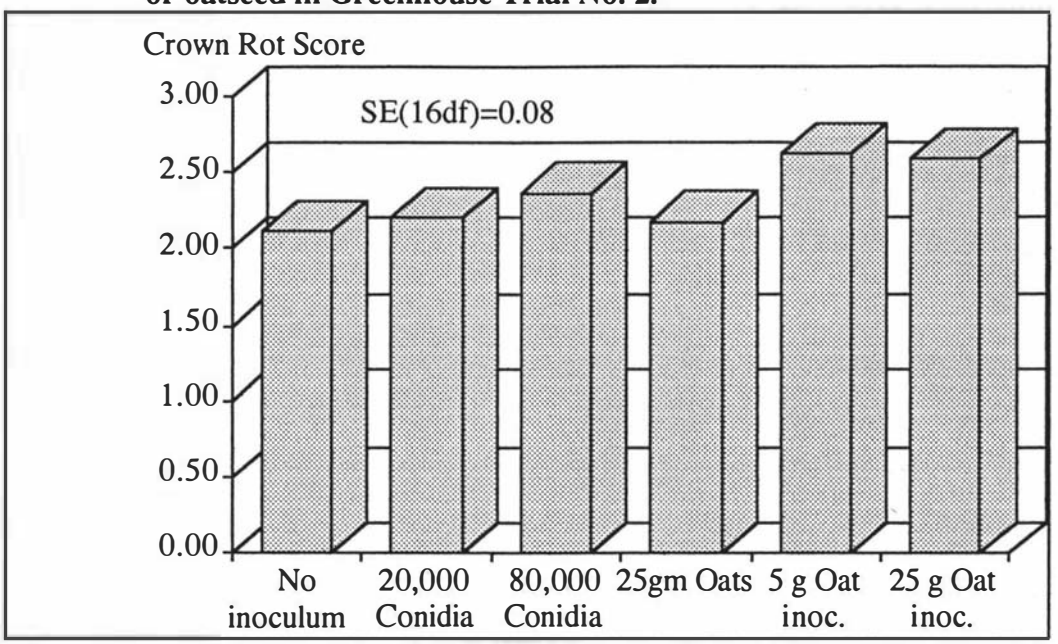
Figure 3.3 Mean crown rot scores (1=low, 5=high) for plants grown in fumigated or non-fumigated, replant or virgin soil in Greenhouse Trial No. 2.



Both rates of conidial suspensions reduced total plant dry weight compared to the no inoculum treatment but the reduction in weight was only significant (P=0.05) at the high rate. (Figure 3.2)

The fumigated virgin soil had the lowest crown rot scores and replant soil the highest. (Figure 3.3) Oatseed inoculum increased the crown rot scores compared with no inoculum on both soils but the differences were only significant (P=0.05) for the high rate on each soil if it had been fumigated and for the low rate on both soils if it had not been fumigated. (Figure 3.4)

Figure 3.4 Mean crown rot scores (1=low, 5=high) for plants grown in presence of two rates of conidia or oatseed inoculum or no inoculum or oatseed in Greenhouse Trial No. 2.



Fusarium root lesion scores were highest in the non-fumigated replant soil and lowest in the virgin soil. (Table 3.5) The oat inoculum at the high rate increased the lesion scores on the fumigated replant and both fumigated and non-fumigated virgin soils. The conidial treatment increased the lesion score on the non-fumigated soils only and did so at both rates in the replant soil but only at the high rate in the non-fumigated virgin soil.

Table 3.5 Lesion scores (1=low, 5=high) for seedlings grown in four soils with six inoculum treatments in Greenhouse Trial No. 2.

	Replant		Virgin	
Inoculum	**Non Fumigated**	**Fumigated**	**Non Fumigated**	**Fumigated**
None	2.38	1.66	1.50	1.44
20,000 Conidia	2.91	1.84	1.63	1.41
80,000 Conidia	3.59	1.88	2.00	1.69
25 g Oats	2.44	2.06	1.65	1.72
5 g Oat inoculum	2.69	2.28	1.69	1.88
25 g Oat inoculum	2.06	2.84	2.00	2.00

SE = 0.22 (4 df)

3.3.4 Discussion

Plant suppression caused by addition of oats alone was probably due to nitrogen depletion of the soil by the microbial population breaking down the large carbon input. The increase in disease scores associated with this treatment can probably be attributed to a reduction in vigour-related tolerance of *Fusarium* due to reduced growth in the presence of depleted nitrogen levels. The effect of the oat grain inoculum was rate dependant with the higher rate having the biggest effect although the difference was only significant (P=0.05) in the fumigated replant soil. Similarly, both rates of conidial application caused some growth suppression and increased the disease scores, on both soils when they had been fumigated, with the higher rate having the greatest effect. Oat seed inoculum at the low rate had significantly more effect than the high rate of conidia. The increased suppression caused by the oatseed inoculum compared with the conidia can be attributed to a greater inoculum level since there was considerably more fungal biomass per pot with the low rate of oatseed inoculum than with the high rate of conidia. These results indicate that the level of *Fusarium* inoculum is an important factor governing the effect of *Fusarium* on asparagus plants.

There was some contamination of all pots with *Fusarium* since a low number of *Fusarium* lesions were detectable on roots from the sterilised soil treatments where no *Fusarium* inoculum had been added. This was most likely due to the transfer of small amounts of inoculum from pot to pot in the greenhouse by insect movement. Small flies were seen in the greenhouse during the course of the experiment and an

insecticide was applied to the pots to try and minimise this cross contamination, however it is doubtful whether the low level of contamination that developed had any significant effect on plant growth during the experiment.

The replant soil suppressed plant growth and led to a higher incidence of *Fusarium* root lesions and crown rot. Fumigating the replant soil greatly reduced lesion scores as the high level *Fusarium* inoculum present in the soil was presumably eliminated or markedly reduced by chloropicrin. Plant growth suppression was not reduced by fumigating the replant soil so it is unlikely that the suppression was due to the presence of plant pathogens but it is more likely that reduced plant growth in the replant soil was due to some form of phytotoxic material.

Fumigating virgin soil led to a lower disease incidence and greater plant biomass production in all treatments except the high level of *Fusarium* oatseed inoculum. These effects can be explained by the asparagus seedling growth being better in an initially "sterile" soil due to the absence of populations of soil micro flora competing for available nutrients. In the presence of high levels of *Fusarium* however, the effect of the *Fusarium* was greater in the "sterile" soil as the introduced disease-causing agents did not have to compete with an existing soil micro-flora.

This pot trial has shown that replant asparagus soil suppresses the growth of asparagus seedlings. The suppression was not reduced by fumigation of the soil so it is unlikely to be entirely due to soil borne pathogens. *Fusarium* inoculum, either as oatseed or conidial suspension, suppressed the growth of asparagus seedlings and this suppression increased with increasing rates of inoculum.

Since the plants grown in the replant soil in the presence of high rates of inoculum were substantially smaller than those in the virgin soil with high rates of inoculum it can be concluded that the suppression due to a phytotoxic substance in the replant soil is additional to any suppression caused by *Fusarium* inoculum. As with other research in this area there is evidence from this experiment that *Fusarium* and toxic substances in replant soil act with some synergy in the suppression of asparagus seedlings (Hartung & Stephens 1983; Peirce & Colby 1987; Hartung et al. 1989). The studies conducted by Hartung et al. (1989) indicate that the autotoxic material in asparagus roots increased electrolyte efflux but decreased peroxidase activity and respiration of excised asparagus roots thus suggesting that the allelochemicals have direct physiological and biochemical effects on asparagus plants that predispose them to *Fusarium*. Alternatively the effect of the allelochemicals could be simply due to the reduction in vigour-related tolerance of *Fusarium* when the seedlings are suppressed by phytotoxic substances in replant soil.

3.4 Greenhouse Trial No. 3

3.4.1 Introduction

Fusarium spp. are a major cause of asparagus decline and the typical symptoms of *Fusarium* infection of asparagus have been well described by various researchers (Elmer 1990; Hartung and Stephens 1983)

Elmer's (1992) research suggests that applications of rock salt to declining stands of asparagus can lead to an improvement in yield and that the main benefit from salt applications to established asparagus comes about through a reduction in the *Fusarium* infections and thus a reduction in the general decline of the bed.

Falloon et al. (1989) showed that applications of thiabendazole as a soil drench on old beds of asparagus improved the yield of spears from those beds. Similar results were obtained in trials in the UK (Giltrap, pers. comm.)

A test capable of predicting severity of *Fusarium* root infections would be extremely valuable to New Zealand growers. Such a test has been developed in Switzerland by Gordon-Lennox and Grindrat (1987) but it has not been evaluated in New Zealand.

3.4.2 Objective

To determine whether the root necrosis potential (RNP) test of Gordon-Lennox and Grindrat (1987) is applicable under New Zealand growing conditions.

3.4.3 Materials and Methods

3.4.3.1 Field trial.

A field trial was established in September 1990 on a declining ten year old block of asparagus cv Beacon (adjacent to Field Trial No.1) to study the effect of salt and fungicide applications over a two year period. (Section 2.6) Spear yield was studied for two years and the levels of infective *Fusarium* spp. present in the soil were assessed by use of the RNP method after two years of soil treatments.

3.4.3.2 Soil Sampling.

Five months after the second application of the field trial treatments (seventeen months after the first application) 500 g soil samples were collected from the rhizosphere (0-150 mm depth) from each plot of the field trial (50 g from ten random sites in each plot). There were six replicate plots for each treatment in the field trial so bulked samples for each treatment gave 3 kg of soil.

Three kilogram samples (bulked from 10 random sites) were also taken from two adjacent areas with the same soil type but with (a) No asparagus history (Virgin

soil) and (b) 10 years in asparagus but the old stand had been destroyed two months earlier by chopping up the plants with a rotary hoe (Replant soil) as described in section 2.2.2.1.

3.4.3.3 RNP Bioassay arrangement

A root necrosis potential (RNP) assay (Gordon-Lennox and Grindrat 1987) was carried out to establish whether any of the treatments in the field trial affected the numbers of *Fusarium* infective propagules in the soil or their ability to cause infection to asparagus plants.

Asparagus cv Syn 4-56 seed was surface sterilised for 60 seconds in 500 ppm sodium hypochlorite/0.01% Tween 20 solution and rinsed three times in sterile distilled water before being germinated between moist sterile paper towels in an incubator at 25°C.

The soil samples were air dried and mixed 50:50 with clean river sand before five germlings with radicles 5-10 mm long were planted in each of five replicate pots holding 500 g of each soil.

The experiment was set out on a bench in the greenhouse in a randomised complete block design.

3.4.3.4 RNP Bioassay Treatments.

Six soils were assayed in the RNP test, four from the field trial (section 2.6, p61) plus a replant soil and a virgin soil as described above.

1. 1120 kg/ha Rock Salt (NaCl)
2. Thiabendazole soil drench.
3. *Trichoderma viride* soil drench.
4. No treatment. (Control)
5. Replant soil.
6. Virgin soil.

3.4.3.5 Growing conditions.

Plants were grown in the greenhouse heated below 15°C and vented above 25°C for 15 weeks. During the growing period a liquid feed containing 150 ppm N, 100 ppm P and 100 ppm K was applied twice a week and the experiment was watered to field capacity three times per week.

3.4.3.6 Assessment procedure and analysis.

After 15 weeks the plants were gently removed from the soil and the roots washed clean. The roots were carefully examined and given a root necrosis severity index ranging from 0 to 3 :

0 = No necrosis, healthy white root system.

1 = some red/brown lesions on up to 33% of root system.

2 = Lesions on 34% to 66% of root system.

3 = Severe necrosis, lesions on more than 67% of root system

The Root Necrosis Potential of each treatment was the mean of the root necrosis index scores of the 25 seedlings obtained from the 5 replicate pots.

The total fresh and dry weight of seedlings grown in each pot was also measured and the mean dry weight for each treatment assessed. Dry weight data were analysed by analysis of variance using general linear models procedure in the SAS statistical package. Differences in root necrosis index scores between treatments were tested non-parametrically using the Kruskal-Wallis test. Treatment separation of the resulting ranks was achieved with a Bon-Ferroni inequality procedure (Haraway, 1993).

3.4.4 Results.

The RNP values of soils exposed to the salt and fungicide treatments in the field trial were slightly lower and the *Trichoderma* treatment was slightly higher than the control. (Table 3.6) The RNP value for the replant soil was not significantly different from that of any of the field trial soil samples, but the virgin soil RNP was significantly lower than for any of the soils that had previously grown asparagus.

Table 3.6 Root Necrosis Potential and mean seedling dry weight for six field soils tested in Greenhouse Trial No. 3.

Soil Treatment	RNP	Dry Wt
1120 Kg Salt	2.2ab	3.83b
TBZ	2.4ab	3.89b
Trichoderma	3.0b	3.54b
Control	2.8b	3.74b
Replant	2.8b	3.73b
Virgin Soil	0.2a	6.26a
	*	SE 30df=0.30

* Critical value of Z=2.72

Means followed by the same letter are not significantly different (P=0.05)

The total plant dry matter was similar for all treatments except the virgin soil, where plants accumulated twice as much dry matter as those grown in any of the soils with a history of asparagus production. (Table 3.6)

3.4.5 Discussion

The RNP test clearly separates virgin soil from soils that had grown asparagus in the past demonstrating that soils which had no history of asparagus production are likely to contain fewer propagules of *Fusarium* spp. pathogenic on asparagus compared with soils that have been in asparagus production.

The salt treatment and the fungicide treatment reduced the soil infectivity slightly but the bio-control agent may have increased the infectivity slightly. The treatments may have had an effect on either the numbers of infective *Fusarium* propagules present in the soil or on the conditions around the roots of the indicator plants so that the *Fusarium* was more or less infective. The TBZ in the fungicide treatment would have reduced the level of *Fusarium* in the soil at each application and this would explain the slightly lower RNP value. The salt treatment may have suppressed *Fusarium* spp. populations also through the fungistatic effect of the NaCl. Alternatively the salt treatment may have changed the host susceptibility through a change in the Na:Cl and/or K:Cl ratios in the host as these mineral ratios are reported to be strongly correlated to disease levels (Schneider 1985; Elmer 1992).

The RNP results and dry weight data from the RNP test are confirmed by the yield data taken from the trial site which indicates that none of the treatments affected plant health or vigour to the extent that numbers or weights of spears harvested were different. (Section 2.6.3) The results obtained in the field trial do not support the findings of Elmer (1992) or Elmer et al. (1996) who achieved increases in marketable yield and a reduction of asparagus decline using spring applications of NaCl under various field conditions in Connecticut, U.S.A. His trials used asparagus cultivars Mary Washington and Syn4-56 and the soils were very much older than those in New Zealand. As discussed in section 2.6.4 the soil pH in the field used for this test was lower than optimal for the successful reduction of *Fusarium* by use of rock salt. Under New Zealand conditions the younger, less developed, soils are prone to structural degradation after annual applications of 1120 kg/ha NaCl as was found in the field trial used as a soil sampling site in this study (Petrie et al. 1992).

The virgin soil had significantly higher indicator plant dry weight than the other soils in the RNP test as would be expected. The asparagus seedlings grown in virgin soil had less disease and were therefore able to grow more vigorously and accumulate more dry matter. The RNP bioassay has clearly shown that soils with

no history of asparagus production will have a lower level of inoculum likely to cause *Fusarium* infections on establishing asparagus. This reinforces similar results obtained by Poll & Huiskamp (1990) where their soil evaluation method using asparagus seedlings as an indicator plant also separated soils with a history of asparagus production from soils that had not been previously planted in asparagus.

The RNP test is a useful tool for indicating the likelihood of replant suppression in old asparagus soils. A useful modification of the test would be to expand the scoring system to a 1-5 scale with $^1/_2$ scores permitted as used in Greenhouse Trials 1 and 2. When the data from the 0-3 scoring system in this study was analysed by analysis of variance and the residuals plotted the residual plots were not normally distributed making this analysis invalid for the data. The non parametric Kruskal-Wallis test and Bon-Ferroni inequality procedure was not a very sensitive method of separating the mean RNP scores for the treatments with only large differences in scores resulting in significant differences between the treatments. If a wider scoring system was used the distribution in residual plots following analysis of variance procedures is more likely to be normal.

3.5 Greenhouse Trial No. 4

3.5.1 Introduction

Three main factors implicated as causes of the replant problem are autotoxins, *Fusarium*, and herbicide residues all of which are likely to be carried over in the soil from the old asparagus beds when they are destroyed prior to planting the new asparagus crop (Hartung & Putnam 1986; Hartung et al. 1989; Blok & Bollen 1993).

The relative importance of each of these factors in a particular field situation has not been well quantified. In order to gain further understanding of the importance of these factors to the growth and development of young asparagus plants a series of small scale assays using seedling cell trays was conducted. By using a small container many large experiments could be conducted quickly and thus more information on the response of asparagus seedlings to the factors involved in the replant problem could be gathered.

3.5.2 Objective.

To establish dose response curves for asparagus seedlings grown in the presence of *Fusarium* inoculum, herbicide and asparagus storage roots.

3.5.3 Materials and Methods.

3.5.3.1 Soils.

Two soils with no history of asparagus production were used in the experiment.

Soil #1 was collected from an area adjacent to Field Trial No. 1, a fertile alluvial Pakowhai silt loam.

Soil #2 was collected from an area adjacent to Field Trial No. 2 and is a peat and pumice based, Paki Paki sandy loam.

Both soils were air dried and passed through a 3 mm sieve before being mixed with an equal volume of sand and stored in sealed 1 kg plastic bags. The soil was sterilised, using gamma irradiation, by Pitman-Moore New Zealand limited in their Upper Hutt plant. Sterilisation was achieved with a dose of 2.5 megarads (25 kGy).

3.5.3.2 Cell Trays

The experiments were conducted in 'Flight 135' cell trays with 135 x 30 ml cells. Each cell was 25 x 25 x 50 mm. New cell trays, sterilised by immersing in 1000 ppm sodium hypochlorite for five minutes, were used.

3.5.3.3 Seedlings

Asparagus cv Syn 4 - 56 'germlings' were raised in seed trays of autoclaved sand in the greenhouse at 25°C. The seed had been surface sterilised by soaking for 60 seconds in a 500 ppm sodium hypochlorite/0.01% Tween 20 solution then rinsing three times in sterile distilled water.

The germlings had one shoot approximately 30 mm long and one root of a similar length when transplanted (one per cell).

3.5.3.4 Asparagus storage root additions.

Root additions were prepared from 7 year old plants of asparagus cv Syn4-56 grown in a sandy loam soil with no history of pesticide use (an organically grown asparagus crop). Storage roots were cut from the rhizomes and washed thoroughly in tap water before being cut into 3-5 mm lengths. The root segments were air dried at 30°C for seven days and sterilised using gamma irradiation at 2.5 megarads (25 kGy) by Pitman-Moore New Zealand limited in their irradiation plant in Upper Hutt.

The rate of root additions for the assays was calculated to represent a range from one tenth to ten times the amount of root material that could be expected to be present in a field after cultivating an old asparagus field. This was calculated by excavating 10 entire 7 year old plants from a bed and assessing the fresh and dry weight of root material per plant. This weight was extrapolated to a dry weight of root material per cubic metre of soil assuming that the asparagus plant residue is cultivated into the top 500 mm of soil.

The dry sterilised root segments were added to the soils at rates to give 0.67- 42 g/kg. (Table 3.7)

3.5.3.5 Herbicide

'Caragard' a residual herbicide which contains equal quantities of terbumeton and terbuthylazine was added to the soil and mixed thoroughly before potting up and planting. The rate of 1 ppm total a.i. of these herbicides used in Greenhouse Trial No. 1 suppressed the indicator plants significantly therefore lower rates were used in this bioassay. (Table 3.7)

3.5.3.6 *Fusarium*

Single spore isolates of *F. moniliforme* (isolate 13) and *F. oxysporum* (isolate 12) isolated from necrotic crown and root material in Field Trial No. 1 (Section 2.2.2.13) were grown on PDA for seven days at 20°C with a twelve hour photoperiod. Spore suspensions were prepared by flooding the plates with sterile distilled water, agitating with a sterile glass rod and filtering through cheese cloth. Conidia numbers were counted with a haemocytometer and the suspensions adjusted before mixing to give 50:50 mixtures of both fungal isolates. The higher rate of conidial suspension in Greenhouse Trial No. 2 caused some suppression of asparagus seedlings. This rate of 160 conidia/g soil or 4800 conidia per 30 ml cell was used as the mid-rate of a range from zero to approximately sixty times higher than the mid rate.

Five millilitres of the adjusted spore suspensions were applied to the top of the cells by pipette after the seedlings had been planted and the experiment set out on the greenhouse bench. (Table 3.7)

Table 3.7 Conidia numbers, Herbicide (ppm a.i.) and Roots (g) added per 30 ml cell in Greenhouse Trial No. 4

Treatment	Conidia	Caragard	Roots
1	nil	nil	nil
2	400	0.05	0.02
3	1500	0.1	0.04
4	5000	0.2	0.08
5	15000	0.4	0.16
6	45000	0.8	0.32
7	100000	1.6	0.64
8	300000	3.2	1.28

3.5.3.7 Experimental design.

Six separate bioassays were done, one for each combination of two soils with eight levels of root material, herbicide or conidial inoculum. Each bioassay was replicated four times with each replicate confined to one cell tray. The cell trays had 15 rows of nine cells and the treatments were each contained in one row of 9

cells with adjacent rows to the treatments being left empty to minimise cross contamination in the trays.
Plots consisted of nine cells (plants) and there were 4 blocks (trays) each containing the eight treatments, in single rows.

3.5.3.8 Management.

The experiment was grown for six weeks in a greenhouse vented when temperatures were above 25°C and heated below 10°C.
During the growing period a liquid feed containing 150 ppm N, 100 ppm P and 100 ppm K was applied twice a week and the experiment was watered three times per week. An insecticide (2 g/l cypermethrin) was applied as a spray over the trays at two weekly intervals to minimise insect traffic on the cell trays.
Each assay was assessed once maximum effect of the treatment was evident on the seedlings as assessed by daily monitoring of the experiment.

3.5.3.9 Assessment procedure and analysis.

Trays were soaked in water for several minutes and the seedlings removed from the cells with the roots intact. The soil was washed from the roots and the following measurements taken for each seedling:

-Fresh weight of shoots and roots
-Dry weight of shoots and roots

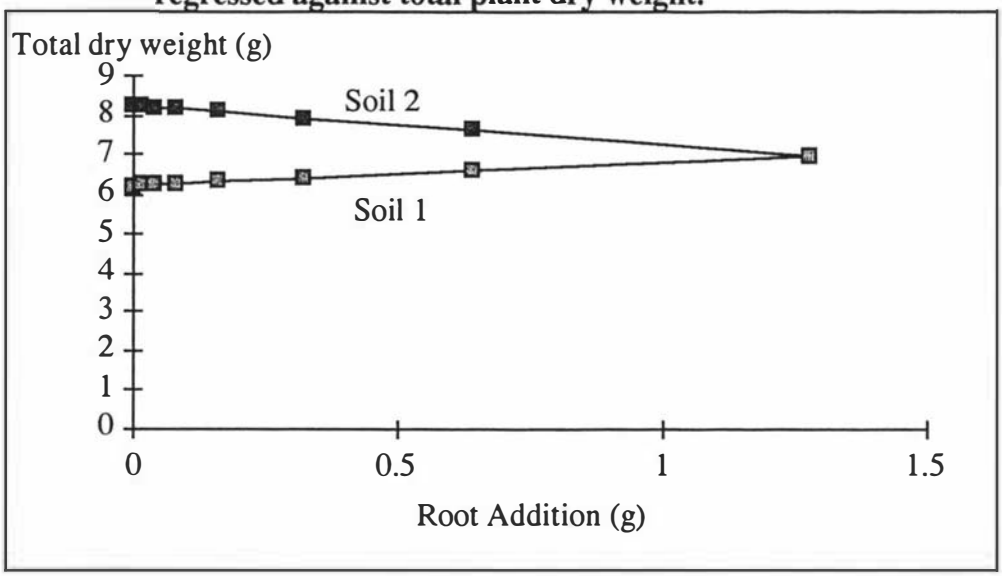
Fusarium infections were assessed as in 3.2.2.8.
The fresh weight and dry weight records for individual plants were summed to give total plot figures and the data were analysed using linear regression analysis procedure in the SAS statistical package. The lesion score data were analysed as described in 3.4.3.6.

3.5.4 Results and Discussion.

Addition of sterilised asparagus storage roots did not significantly (P=0.05) affect the total seedling dry weight in either soil. (Figure 3.5)
Forty two grams of sterilised storage roots per kg dry soil were added at the highest rate and data from previous studies would suggest that this level should strongly inhibit asparagus seedling growth (Peirce and Colby 1987; Hartung et al. 1989; Blok and Bollen 1993). The lack of a response to the storage root treatments could be due to other factors limiting growth in this experiment. The experiment was run in a plastic tunnel house during the months of April and May. The plastic cover was old and did not transmit full light into the tunnel house. Heating was sufficient

to maintain temperatures over 5°C during frosts but on cool days the temperature remained between 10°C and 15°C. The light and temperature conditions for growth of small asparagus seedlings in this situation may have limited the development and growth of the seedlings and masked the effect of the experimental treatments.

Figure 3.5 Eight rates of asparagus storage root additions to two soils regressed against total plant dry weight.



Root lesion scores were similar for the two soils and increased as the rate of storage root increased. (Table 3.8) The increase in disease caused by increasing root additions supports the hypothesis that the root additions inhibited plant growth sufficiently to allow more *Fusarium* infection but other factors suppressed plant growth enough to mask any growth reduction caused by the storage roots. The autotoxic material may have also predisposed the asparagus seedlings to *Fusarium* infection as was found by Hartung & Stephens (1983), Peirce & Miller (1990) and Hazebroek et al. (1989).

Table 3.8 *Fusarium* root lesion scores (1=low, 5=high) for seedlings grown in two soils exposed to eight rates of asparagus storage root addition (g/30 ml cell).

Roots per cell	Soil #1	Soil #2
0	2.00b	1.88b
0.015	2.00b	2.25b
0.04	2.25ab	1.88b
0.08	2.25ab	2.00b
0.16	2.50ab	2.13b
0.32	2.38ab	2.38b
0.64	2.63a	2.13b
1.28	3.13a	2.88a

Means followed by the same letter are not significantly different.
Means separation using a Bon-Ferroni separation procedure. Z critical = 2.91

There was a slight increase in infection level with increasing rates of conidia on both soils (Table 3.9) but this did not result in a subsequent reduction in seedling dry weights.

Table 3.9 *Fusarium* root lesion scores (1=low, 5=high) for seedlings grown in two soils exposed to eight rates of conidial inoculum (000s of conidia/30 ml cell)

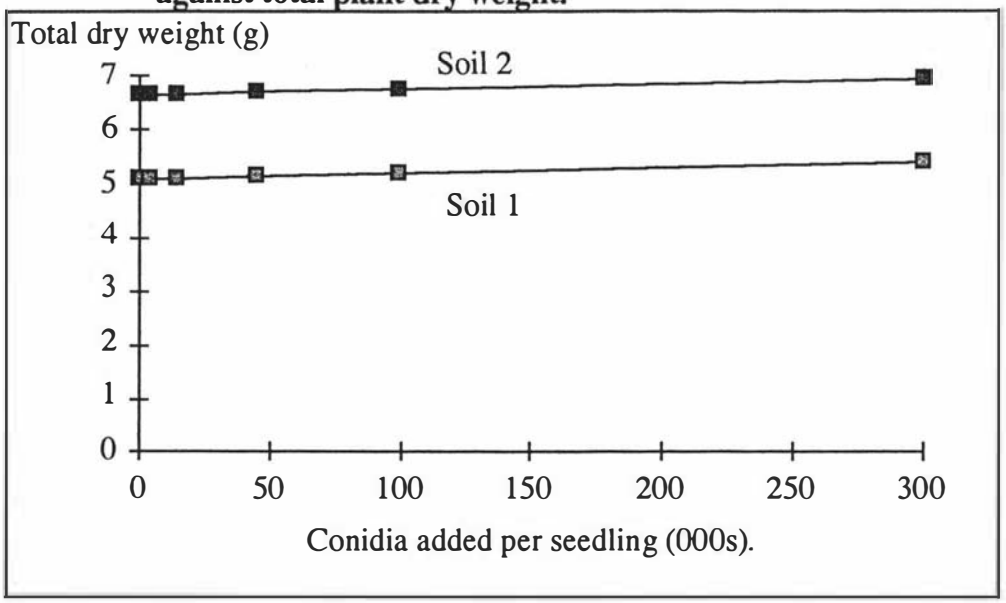
Conidia	Soil 1	Soil 2
0	1.75b	1.93b
0.4	1.88b	1.63b
1.5	1.63b	1.89b
5	2.25ab	2.13b
15	2.13ab	2.38b
45	2.00ab	2.58b
100	2.75a	2.63b
300	2.63a	3.13a

Means followed by the same letter are not significantly different. Means separation using a Bon-Ferroni procedure. Z critical = 2.91

The spore suspensions had no effect on total seedling dry weight in this experiment. (Figure 3.6) Soil 2 produced plants with a mean dry weight approximately 30% higher than Soil 1.

In Greenhouse Trial No. 2 the higher rate of conidia applied (160 conidia per g soil) suppressed seedling growth in virgin sterilised soil so it was expected that addition of up to 10,000 conidia/g would cause considerable suppression in this experiment. The lack of a growth response to conidial suspensions could be attributed to other factors limiting the development and growth of the seedlings thus masking any effect of the disease as described above.

Figure 3.6 Eight rates of conidial inoculum additions to two soils regressed against total plant dry weight.



Total dry weight per seedling was significantly reduced by higher concentrations of herbicide. (Figure 3.7) The effect of the herbicide was greater in Soil #1 than Soil #2 at higher levels of added herbicide however the I_{50} level was similar for both soils. (I_{50} in this case refers to the level of Caragard added that resulted in mean plant dry weight 50% less than the mean dry weight of the zero herbicide control for each soil).

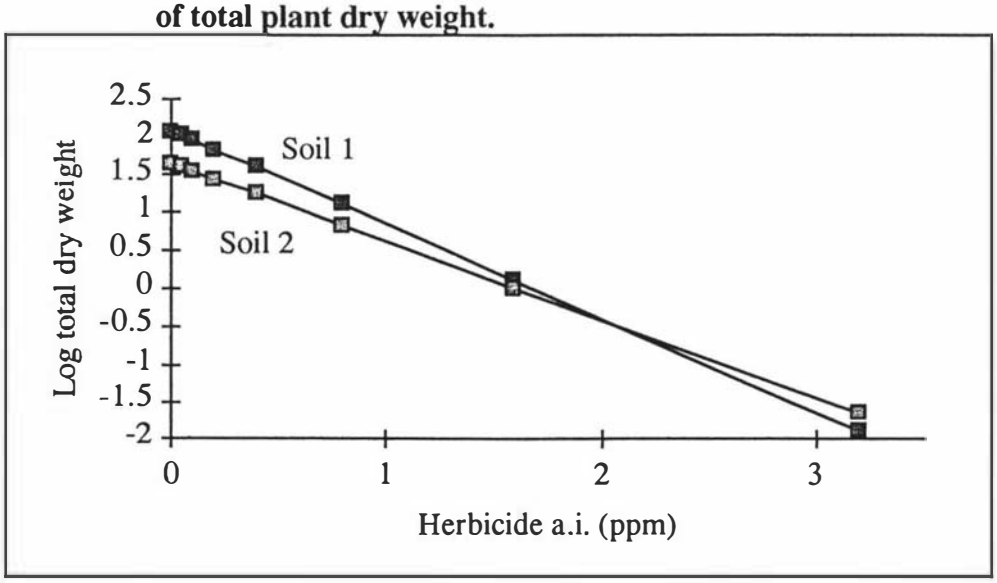
Root lesion scores increased with rate of herbicide applied on both soils (Table 3.10). The disease level was higher for rates of herbicide above 1 ppm in Soil #1 than Soil #2 following the same pattern as the seedling dry weights.

Table 3.10 *Fusarium* root lesion scores for seedlings grown in two soils exposed to eight rates of the herbicide Caragard (ppm a.i./30 ml cell)

Herbicide	Soil 1	Soil 2
0	2.25b	2.25b
0.05	2.13b	2.00b
0.10	2.00b	2.00b
0.20	2.50ab	2.38b
0.40	2.75ab	2.25b
0.80	4.25ab	2.13b
1.60	5.00a	3.13b
3.20	5.00a	4.63a

Means followed by the same letter are not significantly different. Means separation using a Bon-Ferroni procedure. Z critical = 2.91

Figure 3.7 Eight rates of herbicide additions to two soils regressed against log of total plant dry weight.



The differences in herbicide effect can be explained by differences in the organic matter content of the two soils. Soil #1 had an organic matter content of 4.9% and Soil #2 had 9.6% organic matter. The higher level of organic matter in Soil #2 would provide more adsorption sites for the herbicide molecules and thus reduce the herbicide's activity in this soil particularly at higher application rates. In Greenhouse Trial No. 1 the same two soils were used and the herbicide activity at the rate added (1 ppm) was greater on Soil 2. In that trial however much bigger plants were grown for a longer period and in the presence of a lower rate of herbicide. It is likely that the different experimental conditions explain the difference in herbicide activity between Greenhouse Trials 1 and 4.

3.4.5 Conclusions

Although the experiment was conducted with sterilised soil and the root additions had been sterilised there were *Fusarium* lesions evident on all treatments after six weeks of growth. Insecticide was used regularly to minimise insect traffic on the seed trays and the treatments were separated by empty rows of cells to minimise splashing from one treatment to another during watering. It is apparent however that to prevent *Fusarium* cross contamination between plots of experiments such as this each plot must be completely isolated. One method that has been employed to successfully avoid cross contamination (Peirce & Colby 1987) is the placing of individual pots in zip lock plastic bags creating a mini greenhouse for each pot. This method does reduce the need for watering and avoids contamination but would not be suitable for conducting miniaturised experiments with many treatments as was planned in this study.

Section Four
Laboratory Experiments

4.1 Introduction.

Studies by Hartung et al. (1989), Hartung & Putnam (1986) and Young & Chou (1985) have shown that asparagus tissue, asparagus plant residues and soils from old asparagus beds contain substances that are inhibitory to asparagus plant growth. This research indicated that the most toxic material was located in the storage roots of asparagus plants.

The importance of toxins in the replant problem may be related to the effect they have in predisposing asparagus roots and stems to attack by pathogens (Hartung & Stephens 1983; Hazebroek et al. 1989; Peirce & Miller 1990).

Experiments discussed in section three indicate that the toxin may be an important factor in the reduced vigour of asparagus grown in a replant situation however the greenhouse experiments were not sufficiently sensitive to fully explore the effect of the toxin on asparagus seedlings.

4.2 Seedling bioassays to assess the toxicity of *Asparagus officinalis* L root extract using pregerminated *Asparagus officinalis* L seeds.

4.2.1 Objectives

1. To study the effect of asparagus root extract concentration on the growth rate of germinated asparagus seed.

2. To establish whether the toxic material from asparagus roots is heat stable or removed by filtration.

3. To determine whether the suppression in seed bioassays with asparagus root extracts is an osmotic effect.

4.2. 2 Materials and Methods.

4.2.2.1 Root Extract.

Ten year old plants of asparagus cv NZ Beacon were excavated from an old bed adjacent to Field Trial No. 1. After removal from the rhizomes one kilogram of washed storage roots were pulverised using a mortar and pestle and left in 2 l of tap water for 12 h.

The crude root extract obtained was drained off, centrifuged at 12,000 rpm for 15 min., filtered through Whatman #4 filter paper and then finally through a 0.2 μ millepore filter to eliminate fungal spores and bacteria.

One sample of the filtered extract was autoclaved at 120°C (15 psi : 1.04 bars) for 15 min. A further sample was passed through a celite filter. One sample was assayed without further treatment. The remaining extract was stored at -18°C for use in future bioassays.

4.2.2.2 Seed.

Asparagus cv UC 157 seed was surface sterilised by washing in 500 ppm sodium hypochlorite solution with 0.1% Tween 20 for one minute then rinsed three times in sterile distilled water. The seed was pregerminated by placing on blotting paper moistened with sterile distilled water in sterile Petri plates. After 7 days incubation at 20°C in a twelve hour photoperiod, seeds with radicles of uniform length (1-2 mm) were selected for use in the bioassay.

4.2.2.3 Containers

Two sheets of 0.65 mm thick blotting paper 75 mm x 75 mm were placed in each Petri plate and 10 ml of assay solution was applied to the blotting paper in each plate before the addition of 10 pre-germinated seeds.

4.2.2.4.1 Experiment 1 design and Treatments.

Filtered root extract (serially diluted with tap water to give six concentrations for bioassays) was applied to each dish before the asparagus seeds were laid on the papers.

Treatments in Experiment 1 were:

Water (tap)
Undiluted Root Extract
50% Root Extract
25% Root Extract
12.5% Root Extract
6.25% Root Extract
3.13% Root Extract
Autoclaved Root Extract
Celite Filtered Root Extract

There were four replicates arranged in the incubator room as a randomised complete block design. The experiments were carried out at a constant 20°C with 12 h photoperiod.

4.2.2.4.2 Experiment 2 design and Treatments.

A second bioassay was carried using the solid material left after centrifuging and using nutrient solutions with a similar osmotic pressure or conductivity (CF) to the crude root extract.

After autoclaving the root extract a precipitate formed in the autoclave flask. This was resuspended in water and bioassayed. The pellet that remained after centrifuging was resuspended in water and re-centrifuged. The pellet that remained after the second centrifuging was resuspended in water and used in the bioassay to test the insoluble material remaining from the water extraction process.

The treatments in Experiment 2 were:

Water
Undiluted Root Extract
25% Root Extract
6.25% Root Extract
Insoluble material after centrifuging
Autoclaved Root Extract
Precipitate after autoclaving
Nutrient solution CF 23
Nutrient solution CF 12.5

After four days the blotting papers in each plate were re-moistened with 5 ml of the appropriate bioassay solution.

4.2.2.5 Assessment and Data Analysis.

The radicle length and shoot length of each asparagus seedling were measured after seven days. The results were analysed by analysis of variance using the general linear models procedures of the SAS statistical package and the treatment means were separated using Duncan's multiple range test.

Linear regression analysis using the SAS statistical software was employed on the radicle length data from the two dilution series.

4.2.3 Results.

The water control produced the longest shoots but shoot length was not significantly affected by any root extract treatment.

The radicle length of asparagus germlings was significantly reduced by the asparagus root extract with the neat root extract, whether untreated, autoclaved or celite filtered, reducing radicle growth to 25% of that of the water control. In both dilution series radicle length declined as root extract concentration increased. (Table 4.1)

Table 4.1. Radicle and shoot length of asparagus germlings exposed to a range of concentrations of untreated asparagus root extract or autoclaved or celite filtered extract or water in experiment 1.

Treatment	Radicle Length	Shoot length
100% Root Extract(RE)	5.30 **F**	1.17 **AB**
50% RE	7.65 **DE**	1.69 **AB**
25% RE	8.95 **D**	1.61 **AB**
12.5% RE	13.53 **C**	1.86 **AB**
6.25% RE	15.95 **B**	3.75 **AB**
3.13% RE	17.23 **B**	3.11 **AB**
Water	21.38 **A**	4.78 **A**
100% Autoclaved RE	6.40 **EF**	3.36 **AB**
100% Celite filtered RE	7.23 **EF**	2.53 **AB**
SE (24df)	0.68	1.24

Means separation in the table by Duncan's Multiple Range test.
Means with the same letter are not significantly different (P=0.05).

Table 4.2 Radicle and shoot length of asparagus germlings exposed to a range of concentrations of untreated asparagus root extract or nutrient solutions or insoluble material from the extraction or autoclaved extract liquid or autoclaved extract solid or water in experiment 2.

Treatment	Radicle Length	Shoot length
100% Root Extract(RE)	10.95 **D**	7.25 **A**
25% RE	14.55 **C**	9.68 **A**
6.25% RE	20.43 **B**	8.88 **A**
Water	25.18 **A**	9.56 **A**
Autoclave Liquid	5.13 **E**	9.20 **A**
Autoclave Solid	21.58 **B**	9.05 **A**
Insoluble Material	25.03 **A**	6.95 **A**
CF 23	24.73 **A**	7.58 **A**
CF12.5	24.75 **A**	6.80 **A**
SE (24df)	0.78	1.21

Means separation in the table by Duncan's Multiple Range test.
Means with the same letter are not significantly different (P=0.05).

Radicle lengths in the CF 23 and CF 12.5 nutrient solutions were the same as those in the water control as were those in material that precipitated out during autoclaving and the solid material that settled out during centrifuging. (Table 4.2)

Figure 4.1 Log mean radicle length of asparagus germlings exposed to two dilution series of asparagus storage root extract.

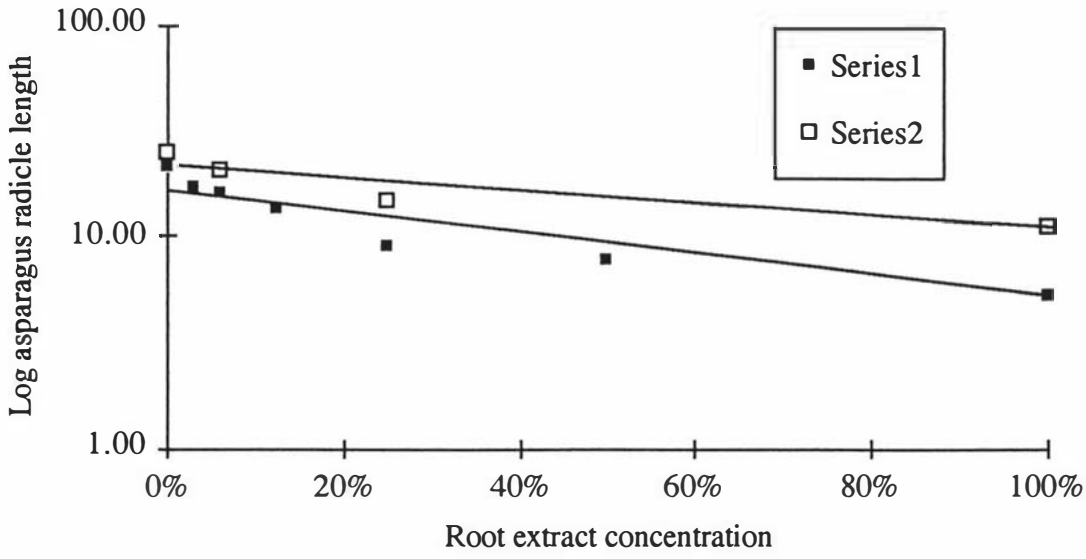


Table 4.3 Regression analysis data (SE in brackets) of log asparagus germling radicle lengths exposed to two dilution series of asparagus root extract.

	Series 1	Series 2
R-squared	0.81	0.72
Slope coefficient	-0.117 (0.022)	-0.138 (0.018)
Intercept	21.63 (1.13)	16.74 (0.78)
df	26	14

The plot of log radicle length against root extract concentration gave straight lines with similar slopes for both dilution series. (Fig 4.1, Table 4.3)

4.2.4 Discussion

The phytotoxic material in asparagus storage roots is very potent and a 3% concentration of the aqueous extract significantly reduced radicle elongation in asparagus germlings. This concentration of crude root extract represents 15 g fresh root material (frw) per litre of water (3.8 g dry weight/l). Estimates of the weight of asparagus crop residues incorporated into the soil during the destruction of an old crop indicate 8 g frw/kg to 20 g frw/kg could be reasonably expected after cultivating an old asparagus bed (section 3.2.4.3). This level of asparagus storage root material in the soil would cause inhibition in the bioassay and may have a phytotoxic effect on plants growing in soil. In a similar bioassay, Young & Chou (1985) showed that water extracts of asparagus stem, root and root litter all inhibited radicle and shoot growth of pregerminated asparagus seedlings with shoots inhibited more than radicles. When the bioassay was conducted on root exudates that had been collected in XAD-4 resin from donor pots, complete inhibition of shoots and some inhibition of radicles occurred (Young 1984). The inhibition of shoots more than roots in the Taiwanese studies contrasts with the results of this bioassay and is likely to be due to the different extraction procedure used or the different growing conditions in the subtropical/tropical climate in Taiwan compared to our temperate growing conditions. In warmer conditions the biochemical content of the water extracts from asparagus tissues are likely to differ from those grown in a temperate climate. The root extract used in this study was from dormant plants whereas asparagus plants do not have a dormant period in Taiwan under normal conditions. Another difference is that the water extraction from roots was done at room temperature (15°C) in this study whereas Young & Chou (1985) extracted their tissue at 5°C and may have extracted different proportions of phytotoxic chemicals from the asparagus tissue.

Hazebroek et al. (1989) exposed germinated asparagus seed to crude water extracts (5 g dw/l to 20 g dw/l) from asparagus storage roots. They also tested germination of lettuce, tomato and cucumber in the presence of asparagus storage root extracts. Their bioassay gave very similar results to this study with radicle elongation inhibited more than shoot growth and the inhibition of radicles occurred at lower concentrations than shoot inhibition. They concluded that asparagus radicle growth was a more sensitive indicator of extract toxicity than either germination or shoot growth.

As radicles are in direct contact with the assay solutions it is likely the phytotoxins involved in the inhibition will affect the elongation of radicles more than shoots as has been found in these bioassays. The phytotoxic material in the root extract may have a localised effect on the radicles that are directly exposed to the extract but this effect may be reduced in the seedling shoots as they are not in direct contact with the assay solution so any inhibitory effect of the phytotoxins would be as a result of the

inhibition of the radicles or through translocation of some of the toxic material from the roots to the shoots.

Sterilisation of the root extract by autoclaving did not reduce the level of suppression in the bioassay. In the second bioassay series an increase in suppression arose after autoclaving but some extract evaporated during the heat treatment and the difference in toxicity is likely to be due to an increased concentration of the extract. These findings support the view that the phytotoxic compounds in asparagus root extracts are heat stable. Alternatively the phytotoxicity could be retained through a heat induced change from one group of toxic compounds to another group of equally toxic compounds. Other studies have also concluded that the autotoxic compounds in asparagus tissue are heat stable (Yang 1986, 1982; Peirce & Colby 1987). The solid material that precipitated out during heat treatment and the pellet of insoluble material recovered after centrifuging were found to have no toxic activity supporting the finding that the toxic compounds in asparagus tissue are both soluble and stable, thus confirming the findings of Peirce and Colby (1987).

The osmotic pressure of the root extract did not explain the suppression found in this bioassay. Radicle and shoot growth in two nutrient solutions, one with the same osmotic pressure and one with half the osmotic pressure of the root extract, had similar growth to germlings in the water controls. Other studies have also found that phytotoxicity of asparagus tissue extracts are not caused by their osmotic pressure (Yang, 1986; Hazebroek et al. 1989).

The dose response lines in the two dilution series had similar slopes (Table 4.3) indicating the radicle growth suppression caused by the root extract was similar for both series. The different Y axis intercepts from the two bioassays result from slightly different radicle lengths of the germlings used in setting up the experiments.

Peirce and Colby (1987) found that root extracts from fresh roots inhibited seedling emergence slightly more than extract from roots that had been stored frozen for $1\frac{1}{2}$ years. The root extract used for the second dilution series had been stored at -18°C for seven days but asparagus germling radicle growth was unaffected by freezing for this relatively short time.

4.3 Seedling bioassays to assess the sensitivity of different crop species to *Asparagus officinalis* L root extract.

4.3.1 Introduction

The bioassay previously described (4.2) used pregerminated asparagus seed and was relatively slow; seven days to pregerminate the seed and another seven days to run the bioassay.

In a Greenhouse Trial Shafer and Garrison (1986) evaluated lettuce, tomato and asparagus seedling emergence in seedling trays with asparagus roots mixed into the growing media. They found that lettuce and tomato were sensitive to the toxic material in asparagus storage roots. Curly cress has also been shown to be suppressed by the toxic compounds in asparagus roots (Hartung et al. 1989).

Evidence from commercial plantings and two pot trials indicated that certain species such as oats, lettuce, tomato and radish are suppressed in soils where asparagus has recently been grown whereas peas, beans and sweetcorn were less affected. By assaying a range of other plant species it was hoped to find an indicator plant species for the bioassay that would be as sensitive or more sensitive than asparagus to the asparagus root extract. This would improve the speed and sensitivity of the bioassay thus obviating the need to top up the assay solutions during the course of the assay and also to reduce the time for bacterial and fungal pathogens to develop in the Petri plates.

Such a bioassay would also provide a means of evaluating various crops' sensitivity to the toxins present in asparagus roots or in old asparagus soil.

4.3.2 Objectives

1. To evaluate the sensitivity of seedlings of a range of vegetable and crop species to the toxins in asparagus root extract.

2. To establish a suitable species for future bioassay experiments.

4.3.3 Materials and Methods.

4.3.3.1 Root Extract.

Asparagus storage root extract was prepared as described in section 4.2.2.1.

The crude root extract was centrifuged at 12,000 rpm for 15 min. before filtration through glass wool. The filtered root extract was refrigerated, until used, at 2°C.

4.3.3.2 Seed.

To improve the uniformity of germination all seed lines were stored at 2°C for at least 24 h before setting up the bioassays.

The asparagus seed was surface sterilised and pregerminated as described in section 4.2.2.2. Seed of other species was sown directly onto the blotters immediately after the bioassay solutions had been applied.

4.3.3.3 Containers

The experiment was carried out on two sheets of 0.65 mm thick 75 mm x 75 mm blotting paper in plastic Petri plates. After treatments were applied to the blotting paper 12 seeds were placed on the paper in each dish.

4.3.3.4 Experimental design and Treatments.

Filtered root extract was diluted with tap water to give three concentrations plus tap water as a control. Ten ml of assay solution was applied to each dish before the seeds were added.

There were four replicates arranged as a randomised complete block design in a 20°C incubator room with a 12 hour photoperiod.

Bioassay solutions:

Water
50% Root Extract
25% Root Extract
12.5% Root Extract

Species bioassayed:

Cucumber	*Cucumis sativum* cv Crystal Apple
Beans	*Phaseolus vulgaris* cv Espada
Broccoli	*Brassica oleracea* var *italica* cv Shogun
Lettuce	*Lactuca sativa* cv Gloria
Oats	*A vena sativa* cv Greenfeed
Peas	*Pisum sativum* cv WF Massey
Radish	*Raphanus sativus* cv Champion
Sweetcorn	*Zea mays* cv Honey'n Pearl
Tomato	*Lycospersicon esculentum* cv Temprano
Asparagus	*Asparagus officinalis* cv UC157

4.3.3.5 Assessment and Data Analysis.

Each species was assessed when treatment differences were evident and bioassays still running after four days were re-moistened with 5 ml of the bioassay solutions. Bioassays were assessed by measuring the radicle length and shoot length on the biggest 10 seedlings in each of the four replicate Petri plates. Data were subject to analysis of variance and regression analysis using the SAS statistical package. Mean radicle lengths and shoot lengths were plotted against concentration of root extract to arrive at I_{50} values for root and shoot growth of each species. The I_{50} value was the concentration of root extract that reduced growth of the root or shoot to 50% of that in the water control for that species.

4.3.4 Results.

Plots of radicle length and shoot length of each genera against root extract concentration are given in Appendix 2.

Shoot and radicle growth of peas and shoot growth of sweetcorn were not affected by the asparagus storage root extract (RE). (Table 4.4) Radicle growth of beans and sweetcorn and the shoot growth of asparagus were slightly suppressed at the highest concentration of RE used. Radicles and shoots of tomato and broccoli were both inhibited by similar amounts in RE. Shoot growth of cucumber and oats were suppressed more than radicle growth at higher concentrations of RE. Asparagus radicles were increasingly suppressed by RE as concentration increased. At the lower concentrations of RE lettuce radicle growth was inhibited more than that of the other genera. Cucumber and oats were the only plants tested where shoot growth was inhibited more than that of lettuce.

Table 4.4 Radicle length, shoot length and number of days to complete the bioassay for ten plant genera grown in the presence of four concentrations of asparagus root extract. (SE 4df in brackets)

Treatment	Cucumber	5 days	Bean	7 days
	Radicle	Shoot	Radicle	Shoot
50% RE	16.28 (1.84)	0.0	31.3 (1.21)	0.0
25% RE	15.85 (3.87)	0.1 (0.05)	23.2 (1.08)	0.0
12.5% RE	20.95 (3.19)	1.5 (1.16)	32.6 (1.84)	0.0
Water	54.03 (2.82)	11.9 (2.08)	54.9 (2.13)	0.0
	Lettuce 1	3 days	**Oat**	7 days
	Radicle	Shoot	Radicle	Shoot
50% RE	1.8 (0.20)	4.0 (1.03)	3.4 (0.06)	0.1 (0.08)
25% RE	3.7 (0.36)	8.1 (1.03)	14.9 (1.79)	0.6 (0.55)
12.5% RE	6.7 (0.52)	13.1 (0.93)	23.9 (1.12)	2.8 (0.82)
Water	25.3 (1.06)	28.7 (1.19)	33.2 (1.29)	8.7 (0.95)
	Lettuce 2	3 days	**Tomato**	4 days
	Radicle	Shoot	Radicle	Shoot
50% RE	1.0 (0.10)	5.5 (0.53)	6.3 (0.90)	0.8 (0.60)
25% RE	3.5 (0.29)	7.5 (0.46)	11.2 (1.17)	4.2 (0.49)
12.5% RE	6.5 (0.28)	9.8 (0.16)	17.7 (1.16)	6.8 (1.16)
Water	17.1 (0.89)	19.4 (1.17)	28.2 (2.29)	10.2 (1.19)
	Asparagus	8 days	**Sweetcorn**	8 days
	Radicle	Shoot	Radicle	Shoot
50% RE	7.3 (0.63)	2.4 (0.18)	15.5 (1.21)	6.9 (0.94)
25% RE	8.9 (1.12)	2.5 (0.49)	21.4 (2.27)	4.8 (0.61)
12.5% RE	9.9 (0.47)	3.3 (0.40)	24.0 (1.73)	5.0 (0.90)
Water	14.2 (0.83)	3.9 (0.43)	26.1 (4.45)	11.4 (1.28)
	Broccoli	3 days	**Pea**	5 days
	Radicle	Shoot	Radicle	Shoot
50% RE	4.4 (0.54)	4.1 (0.35)	20.7 (4.73)	8.4 (0.56)
25% RE	20.4 (3.08)	11.9 (0.78)	18.8 (5.55)	5.6 (1.00)
12.5% RE	21.1 (1.33)	19.9 (0.65)	29.6 (4.49)	7.4 (0.58)
Water	33.8 (1.11)	23.8 (0.56)	20.1 (7.92)	6.0 (1.31)

I_{50} values (Table 4.5) indicate that cucumber and lettuce radicles and shoots and oat shoots are highly sensitive to RE with I_{50} values of 12% or less. Comparison of the standard error values for the lengths (Table 4.4) indicate that lettuce radicle measurements gave the lowest error rates whereas the experimental error for cucumber shoots was relatively high.

Table 4.5 I_{50} values (%RE) for root and shoot growth of ten genera exposed to asparagus storage root extract.

	I_{50} Radicle	I_{50} Shoot
Cucumber	11.0	5.8
Bean	NA	NA
Broccoli	29.5	25.0
Lettucel	8.8	11.5
Lettuce 2	8.5	11.8
Oat	22.8	9.0
Pea	NA	NA
Tomato	19.0	21.0
Sweetcorn	NA	11.8
Asparagus	55.0	NA

NA = Growth suppression was not sufficient to allow the I_{50} value to be assessed
Refer to plots of radicle length and shoot length of each species against root extract concentration in Appendix 2.

4.3.5 Discussion.

The inhibitory effect of RE on the germination and growth of radicles and shoots varies greatly between different plant species. Peas and beans were both relatively unaffected in this study and also in pot trials conducted on replant soils in the greenhouse. This supports evidence from the our commercial fields which indicated that legume crops grown immediately after removal of asparagus tended to perform as well as where those crops were grown on ground that had not been in asparagus production. Although the bioassay indicates sweetcorn shoots are suppressed in the presence of RE, pot trials and field studies have shown that sweetcorn crops have also performed as well in asparagus soils as in sites where asparagus has not been previously grown. Sweetcorn shoot growth was probably reduced in this study by contact of the Petri plate lids by the shoots.

Broccoli, cucumber, tomato, lettuce, oats and asparagus shoot and root growth were inhibited in the bioassay indicating that these crops are likely to perform poorly in fields where asparagus crops have recently been removed.

This study confirms the findings of Hazebroek et al. (1989) who found that RE reduced radicle elongation of barley, lettuce and asparagus with lettuce radicles being the most sensitive to low concentrations of RE. Yang (1986) found crude aqueous extracts (1 g dw/100 ml water) of asparagus storage roots significantly reduced the radicle growth in seedlings of cucumber, carrot, radish, tomato and asparagus in growth pouches and at higher concentrations (5 g dw/100 ml water) the RE killed these seedlings. The lowest rate of RE used in this study was 1.5 g dw/100 ml water and the suppression of radicles in this bioassay is similar to that found by Yang (1986) and by Hazebroek et al. (1989) at similar rates of RE. Germination

studies (Shafer & Garrison 1986) also show inhibition of lettuce, tomato and asparagus emergence after adding asparagus storage root tissue to soil at a rate of 2 g dw/100 g dry soil. Poor growth of oats in fields that have recently had asparagus crop residues cultivated into the soil have been reported by Lake et al. (1993) also confirming the findings of this bioassay.

On the basis of this study, lettuce was chosen as a suitable species for future bioassay studies.

The reasons for this choice were:

1. Lettuce radicle growth is sensitive to low concentrations of RE.
2. Growth was uniform leading to low experimental error.
3. The germination rate of the seed line was 99% and the seed was inexpensive.
4. Germination occurred within 24 h and treatment differences were obvious after 72 h.

4.4 Bioassays to assess the toxicity of asparagus root extract from asparagus storage roots of different ages.

4.4.1 Introduction

During the life of an asparagus plant there is a constant turn over of storage roots. New roots develop in association with developing shoots while older roots decay after an unknown period. A three to six year life of storage roots has been reported (Hughes 1992).

The bioassays described in sections 4.2 and 4.3 were performed using root extract derived from storage roots that were relatively mature. Hartung et al. (1989) compared the activity of water extracts prepared from dried, ground storage roots from 5, 12 and 20 year old asparagus plants. In that study it is likely that the roots used from the different aged plantings were of a similar age due to the turn over of storage roots during the life of the crop whereas in this experiment much younger roots were evaluated for differences in activity between extracts from roots less than one year old compared with those five to six years old.

4.4.2 Objective

To assess the toxicity of root extracts derived from asparagus storage roots of different age

4.4.3 Materials and Methods.

4.4.3.1 Root Extracts.

Asparagus storage root extract was prepared as described in section 4.2.2.1.

Young roots produced in the last six months (the last growing season) were very clean and white and connected to the rhizome beside newly formed buds.

Medium aged roots (2 to 3 years old) were a light brownish colour due to the presence of *Fusarium* lesions on the feeder roots and epidermis and were generally associated with the more recently formed bud clusters.

Old roots (5 to 6 years old) were a dark reddish/brown colour due to the numerous *Fusarium* lesions present on the epidermis. The old roots were connected to the used bud sockets on old parts of the rhizome.

Five hundred grams of each of the three fresh root samples were pulverised using a mortar and pestle and placed in 1 l of tap water for 12 h before the liquid was drained off. Crude root extract was centrifuged at 12,000 rpm for 15 min., the extract decanted off and filtered through glass wool before being serially diluted to make up the assay solutions.

4.4.3.2 Seed.

Lettuce cv Gloria was used as the bioassay species. Lettuce seed was stored at 2°C and 11 seeds were sown in each dish after the bioassay solutions had been applied.

4.4.3.3 Containers

The experiment was run on $1^1/_2$ sheets of 0.65 mm thick blotting paper 75 mm x 75 mm in plastic Petri plates. The half sheet of blotting paper was folded in half and placed under the square of blotting paper in the centre of the Petri plate to provide a storage area for assay solutions. The paper square had the corners bent up the side of the Petri plate to hold the lid just above the rim thus allowing some ventilation during the bioassay.

4.4.3.4 Experimental design and Treatments.

Each filtered root extract was diluted serially with tap water to give seven concentrations plus a water control. Six millilitres of assay solution was applied to each plate before the seeds were laid on the papers.

The assay solutions for each age of root were:

Undiluted Root Extract
75% Root Extract
50% Root Extract
25% Root Extract
12.5% Root Extract
6.25% Root Extract
3.13% Root Extract

The experiment was replicated four times and was laid out as a randomised complete block design in the incubator room at 20°C with 12 h photoperiod.

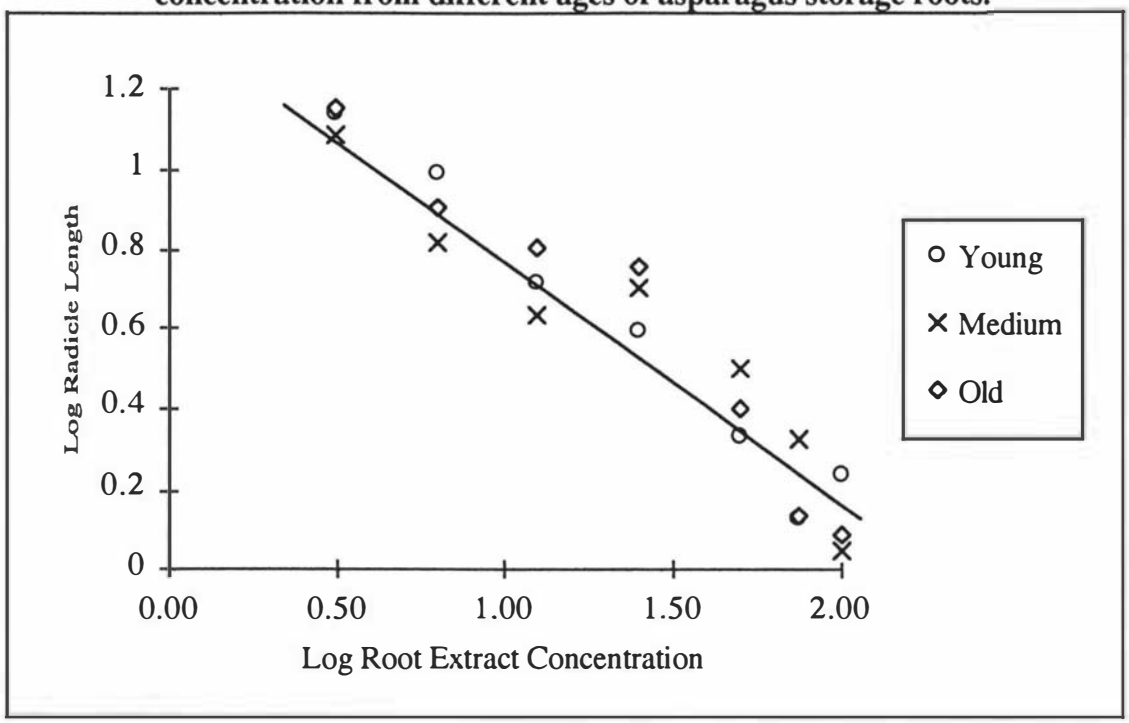
4.4.3.5 Assessment and Data Analysis.

After three days treatment differences were obvious and the bioassay was assessed by measuring the radicle length of ten seedlings in each dish. If all 11 seeds had grown the smallest radicle for the dish was ignored. Data were analysed by analysis of variance and regression analysis using the SAS statistical software. Slope coefficients for regression lines after transformation using the power function (Fernandez, 1992) were subject to analysis of variance. Power function transformation (log RE concentration = $ß_0+ß_1$ log radicle length) was employed as it provided the best fit for regression lines.

4.4.4 Results.

Lettuce radicle growth was strongly inhibited by extracts of all three ages of asparagus storage roots and the inhibition followed the same pattern for the three ages of roots (Fig 4.2).

Figure 4.2 Log lettuce seedling radicle length plotted against log RE concentration from different ages of asparagus storage roots.



Regression analysis of the dose response lines for extracts from the three ages of asparagus storage roots showed the lines had R squared values of 0.82 to 0.93. The slopes on the lines were similar for each age.

4.4.5 Discussion

Root extracts derived from three age classes of roots were equally inhibitory at all concentrations. Comparisons between toxicity of extracts from newly formed asparagus storage roots and those from older roots that have undergone one or more cycles of carbohydrate storage and subsequent depletion have not previously been reported.

Young (1984) demonstrated that root exudates from 1 year old donor plants exhibited autotoxic activity on germinated asparagus seed. Other studies have shown that extracts from dried, frozen and fresh asparagus storage roots from plantings of various ages inhibit germination and growth of asparagus, lettuce, tomato, barley and curly cress. (Shafer & Garrison 1986; Hazebroek et al. 1989; Hartung et al. 1989).

One study using extracts from roots excavated from 5, 12 and 20 year old plantings did show that the extract from the 5 year old plantings inhibited curly cress root growth slightly more at lower concentrations than that from the older plantings but this difference was small (Hartung et al. 1989). When roots are excavated from older plantings a higher percentage of dead or inactive root material would be recovered than where the roots were excavated from a five year old planting where all the roots are likely to be involved in the turnover of carbohydrate. This could explain the results of Hartung et al. (1989) and is reinforced by this study where all the roots used were from plants of the same age but were graded for age on the basis of their colour and location on the rhizomes.

That live asparagus storage roots of all ages contain similar levels of toxic material has some implications for the asparagus replant problem. The level of toxin in an old asparagus soil after removing a crop will depend more on the total mass of root material present than on the age of the crop that was taken out of production. The volume or weight of storage roots present in an asparagus soil is dependant on the age of the crop, the rate of growth of the crop and the depth of topsoil the crop is grown in. These factors along with the rate of degradation of the toxic material in soil will determine the level of toxicity likely to be encountered following termination of the previous asparagus crop.

4.5 Bioassays to assess the toxicity of asparagus storage root extract from intact roots compared with pulverised roots.

4.5.1 Introduction.

The phytotoxin present in asparagus storage roots appears to be readily extracted from pulverised roots using water as demonstrated in earlier experiments. Young (1984) demonstrated that root exudates from living donor plants in vermiculite culture inhibited the growth of asparagus seedlings in acceptor pots. The exudation of autotoxic material from root systems of asparagus plants may explain partially the decline of asparagus beds as they age.

4.5.2 Objectives.

1. To assess whether the toxins under study diffuse out of asparagus storage roots.

2. To assess the toxicity of root extracts derived from pulverised or intact asparagus storage roots.

4.5.3 Materials and Methods.

4.5.3.1 Root Extracts.

Ten year old plants of asparagus cv NZ Beacon were excavated from an old bed adjacent to Field Trial No. 1 and the soil washed off before 200 g of the fresh root sample was pulverised using a mortar and pestle and left to stand in 400 ml of water for 12 h.

A second extract was prepared from 400 g of roots in 300 mm lengths by immersing them in 400 ml of water so that the cut ends of the roots were clear of the water. Approximately half the length of the roots was immersed in the water. After 24 h the water was poured off and the crude root extracts were centrifuged at 12,000 rpm for 15 min. then filtered through glass wool before being diluted serially with tap water to make up the bioassay solutions.

4.5.3.2 Seed.

Eleven seeds of Lettuce cv Gloria seed were sown in each dish after the bioassay solutions had been applied.

4.5.3.3 Containers.

The experiment was run on blotting paper in Petri plates as described in 4.4.3.3.

4.5.3.4 Experimental design and Treatments.

Each filtered root extract was serially diluted with tap water to give seven concentrations for bioassaying plus tap water control. Six millilitres of assay solution was applied to each dish before the seeds were laid on the papers.

Dilution series of the root extracts:

Undiluted Root Extract
75% Root Extract
50% Root Extract
25% Root Extract
12.5% Root Extract
6.25% Root Extract
3.13% Root Extract

The experiment was replicated four times and laid out as a randomised complete block design in the incubator room at 20°C with 12 h photoperiod.

4.5.3.5 Assessment and Data Analysis.

Carried out as described in 4.4.3.5.

4.5.4 Results.

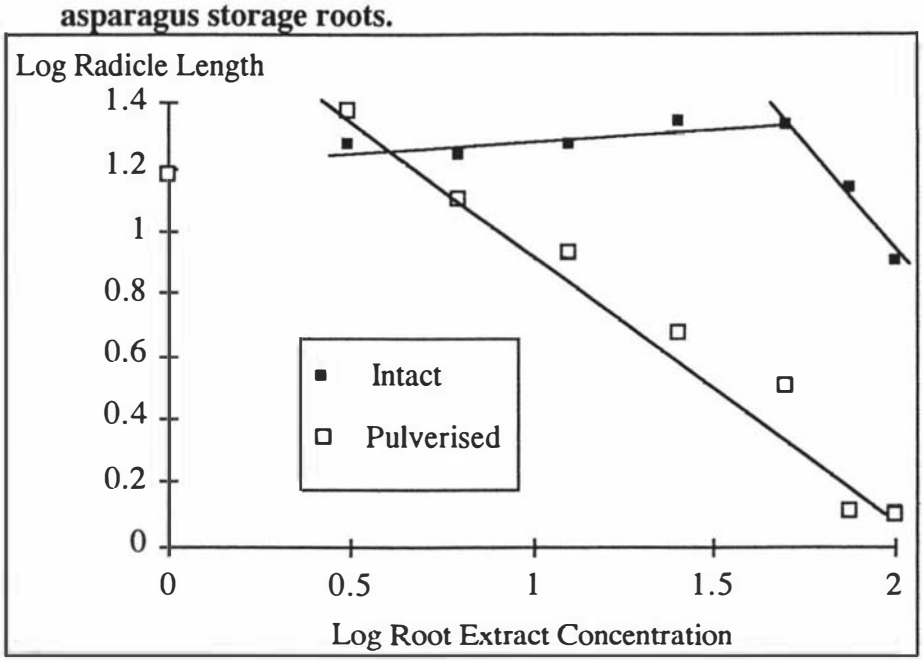
Table 4.6 Radicle lengths of lettuce seedlings exposed to seven concentrations of extracts from pulverised or intact asparagus storage roots or water.

Root extract	Lettuce Radicle Length	
Concentration	Intact Roots	Pulverised Roots
100% RE	8.0	1.3
75% RE	13.8	1.3
50% RE	21.5	3.2
25% RE	22.3	4.7
12.5% RE	18.9	8.6
6.25% RE	17.3	12.7
3.13% RE	18.7	23.7
Water	15.3	15.3
SE 32 df	1.23	1.02

The extract from pulverised asparagus storage roots was more active than that from the intact storage roots. (Table 4.6) Immersing intact storage roots in water for 24 h resulted in an extract that inhibited lettuce radicle growth to approximately 50% of that in the water control while pulverised root extract reduced lettuce radicle growth to 8% of that in the water control treatment.

The 12.5% concentration from pulverised roots caused a 50% reduction in lettuce radicle lengths whereas the 100% extract from intact roots caused a similar reduction in lettuce radicle growth. The 3.13% concentration of pulverised root extract enhanced the growth of lettuce radicles while the 25% and 50% concentration from intact roots had a similar effect.

Figure 4.3 Log radicle length plotted against log root extract concentration for lettuce seedlings exposed to extracts from pulverised or intact asparagus storage roots.



Plotting the transformed data provided a comparison of the activity of the two root extracts over the range of concentrations assayed. (Figure 4.3) The extract from intact roots was not inhibitory at lower concentrations but the two highest concentrations did inhibit lettuce radicle elongation in a similar manner to the lower concentration of root extract from pulverised storage roots.

4.5.5 Discussion

The toxic material present in asparagus storage roots is more readily extracted where the roots have been pulverised but toxin will diffuse out of intact roots at detectable levels. Evidence from this bioassay suggests that approximately 12% of the toxin that is extracted by soaking pulverised roots in water can be extracted by immersing intact roots in water for 24 h.

Most studies of the toxic material from asparagus have extracted the toxin from dried ground storage roots (Yang 1982; Hartung et al. 1989; Blok & Bollen 1993) or macerated fresh roots (Lake et al. 1993; Peirce & Colby 1987).

One study of the toxicity of exudates from intact asparagus root systems of asparagus seedlings grown in donor pots (Young 1984) shows that phytotoxic material is exuded from intact root systems of one year old asparagus seedlings in pots. It is not possible to compare the level of inhibition of the asparagus germlings in Young's (1984) paper bioassay with the results of this bioassay as the exudate Young (1984) collected from donor pots was trapped in an XAD-4 resin column and concentrated an unknown amount after methanol elution of the resin.

It is apparent from this bioassay and from Young's (1984) work that toxic material in asparagus storage roots will leach, diffuse or be exuded into the soil where asparagus plants are growing. The amount of toxic material from asparagus roots in the soil will depend on the mass of roots present and the rate at which it is released from the root system. The rate of diffusion of toxic material out of the roots is likely to be dependant on the soil moisture level and temperature (Rovira 1969). An equilibrium is likely to be reached between the toxic material in the soil solution, the soil and in the root system with the mechanisms of adsorption, decomposition, polymerisation, mineralisation and protection by soil components all likely to be involved in the balance of the phytotoxins in the soil.

Lettuce radicle growth in this bioassay was enhanced at low (3.13%) concentration of the extract from pulverised root and at 25% to 50% concentration from intact roots. This effect was not apparent in other bioassays and may indicate the presence of material in asparagus roots that is phytotoxic at some concentrations but actually enhances growth at very low concentrations. It is also possible that the enhanced growth at lower concentrations of root extract in this bioassay was caused by biologically active material from the storage roots that is unrelated to the phytotoxic compounds which override the growth enhancers when present at sufficiently high concentration. In an asparagus replant situation the level of toxic material in the soil will depend on the amount of root material that was present in the old asparagus crop and the cultivation that was carried out at the termination of the old crop. As found in Field Trial No. 1, when an old asparagus planting has been recently abandoned and the new crop is planted with minimal cultivation of the old plants' root systems,

the level of autotoxic material present is likely to be less than where the root system of the old crop has been chopped up with cultivation equipment. If the old crop is cultivated into the soil the level of toxic material present at replanting time is likely to be dependant on the time since the cultivation took place and the amount of rainfall that has occurred to leach the toxins out of the root zone of the new planting. Where the old planting is left intact a low level of toxic material is likely to be present for a longer time period than where cultivation is used to destroy the old crop in which case the toxin level is likely to be higher initially but then decline over time.

4.6 Bioassays to assess the toxicity of root extracts from five green and one purple asparagus cultivars.

4.6.1 Introduction

There has been little research into the relative toxicity of different cultivars of asparagus. Young (1984) showed that there was no difference between the suppression caused by root exudates from three cultivars or in the suppression caused to the three cultivars in donor pot/acceptor pot experiments. Differences in the activity of the toxins from different cultivars could have wide implications for the replant problem in the field.

All reported research has been conducted with green cultivars of *Asparagus officinalis* L. In this study the toxicity of root extract from a tetraploid purple cultivar *A. officinalis* cv violetto d'albenga was also measured.

4.6.2 Objective

To assess the toxicity of storage root extracts from six asparagus cultivars.

4.6.3 Materials and Methods.

4.6.3.1 Root Extracts.

Fresh storage root samples from six asparagus cultivars were collected from cultivar trials in the field. After all soil was washed from the roots, 150 g of each fresh root sample was pulverised using a mortar and pestle and placed in 300 ml of water for 12 h before the liquid was drained off. Extracts were centrifuged at 12,000 rpm for 15 min., filtered through glass wool and serially diluted with tap water to make up bioassay solutions.

4.6.3.2 Seed.

Eleven seeds of lettuce cv Gloria seed were sown in each dish after the bioassay solutions had been applied.

4.6.3.3 Containers

The experiment was run on blotting paper in Petri plates as described in 4.4.3.3.

4.6.3.4 Experimental design and Treatments.

Each filtered root extract was serially diluted with tapwater to give four concentrations plus a water control. Six millilitres of bioassay solution was applied to the papers in each dish before the seeds were added.

Asparagus cultivars evaluated:

NZ Beacon
Taramea
UC157
Franklim
Jersey Giant
Purple Passion (violetto d'albenga)

Root extract concentrations used:

Undiluted Root Extract
33% Root Extract
16% Root Extract
5% Root Extract

The experiment was replicated four times and was laid out as a randomised complete block design in the incubator room at a constant 20°C with 12 h photoperiod.

4.6.3.5 Assessment and Analysis

After three days growth the bioassay was assessed by measuring the radicle length of ten seedlings in each dish. If all 11 seeds had grown the smallest radicle for the dish was ignored. After power function transformation (Fernandez, 1992) the data were analysed using regression analysis of each cultivar block combination and analysis of variance was carried out on the slope coefficients ($ß_1$) using the SAS statistical package.

Formula for the power function is : Log RE concentration = $ß_0+ß_1$log radicle length.

4.6.4 Results.

Table 4.7 Radicle length (mm) of lettuce seedlings exposed to four concentrations of root extract from six asparagus cultivars or water.

	Root Extract Concentration				
Cultivar	**100%**	**33%**	**16%**	**5%**	**Water**
Beacon	1.1	2.8	8.9	16.3	15.2
Taramea	1.1	2.9	5.3	13.5	15.2
UC157	2.2	4.3	6.7	16.1	15.2
Franklim	1.4	3.3	6.3	14.5	15.2
Syn 4-56	1.2	3.0	5.7	15.9	15.2
Purple	1.9	5.6	8.0	18.4	15.2

SE 24df=0.82

There were only small differences in the radicle lengths of lettuce seedlings exposed to storage root extracts from the six different cultivars. (Table 4.7) The power function transformation of the data gave regression lines with a good fit (range of r^2 was 0.89 to 0.99). Analysis of variance of the regression line slope coefficients showed statistically significant differences between the cultivars.

Table 4.8 Slope coefficient ($ß_1$) of regression lines of radicle lengths of lettuce seedlings exposed to four concentrations of root extracts from six asparagus cultivars.

Cultivar	Slope Coefficient ($ß_1$)	
Beacon	0.94	C
Taramea	0.85	BC
UC157	0.65	A
Franklim	0.80	BC
Syn 4-56	0.88	BC
Purple	0.75	BC

Means followed by the same letter are not significantly different at P=0.05

The slope of Log radicle length vs Log root extract concentration plot was steeper for Beacon and flatter for UC 157 compared with the other four varieties. (Table 4.8)

4.6.5 Discussion

Although the bioassay showed only small differences in the growth of lettuce seedlings exposed to root extracts from different asparagus cultivars analysis of the slopes of the transformed dose response curves (Table 4.8) showed that the root extract from asparagus cv Beacon suppressed lettuce radicle growth more and that from cv UC157 less than those from other cultivars.

The differences in toxicity of the storage root extracts as expressed by this bioassay can be explained by the different morphology of the storage roots used in the root extract preparation.

UC157 typically has smaller diameter storage roots than the other cultivars. For a given weight of roots UC157 will have more epidermal and connective tissue present and less storage carbohydrate than the other cultivars. As the most toxic material in asparagus storage roots is located in the amino acid/carbohydrate material (Lake et al. 1993) the difference in storage root diameter and consequent difference in weight of storage carbohydrate for a given weight of roots could explain why the UC157 root extract was slightly less inhibitory than the extract from other cultivars. Similarly the storage roots used to obtain the asparagus cv Beacon root extract were slightly greater in diameter than storage roots used from the other cultivars. Storage root diameter then could explain the slight differences in lettuce radicle growth caused by storage root extracts in this bioassay.

This experiment evaluated the storage root extracts from a diverse range of cultivars from different breeding lines and showed they all contained phytotoxic material at similar levels. These results agree with the findings of Young (1984) who showed that there was no difference in the response of acceptor plants exposed to exudates from one year old plants of three asparagus cultivars: Mary Washington, California 309 and California 711.

In an asparagus replant field it is unlikely that the cultivar that was previously grown will influence the amount of autotoxin present in the soil except that some cultivars may have a greater mass of storage root accumulated in the old asparagus soil and thus release more autotoxin into the soil through the presence of more roots rather than production of more or stronger autotoxins.

4.7 Bioassays to assess the susceptibility of different asparagus cultivars to asparagus storage root extract.

4.7.1 Introduction

There has been little research into the relative susceptibility of different asparagus cultivars to the autotoxic material found in asparagus tissue. Young (1984) showed that there was no difference between the suppression caused by root exudates from three cultivars or in the suppression caused to the three cultivars in donor pot/acceptor pot experiments.

Differences in the susceptibility of asparagus cultivars to the autotoxins could have wide implications for the replant problem in the field.

4.7.2 Objective

To assess the susceptibility of the four commercial cultivars of asparagus currently used in New Zealand to asparagus storage root extracts.

4.7.3 Materials and Methods.

4.7.3.1 Root Extract.

Was prepared as described in section 4.2.2.1.

4.7.3.2 Seed.

Seed of the four asparagus cultivars was surface sterilised and pregerminated as described in 4.2.2.2.

As the different cultivars had differing rates of germination it was not possible to set up the experiment with uniform radicle lengths for all cultivars. Each replicate dish was set up with radicles of a relatively uniform length for each cultivar but there were differences between the replicates and between the cultivars at the start of the experiment.

4.7.3.3 Containers

The experiment was run on two sheets of 0.65 mm blotting paper in plastic Petri plates as described in 4.2.2.3.

4.7.3.4 Experimental design and Treatments.

Filtered root extract was serially diluted to give five concentrations for bioassaying plus water as a standard. Ten millilitres of assay solution was applied to the papers in each dish before 6 pregerminated asparagus seeds were placed on the blotting paper.

Asparagus cultivars evaluated:

Taramea
UC157
Syn 4 - 56
JWC1

Root Extract concentrations:

Undiluted Root Extract
50% Root Extract
25% Root Extract
12.5% Root Extract
6.25% Root Extract
Water

The experiment had four replicates and was laid out as a randomised complete block design in the incubator room at 20°C with 12 h photo period.

4.7.3.5 Assessment and Analysis

After seven days growing the treatment differences were obvious and the bioassay was assessed by measuring the radicle length of the six germlings in each dish. Mean radicle lengths for each replicate dish were calculated and data analysed by analysis of variance using the SAS statistical software. The power function transformation was employed as described in 4.6.3.5.

4.7.4 Results.

Radicle growth of all four cultivars was significantly inhibited by RE. Syn 4-56 was inhibited more by higher concentrations of RE than Taramea, UC157 or JWC1.

There were large block effects evident when the transformed data were regressed. After omitting data from one block for two of the cultivars the R squared values for individual replicate cultivar combinations were in a range of 0.82 to 0.98.

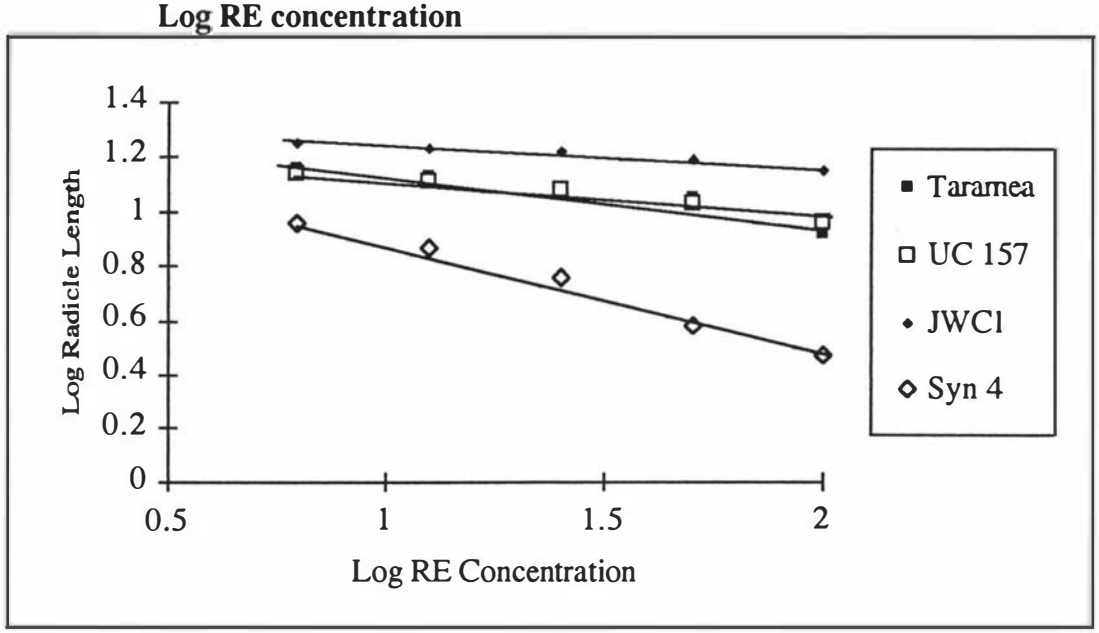
Table 4.9 Slope coefficient ($ß_1$) of the regression lines of radicle lengths of four cultivars of asparagus germlings exposed to five concentrations of asparagus RE. (Transformation: Logy = $ß_0$ +$ß_1$logx)

Cultivar	Slope Coefficient	
JWC1	-0.37	A
UC157	-0.48	A
Taramea	-0.63	A
Syn 4	-1.25	B

Means followed by the same letter are not significantly different at P=0.05

Analysis of the regression lines (Table 4.9) showed that UC157, JWC1 and Taramea were equally inhibited by RE while Syn 4-56 was inhibited more than the other three cultivars. (Figure 4.4)

Figure 4.4 Log radicle length of four asparagus cultivars plotted against Log RE concentration



4.7.5 Discussion

Radicle elongation of all four asparagus cultivars was inhibited by exposure to asparagus storage root extracts in this assay. This experiment also confirms the findings of Young (1984).

In this study the cultivar Syn 4-56 was suppressed more than the other three cultivars. This difference is likely to be due to the different stage of development of the germlings at the time the assay was set up. The inhibition of asparagus radicle growth by increasing concentrations of RE in the bioassay shows a typical asymptotic pattern which is transformed to a linear relationship using log/log transformation. Germination of the four cultivars used in the bioassay took varying lengths of time and the bioassay was begun once there were sufficient germlings of each cultivar with uniform radicle length. As Syn 4-56 was slower than the other three cultivars to reach a stage where there were sufficient germlings to set up the bioassay germlings of three cultivars were held at 10°C for three days while the Syn 4-56 germlings were incubated at 20°C while they caught up. The bioassay was then set up with germlings of differing physiological age and variable radicle length. The Syn 4-56 germlings were the youngest with the shortest radicles and it is likely that these differences account for the response observed in the bioassay.

The field experiment reported in section 2.5 showed that all 20 cultivars evaluated were suppressed in a replant situation with the poorest performing cultivars those that were smallest at establishment of the trial. Anecdotal evidence from asparagus field plantings in many asparagus producing areas provides little evidence of asparagus cultivars that survive or grow better in a replant situation. The evidence from this study suggests that all asparagus varieties are likely to be inhibited by any phytotoxic material carried over from the previous asparagus crop in an asparagus replant situation.

4.8 Bioassays to assess the toxicity of storage organ extracts from four different plant species.

4.8.1 Introduction

There is evidence of phytotoxic or allelopathic activity from the exudates or residues of many different plant species (Rice 1984). Root residues of many weed species have been shown to be phytotoxic (Schreiber & Williams 1967; Wilson 1981). Although allelopathic activity of plant root extracts or exudates has been reported for some species it is not common so the lettuce seed bioassay was used to evaluate four plant species for the presence of toxins in water extracts from their storage organs.

Plants evaluated:

Lily. *Lillium hollandicum* Lilliaceae family
Storage roots similar to asparagus.

Convolvulus. *Convolvulus sepium* L. Convolvulaceae family
Stolons form fleshy storage organs similar in appearance to asparagus storage roots.

Carrot. *Daucus carota var. sativus* Apiaceae family
Large fleshy tuberous storage organ.

Asparagus *Asparagus officinalis* L. Lilliaceae family
Large storage roots.

4.8.2 Objective

To assess the toxicity of storage organ extracts from four plant species as measured by the lettuce seed bioassay.

4.8.3 Materials and Methods.

4.8.3.1 Storage organ Extracts.

Stolons were collected from convolvulus plants growing as a weed in an asparagus field. Fleshy storage roots were collected from lily plants growing alongside the asparagus beds. Carrots were harvested from a crop adjacent to the asparagus field used in Field Trial No. 1. Asparagus storage roots were collected from a 10 year old plant of asparagus cv NZ Beacon

After washing all the soil from the fresh samples 150 g of each sample was pulverised using a mortar and pestle and immersed in 300 ml of tap water for 12 h before the liquid was drained off. Extracts were centrifuged at 12000 rpm for fifteen min. and filtered through glass wool before being diluted to make up the bioassay solutions.

4.8.3.2 Seed.

Lettuce cv Gloria was used as the bioassay species as in section 4.4.3.2.

4.8.3.3 Containers

The experiment was run on blotting paper in Petri plates as in 4.4.3.3.

4.8.3.4 Experimental design and Treatments.

Bioassays were done on water as a control and each filtered plant extract was tested undiluted and diluted to one third strength. Six millilitres of the appropriate bioassay solution was applied to each dish before the seeds were laid on the blotting papers. The experiment was replicated four times and set out as a randomised complete block design in the incubator room at 20°C with 12 h photoperiod.

4.8.3.5 Assessment and Analysis

After three days treatment differences were obvious and the bioassay was assessed by measuring the radicle length of ten seedlings in each dish. Data were analysed by analysis of variance using the SAS statistical package.

4.8.4 Results.

Table 4.10 Radicle length (mm) of lettuce seedlings exposed to two concentrations of extracts from the storage organs of four plant species and water.

Extract	Source of Extract			
Concentration	Asparagus	Carrot	Convolvulus	Lily
100 %	1.2	15.8	1.0	8.5
33 %	3.3	19.3	4.8	15.2
Water	15.6	15.6	15.6	15.6

SE 11df = 0.60

There were significant differences in the suppression of lettuce seedling radicle growth by the extracts from the four species evaluated. Asparagus and convolvulus extracts inhibited lettuce seedling growth by a similar amount while the dilute carrot extract actually enhanced the growth of lettuce radicles slightly.

Extract from the lily storage roots suppressed lettuce seedling growth at the highest concentration only (Table 4.10).

4.8.5 Discussion

Allelopathy refers to interference through production of chemicals by living or decaying plant tissues which inhibit the growth of a neighbouring plant. Generally the term refers to the detrimental effects of one species (donor) on the growth of another species (recipient), however the interference can be intra specific and the term autotoxicty is often used in that case (Rice 1984). There have been many reports of plants exhibiting allelopathic activity (Schreiber & Williams 1967; Wilson 1981) and this bioassay provides evidence of allelopathic potential of asparagus, convolvulus and lily. This bioassay provides further confirmation of the presence of allelochemicals in storage roots of asparagus (Hartung et al. 1989; Hazebroek et al. 1989; Blok & Bollen 1993). The lower rate assayed was equivalent to a storage root addition of 0.05 g dw /ml water and as approximately 2 to 5 g dw of asparagus storage roots per kg of soil may be present in an asparagus replant soil (4.2.4) these levels could be biologically active in a natural situation.

The presence of allelochemicals in storage roots of other species of asparagus has been reported with Hazebroek et al. (1989) demonstrating inhibition of lettuce radicle growth with crude extract from the storage roots of *A. racemosis*. Other species that have been shown to display inhibitory activity include *A.curillus* and *A. sprengeri* (Sharma et al. 1983) and *A.plumosus* (Sati & Pant 1985).

Other studies have shown that the weed species *Agropyron repens* (Hold et al. 1977) and *Cirsium arvense* (Stachan & Zimdahl 1980) contain allelochemicals and this bioassay has demonstrated the presence of phytotoxic material in the storage organs of another weed species *Convolvulus sepium* L. There is evidence from this bioassay that storage roots of *Lillium hollandicum* contain phytotoxic material and that the toxic activity of extracts from asparagus storage roots is not unique to this species with other plants containing compounds with similar activity.

Although this experiment has shown the potential of residues of convolvulus or lilies to inhibit the growth of lettuce further studies would be required to ascertain whether residues of these plants or others known to contain allelochemicals such as couch or californian thistle would affect newly planted crops such as asparagus in a field situation.

4.9 Bioassays to monitor the toxicity of soil over a twelve month period following the removal of an old asparagus crop.

4.9.1 Introduction

The normal economic life of an asparagus bed in New Zealand is 12 to 20 years (Falloon et al. 1989) and the removal of the old asparagus plants is not practical so it is normal practice to cultivate the old plants into the soil using a rotary hoe or by ploughing.

Estimates of the weight of asparagus crop residues incorporated into the soil during the destruction of an old crop are in the range of 12 to 34 tonnes fresh weight per hectare. These estimates are based on excavating plants to a depth of 500 mm in the trial sites of the two field trials described in section 2. Estimates done in the Netherlands by Blok & Bollen (1993) indicate similar levels of asparagus residues are found in old asparagus soils there and that even after 10 years approximately 10% of this material was still present.

There have been pot trials done to assess the inhibition caused by growing asparagus seedlings in old asparagus soils (Section 3, Huiskamp & Poll 1990) but little work has been reported on other means of assessing the toxicity of these soils. Lake et al. (1993) developed a bioassay using asparagus seed as an indicator to assess toxicity in soil extracts and extracts from asparagus storage roots. Their bioassay took up to 14 days incubation and therefore required very clean bioassay solutions and regular topping up of the assay solutions during the bioassay.

A bioassay that is fast, easy to perform and uses a simple extraction procedure would be a useful tool in assessing the likelihood of a replant problem in a particular site.

4.9.2 Objectives

1. To establish a simple bioassay procedure for indicating the likely toxicity problems in old asparagus soils.

2. To monitor the toxicity of the soil, over a twelve month period, from two sites where the old asparagus crop had been terminated compared with adjacent sites without a history of asparagus production.

4.9.3 Materials and Methods.

4.9.3.1 Soil samples.

Soils were sampled at two sites for this series of bioassays.

Site 1 A 7 year old planting of asparagus cv Syn4-56 grown in an alluvial silt loam soil with no history of herbicide use (an organically grown asparagus crop). The soil type was similar to the soil in Field Trial No. 1 (section 2). An area of 20 m x 3 m of the asparagus planting was destroyed on 20 July 1995 by cultivating to a depth of 500 mm by two successive passes with a rotary hoe followed by deep ripping and a final pass with the hoe as described in 2.2.2.1.

Site 2 A 10 year old planting of asparagus cv Limbras grown in a pumice based sandy loam soil adjacent to Field Trial No. 2 (section 2). Approximately 1.5 ha of the planting had become uneconomic to continue farming and the planting was destroyed by cultivation on 18 August 1995 as described in 2.2.2.1. There had been no residual herbicide applied to the area in the 22 months prior to terminating the old crop.

Soil samples of approximately 5 kg were collected from the cultivated replant areas, at both sites, monthly commencing five days after the initial cultivation. Virgin soil samples were also collected at both sites on each sampling date from adjacent areas that had no history of asparagus production. The virgin sampling area at site 1 was at the side of the paddock 10 m from the replant area. At site 2 the virgin sampling area was an area of pasture immediately beside the old asparagus field. Each 5 kg sample was made up of 20 sub samples taken from 0 to 300 mm depth at random sites across the sampling area.

The soil samples were air dried at room temperature (15°C to 20°C) for four days before being stored in plastic bags at -18°C until the bioassays were carried out.

Table 4.11 Sampling dates and number of days since the asparagus crop was destroyed at the two sampling sites.

	Days since crop was terminated	
Sample date	**Site 1**	**Site 2**
25 July 1995*	5	
23 August 1995	34	5
20 September 1995	61	32
16 October 1995	87	58
13 November 1995	115	86
15 December 1995	147	118
28 January 1996	191	162
7 March 1996	229	200
15 April 1996	268	239
24 May 1996	307	278
25 June 1996	339	310

* Site 1 only

4.9.3.2 Soil Water extracts.

Soil samples were taken from the deepfreeze twelve hours before the extractions were done to allow them to reach room temperature. Two kilograms of each soil sample was placed in 2 l of water and mixed thoroughly every 8 h. After 24 h at 20°C a 1 litre sample was decanted off and centrifuged (12,000 rpm for 15 min.) before being concentrated to 100 ml *in vacuo*, using a rotary evaporator at 45°C to avoid possible unwanted chemical reactions that may occur at higher temperatures.

The concentrated soil water extract was serially diluted to give four (1, 2.5, 5 and 10 times) concentrations for the bioassay.

4.9.3.3 Containers / Seeds

The experiment was run on blotting paper in plastic Petri plates as described in 4.4.3.3 using lettuce seed. (4.4.3.2.)

4.9.3.4 Experimental design and Treatments.

Bioassays were carried out on replant soil and virgin soil extracts for each sampling date and site as single experiments with a water control treatment in each experiment.

Six millilitres of each bioassay solution was applied to each dish before the seeds were laid on the blotting papers. Each experiment was replicated four times and laid out as a randomised complete block design in the incubator room at 20°C with 12 h photoperiod.

4.9.3.5 Assessment and Data Analysis

After three days bioassays were assessed by measuring the radicle length of ten seedlings in each dish. If all 11 seeds had grown the smallest radicle for the dish was ignored. Data were analysed using the SAS statistical package. Radicle length was plotted against soil extract concentration for each site at each sample time in non linear regression analysis. The quadratic equations that describe the curves were estimated and analysed by analysis of variance.

The difference in radicle length between the virgin soil and replant soil samples was calculated by subtracting the predicted replant soil radicle length from the predicted virgin soil radicle length for each replicate, extract concentration, sample time and site combination and these data were analysed by analysis of variance. The virgin-replant radicle length difference was termed the 'Radicle Inhibition'. It was attributable to toxicity in the replant soil and was plotted over time for each soil extract concentration and for each site to show the pattern of the toxicity of the replant soil over time as measured by this bioassay method.

4.9.4 Results.

The weight of asparagus plant residues in the replant soil was assessed at both sites on the first and last sample dates.

At site 1 there was 9 g fresh weight of asparagus plant residues per kg dry soil (9 g frw/kg) 5 days after destroying the plants. After 340 days the fresh weight of asparagus residues had fallen to 3.5 g frw/kg of partly decomposed asparagus residues.

At site two 5.5 g frw/kg dry soil was found five days after cultivating the plants and this fell to 2.5 g frw/kg after 310 days.

Radicle length was increasingly suppressed at higher concentrations of water extracts from both virgin soil and replant soil at both sites compared with the tapwater control treatment. This pattern followed throughout the twelve months of sampling on both sites although at some sampling dates, such as the 23 August 1995 sample from both sites, there was an enhancement in lettuce radicle growth compared to the control treatment at the lower soil water extract concentrations particularly from the virgin soil. Replant soil extracts generally suppressed growth more than corresponding virgin soil extracts however water extract from the virgin soil sample from both sites on the 20 September sampling date inhibited lettuce radicles an equal amount to the replant soil extract. This also occurred with the sample taken 147 days after the crop was terminated at site 1.

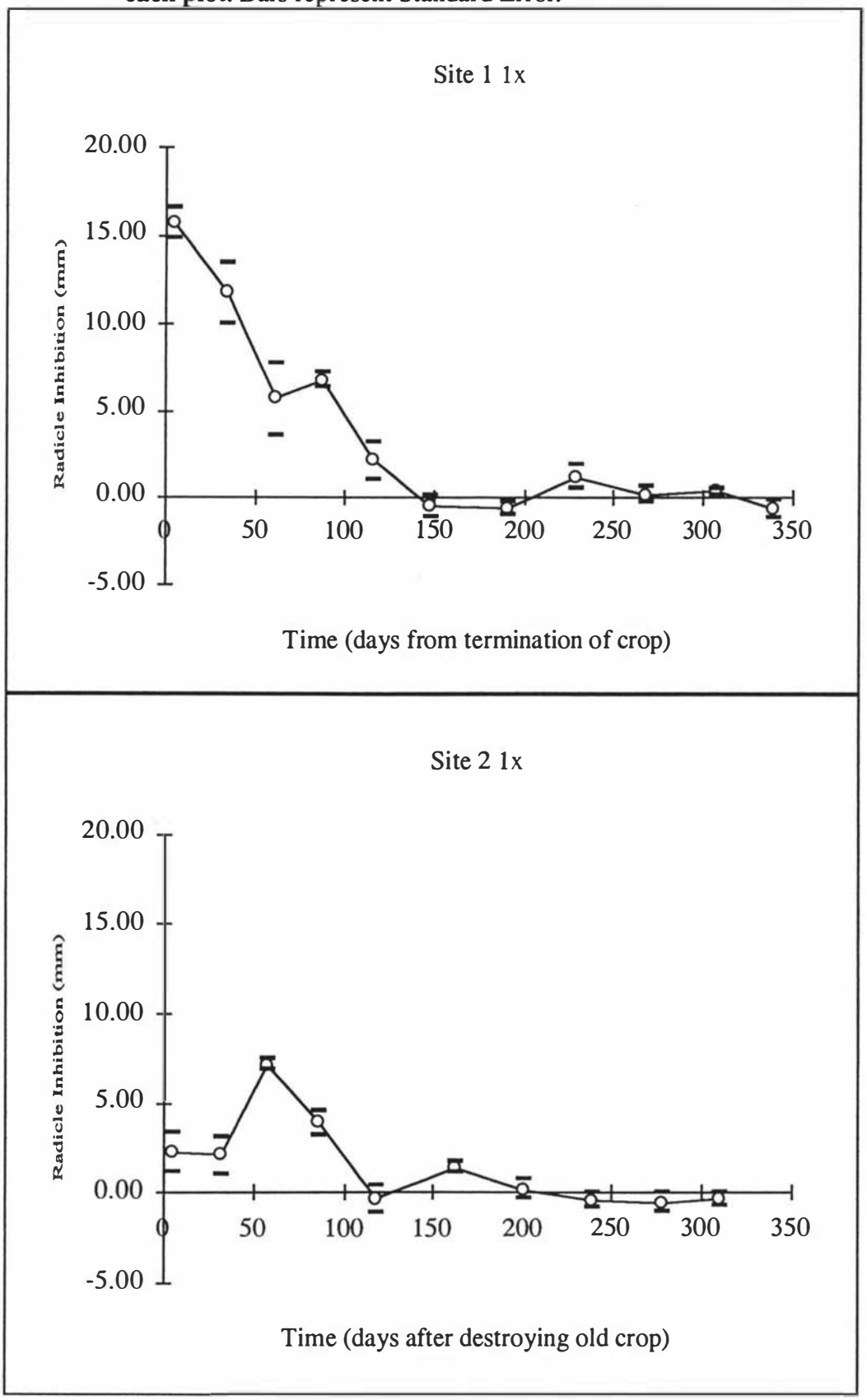
The difference between the radicle length of the virgin soil extracts and the replant soil extracts at each concentration for each sample date represents the inhibition of the lettuce seedling radicle growth due to toxicity in the replant soil. This value is referred to as the Radicle Inhibition and was plotted over time for each site and extract concentration. (Figure 4.5)

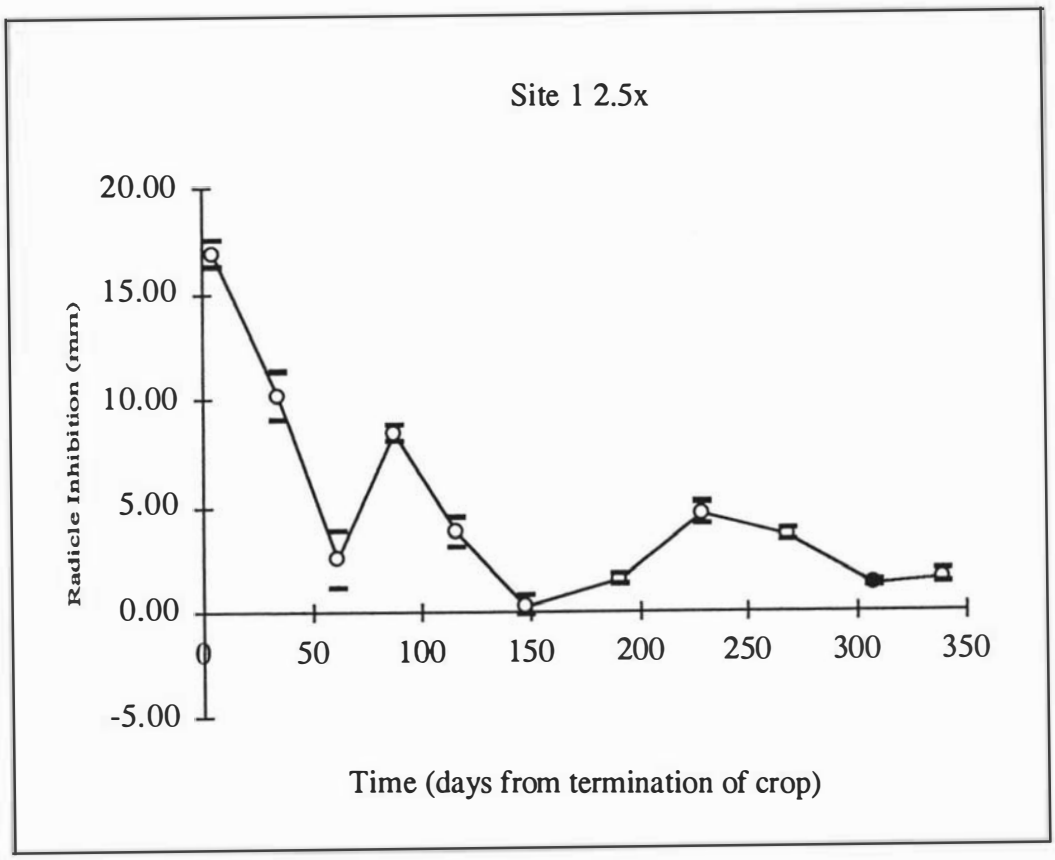
Bioassays of the neat soil extract from site 1 showed significant inhibition of lettuce radicle growth for the samples taken over the first five months. From the sixth month onwards the neat extract did not inhibit lettuce seedlings. The inhibition detected by neat soil extract was greatest at the first sample taken five days after the old crop was destroyed and was progressively lower at each sample date until no inhibition was detected from the sample 147 days after the old asparagus crop was destroyed.
When the soil water extract from site 1 was concentrated either 2.5x, 5x or 10x there was significant inhibition of lettuce seedling radicles at all sample dates except after 61 days and 147 days from destruction of the crop. (Figure 4.5)

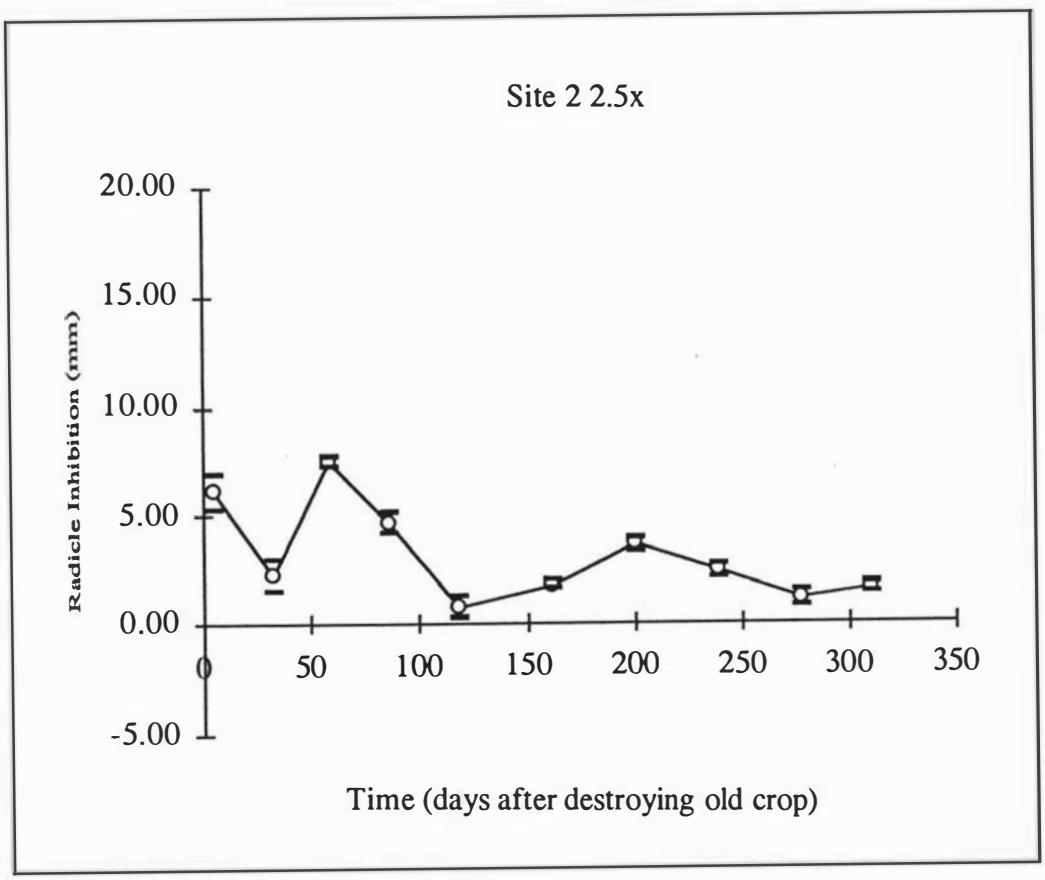
There was significant inhibition of lettuce radicle growth by the concentrated virgin soil samples taken 61 days after the crop was destroyed. The inhibition caused by the concentrated virgin soil extract was similar to that of the replant soil extract and as a result there was no significant inhibition attributable to the replant soil toxin. After 147 days, concentrated virgin soil extract again suppressed lettuce radicle growth a similar amount to the replant soil extract. (Figure 4.5)

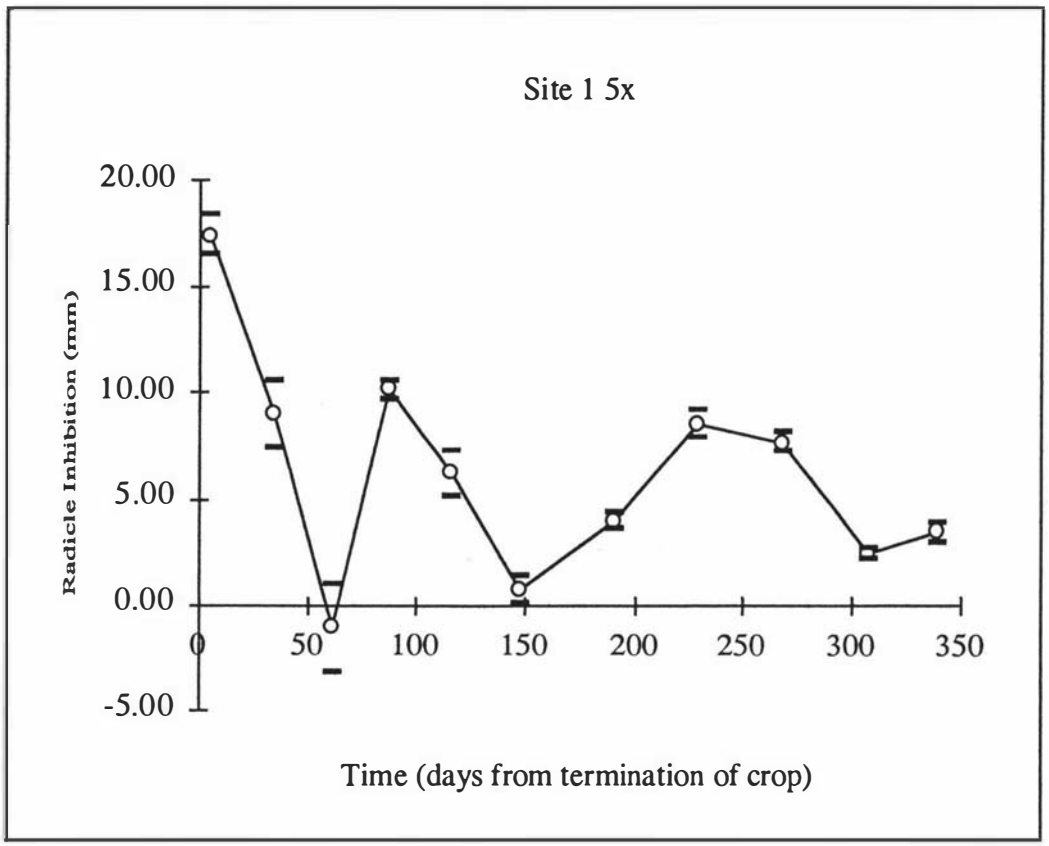
As the concentration of the soil water extract increased at site 1 the level of inhibition detected by the bioassay increased particularly at the later sampling dates. The greatest inhibition at site 1 was at the first two sample dates 5 days and 34 days after the old crop had been cultivated into the soil. The tendency for the inhibition to fall over time was evident with the lower concentrations of assay solutions but was less evident at 5x and 10x concentration.

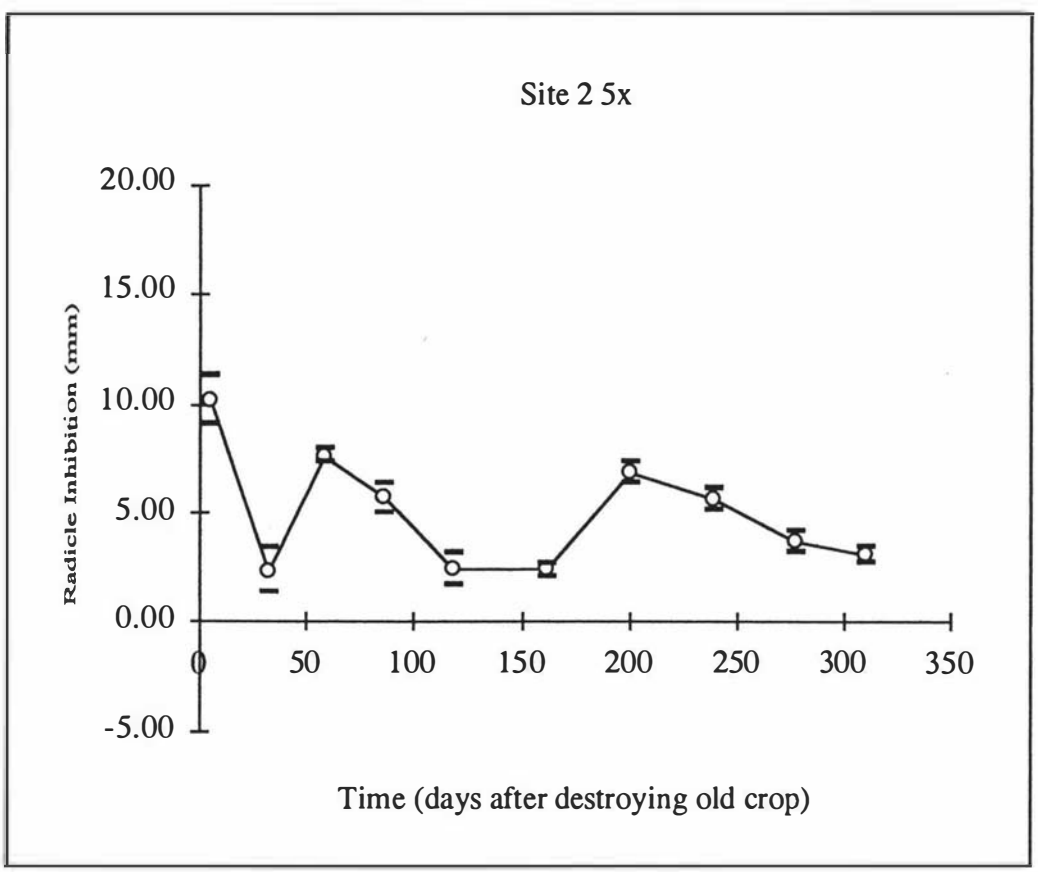
Figure 4.5 Radicle Inhibition of lettuce exposed to extracts from virgin and replant soils at two sites plotted against time (days from destruction of the old asparagus crop) . Site and extract concentration given on each plot. Bars represent Standard Error.

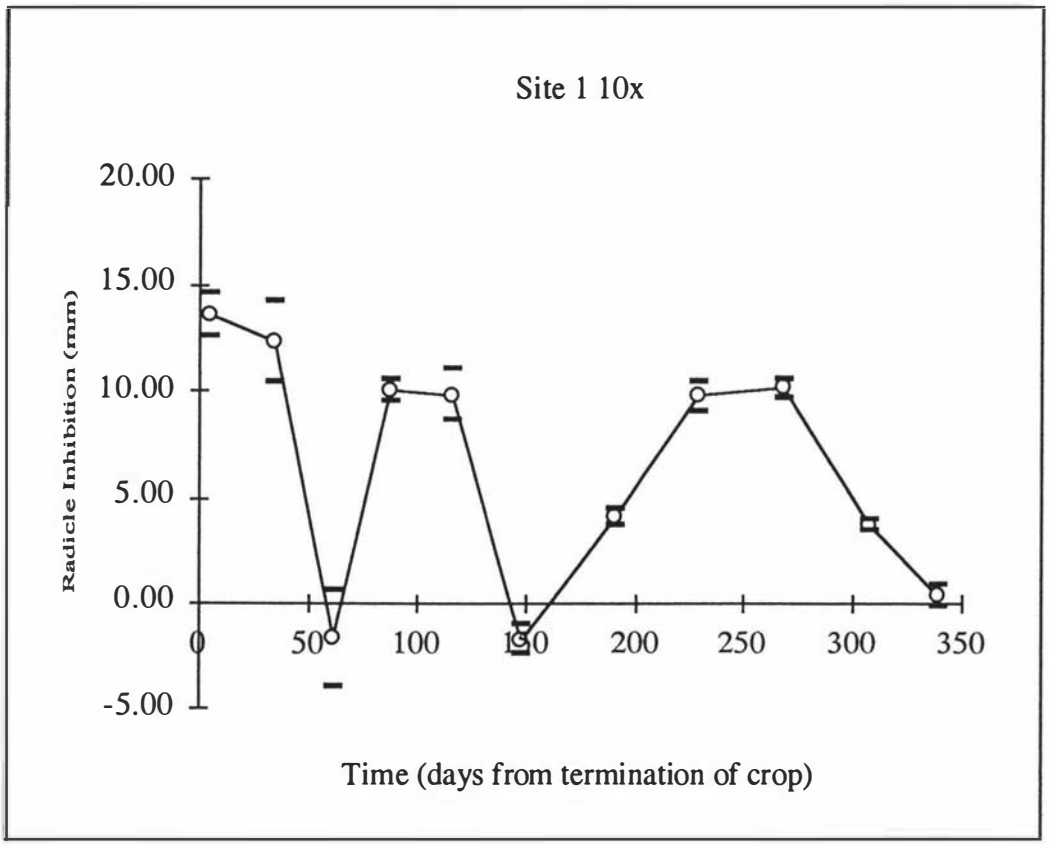


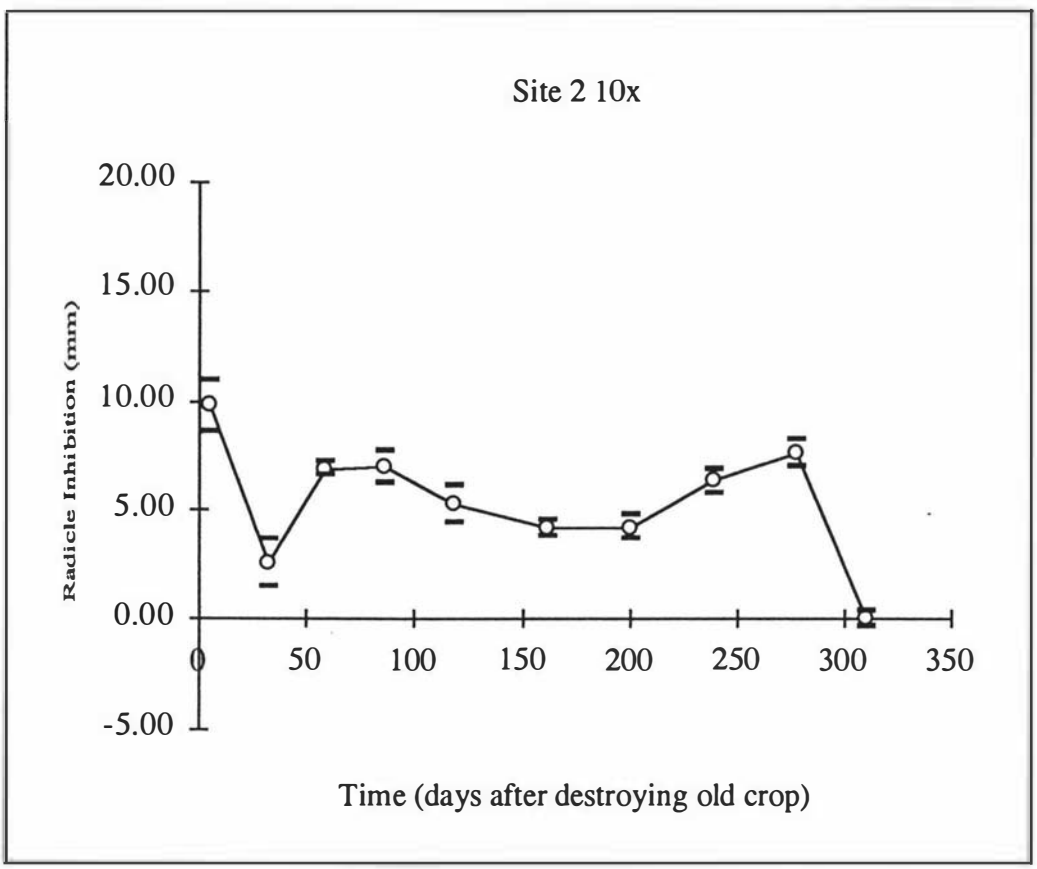

Site 1 2.5x
20.00
15.00
10.00
5.00
0.00
-5.00
Radicle Inhibition (mm)
0
50
100
150
200
250
300
350
Time (days from termination of crop)


Site 2 2.5x
20.00
15.00
10.00
5.00
0.00
-5.00
Radicle Inhibition (mm)
0
50
100
150
200
250
300
350
Time (days after destroying old crop)


Site 1 5x
Radicle Inhibition (mm)
20.00
15.00
10.00
5.00
0.00
-5.00
0
50
100
150
200
250
300
350
Time (days from termination of crop)


Site 2 5x
Radicle Inhibition (mm)
20.00
15.00
10.00
5.00
0.00
-5.00
0
50
100
150
200
250
300
350
Time (days after destroying old crop)


Site 1 10x
20.00
15.00
10.00
5.00
0.00
-5.00
Radicle Inhibition (mm)
0
50
100
150
200
250
300
350
Time (days from termination of crop)

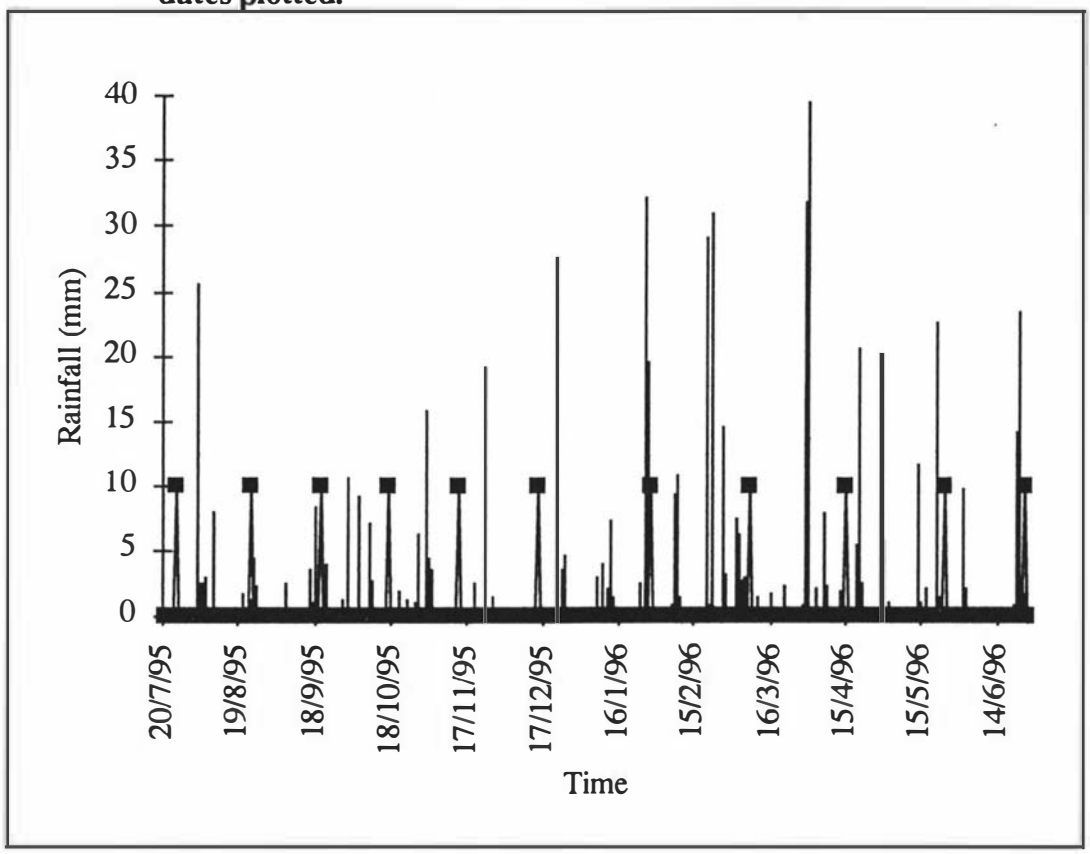

Site 2 10x
20.00
15.00
10.00
5.00
0.00
-5.00
Radicle Inhibition (mm)
0
50
100
150
200
250
300
350
Time (days after destroying old crop)

The greatest inhibition measured from the neat soil water extracts at site two was from the samples taken 58 and 86 days after the old crop had been cultivated into the soil. The first two samples and the sample taken 162 days after cultivation were also slightly inhibitory while all other samples showed no significant inhibition when the soil extract was not concentrated.

When the soil water extract was concentrated 2.5x lettuce radicle growth was significantly inhibited by toxins in the replant soil for all sample times except those taken 118, 278 and 310 days after the old crop had been destroyed. At 5x concentration the soil water extract was inhibitory at all sampling dates and at 10x concentration only the last sample taken 310 days after the initial cultivation did not show a significant inhibition of lettuce radicle growth.
There was a general trend for the inhibition to fall over time at all concentrations assayed.

Sampling dates of 20 September 1995 (15 mm), 28 January 1996 (71 mm), 24 May 1996 (23 mm) and 20 June (41 mm) were preceded by significant rainfall in the four days before sampling (Figure 4.6).
The virgin soil samples taken from both sites on 20 September 1996 significantly inhibited lettuce radicle growth at all concentrations assayed and consequently the inhibition attributable to the replant soil toxicity was low compared with other sampling dates.
The level of inhibition due to replant soil toxicity detected by the bioassay was lowest at the sampling dates where significant rain fell in the few days prior to sampling.

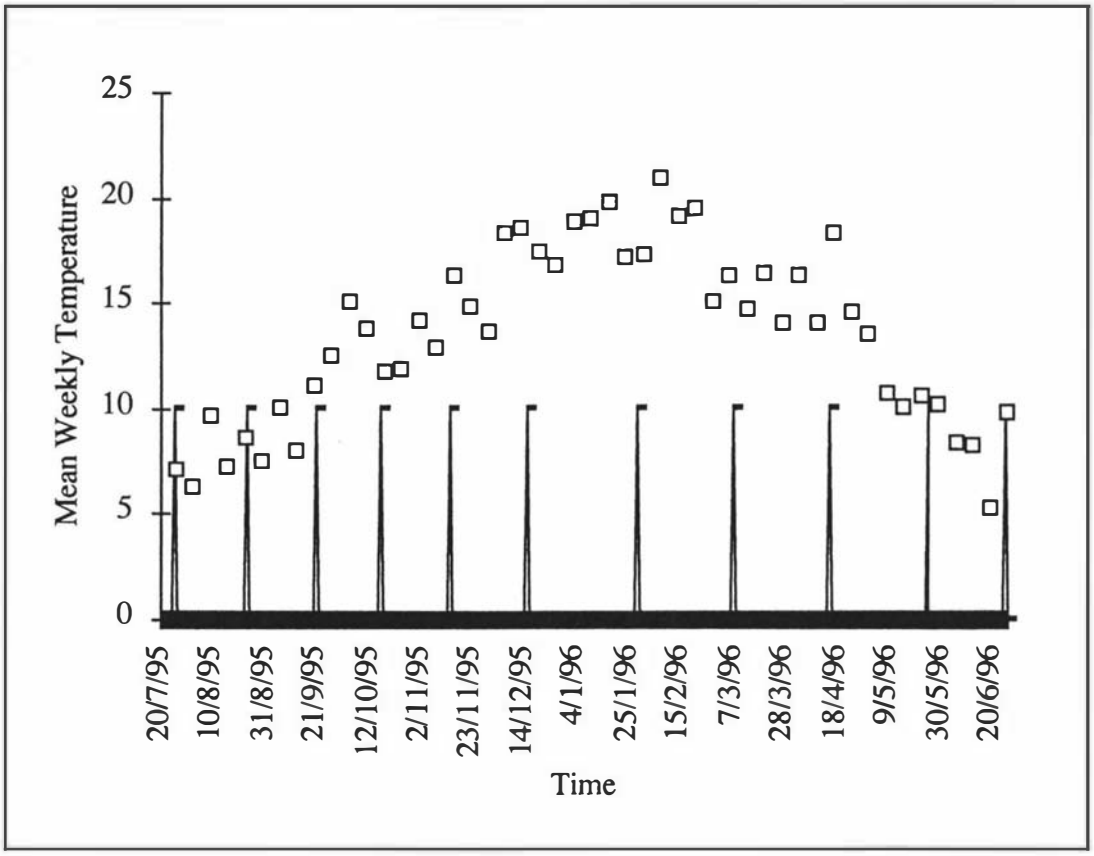
Figure 4.6 Daily rainfall over the twelve month sampling period with sampling dates plotted.



Weekly mean air temperatures were plotted for the twelve month sampling period. Temperatures followed a normal seasonal fluctuation with highest temperatures (18°C to 25°C) occurring during December 1995 through until March 1996. Lowest temperatures (5°C to 12°C) were recorded during July 1995 and June 1996 (fig 4.7).

The highest temperatures over the period December 1995 to March 1996 corresponded to a time when asparagus toxicity as measured by the bioassay was lower than at earlier or later measurements on both sites. This was particularly evident at the 2.5 times concentration at site 1 and the 5 and 10 times concentration at site 2.

Figure 4.7 **Mean weekly air temperature (^{o}C) over the twelve month sampling period. Sampling dates plotted.**



4.9.5 Discussion.

The bioassay method developed in the course of this study readily detected suppression of lettuce radicle growth when seedlings were exposed to water extracts from both replant and virgin soils.

4.9.5.1 Phytotoxins in virgin soil

On some sample dates virgin soil extracts enhanced growth at low concentration and suppressed growth as concentration increased indicating that the biological activity was dependant on concentration. It is possible that allelochemicals present in the virgin soil were capable of enhancing growth at low concentrations but were phytotoxic at higher concentrations. Alternatively the material responsible for enhanced growth at the lower concentrations in the virgin soil samples may have been masked by other, phytotoxic compounds as the concentration increased. Bioassays of soil extracts using asparagus germlings (Lake et al. 1993) also demonstrated significantly enhanced growth of seedlings exposed to virgin soil extracts while replant soil extracts suppressed growth at approximately 2x concentration. The extraction procedure in that work used a 50:50 mixture of methanol and water and was likely to extract different proportions of biologically active material than the water extraction process employed in this study.

Extracts from virgin soil samples taken from both sites caused some inhibition of the lettuce seedlings, particularly at higher concentrations, indicating the presence of phytotoxic material in the virgin soil. The phytotoxic material was generally only detected at higher concentrations of the extract (5x and 10x) and as there had been no herbicides used at site 1 for more than five years and at site 2 for 22 months prior to cultivating the old asparagus beds herbicide residues were unlikely to affect the bioassay results. Phenolic compounds, saponins, amino acids and carbohydrate material are most often implicated in the reports of naturally occurring phytotoxins or allelochemicals found in field soils (Lake et al. 1993; Hazebroek et al. 1989; Young & Chou 1985; Rice 1984). It is likely that these compounds, released from plant roots or the products of decomposing organic matter in the soil, are naturally present in the soil at low levels which vary according to the soil moisture level, temperature and plant species. The 5x and 10x concentration of extracts in this study increased the concentration of the biologically active materials in the virgin soil to levels high enough to inhibit the growth of lettuce radicles.

The virgin soil sample area at site 1 was an area of fallow ground close to the edge of the field and grass weeds including paspalum, bristle grass and prairie grass were

the main species present. The same weed species colonised the old asparagus soil area
during the experiment. At site 2 the virgin soil area was an old pasture immediately beside the former asparagus bed with the main plant species present being perennial rye grass, white clover and couch. The replant asparagus soil at site 2 became infested with annual weeds during the course of the experiment. The suppression found in the virgin soil samples is most likely due to phytotoxins exuded by the weeds or that occur as breakdown products during the decomposition of the weeds present. Couch (*Agropyron repens*) is known to produce allelochemicals along with many other plants as discussed in 4.8.5 (Hold et al. 1977; Rice 1984). Particularly strong inhibition by the virgin soil samples taken on 20 September 1995 soon after 15 mm of rain may have been caused by the release of allelochemicals by the weeds during the first significant rain that had fallen for five weeks.

4.9.5.2 Phytotoxins in replant soil

Significant differences in suppression caused by virgin soil and replant soil extracts were due to differences in the levels of phytotoxins in the two soils. As the pesticide history of the virgin and replant areas at both sites was the same any difference in the levels of phytotoxic activity in the replant compared with virgin soils was due the toxic material released from the asparagus plants following their incorporation into the replant soil.

The presence of phytotoxic material in the virgin soil made the detection of asparagus toxins in the replant soil more difficult and added to the variability in the amount of asparagus toxin detected at different concentrations of the soil extracts and at different sampling dates. The phytotoxic material in the virgin soil was likely to inhibit the lettuce seedlings at different concentrations to the inhibition caused by asparagus toxins in the replant soil. This was demonstrated by the changes in slope of the virgin soil dose response curves when the phytotoxin levels were at their highest in the virgin soil. The last virgin soil sample from both sites showed strong inhibition of the lettuce seedlings at a 10x concentration only. The replant soil extracts were slightly inhibitory at 5x concentration and more so at 10x concentration so inhibition by asparagus toxins at this time was only detected at the 5x concentration and not the 10x, as the phytotoxins in the virgin soil became as active as the phytotoxins in the replant soil at 10x concentration.

Added evidence of the activity of toxic material, other than the asparagus toxins in this study, was given by the shape of the dose response curves for both replant and virgin soil extracts. In the bioassays using root extracts described in section 4.3 the inhibition of lettuce seedling radicles by increasing concentrations of RE was asymptotic and log transformations or log/log transformations were able to give a

linear response curve. Exposure of the lettuce seedlings to the soil extracts however did not produce the same logarithmic inhibition and transforming the data using logs, inverse relationships or combinations of these did not provide straight line fits for the dose response curves. It is likely that this sort of pattern would arise when the lettuce seedlings were inhibited by more than one toxin or group of toxins that were active at different concentrations of the assay solutions.

4.9.5.3 Influence of sample date, temperature and rainfall on asparagus toxicity.

At both sites the non-concentrated water extract detected asparagus derived toxicity in the replant soil for approximately five months following the destruction of the previous asparagus crop. By concentrating the soil water extracts, the levels of toxicity observed in both replant and virgin soils from both sites increased. As the replant soil extract toxicity increased more than virgin soil for most sample dates, the toxicity due to asparagus toxins was detected more readily when the extracts were concentrated. Concentrating the extracts however did not improve the detection of asparagus toxins in replant soil when the virgin soil extracts contained phytotoxins at similar levels to the replant soil. The samples taken on 20 September 1995 and 15 December 1995 at both sites had levels of phytotoxic material present in the virgin soil approximately equal to the replant soil, therefore increasing the extract concentration did not improve the detection of asparagus toxins. The presence of increased levels of toxic material in the virgin soil at both sites which were 10 km apart, on two sampling dates, indicates that this was possibly due to climatic factors rather than a local effect. The rainfall event that preceded the September sampling date may explain the virgin soil toxicity on this occasion as previously discussed. The increased virgin soil toxicity at both sites in the December samples could be explained by the increased temperature. Temperatures in the seven days before the December samples were taken were an average of 6^{o}C and up to 12^{o}C higher than they had been since the asparagus plants were cultivated into the soil. This increase in temperature may have led to an increase in the release of phytotoxins from plants and plant litter present in the virgin sites. This is supported by the findings of Chou et al. (1981) who found that the presence of phytotoxins was greater in soils incubated at temperatures of 25^{o}C than at 15^{o}C to 20^{o}C.

When the toxin level was high in the replant soil, as with the first sample taken at site 1 and the third sample at site 2, the level of toxicity due to asparagus toxins detected was similar for all extract concentrations. Increased concentration of the soil extracts did improve the detection of asparagus toxin from the later soil samples however this effect was masked at some sample dates by phytotoxic material in the virgin soil samples which also became more evident at increased concentration.

The highest levels of asparagus toxins were detected at the earlier sampling dates and tended to be lower at later sampling dates. At site 2 the asparagus toxin level was highest at the third sampling date for the lower concentrations of extracts and at the first sampling date for the higher concentrations whereas at site 1 the highest levels of asparagus toxin were detected at the first sampling date for all concentrations assayed. This difference might be explained by the different timing of the cultivation of the two sites and the different textural classes of the two soils. Site 2 was cultivated one month later than site 1. The silt loam soil at site 1 has a high water holding capacity whereas the sandy soil at site 2 had a very low moisture holding capacity. Cultivation was performed at site 1 when the soil was damp and 55 mm of rain fell in the ten days after cultivation. At site 2 cultivation was performed on dry soil (4 mm rain in the 14 days prior to cultivation) and only 25 mm of rain fell in the 30 days following cultivation. As the soil conditions were drier at site 2 during cultivation and remained dry for some time following the cultivation, release of toxins may have been slower. The mean temperatures over July and August were similar and would probably not have caused any difference in the rate of release of toxic material from the previous asparagus crop.

The presence of reasonably high levels of phytotoxic material in the first four virgin soil samples taken at site 2 could have been due to the presence of couch in the pasture of the virgin soil sampling area as this grass species is known to produce allelochemicals. The virgin soil toxicity at site 2 reduced the ability to assess the toxicity due to asparagus in the replant soil particularly at the first two sampling dates and partly explains the low levels of asparagus toxin measured in the non concentrated extracts.

4.9.5.4 Level of asparagus toxicity relative to the asparagus crop residue.

The inhibition of lettuce radicle growth due to asparagus toxins in the soil at the early sampling dates at both sites was high when compared to the inhibition seen when lettuce seedlings were exposed to asparagus storage root extracts in earlier experiments (section 4.3). Immediately after cultivating the asparagus into the soil there was 9 g frw/kg of asparagus residues in the dry soil at site 1 and 5.5 g frw/kg at site 2. These levels of crop residues were present in the soil samples and soaked for 24 h in 1 l of water. The storage root extract concentration that gave rise to a similar inhibition of lettuce radicle growth as seen in the first sample at site 1 and the third sample at site 2 contained approximately 50 g of fresh storage roots per l of water. This was up to 8 times more storage root material than needed to give rise to similar suppression in the soil extract experiments. The longer extraction time of 24 h for the soil extracts compared with 12 h for the storage root extracts is unlikely to explain the high level of toxicity measured in the replant soils. It is likely that the

toxic material from the asparagus crop residues diffuses into the soil and remains in the soil solution or is protected by the soil particles in some way. Many biologically active organic substances are adsorbed by humus or clay colloids in soil and are protected from leaching or microbial decomposition by being associated with the soil particles (Young & Chou 1985) The ability to extract toxic material from asparagus soils by the use of resins such as XAD-4 demonstrates the propensity of the toxic materials from asparagus to bind with other substances. Young and Chou (1985) found the XAD-4 resin extraction process superior to any of the water or solvent extraction processes they employed. Studies have shown that the allelochemicals or autotoxins from asparagus crop residues are polar in nature which would facilitate the sorption processes in the soil (Hartung & Stephens 1983). Such an association with the soil would explain the high levels of asparagus toxin measured in the earlier sample dates in this study as the toxic material would be released from the asparagus crop residue over time and build up in the soil by being associated with soil particles and thus protected from leaching or microbial decomposition.

The toxicity measured in the replant soils at both sites eleven and twelve months after the asparagus plants had been cultivated is low in relation to the crop residues that were still present. At the final sampling date asparagus toxins were detected in the 5x concentrated assay solutions but not the 10x concentration as previously discussed. There were 3.5 g frw/kg and 2.5 g frw/kg of decomposing asparagus residue in the dry soil at sites 1 and 2 respectively at the final sampling date. In the fresh storage root extract experiments this weight of material would have caused approximately three times more suppression of lettuce radicle growth than was observed in the soil extract experiments. The fact that the asparagus storage roots and rhizomatous material recovered from the replant soils at the final sampling date was mainly decomposed would explain the small amount of phytotoxin recovered from this material. Most of the phytotoxic material from the asparagus residues would have been released into the soil by the final sampling date and any asparagus toxins detected were more likely extracted from the soil than from the crop residues present in the soil sample. The low levels of phytotoxins presented in asparagus root litter compared with fresh asparagus roots was demonstrated by Young & Chou (1985). In a paper bioassay using germinated asparagus seed they found that root extracts inhibited asparagus radicle elongation up to ten times more than root litter extracts.

4.9.5.5 Level of asparagus toxins measured by the different extract concentrations.

The levels of asparagus toxin measured by the non-concentrated and 2.5x concentrated soil extracts decreased as time from destroying the asparagus plants increased. At both sites the non-concentrated assays failed to detect asparagus toxins after five months. The 2.5x concentrated extracts measured asparagus toxins for seven months following the termination of the asparagus crops. Lake et al. (1993) found samples taken four months after the termination of an asparagus planting were strongly inhibitory to asparagus germlings whereas samples taken after five months were less inhibitory. The asparagus planting in that study was terminated by ploughing and as that would have resulted in leaving the plants much more intact than the rotary hoeing employed in this study it is likely that asparagus toxins would be released more slowly and over a longer period of time.

The 5x and 10x concentrations of soil extracts in this study were able to detect asparagus toxins in the replant soil throughout the twelve month sampling period and the general trend was for the toxicity in the replant soil to decline with increasing time from the termination of the asparagus crop. Because of the sensitivity of the assay procedure and the activity of phytotoxins other than those from the asparagus plants the higher concentrations assayed did not show a clear pattern of reduction in asparagus toxicity over time as the lower concentrations did.

4.9.5.6 Importance of autotoxins in replant soils.

The importance of asparagus autotoxins in a replant field situation will depend on the level at which they are present in the soil and whether their level is sufficient to directly inhibit the growth of a new crop. It is likely that within five to six months of destroying an asparagus planting by rotary hoeing and deep ripping (chopping the old plants into small pieces to aid their breakdown) that the levels of toxin are not high enough to directly inhibit a new asparagus planting. This is supported by field trials done in New Zealand where fields that were replanted immediately after terminating the old crop had poor establishment whereas the survival and vigour of the new planting was improved when the planting was delayed for twelve months after terminating the old crop (Nichols 1989; McCormick & Thomsen 1989).

After the toxins have fallen to a level where they are unlikely to have a direct affect on new plantings the interaction of asparagus autotoxins with *Fusarium* spp. is likely to be more important in reducing the survival or vigour of a new planting (Peirce & Colby 1987; Hartung & Stephens 1983). Residues of the previous asparagus crop are likely to be present for 20 years or more after the termination of an asparagus bed and *Fusarium* spp. pathogenic to asparagus will survive in association with this

material or other organic matter in the soil (Blok & Bollen, 1993; 1996). Evidence from the assays of concentrated soil extracts in this study suggest that low levels of asparagus toxin will be present for twelve months or more after destroying the previous asparagus planting with a rotary hoe. It is likely that low levels of autotoxic material will exist for much longer periods than this, either protected in the soil by adsorption processes, or in association with soil colloids, or in the crop residues that still remain in the soil for at least ten years after the termination of the previous crop (Blok & Bollen 1993, 1996(c); Young & Chou 1985). Asparagus root litter recovered from asparagus fields ten years after the termination of the crop was shown to inhibited root growth of asparagus seedlings and was inhibitory to curly cress in a paper bioassay (Blok & Bollen 1993). The low levels of toxin likely to exist in asparagus soils, although not directly inhibiting the new plants in a replant site, could have a more significant affect by increasing their susceptibility to attack by *Fusarium*. spp. and reducing the survival and vigour of the replanted stand in this way.

The performance of a new asparagus planting in a replant site might be affected in two ways by the autotoxic material that is present in the soil and asparagus crop litter. Firstly at high levels the autotoxin will have a direct inhibitory effect on the growth of the new plants. The autotoxin will also bring about an increased level of disease incited by *Fusarium* spp. (Hartung & Stephens 1983; Peirce & Colby 1987; Wacker et al. 1990). It is likely that both paths of action of the autotoxins occur simultaneously and they would most likely act together with synergy. *Fusarium* spp. infections are known to increase as plant vigour decreases so once the cycle of suppression by the autotoxins and infection by *Fusarium* spp. starts it would snowball as more infections occur causing more root death and release of more autotoxin.

As the infection of asparagus by *Fusarium* spp. is increased in the presence of the autotoxic material and the performance of the new planting is reduced through this increased disease level or through the direct plant suppression by the autotoxins, it is probably not necessary to differentiate the way the autotoxin is reducing the performance in a replant field situation.

4.9.6 Conclusions

The paper bioassay procedure using lettuce seed exposed to water extracts from the soil developed in this study has proved to be very useful in monitoring the levels of asparagus toxin in soil following the termination of the old asparagus crop. The assaying of neat soil water extracts or 2.5x concentrated extracts, detected levels of toxicity in the soil that are probably high enough to cause direct inhibition to subsequent crops. When the soil extracts were concentrated 5x or 10x the bioassay detected a range of materials from soil which become phytotoxic at increased concentrations but increased the detection of asparagus toxins present at low levels.

The bioassay procedure would be useful for testing individual fields for asparagus toxins. Care would be needed to ensure a representative soil sample is taken both from the asparagus field and from adjacent virgin soil preferably with the same vegetative cover and similar pesticide history.

Bioassaying the neat soil water extract only will identify levels of toxin that are likely to directly inhibit sensitive crops. As the bioassay procedure is fast and inexpensive to perform then concentrating the extracts and assaying three or four concentrations as in this study would provide more information on which to base decisions on the possible uses of the area being tested.

Section Five
General Discussion & Conclusions

5.1 Importance of herbicide residues in the Asparagus Replant Problem.

The persistence of residual herbicides is influenced by the following processes in soil (Anderson 1977).

1.	Physical.	- Volatility - Leaching - Soil Erosion
2.	Chemical.	- Adsorption - Photochemical decomposition - Reactions with soil constituents.
3.	Biological	- Decomposition. - Uptake by plants and micro-organisms.

The main factors that influence each of these processes are the climatic conditions, the soil type and the effect these have on soil temperature and moisture (Ross & Lembi 1985).

The carry over of residual herbicides in the soil to cause replant problems in subsequent crops has not been widely reported but was hypothesised as a likely factor in the replant problem of fruit trees (Utkhede & Smith 1994) and other crops (Davison & Clay 1972).

In New Zealand long lasting residual herbicides such as bromacil, terbuthylazine and terbumeton are often used on asparagus beds to control weeds during harvest and fern growing period. After several years levels of applying such compounds they may build up to levels high enough to affect the growth of subsequent crops. Anecdotal evidence of asparagus replant failures believed to be caused by herbicide residues exists in New Zealand where replanting was carried out after several years of the use of either bromacil or terbuthylazine.

Two of the Greenhouse Trials reported in this thesis demonstrate the possibility of herbicide residues being involved in asparagus replant problems under New Zealand conditions.

Of the factors considered to be involved in the replant problem that were evaluated in Greenhouse Trials the residual herbicide Caragard had the greatest effect, significantly reducing growth of asparagus and resulting in increased disease levels on the roots of asparagus seedlings.

In Greenhouse Trial 4 rates of 0-3.6 ppm a.i. terbuthylazine and terbumeton were evaluated to establish dose-response relationships between the herbicide and asparagus seedlings in two soils. In that experiment a rate of approximately 0.6 ppm total a.i. was sufficient to halve the growth of asparagus seedlings in both soils. This level of herbicide could arise in old asparagus soils after several years of residual herbicide applications.

Both Greenhouse Trials therefore support the view that under New Zealand conditions it is possible to build up residues of herbicides in soils that are high enough to significantly inhibit the growth of replanted asparagus however provided herbicides are used with a sound knowledge of their likely adsorption, movement and degradation in the particular soil to which they are applied the appropriate rates to use can be assessed and their likely movement and persistence can be predicted. Careful consideration should be given to the use of different herbicides rather than the annual use of the same herbicide in an asparagus crop.

It is apparent that where an asparagus crop is in decline and termination of the crop is planned, the likely carryover of herbicides in the soil should be taken into account when using herbicides several years before terminating the crop. If the herbicide program on an asparagus crop is based on knowledge of the products used and their likely persistence in the particular soil type and climate there is no reason for herbicide residues to cause problems with subsequent crops.

5.2 Importance of autotoxins in the Asparagus Replant Problem.

Many studies have shown that asparagus tissue, asparagus plant residues and soil from old asparagus beds contain substances that are inhibitory to asparagus plant growth (Blok & Bollen 1993; Hartung et al. 1989; Hartung & Putnam 1986; Young & Chou 1985). The importance of autotoxins in the replant problem may be related to an interaction with *Fusarium* spp. (Hartung & Stephens 1983; Pierce & Miller 1990; Hazebroek et al. 1989).

Research to identify the autotoxin in asparagus roots has shown that the inhibition is not likely to be caused by one compound but rather a group of compounds acting together. Hartung et al. (1990) and Miller et al. (1991) indicated caffeic acid, methylenedioxycinnamic acid (MDCA), asparagusic acid, fumaric acid, malic acid, isoferulic acid, ferulic acid, dihydrocitric acid and citric acid were present in asparagus storage root extracts. Lake et al. (1993) found that tryptophan was present in root extracts at levels high enough to cause inhibition. Many of the chemical components of asparagus root extracts have been shown to be biologically active in the laboratory although often the levels of individual compounds present in nature may not be high enough to cause inhibition on their own. It is more likely that the autotoxic effect of asparagus crop residues arises from a complex interaction between many chemical components acting in different ways depending on soils, environmental conditions and microbial populations in the asparagus rhizosphere.

It is not practical to physically remove the residues of an old asparagus planting prior to replanting because of the volume of material involved and the depth to which the storage root system grows. Estimates of the amount of asparagus plant material present in the soil of asparagus beds in the Netherlands were 4180 to 11060 kg dw/ha (Blok & Bollen 1993). During experiments described in this thesis (Field Trials 1 and 2 and the two sampling sites for soil water extract experiments in section 4.9) samples taken support these figures with between 6875 and 14850 kg dw/ha found at the sites where total crop residue was estimated.

In greenhouse experiments asparagus seedlings were not significantly suppressed in the presence of asparagus storage root additions to the soil. In Greenhouse Trial 1 this was most likely because the herbicide additions severely inhibited growth in the experiment to the extent that the effect of other treatments was influenced by the herbicide additions.

In Greenhouse Trial 4 the temperature and light conditions were likely to have limited growth in the trial and thus masked the effect of the storage roots.

In other studies where up to 20 g of dried roots were added per litre of soil (Hartung et al. 1989; Blok & Bollen 1993) asparagus seedlings were inhibited but at rates that approximate the levels found in field soils (2 g/l) there was no inhibition. These studies suggest that autotoxins may not be an important factor in direct growth inhibition in asparagus replant situations because the amount of material likely to be present in a replant soil is too low to cause such an effect as was concluded by Blok & Bollen (1993).

Asparagus seedlings were inhibited by replant soils compared to the same soils with no history of asparagus production in Greenhouse Trial 2. As the inhibition was not affected by soil sterilisation it is likely that autotoxic material in the replant soil was responsible for the inhibition.

The bioassays of soil water extracts from replant soils, described in section 4.9, showed that, for up to five months after the destruction of the previous crop, autotoxin levels in replant soils were much higher than would be expected, based on the levels of crop residue present. The autotoxic material is likely to accumulate in the soil by being adsorped or associated in some way with soil particles and thus protected from leaching or biochemical breakdown. The level of inhibition due to autotoxins found in the soil water extracts from two replant sites suggest that direct inhibition of replanted asparagus is possible if replanting is undertaken within a few months of terminating the previous crop. This finding is supported by field trials undertaken in New Zealand where fields replanted within three months of destroying the previous planting had poor establishment whereas by delaying planting for twelve months survival and vigour of the new planting was greatly improved (Nichols 1989; McCormick & Thomsen 1989). In Field Trial No. 1 leaving the old plants intact and thus reducing the initial release of phytotoxic material was compared with cultivating the old plants into the soil. Leaving plants intact did not affect initial plant growth or survival however the spear numbers and total weight of spears produced over three successive harvest seasons was higher where the old plants were left intact. The effect was significant at $P=0.0735$ for total spear numbers and at $P=0.1014$ for total weight of spears and such a difference could be important in a commercial situation as the total accumulated yield over the three harvests was 20% higher where the old plants were left intact. The difference in yield between chopping up the old plants and leaving them intact in field trial 1 may have been due to the difference in levels of autotoxic material released or difference in the *Fusarium* inoculum levels as cultivating of the old plants into the soil would spread inoculum through the soil and provide more substrate for *Fusarium* growth. It is likely that both autotoxins and increased inoculum levels contributed to the effect observed in the field trial with the likely interaction of autotoxins and *Fusarium* also implicated.

5.2.1 Interactions between autotoxins and *Fusarium* spp.

There have been many studies of the interaction between asparagus autotoxins and *Fusarium* spp. An interaction of asparagus root autotoxin with the two common *Fusarium* spp. affecting asparagus was first reported by Hartung and Stephens (1983). These workers also described the ability of autotoxins to inhibit the growth of a range of fungi. More recent reports have suggested several possible mechanisms for the interaction. Peirce & Colby (1987) suggested that *Fusarium* spp. are stimulated by the soluble solids content of the autotoxin and also found that young asparagus seedling radicles were predisposed to *Fusarium* infection by exposure to root extracts. Hazebroek et al. (1989) indicated that the autotoxic components of asparagus tissue are biologically active against a wide range of organisms, reporting that extracts of asparagus tissue reduced the growth of some species of bacteria, fungi and nematodes. Decreased peroxidase activity and increased electrolyte reflux in asparagus roots exposed to autotoxins was demonstrated by Hartung et al. (1989). Nigh (1990) described the importance of stress factors such as autotoxins in weakening asparagus plants defence mechanisms to *Fusarium* infections. Peirce and Miller (1993) exposed young asparagus seedlings to cinnamic acids and found they damaged radicle epidermal cells and caused precocious root hair development while Wacker et al. (1990) found ferulic acid reduced the symbiotic effectiveness of *Glomus-fasciculatum* colonisation of asparagus roots.

In contrast to other reports, recent studies by Blok and Bollen (1996b) found that *Fusarium* root rot or *Fusarium* soil populations were not increased by the presence of root residues. They argued that the studies that showed increases in *Fusarium* infection of seedlings in the presence of root residues were done in sterilised soil or used unrealistically high levels of root material whereas in unsterilised soil they found no interaction between *Fusarium* and levels of asparagus residues likely to be encountered in the field. In this thesis, Greenhouse Trial 2 (3.2) provides evidence of an interaction between *Fusarium* inoculum additions and toxic material in replant soils. Seedling growth was inhibited and disease levels were significantly higher in replant soil compared to virgin soil. When both soils were sterilised the differences in growth and disease levels were accentuated, supporting the findings of Blok and Bollen (1996b). Results of Greenhouse Trial 2 suggest an interaction between *Fusarium* and autotoxic material in the replant soil when neither soil was sterilised in contrast to Blok and Bollen's (1996b) findings however, the increased disease levels and reduced growth in non-sterilised replant soil in the trial may have been due to a high level of pathogenic *Fusarium* spp. inoculum when the soil was not fumigated.

The levels of autotoxic material in a replant site are only likely to be high enough to cause direct inhibition of a new planting if they accumulate in the soil following termination of the previous crop. Such a build up of autotoxic material would be dependant on the soil type and environmental conditions but it is most likely that within six months of terminating the crop the autotoxin levels would be too low to cause direct inhibition. Many reports of the presence and likely mechanisms of an interaction between *Fusarium* spp. and autotoxins and the likelihood of a very complex interaction dependant on soil type, environmental conditions and soil microbial populations indicate that while there are conflicting reports of the importance of the interaction it is likely that such an interaction is a contributing factor in the asparagus replant problem.

5.3 Importance of *Fusarium* spp. in the Asparagus Replant Problem.

The four *Fusarium* spp. known to infect *Asparagus officinalis L* in New Zealand are (Falloon & Tate 1986) ;

-*Fusarium oxysporum* (Schlecht) emend. Snyder and Hansen *f. sp. asparagi* Cohen *(FOA).*

-*Fusarium moniliforme* (Schlecht.) emend. Snyder and Hansen *(FM).*

-*Fusarium solani* (Mart.) App. and Wr. emend Snyder and Hansen *(FS).*

-*Fusarium redolens* (Wollenw.) *(FR).*

Overseas research suggests that both *FOA* and *FM* are the major causal agents associated with both asparagus decline and the asparagus replant problem (Grogan & Kimble 1959; Johnston et al. 1979; Gordon-Lennox & Gindrat 1987; Elmer 1990; Schreuder & Lamprecht 1995; Blok & Bollen 1996c), with *FOA* normally identified as the main pathogen because to its prevalence and virulence. In the Netherlands Blok & Bollen (1996b) characterised a condition they termed asparagus replant-bound early decline (ARED) incited by *FOA* in replant soils and concluded that the main causal factor of ARED and early decline on fresh soils was *FOA*. U.S.A. research also suggests that in young planting *FOA* is the predominant pathogen isolated and as the stands age the prevalence of *FM* increases (Elmer et al. 1996). In South Africa, Schreuder & Lamprecht (1995) found that highly virulent strains of *FOA* and *FM* were the most frequently isolated pathogens in a declining asparagus planting and concluded that both pathogens were involved in the early decline of asparagus stands.

The isolations of *Fusarium* spp. from plants in the field trial 1 in this thesis support the findings from other asparagus producing countries in which predominantly *FOA* was isolated but some isolates of *FM* were also obtained. Of the 65 pathogenic *Fusarium* spp. isolates obtained from Field Trial 1, 61 were identified as *FOA* and 4 were *FM*.

Observational results in Field Trial 1 indicated that replant failures were caused by *FOA* and *FM* infections and control by the TBZ treatment was not sufficient to improve plant vigour or yield in the replant area. In Field Trial 2 however the vigour and yield difference between replant and virgin sites was significantly reduced by the TBZ treatment suggesting that *Fusarium* spp. infections were a major factor in the reduced vigour and yield in the replant site in this trial.

The results of the greenhouse trials (section 3) confirm the importance of *FOA* and *FM* in the replant problem. In Greenhouse Trial 1 conidial inoculum at the rate used

did not suppress growth but did give rise to increased disease levels in the experiment. With higher rates of a mixed *FOA* and *FM* inoculum in Greenhouse Trial 2 disease levels and suppression of asparagus growth increased as the level of inoculum increased. This experiment demonstrated the importance of inoculum levels in *Fusarium* infections of asparagus. The greenhouse trials showed that *FOA* and *FM* readily infected asparagus seedlings under experimental conditions even in pots where inoculum treatments were not applied. Sterile growing media, disease free plant material, and complete isolation of individual plots is necessary to conduct pot trials if complete freedom from *FOA* and *FM* is required during the conduct of pot trials. The fact that *FOA* and *FM* readily infected asparagus seedlings in the Greenhouse Trials and also appeared to be the primary cause of replant suppression and deaths in the field trials supports the findings of Blok & Bollen (1996b) that *Fusarium* infections are the major factor in the asparagus replant problem.

Old asparagus soils contain high levels of *FOA* and *FM* in association with asparagus root and crown residues. Blok & Bollen (1996c) retrieved $2x10^6$ colony forming units of *FOA* per gram of dry asparagus tissues from standing asparagus crops. The population fell to $1x10^5$ cfu/g during the first 10 years after asparagus plantings were destroyed and after 25 years populations were still as high as $1x10^4$ cfu/g. Asparagus crop residues break down slowly in the soil after asparagus cropping has ceased and Blok and Bollen (1996c) concluded that the persistence of *FOA* in asparagus root residues is the main reason for the long term survival of *FOA* in asparagus soils. *FM* is also likely to persist in old asparagus soils in the same way as both pathogens are able to survive as saprophytes on crop residues. *FOA* and *FM* are also known to occur as natural residents in soils with no history of asparagus cropping (Stephens & Elmer 1988; Gordon-Lennox & Gindrat 1987). The ability for these pathogens to persist for 25 years or more in soils where asparagus has grown and the ubiquitous nature of the pathogens explains the lack of progress in finding a solution to the asparagus replant problem or ARED.

Soil sterilisation to prevent the carryover of *Fusarium* spp. from one asparagus crop to the next has been largely unsuccessful. Damicone and Manning (1985) concluded that some *Fusarium* propagules may have survived fumigation with 80% chlorinated hydrocarbons and 20% methylisothiocyanate and rapidly recolonised the soil or new infections may have arisen through airborne conidia formed on sporodochia in nearby fields. Damicone et al. (1981) and Manning & Vardaro (1977) also report that fumigation of asparagus soils which either did not completely eradicate *Fusarium* or that rapid reinfestation occurred.

As *FOA* forms chlamydospores and *FM* can survive as thickened mycelium then these structures, when embedded in asparagus tissues deep in a field soil, are likely

to survive fumigation in field soils. The infection of asparagus seedlings grown in uninoculated sterilised replant and virgin soils in Greenhouse Trial 2 in this thesis also provides evidence that fumigation of soils, even in sealed containers, may not completely eliminate the disease. Asparagus storage roots have been found at depths of over 2 m in deep alluvial soils used for asparagus cropping in New Zealand (Reid pers. comm.) and it is likely that at such depth *Fusarium* spp. associated with the roots would be unaffected by fumigation. The ubiquitous nature of *FOA* and *FM* also suggests that soil sterilisation is unlikely to provide long term control of these diseases in a replant soil. Nearby soils with no asparagus history or nearby asparagus crops may readily provide inoculum for the reinfestation of a sterilised replant site.

The use of fungicides to control *Fusarium* in newly planted and established asparagus beds has been widely evaluated, however no completely successful control measures currently exist (Yang 1982; Gordon-Lennox & Grindrat 1987; Elmer 1996). Field Trial 2 in this thesis indicated that treatment of planting material with TBZ may give some measure of control during establishment in a replant soil and result in higher yields from the replanted crop. Falloon & Fraser (1989) also reported improved establishment and vigour from replanted asparagus treated with TBZ. In Field Trial 1 however the TBZ treatment did not improve establishment or yield either alone or in combination with metalaxyl. Similarly the use of TBZ as a soil drench on established asparagus beds did not improve the yield of a declining asparagus stand and had no affect on the Root Necrosis Potential or on asparagus seedling weight grown in the TBZ treated soil in Greenhouse Trial 3. Although the use of fungicides to control *Fusarium* in replant sites may improve initial establishment in some instances they are unlikely to provide a long term solution to the replant problem for similar reasons to the lack of success with soil sterilisation.

Elmer (1989, 1990, 1996) found that increased yields were achieved in declining asparagus stands using 560 to 1120 kg/ha of rock salt and the yield increases were associated with suppressed *Fusarium* spp. infections. Elmer (1996) concluded that NaCl may reduce *Fusarium* crown and root rot through a fungistatic effect and/or through manipulation of host resistance. Falloon and Fraser (1990) have also shown that slight yield increases are achieved by applying 560 kg/ha rock salt pre-harvest on established asparagus. Rates of 560 and 1120 kg/ha applied at two sites in Hawkes Bay for two seasons had no effect on the yield in either season and the higher rate at one site did not effect the Root Necrosis Potential or asparagus seedling weight grown in the treated soil in Greenhouse Trial 3. Furthermore, as was found in the Hawkes Bay trial sites, annual applications of 1120 kg/ha NaCl to the recent soils used for asparagus cropping in New Zealand may give rise

structural degradation of the soil. The use of rock salt is therefore not likely to be a successful control measure for *Fusarium* associated with the replant problem or ARED in New Zealand and is likely to cause serious structural degradation of the soil.

Biological control measures appear to offer the most potential for the control of *Fusarium* infections in asparagus for prolonged periods. Damicone and Manning (1982) were able to protect plants against crown rot development in the field for up to eight weeks by inoculating with a non-pathogenic strain of *F. oxysporum.* Armstrong and Armstrong (1969) identified several races of *Fusarium* that showed cross pathogenicity for various crops but were non-pathogenic on asparagus, and showed potential for the protection techniques demonstrated by Damicone and Manning (1982). Soil amendments have shown some promise in reducing the incidence and severity of the disease (Sun & Huang 1985; Tu et al. 1990). Scher and Baker (1980) found a species of *Pseudomonas* that suppressed *Fusarium* infections in soils with high inoculum levels. Exposure of the pathogen to other fungal communities has shown some promise (Marrois et al. 1981; Marrois & Mitchell 1981) however in this thesis and in other New Zealand trials use of *Trichoderma viride* conidia and mycelium as a crown dip or soil drench did not reduce *Fusarium* incidence or improve establishment in a replant soil. If an antagonist or non-pathogenic strain can be found that will be stable in an asparagus stand or that can be favoured by certain soil management techniques then at least a partial solution to the replant problem may be found.

Breeding or cloning *Fusarium* resistant cultivars offers a means of avoiding this aspect of the replant problem. Breeding efforts have been unsuccessful to date and many years of breeding effort have gone into locating some measure of genetic resistance to the disease (Bussell & Ellison 1987). It appears that the greater tolerance to crown and root rot of parents selected for *Fusarium* resistance in the field is due to increased vigour-related tolerance of the disease rather than to any genetic resistance or specific defence mechanism. Elmer et al. (1990) attributed failure to breed resistant lines to the existence of many genetically distinct strains of *FOA* and *FM* found infecting asparagus. As some *Asparagus* spp. are immune to *Fusarium* the use of genetic transformation provides the most likely means of developing resistant *A. officinalis* cultivars in the future.

5.4 Conclusions.

Of the factors involved in the asparagus replant problem that have been investigated in this study *Fusarium* spp. appear to be the major factor involved and it is most likely that if *FOA* and *FM* infections of asparagus plants could be avoided the asparagus replant problem would not exist.

Herbicide residues in asparagus soils may cause specific replant problems in some instances but as with soil structural problems or imperfect drainage, herbicide residues need not cause a replant problem if appropriate management techniques are employed in the planning of the new asparagus development.

Autotoxins are involved in asparagus replant problems when a new planting is made within a few months of terminating the previous planting. The levels of autotoxins in a replant soil fall rapidly following the termination of the previous crop and within six months of destroying the old asparagus planting any involvement of autotoxins would be mainly through an interaction with *FOA* and *FM*.

The asparagus replant problem would be better termed a replant disease as the primary and possibly the only cause in many instances is infection by *FOA* and *FM*. Blok & Bollen (1996b) have named the disease ARED (asparagus replant-bound early decline) and as the causal organisms are the same as asparagus decline suggest it is probably not necessary to discriminate between the two. As early decline in a site with no history of asparagus production is more readily avoided than in a replant site because of the levels of inoculum likely to be present, discrimination between ARED and asparagus decline and the reason for their occurrence is important.

5.5 Recommendations for Growers.

Planting asparagus in soils where asparagus has been previously grown should be avoided. In some countries suitable soils for asparagus production are limited in area so if the crop is continued to be grown replanting declining stands is inevitable however in New Zealand this is not the case and replanting should be discouraged.

Often replanting is undertaken because an existing grower wants to continue to grow the crop but does not have any land which has not previously grown asparagus. In these situations other options such as replacing the old asparagus land by leasing or buying new land or going out of asparagus production should be considered. The studies undertaken in this thesis provide base information on which to make decisions on whether or not to replant asparagus and on how to manage a replant development if it is, indeed, undertaken.

The field trials demonstrated that planting material treatments do not overcome the replant problem but may reduce it's effect to some extent. They clearly demonstrate (along with other research) that when asparagus soil is replanted a yield 20% to 30% lower than the same development on virgin soil can be expected. This yield differential between virgin and replant soil is likely to grow over time and the commercial life of a replanted stand will be shorter than a development on similar virgin soil.

The field and laboratory experiments with different asparagus cultivars showed that the likelihood of finding cultivars that are immune to ARED is remote. In a replant situation the most vigorous varieties for the particular growing area should always be used to take advantage of the vigour-related tolerance asparagus has to *FOA* and *FM*.

Replant establishment problems can be minimised by destroying the old asparagus planting at least twelve months before planting the new crop or by leaving the old plants intact in the soil and replanting between the old rows. Drenching plant material with TBZ at planting and treating the replant area with metalaxyl is also recommended. Production of virus indexed, disease free plant material by using disinfested seed grown in clean soil will also help improve establishment in a replant site.

It is not clear how long after an old crop is taken out the replant problem persists but indications are that ARED is most likely to still affect sites, to some extent, 25 years or more after the previous asparagus crop. The RNP test described in this thesis would be an excellent means of assessing the likelihood of ARED at a replant site. Similarly the soil bioassay developed in this study will give an indication of the presence of asparagus toxins in fields where the previous asparagus crop has been recently destroyed. The bioassay will be equally useful in determining the likelihood of problems when growing any crop sensitive to asparagus toxins in a field where asparagus beds have been destroyed.

5.6 Research Directions.

As the asparagus replant problem is generally a replant disease (ARED) incited by *FOA* and *FM* future research of the replant problem should concentrate on control measures for the crown and root rot complex that is caused by these pathogens.

The existence of very high inoculum levels of genetically distinct strains of *FOA* and *FM* and their long term persistence in asparagus soils presents a considerable challenge to finding successful and lasting control measures.

Chemical control or elimination of the pathogens by soil treatments are unlikely to provide a lasting solution.

Development of asparagus clones or cultivars with resistance to *FOA* and *FM* may be possible using genetic transformation but is also likely to be a short term solution as the genetic diversity in pathogenic *Fusarium* strains implies they can readily adapt and could do so to avoid the mechanism of resistance.

Biological control probably presents the most promising avenue of research. Successful control of *FOA* and *FM* would most likely involve manipulation of the complex environment, pathogen, host interactions to tip the balance in favour of the host and take advantage of the hosts' natural ability to tolerate and wall off *Fusarium* infections. Investigation of the environment, pathogen, host interactions at sites where asparagus plantings are highly productive and have a long commercial life would provide clues as to the more fruitful avenues of research into the biological control of ARED.

APPENDICES

Appendix 1.

Media used in isolating and identifying *Fusarium* spp.

Potato Dextrose Agar.(PDA)

PDA was prepared using Difco PDA preparation at 21.5 g per litre of water. After autoclaving for 15 min. at 121°C and cooling to 45°C, plastic Petri plates were poured with 25 ml of media per plate.

Carnation Leaf Agar.(CLA)

CLA was prepared using young carnation (*Dianthus caryophylus L*) leaves from actively growing pesticide free plants. The leaves were cut into 5 mm square pieces and oven dried at 40°C for 48 h. The dry pieces were wrapped in aluminium foil packages and sterilised using a 2.5 Megarad (25kGy) dose of gamma radiation.

Several sterile leaf pieces were placed in each Petri plate and 25 ml of cooled (45°C) autoclaved 2% water agar poured into each plate (Nelson et al 1983).

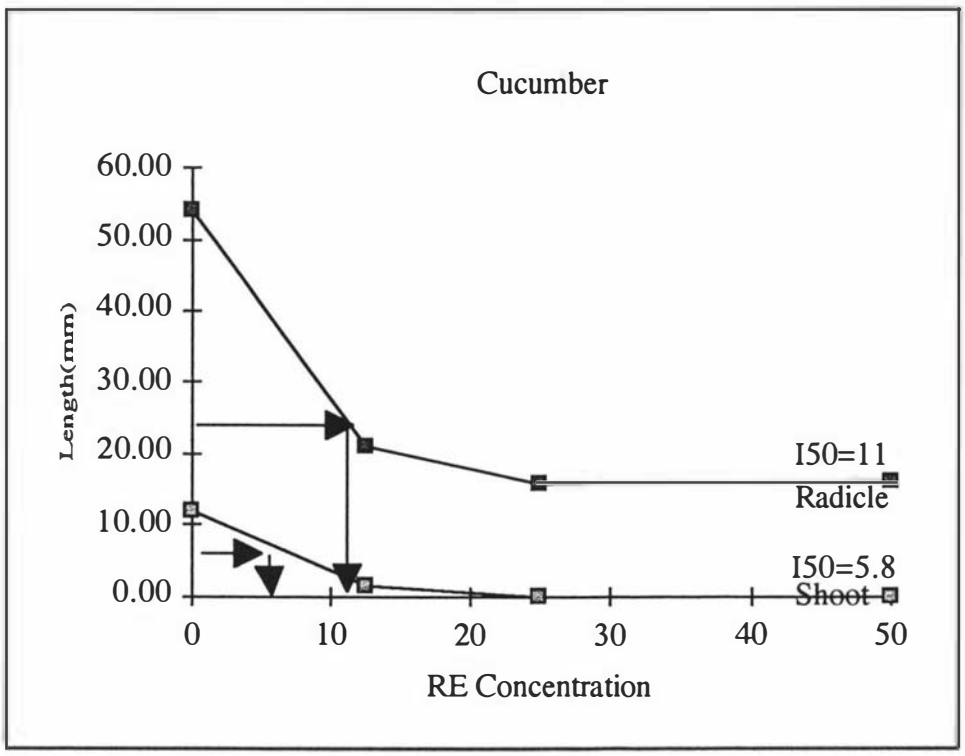
Fusarium selective media.(FSM)

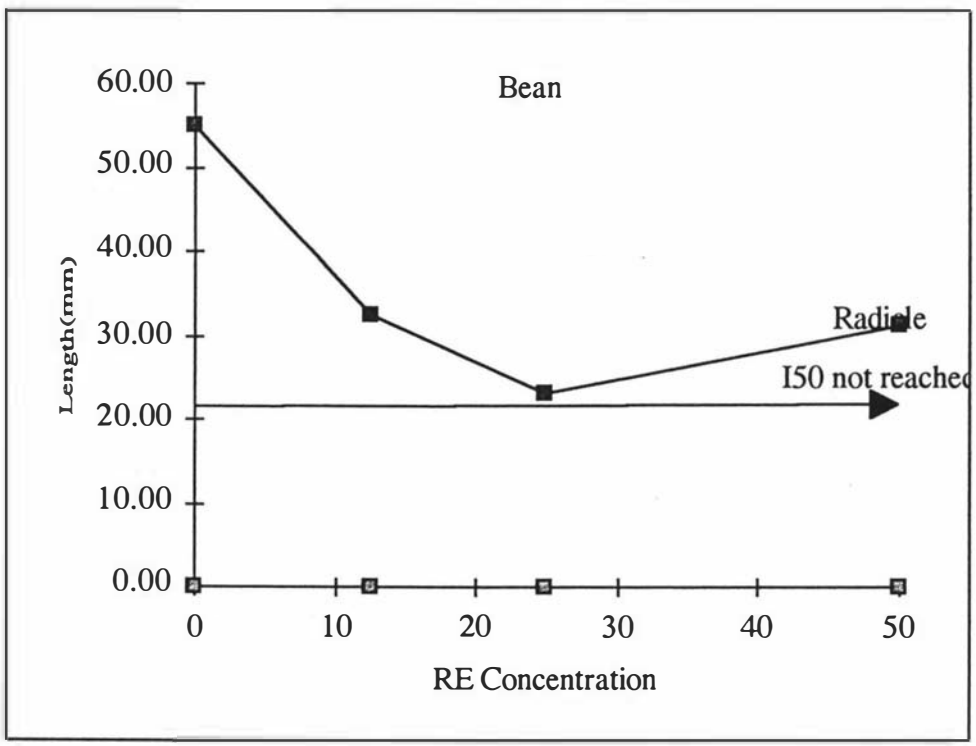
FSM was a modified Nash-Snyder medium. The formula is 15.0 g Difco peptone, 1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 20.0 g of agar, 1.0 g of pentachloronitrobenzene (PCNB) and 1.0 l of water adjusted to pH 5.5 - 6.5 before autoclaving. Twenty millilitres of a streptomycin sulfate stock solution and 12 ml of a neomycin sulfate stock solution were added to each litre after cooling to 45°C and just before being poured into Petri plates.

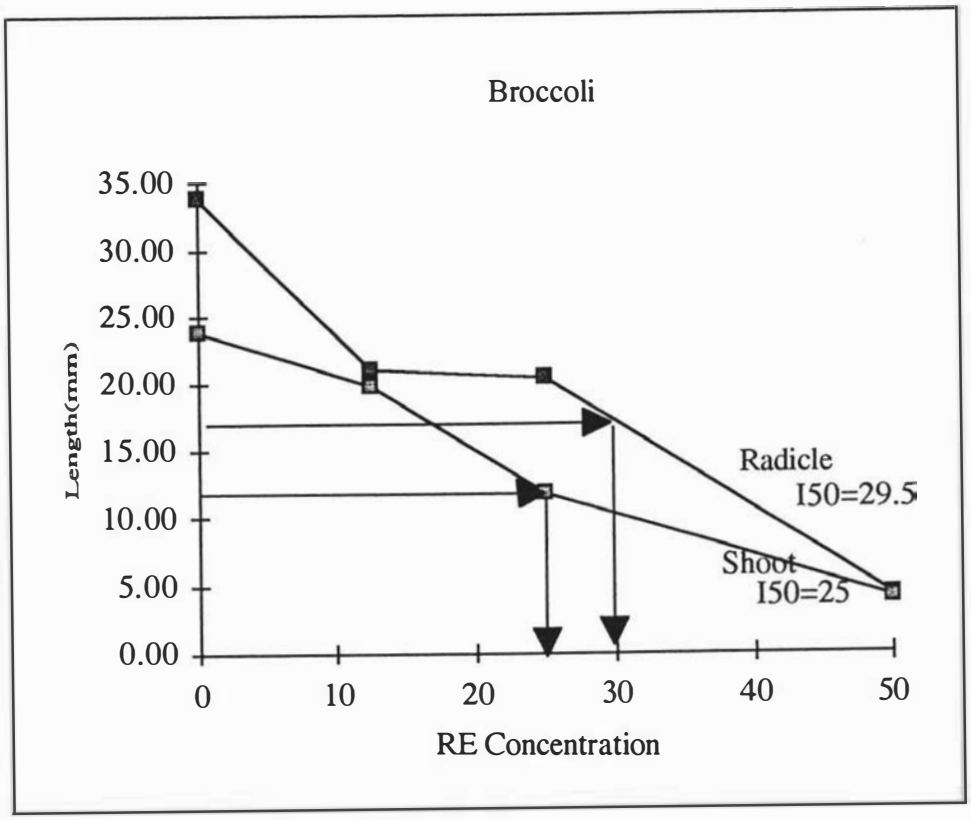
The stock solutions were prepared by adding 5 g of streptomycin sulfate to 100 ml of water and 1 g of neomycin sulfate to 100 ml of water.

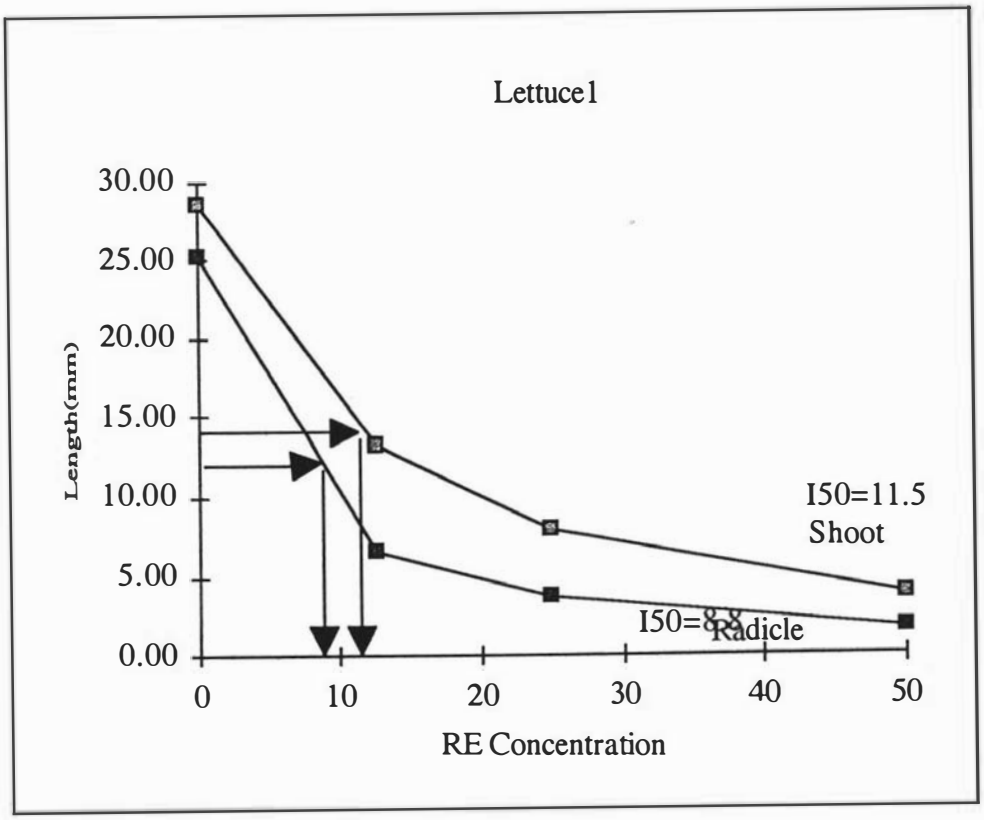
Appendix 2

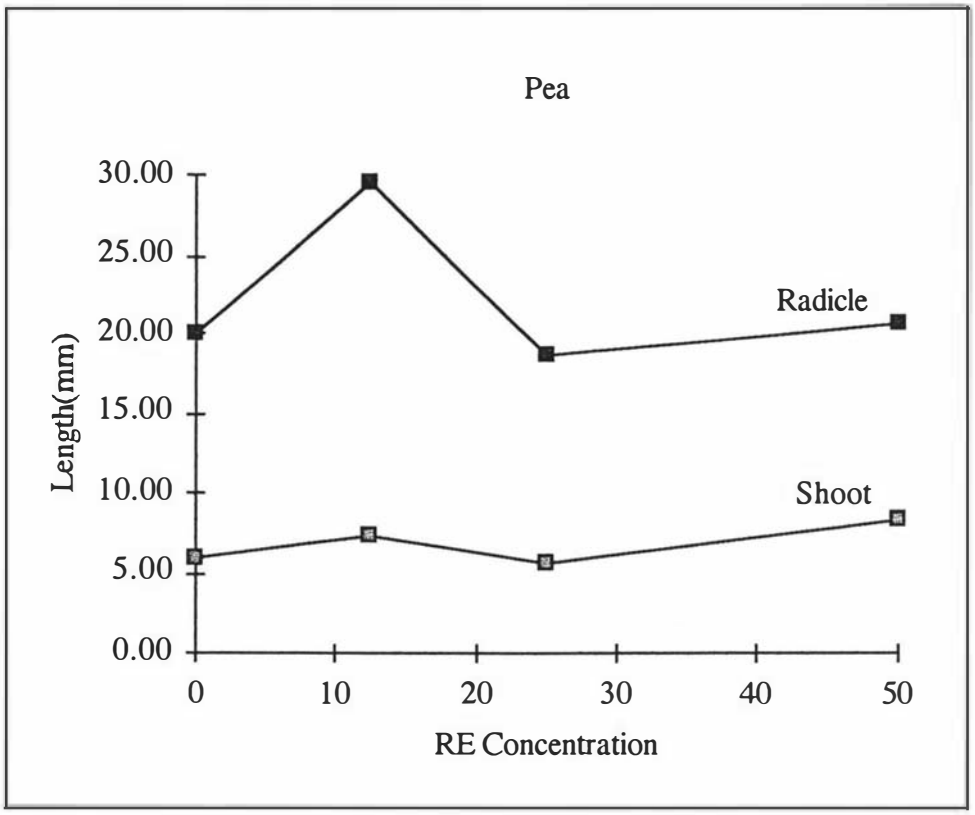
Plots of radicle length and shoot length of ten plant species exposed to four concentrations of asparagus storage root extract. Plant species and I50 values if obtained are given on each plot.

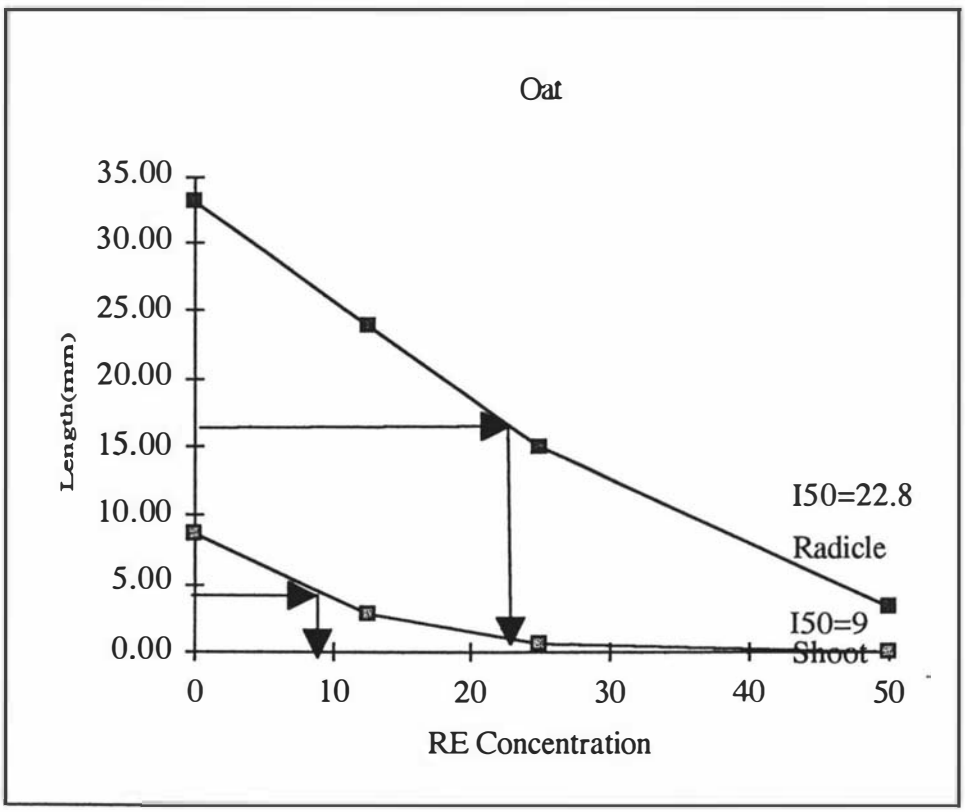


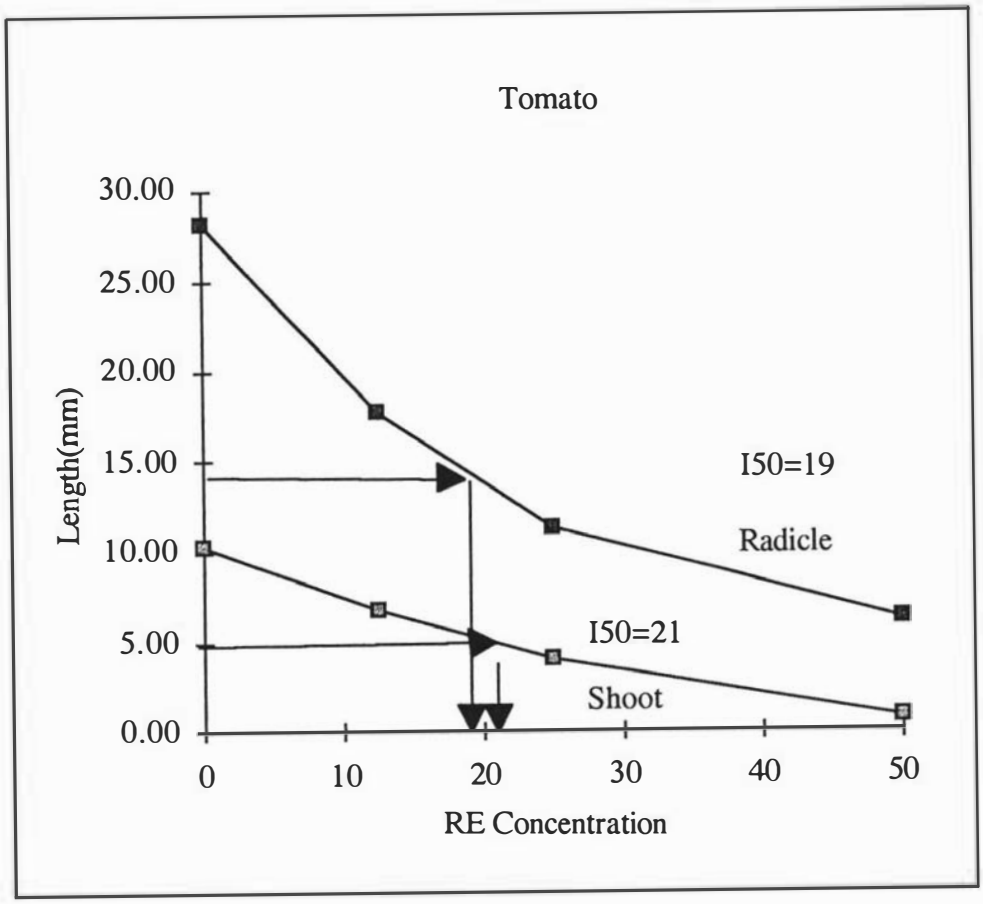


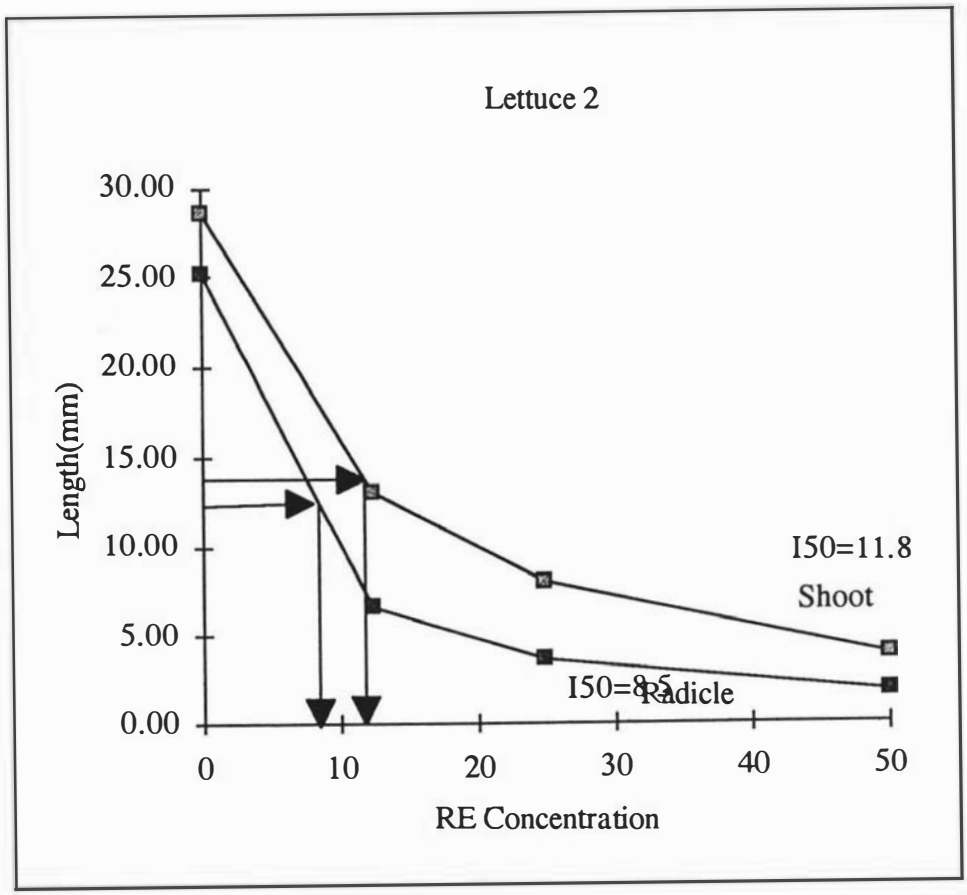

Broccoli
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Length(mm)
Radicle
I50=29.5
Shoot
I50=25
0
10
20
30
40
50
RE Concentration

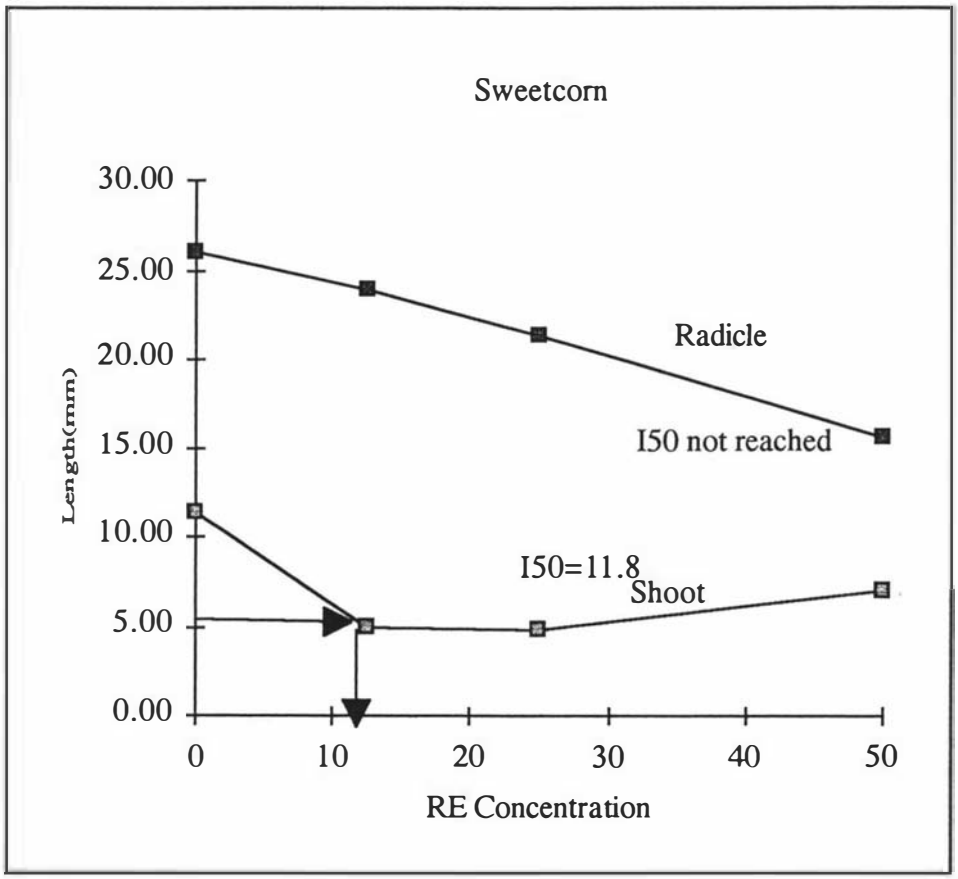

Lettuce1
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Length(mm)
I50=11.5
Shoot
I50=8.8
Radicle
0
10
20
30
40
50
RE Concentration

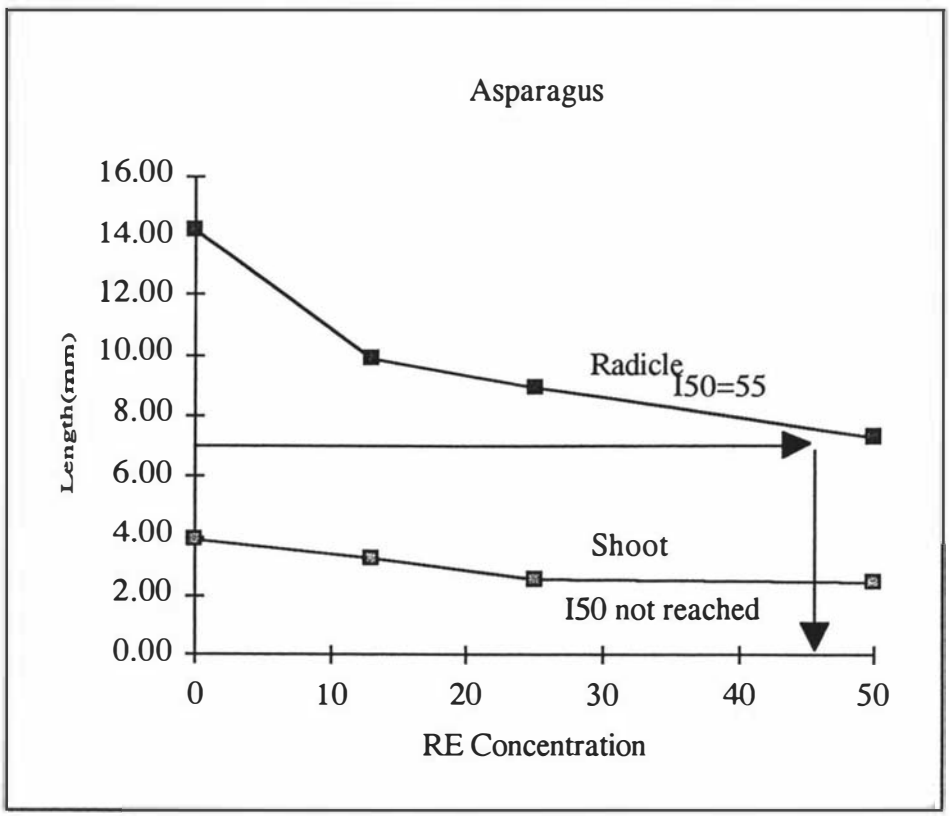

Pea
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Length(mm)
Radicle
Shoot
0
10
20
30
40
50
RE Concentration


Oat
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Length(mm)
I50=22.8
Radicle
I50=9
Shoot
0
10
20
30
40
50
RE Concentration


Tomato
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Length(mm)
I50=19
Radicle
I50=21
Shoot
0
10
20
30
40
50
RE Concentration


Lettuce 2
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Length(mm)
I50=11.8
Shoot
I50=8.5
Radicle
0
10
20
30
40
50
RE Concentration


Sweetcorn
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Length(mm)
Radicle
I50 not reached
I50=11.8
Shoot
0
10
20
30
40
50
RE Concentration


Asparagus
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Length(mm)
Radicle
I50=55
Shoot
I50 not reached
0
10
20
30
40
50
RE Concentration

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