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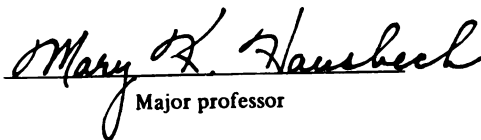
Evaluation of Strategies for the Control of Fusarium Crown
and Root Rot of Asparagus

presented by

Taylor C. Reid

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Masters degree in Science


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**EVALUATION OF STRATEGIES FOR THE CONTROL OF FUSARIUM CROWN
AND ROOT ROT OF ASPARAGUS**

By

Taylor C. Reid

A THESIS

**Submitted to
Michigan State University
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ABSTRACT

EVALUATION OF THE STRATEGIES FOR THE CONTROL OF FUSARIUM CROWN AND ROOT ROT OF ASPARAGUS

By

Taylor C. Reid

Potential strategies for the control of Fusarium crown and root rot of asparagus, caused by the fungal pathogens *Fusarium proliferatum*, and *Fusarium oxysporum* f. sp. *asparagi* were explored. The effectiveness of sodium chloride (NaCl), and lime applications were investigated in a research field and two fields (one healthy, one declining) in commercial asparagus production. NaCl applications (1120 kg/ha) were effective in significantly increasing yield and fern health in the severely declined research plot, but neither NaCl or lime applications had a significant effect in commercial fields. Alternative forms of chloride salt were tested for their ability to control Fusarium disease on seedlings in growth chamber and greenhouse studies. No salt treatment was more effective than NaCl at reducing disease in these tests. The effectiveness of fungicides and biological controls in controlling Fusarium disease was tested in growth chamber, greenhouse and field studies. Five non-pathogenic strains of *F. oxysporum* significantly reduced disease caused by both *F. proliferatum*, and *F. oxysporum* f. sp. *asparagi* in growth chamber tests on asparagus seedlings in culture tubes, but not in greenhouse studies with seedlings in soil. The fungicide fludioxonil was effective in controlling seedling death in greenhouse tests with high pathogen inoculum, and fludioxonil, benomyl, and *Trichoderma harzianum* significantly reduced disease compared to the control in greenhouse tests with a low pathogen inoculum. Field experiments with fungicides and biological controls were not successful due to low disease pressure.

DEDICATION

To my father,
for instilling in me
a passion for the natural world,
and for giving me the freedom
to explore it;
and to the memory of
my fiend
Jeph Sternheim

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TABLE OF CONTENTS

LIST OF TABLES	vi
LIST OF FIGURES	vii
LITERATURE REVIEW	1
Asparagus	2
Fusarium Crown and Root Rot	13
Literature Cited	42
SECTION I.	
EFFECT OF SODIUM CHLORIDE AND LIME APPLICATIONS ON COMMERCIAL ASPARAGUS AND THE EFFECT OF ALTERNATE FORMS OF CHLORIDE SALT ON FUSARIUM CROWN AND ROOT ROT	62
Abstract	63
Introduction	64
Materials and Methods	65
Results	71
Discussion	84
Literature Cited	89
SECTION II.	
THE USE OF FUNGICIDES AND BIOLOGICAL CONTROLS IN THE SUPPRESSION OF FUSARIUM CROWN AND ROOT ROT OF ASPARAGUS ...	92
Abstract	93
Introduction	94
Materials and Methods	95
Results	102
Discussion	111
Literature Cited	114
APPENDIX	120
Section I.	120
Section II	126

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Mean data for soil samples from 4/98 (before any treatments were applied) and 5/00 (after two years of treatment) in Oceana County field plots, tables show combined data for both fields unless significant field by treatment effects were found, letter notation indicates significant differences between treatment means from 2000 with 1998 data used as a covariate.	114
2. Mean data for soil samples from 4/98 (before any treatments were applied) and 5/00 (after two years of treatment) at MSU Botany and Plant Pathology Farm, letter notation indicates significant differences between treatment means from 2000 with 1998 data used as a covariate for top and bottom data separately.	115

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
I. EFFECT OF SODIUM CHLORIDE AND LIME APPLICATIONS ON COMMERCIAL ASPARAGUS AND THE EFFECT OF ALTERNATE FORMS OF CHLORIDE SALT ON FUSARIUM CROWN AND ROOT ROT	
1. Effect of different forms of chloride salt on root rot (%) in two experiments with asparagus seedlings grown in Hoagland's agar in test tubes and infested with <i>F. proliferatum</i> .	73
2. Effect of different forms of chloride salt on root rot (%) in two experiments with asparagus seedlings grown in Hoagland's agar in test tubes and infested with <i>F. oxysporum</i> f. sp. <i>asparagi</i> .	74
3. Effect of different forms of chloride salt on root rot (%) in two greenhouse experiments with asparagus plants grown in soil infested with <i>F. oxysporum</i> f. sp. <i>asparagi</i> and <i>F. proliferatum</i> .	75
4. Effect of different forms of chloride salt on root weight (g) in two greenhouse experiments with asparagus plants grown in soil infested with <i>F. oxysporum</i> f. sp. <i>asparagi</i> and <i>F. proliferatum</i> .	76
5. Effect of different forms of chloride salt on shoot weight (g) in two greenhouse experiments with asparagus plants grown in soil infested with <i>F. proliferatum</i> and <i>F. oxysporum</i> f. sp. <i>asparagi</i> .	78
6. Mean harvest weight of asparagus spears from commercial field plots in Oceana County, MI 1998-2000.	79
7. Percentage of asparagus stalks > 0.79 cm in diameter in commercial field plots in Oceana County, MI 1998-2000.	80
8. Mean harvest weight of asparagus spears from research plot at M.S.U. Botany and Plant Pathology Farm 1998-2000.	81
9. Percentage of asparagus stalks > 0.79 cm in diameter from research plot at M.S.U. Botany and Plant Pathology Farm 1998-2000.	82

LIST OF FIGURES (Continued)

<u>Figure</u>	<u>Page</u>
 II. THE USE OF FUNGICIDES AND BIOLOGICAL CONTROLS IN THE SUPPRESSION OF FUSARIUM CROWN AND ROOT ROT OF ASPARAGUS	
10. Average number of asparagus plants dead in two greenhouse experiments when grown in soil infested with a high level of <i>F. proliferatum</i> and <i>F. oxysporum</i> f. sp. <i>asparagi</i> and treated with a chemical or biological control product.	103
11. Shoot weight of asparagus seedlings from two greenhouse experiments when grown in soil infested with a low level of <i>F. proliferatum</i> and <i>F. oxysporum</i> f. sp. <i>asparagi</i> and treated with a chemical or biological control product.	105
12. Root weight of asparagus seedlings from two greenhouse experiments when grown in soil infested with a low level of <i>F. proliferatum</i> and <i>F. oxysporum</i> f. sp. <i>asparagi</i> and treated with a chemical or biological control product.	106
13. Roots of asparagus seedlings exhibiting lesions (%) in two greenhouse experiments when grown in soil infested with a high level of <i>F. proliferatum</i> and <i>F. oxysporum</i> f. sp. <i>asparagi</i> and treated with a chemical or biological control product.	107
14. Root system of asparagus seedlings exhibiting lesions (%) in two growth chamber experiments when grown in culture tubes infested with <i>Fusarium oxysporum</i> f. sp. <i>asparagi</i> and treated with non-pathogenic <i>Fusarium oxysporum</i> .	109
15. Root system of asparagus seedlings exhibiting lesions (%) in two growth chamber experiments when grown in culture tubes infested with <i>Fusarium Proliferatum</i> and treated with non-pathogenic <i>Fusarium oxysporum</i> .	110
 APPENDIX I.	
16. Mean sodium level in soil samples from MSU Botany and Plant Pathology Farm field 4/98-5/00 in parts per million.	123

LIST OF FIGURES (Continued)

<u>Figure</u>	<u>Page</u>
17. Mean sodium level in soil samples from healthy field in Oceana County between 4/98 and 5/00 in parts per million.	124
18. Mean sodium level in soil samples from declined field in Oceana County between 4/98 and 5/00 in parts per million.	125
APPENDIX II.	
19. Mean fern height in two seedling nursery trials with chemical and biological controls.	127
20. Number of shoots per plant in two seedling nursery trials with chemical and biological controls.	128
21. Fresh weight of shoots in two seedling nursery trials with chemical and Biological controls.	129
22. Fresh weight of roots in two seedling nursery trials with chemical and Biological controls.	130
23. Percentage of root system exhibiting lesions in two seedling nursery trials with chemical and biological controls.	131

LITERATURE REVIEW

ASPARAGUS

Asparagus (*Asparagus officinalis* L.) is an important vegetable crop throughout the world. A member of the Liliaceae (lily) family, the genus has 150 species, many of which are grown for ornamental purposes (Kotecha and Kadam, 1998). Although its center of origin is not known with certainty, it is generally considered to be native to the eastern Mediterranean region (Nonnecke, 1989). Reports of asparagus cultivation date back as far as Cato (200 B.C.), and its name comes from the ancient Greeks (Kidner, 1959). Pliny, writing in the time of Christ, described the plant much as we see it today (Kidner, 1959). In ancient Europe asparagus was used as a diuretic, sedative, and pain killer (Drost, 1997). Cultivation was perfected by the Romans who grew their asparagus in trenches, the favored method of culture until the 1800's (Kidner, 1959).

Asparagus is a dioecious, herbaceous perennial, the below ground parts of which are collectively referred to as the crown, while the above ground portion is called either spears or fern, depending on its stage of growth (Drost, 1997). The crown is composed of the rhizome which produces the buds, adventitious roots, and storage roots (Drost, 1997). Each crown produces shoots throughout the season from the buds borne on the rhizome. These shoots, often called spears, are harvested when they are young and tender (Kotecha and Kadam, 1998). When left to grow after the harvest season, the shoots grow into bushy plants up to two meters in height which are referred to as ferns. The true leaves of the plant are inconspicuous flat scales pressed close to the stalk, while the major photosynthetic organs are the myriad needle-like cladodes (cladophylls, phylloclades) that proliferate along the stems (Anonymous, 1960). The flowers of the plant are greenish-

white or yellow, and male and female flowers are borne on separate individuals, though some may be perfect, having both pistils and stamens (Green, 1896). Female and perfect flowers will produce red berries in the fall, bearing a single hard black seed.

North America and Europe are the largest asparagus producers, though world production has increased greatly in the last few years. Taiwan, Japan, China, Peru, Chile, Australia, New Zealand, and South Africa all have significant production acreage (Drost, 1997). Europeans are partial to white asparagus, etiolated by mounding earth over the beds, while most U.S. consumers prefer green asparagus, grown aboveground. In 1997, 29,800 ha of asparagus were grown in the U.S., with a production value of over 180 million dollars (National Agricultural Statistics Service, 1998). This represents just a fraction of world production, which is estimated at 218,395 ha (Benson, 1997). The U.S. is the world's second largest producer after China whose estimated production is 55,000 ha and is increasing (Benson, 1997). Peru and Spain follow with about 20,000 ha each, while Germany, France, and Mexico each produce over 10,000 ha (Benson, 1997). Most U.S. asparagus is grown in California (12,180 ha), Washington (9,310 ha), and Michigan (7,085 ha), with other states growing a significantly smaller fraction for local fresh markets (Michigan Department of Agriculture, 1998). In Michigan, 79% of the total production value was from processing in 1997, though the fresh market has seen growth in recent years (Michigan Department of Agriculture, 1998).

Asparagus grows best on a light, well drained soil like the loamy sands of Western Michigan (Kotecha and Kadam, 1998). Some asparagus is direct seeded, but most is transplanted from seedling nurseries after a year of growth. Seeds are planted 2.5-5 cm

deep, at a rate of 7-9 kg per ha, with rows spaced 60-76 cm apart (Kee et al., 1999; Kotecha and Kadam, 1998; Ware and McCollum, 1975). In the spring of the second year, before growth commences, crowns are dug from these seedling nurseries for planting in the field. One ha of seedlings will produce enough crowns for approximately 5 ha of production field (Zandstra et al., 1992). Furrows for crown planting are set 1.22-1.525 m apart, and are plowed to a depth of 15-25 cm (Kee et al., 1999; Kotecha and Kadam, 1998; Ware and McCollum, 1975). Most publications recommend a crown spacing of 30.5-45.7 cm apart within the furrows, although recent research has shown a closer spacing to be favorable, and Michigan farmers are beginning to adopt this practice (Kelly et al., 1997). Crowns are covered by about 5 cm of soil, and trenches are filled in gradually as plants emerge (Ware and McCollum, 1975). The older, open pollinated cultivars such as 'Viking KB3' and 'Mary Washington', have been replaced with the more vigorous all male hybrids, such as 'Jersey Giant' and 'Jersey Knight', or with other hybrids such as 'Synthetic 4-56', which is about 85% male (Foster et al., 1999).

Harvesting of asparagus typically begins in the third season of growth, but is limited to two weeks (Kotecha and Kadam, 1998; Zandstra et al., 1992). Beginning the fourth year, harvesting occurs for approximately six weeks or until the majority of spears become thin and unmarketable (Zandstra et al., 1992). In Michigan and Washington, harvest usually begins in early May, while California's season begins in February (Ware and McCollum, 1975; Zandstra et al., 1992). Spear harvest varies among regions, and is treated differently for the fresh or processing markets. In some areas, spears are mechanically harvested or hand cut below the soil surface (Kotecha and Kadam, 1998). In

Michigan, spears are harvested once they reach approximately 12.7 cm in height by hand snapping them at the base (Zandstra et al., 1992). Workers ride on motorized carts that span 3-7 rows, and harvest the asparagus in front of them as they pass. Fresh market asparagus is picked longer to allow trimming for uniformity. Asparagus is capable of rapid growth (up to 1.6 cm/day) during warm weather (Kotecha and Kadam, 1998). Thus, the crop must be harvested every day when temperatures are high. Hot weather can also cause premature opening of heads which will result in a decrease in quality (Zandstra et al., 1992).

Two federal grades (U.S. No. 1, and U.S. No. 2) apply to fresh market asparagus, and these are further divided by spear diameter into Colossal, Fancy and Choice sizes, (Nonnecke, 1989). The Michigan asparagus industry has established guidelines for processing asparagus which include a minimum spear diameter of 0.79 cm (5/16 inches) 12.7 cm (5 inches) below the tip, no white butts, no long spears, and no beetle eggs (Zandstra et al., 1992). The tolerance of the industry often depends on the time of year and the demand for product.

It is recommended that fresh market asparagus be cooled as soon as possible after harvest (Zandstra et al., 1992) (Kotecha and Kadam, 1998). Hydrocooling or vacuum-cooling are both feasible, though the latter is less effective (Kotecha and Kadam, 1998; Wills et al., 1989). Warm asparagus will very quickly develop increased fiber, open tips, wilted stalks, a loss of flavor, and a loss of vitamin C (Kotecha and Kadam, 1998; Zandstra et al., 1992). Asparagus can be kept for no longer than three weeks at 20⁰ C (Kotecha and Kadam, 1998; Zandstra et al., 1992). Biochemical and physiological

changes in storage can include spear lengthening, increased fiber, and decreased sugar (Kotecha and Kadam, 1998). Lower temperatures will decrease these changes, and asparagus can be stored at 0° C for up to 10 days, but is subject to chilling injury if held longer at this temperature (Kotecha and Kadam, 1998; Wills et al., 1989). A high relative humidity must also be maintained in order to prevent dessication (Kotecha and Kadam, 1998; Zandstra et al., 1992).

Since most Michigan asparagus is grown for the processing market, several local and national processing plants, are set up in the major production areas. Most processing asparagus is dumped into bulk boxes in the field, and taken to the processing plants at the end of each day (Zandstra et al., 1992). Most processing asparagus is canned rather than frozen (Kotecha and Kadam, 1998). From the conveyer belts asparagus is sorted for size and color and placed in the cans, to which a dilute brine of 2% salt is added (Kotecha and Kadam, 1998). The cans are exhausted and sealed in an atmosphere of live steam (Kotecha and Kadam, 1998). Sealed cans are processed at 115-120° C for 25-35 minutes depending on their size (Kotecha and Kadam, 1998).

The nutritional requirements of asparagus are complicated somewhat by the fact that it is an extremely deep rooted, perennial crop. Nitrogen applications, for instance, have been shown to be more effective when applied after harvest than before, and phosphorus is commonly applied in furrows before planting (Drost, 1997; Kotecha and Kadam, 1998; Nonnecke, 1989). An asparagus crop will annually remove 7, 2, and 5% of applied nitrogen, phosphorus, and potassium, respectively (Drost, 1997). Most of the plant's nutrient utilization occurs post-harvest during fern and root growth (Drost, 1997).

Commercial recommendations for fertilizer applications call for the band application of phosphorus at 33.9 kg/ha in the trench furrow during crown planting, an application of not more than 90.4 kg/ha of nitrogen, split between pre- and postharvest, and potassium applied every second year at 67.8 kg/ha K₂O (Warncke et al., 1992). There has been no yield response shown to higher rates of N, and an excess could potentially contribute to groundwater contamination (Zandstra et al., 1992). For asparagus crown nurseries, commercial recommendations call for 56.5 kg/ha of nitrogen to be disced in before seeding, and 56.5 kg/ha to be sidedressed when plants are about 6 inches high (Warncke et al., 1992). Phosphorus and potassium should be applied so that 226 kg/ha of P₂O₅ and K₂O are available to the crop (Zandstra et al., 1992). Most commercial growers in Michigan apply their nitrogen as ammonium nitrate.

Another important component of asparagus production is maintaining proper pH. Commercial recommendations call for growers to maintain a soil pH of 6.8, both in the crown nursery and harvested fields (Warncke et al., 1992). Research has shown that asparagus produces best at a soil pH of 7.5, and that low pH can increase the potential for early decline (Hodupp, 1983). Most asparagus growers are aware of this factor, and many test their soil and treat regularly with lime. However, a 1983 study indicated that commercial fields in Michigan showed soil pH levels as low as 3.7 and averaging 5.6 (Hodupp, 1983).

Asparagus is one of the most salt tolerant crops known, and historically salt has been considered an important element in healthy asparagus growth (Elmer, 1989). References to, and recommendations of salt applications on asparagus can be found in

many old gardening books, and books on asparagus culture (Anonymous, 1960; Barnes and Robinson, 1881; Bennett, 1910; Brill, 1884; Buist, 1855; Burr, 1865; Green, 1896; Greiner, 1890; Harris, 1883; Kidner, 1959). Some of the references include using salt as a weed control, but most suggest using it for its growth benefits, or simply note that its use is common practice. Interestingly, some authors indicate that, while salt will not harm the plant, is not specifically beneficial to growth (Bennett, 1910; Green, 1896; Greiner, 1890). Barnes (Barnes and Robinson, 1881), however, suggests that salt may be beneficial only inland where saltwater is not present. Green (Green, 1896) specifically cites experiments that indicate that salt is not of value as a manure.

Early references to salt application vary, and may include personal observations, folk wisdom, or scientific studies. Barnes and Robinson (1881) suggest that salt is most beneficial on older plantings, and claim that it has insecticidal properties. They also recommend that it not be applied while the roots are in a dormant state, while it is dry and sunny, or to recently transplanted crowns. Brill (1884) comments that some people assert that a layer of salt $\frac{1}{2}$ inch thick will be beneficial, and notes that asparagus can tolerate almost any amount of salt, though using more than 5 bushels/acre is not necessary. According to Bennett (1910), salt is commonly applied at a rate of 600 lb/acre, and may serve as a weed killer for a short time. According to Harris (1883), salt was usually broadcast in early spring at a rate of 10 bushels/acre, though he also notes that it has been recommended to apply it at much higher rates (120 bushels/acre). Rates of up to 2 lb. per square yard (9680 lb/acre) are suggested in some texts, split into two applications (Barnes and Robinson, 1881). Burr (1865) suggests mixing salt into manure to apply both in the

planting furrow and each autumn, but does not indicate an appropriate rate. Kidner (1959) opines that sodium liberates more available potash and thus is useful in nutrition, and goes on to note some of the negative effects of salt on soil structure, concluding that salt use is excellent in moderation but dangerous in excess.

In 1905, Walker (1905) demonstrated a benefit from salt applications (1000 lb. per acre) on 5 varieties, showing a 13.5% increase in growth in the third year after planting, resulting in an increase of 100 lb/acre of harvested asparagus. Rudolfs (1921) also demonstrated the benefit of salt to asparagus growth and determined an optimum rate of application. It is interesting to note that Rudolfs made applications during rainy weather, a practice that at least one of the early references recommends (Barnes and Robinson, 1881). Better fern vigor and a higher yield resulted from increasing amounts of salt (up to 500 lb./acre) following the second year of application on both 12 year old and 3 year old fields (Rudolfs, 1921). No weed control benefit was attributable to any of the treatment rates (150, 300, and 500 lb./acre) (Rudolfs, 1921).

Weeds can be a serious problem on asparagus. Because research has shown increased yields when asparagus fern is mowed, rather than disced in the fall or spring (Putnam and Lacy, 1977), most Michigan asparagus is produced in a no-till system. Generally, a cover crop of winter rye is planted in order to combat soil erosion. Weed control is obtained solely by chemical means. A broad spectrum herbicide is applied before harvest, and again after harvest is completed. Spears are mowed or picked close to the ground before applications in order to minimize herbicide damage to the crowns. Greenhouse studies have shown that the herbicides diuron, metribuzin, simazine, and

terbacil all cause damage to young asparagus crowns, and reduce yield (Hodupp, 1983). Other research has shown that the herbicide terbacil, despite providing good, season long control of weeds in asparagus plantings, causes injury to the fern when applied at a rate of 2.2 kg/ha or greater (Welker and Brogdon, 1972). Simazine has also been shown to reduce yield in field plots (Damicone et al., 1987).

Weeds can cause damage to asparagus plantings by competing for water and nutrients and by producing allelopathic substances (Putnam et al., 1983). Perennial weeds, including horsenettle (*Solanum carolinense*), quackgrass (*Agropyron repens*), field bindweed (*Convolvulus arvensis*), common milkweed (*Asclepias syriaca*), swamp smartweed (*Polygonium lapathifolium*), northern dewberry (*Rubus fragellaris*), field horsetail (*Equisetum arvense*), and yellow nutsedge (*Cyperus esculentus*) are the most problematic in asparagus because it is a perennial crop (Putnam et al., 1983) (Zandstra et al., 1992). It is recommended that asparagus growers kill perennial weeds with a registered herbicide the year before planting a field (Zandstra et al., 1992). Volunteer asparagus, which grows from seeds dropped by the female plants in the fall, can be a serious pest in fields that are not planted with the all-male varieties (Zandstra et al., 1992). Common annual weeds include crabgrass (*Digitaria sanguinalis*), yellow foxtail (*Setaria glauca*), redroot pigweed (*Amaranthus retroflexus*), common lambsquarters (*Chenopodium album*), fall panicum (*Panicum dichotomiflorum*), barnyardgrass (*Echinochola crusgalli*), stinkgrass (*Eragrostis major*), and field sandbur (*Cenchrus tribuloides*) (Putnam et al., 1983). Fall panicum, in particular, has become a problem in no tillage fields, and is a vigorous competitor with asparagus fern (Putnam et al., 1983).

A number of insects are pests in asparagus. Cutworms (*Euxoa messoria* and *E. scandens*) can cause damage to growing spears by feeding on the tips or sides of new shoots, causing losses of up to 90% of the harvestable crop (Grafius, 1983; Zandstra et al., 1992). Most farmers control this pest with a pre-emergence insecticide application at the time of spring mowing. Common asparagus beetles (*Crioceris asparagi*) can be a major pest on spears, where they feed and lay eggs, and on fern, where larvae can cause severe defoliation (Putnam et al., 1983; Zandstra et al., 1992). Farmers often spray during harvest to control these insects because the presence of eggs on spears can lead to rejection by processors. Spotted asparagus beetles (*C. duodecimpunctata*), which appear later in the season, are usually a lesser problem. They feed on spears, but lay their eggs on fern. Larvae feed only on fruit, and do not cause defoliation (Putnam et al., 1983; Zandstra et al., 1992). Plant bugs (*Adelphocoris lineolatus* and *Lygus lineolaris*) can cause severe fern damage late in the season by injecting toxic saliva into the plant and causing tip dieback and tissue collapse (Putnam et al., 1983; Zandstra et al., 1992). Asparagus aphids (*Brachycorynella asparagi*) are a common inhabitant of asparagus fields, though they are of minor economic importance in Michigan where their numbers are generally kept low by predators (Putnam et al., 1983; Zandstra et al., 1992). Aphids tend to be a problem in young fields, and can be very damaging in the asparagus growing areas of Washington (Putnam et al., 1983; Zandstra et al., 1992). Asparagus miners (*Ophiomyia simplex*) are Dipteran pests that lay two generations of eggs each year (Putnam et al., 1983). Although they are not particularly damaging alone, they are closely associated with stem rot caused by *Fusarium* spp. (Damicone et al., 1987; Gilbertson et

al., 1985). Insecticide treatments used to control the miner have led to decreased incidence of stem and crown rot, and increased survival and yield of asparagus plantings (Damicone et al., 1987).

Nematode problems have been sporadically reported in asparagus (Crittenden, 1952; Putnam et al., 1983; Rohde and Jenkins, 1958). Most research, however, indicates that asparagus is not very susceptible to nematodes, presumably because of toxic substances excreted by the roots (Crittenden, 1952; Rohde and Jenkins, 1958). Crittenden (1952) observed very low infection rates and no hypertrophy when asparagus was planted in soils that were heavily infested with root-knot nematodes. Similarly, Rohde and Jenkins (1958) showed that populations of *Trichodorus christiei* died out in the soil around asparagus plants, and isolated a root exudate that was antagonistic to several species of plant parasitic nematodes.

There are several disease problems that cause losses on asparagus in Michigan each year. Purple spot is a disease caused by the fungus *Stemphylium vesicarium* and was first described in Michigan in 1982 (Lacy, 1982). The disease causes small purple lesions with a necrotic center on asparagus stems and cladophylls that can build up in numbers great enough to cause premature defoliation and fern death. The disease also attacks spears and can cause processors to reject asparagus because of aesthetics. The fungus enters the plant through wounds, or natural openings, and is favored by warm, wet conditions (Meyer, 1997). The causal organism overwinters on plant debris, and consequently is a much more severe problem in no tillage systems (Zandstra et al., 1992). Control of the disease is attained by the use of fungicide sprays, and the development of a

disease forecasting system has allowed for better application timing in Michigan (Meyer, 1997). *Asparagus rust* is a fungal disease caused by *Puccinia asparagi* (Zandstra et al., 1992). Several spore stages are produced during the growing season, that cause damage to all aboveground plant parts (Putnam et al., 1983). The disease is most prevalent in wet years, and severe outbreaks can cause premature fern death and yield loss (Putnam et al., 1983; Zandstra et al., 1992). *Asparagus virus I* (AV-I), a potyvirus, *asparagus virus II* (AV-II), an ilarvirus, and tobacco streak virus, an ilavirus, have been reported to occur in asparagus throughout the world (Evans et al., 1990; Falloon et al., 1986). These viruses, common in commercial asparagus plantings, have been associated with a decline in field productivity of up to 50%, and a diminished rooting capacity (Evans et al., 1990; Jaspers and Falloon, 1997). These viruses have also been linked with an increased susceptibility to *Fusarium* diseases (Evans and Stephens, 1989). All are mechanically transmitted, and *asparagus virus II* is seedborne (Evans and Stephens, 1989; Falloon et al., 1986). No measures to control virus are currently practiced by growers in Michigan.

FUSARIUM CROWN AND ROOT ROT

One of the major limitations to asparagus production, both in Michigan and throughout the world, is asparagus decline syndrome. This condition was first described in California as “a slow decline in the productivity of old asparagus plantings, and the inability to re-establish productive plantings” (Grogan and Kimble, 1959). Fields afflicted with this disorder exhibit a premature reduction in the size and number of spears, death of crowns, and eventually become unprofitable to harvest (Elmer et al., 1996;

Grogan and Kimble, 1959). In addition, fields replanted in asparagus after an asparagus crop is removed often exhibit reduced emergence, yellowing, wilting, and death (Grogan and Kimble, 1959). This phenomenon is commonly referred to as “replant bound early decline” (Elmer et al., 1996). These problems often have serious economic impacts on farmers, and are responsible for the abandonment of asparagus production in Western Massachusetts, Connecticut, and New Jersey (Hartmann, 1996; Damicone et al., 1988; Taylor and Rathier, 1985; Johnston et al., 1979). There are a number of cultural and biological factors that contribute to asparagus decline syndrome. Acidic soils (Hodupp, 1983), allelopathic root exudates (Blok and Bollen, 1993), over-harvesting (Shelton, 1978), and other abiotic factors that weaken the plant can contribute significantly to the problem (Elmer et al., 1996). Although insects, foliar diseases, and weeds all play a role (Elmer et al., 1996), the single most important factor contributing to decline is *Fusarium* crown and root rot (Grogan and Kimble, 1959; Fantino, 1990; Schofield et al., 1996; Taylor and Rathier, 1985).

The fungal genus *Fusarium* was first described by Link in 1809 as containing species with fusiform spores borne on a stroma (Booth, 1971). The genus was validated in the International Botanical Code by Fries in 1821, who placed it in the order Tuberculariae (Booth, 1971). *Fusarium* spp. may produce both macroconidia and microconidia, so named for their respective sizes (Alexopoulos et al., 1996). Macroconidia are crescent shaped spores that are often borne on sporodochia, and have a foot-shaped basal cell (Windels, 1992; Alexopoulos et al., 1996). Microconidia are small, aseptate spores that are generally round or oval in shape (Alexopoulos et al., 1996). In

many species, they are produced in chains or false heads on aerial mycelia (Nelson et al., 1983). Some species may also produce thick-walled chlamydospores, and some produce sclerotia (Alexopoulos et al., 1996; Windels, 1992). The perfect stage of the fungus, though only known in some species, produces perithecia and belongs to the Hypocreales (Booth, 1971). The taxonomy of the genus *Fusarium* is the subject of some debate, and different systems separate the genus into as few as nine or as many as 90 species (Toussoun, 1981; Windels, 1992; Wollenweber, 1913; O'Donnell et al., 1998). More recently, molecular techniques have been employed in an attempt to elucidate evolutionary relationships, and to organize species along these lines (O'Donnell et al., 1998).

Fusarium spp. are among the most common fungi in the world, and are widely distributed in soil and on organic substrates (Booth, 1971). They have been isolated from permafrost in the arctic, and from the sand of the Sahara, and can be pathogens, parasites, or saprophytes (Booth, 1971; Windels, 1992). *Fusarium* can be found on almost every plant species in most parts of the world, and can cause disease in humans and animals as well as plants (Booth, 1971; Windels, 1992). Some species of the fungus produce mycotoxins that can be contaminants of human and animal food, and were reportedly used as biological warfare agents in both Vietnam and Afghanistan (Alexopoulos et al., 1996; Booth, 1971). Well adapted for survival in a broad range of environments, tenacious isolates have been found in many preserved foods, stored chemicals, and in airplane fuel tanks (Booth, 1971). Though generally considered a soil-borne fungus, many species are also efficient at airborne dispersal (Matos et al., 1997; Burgess, 1981).

The main interest in the genus *Fusarium*, however, is not strictly mycological, but rather in its role as a plant pathogen. *Fusarium* causes two distinct types of disease on plant roots: cortical rots, and vascular wilts (Toussoun, 1981). Several economically devastating diseases on important agricultural crops have made *Fusarium* infamous in the last 100 years. Cotton wilt, pea wilt, tomato wilt, and banana wilt, to name just a few, have been so catastrophic that they threatened to eliminate entire agricultural industries (Toussoun, 1981). Today, *Fusarium* diseases affect an astoundingly broad range of crops, and *Fusarium* is among the most economically important genera of plant pathogens worldwide.

Fusarium crown and root rot of asparagus is caused by the pathogens *Fusarium oxysporum* (Schlect.) f. sp. *asparagi* (Cohen & Heald) and *Fusarium proliferatum* (T. Matsushima) Nirenberg (formerly *Fusarium moniliforme* Sheld.). The disease was first described in Massachusetts in 1908 as a wilt of young shoots during the cutting season, followed by a yellowing and rot of the mature stalks, and was associated with an unidentified *Fusarium* sp. (Cohen and Heald, 1941). Cook (1923) described two cases in New Jersey where circular areas in asparagus fields were dwarfed and dying. The disease was most prominent in the center of the circular area, becoming less severe toward the margins, and the plants were characterized by “a pronounced brown or rusty discoloration on the stems below the surface of the ground”. Cook went on to satisfy Koch’s postulates with an organism he identified as *Fusarium* sp. Between the time of Cook’s observations and 1940, root rot, crown rot and wilt of asparagus were reported from New York, New Jersey, North Carolina, South Carolina, Pennsylvania, Illinois, Massachusetts, Missouri,

California, and Washington (Cohen and Heald, 1941). Cohen and Heald (1941) described the symptoms of the disease in depth and noted both vascular discoloration, wilt, and death of the stalks, dried out roots, red-brown lesions on the cortical areas of the root, and discoloration extending through the vascular system of the roots from the site of the lesion. They also noted that it was often possible to trace vascular discolorations down through an injured or harvested shoot, into the crown, and through the root system, suggesting that the point of infection may actually be the injured above-ground parts. These researchers isolated *F. oxysporum* from diseased plant material, and satisfied Koch's postulates with single spored isolates of the fungus. Investigations of asparagus material throughout the country led to the conclusion that the disease-causing organism was present in all asparagus growing areas. Cohen and Heald (1941) also observed disease affecting asparagus seedlings, a problem that was later described by Graham (1955) as poor seedling emergence, stunting, yellowing, and wilting of the older seedlings, collapse of sections of primary root, and elliptical root lesions of varying size. Graham also described an inability to establish crown plantings in a field that had been afflicted with the disease, a situation that Grogan and Kimble (1959) would later refer to as the asparagus replant problem. Graham (1955) confirmed Cohen and Heald's (1941) observation that the pathogen is widespread, and noted that it could survive for long periods of time without its preferred host, was efficient at colonizing dead organic matter, and could be found on wild asparagus plants. Grogan and Kimble (1959) were the first to call the causal agent of decline *F. oxysporum* f. sp. *asparagi*.

Both Grogan and Kimble (1959) and Graham (1955) reported finding pathogenic

isolates of *F. moniliforme*. The former found 21 isolates in their studies, only two of which killed fewer than 25% of their test plants, while most killed many more. Graham (1955) implicated *F. moniliforme* as a contributor to asparagus decline, and described it as pathogenic on seedlings, but was unable to reproduce the disease symptoms observed in the field. The first mention of *F. moniliforme* as a primary disease-causing organism was in 1971 when Endo and Burkholder (1971) linked the fungus to a dry, brown crown rot and stand decline in California. Johnston et al. (1979) determined the role of *F. moniliforme* in asparagus decline, and compared its pathogenicity with that of *F. oxysporum* f. sp. *asparagi*. This study showed that *F. moniliforme* was more commonly associated with crown and stem tissue than with root tissue, and that its virulence was similar to that of *F. oxysporum* f. sp. *asparagi*. Further, *F. moniliforme* was dominant in older plantings while *Fusarium oxysporum* f. sp. *asparagi* tended to predominate in younger ones. Damicone and Manning (1985), however, found that *F. moniliforme* was more virulent than *F. oxysporum* f.sp. *asparagi*, and was dominant in first year asparagus from disease free crowns in Massachusetts. After *F. proliferatum* was differentiated from *F. moniliforme* based on the presence of polyphialides (Nelson et al., 1983), Elmer (1990b) found that *F. proliferatum* isolates were highly virulent on crown tissue and were commonly isolated from plants in the field. It is now generally accepted that *F. proliferatum*, not *F. moniliforme* is the primary cause of crown and stem rot of asparagus, though the latter is also a pathogen (Elmer and Ferrandino, 1992).

Other *Fusarium* spp. have also been shown to be pathogenic on asparagus.

Fusarium culmorum can be an important stem pathogen in Europe, while *F. proliferatum*

is generally absent (Blok and Bollen, 1995). Schreuder et al. (1995) found *F. solani* caused root lesions on seedlings in South Africa, though it was less virulent than either *F. proliferatum* or *F. oxysporum* f. sp. *asparagi*. Damicone and Manning (1985) also reported finding this species in 1.4% of sampled crowns and found it was a mild pathogen on seedlings. Elmer and Ferrandino (1992) found that *F. subglutinans* could be slightly pathogenic to asparagus. Armstrong and Armstrong (1969) reported that *F. oxysporum* f. sp. *apii*, which causes celery wilt, as well as formae speciales' *cubense* (banana, some grasses), and *medicaginis* (alfalfa, pea, vetch) could cause symptoms on asparagus. Elmer and Stephens (1989) observed root lesions on asparagus caused by *F. oxysporum* f. sp. *cepa*e (onion) and *F. oxysporum* f. sp. *gladioli* (gladiolus). Blok and Bollen (1997), in contrast reported that asparagus was not susceptible to *F. oxysporum* f. sp. *pisi* (pea), *lupini* (lupine), *cepa*e (onion), *lilii* (lily), or *gladioli* (gladiolus). In general, most other species are considered to be of minor importance in comparison to *F. proliferatum* and *F. oxysporum* f. sp. *asparagi*.

Early reports of disease caused by *Fusarium* recognized that the pathogen is widely distributed (Grogan and Kimble, 1959; Cohen and Heald, 1941; Graham, 1955). Graham (1955) suggested that the pathogen may be indigenous to soil never planted in asparagus. Gilbertson et al. (1981) isolated pathogenic *F. oxysporum* from a meadow, a pasture, and a woodland. Hartung et al. (1990) confirmed that, although pathogen populations were higher in the vicinity of asparagus plants, soils that had no history of or proximity to asparagus plantings still contained pathogenic isolates of both *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*.

Although *F. oxysporum* is not commonly thought of as being wind dispersed, this can be an important factor in some situations (Burgess, 1981). One report has linked air-borne microconidia of *F. oxysporum* f. sp. *radicis-lycopersici*, an important pathogen of tomato, with re-colonization of steamed greenhouse soil (Burgess, 1981). Air dispersal of the fungus on wind-blown dust and soil particles has been suggested as well (Burgess, 1981). The extent to which this is a factor with *F. oxysporum* f. sp. *asparagi* is unknown. *F. proliferatum*, in contrast, is known to be wind dispersed (Burgess, 1981), and airborne spores of this fungus are common in fields, often infecting asparagus flowers and fruit (Gilbertson and Manning, 1983). Damicone and Manning (1985) have also reported finding the pathogen sporulating prolifically on asparagus stubble in the spring, confirming that air-borne dispersal may be very common.

Both *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* may be seed borne. Contrary to published reports (Damicone et al., 1981; Inglis, 1980), Graham (1955) did not find any contamination of seed with pathogenic *Fusarium* despite repeated attempts. Grogan and Kimble (1959), however, did find that unsterilized seeds planted in steamed soil could sometimes (though rarely) result in wilt and death of seedlings from *F. oxysporum* f. sp. *asparagi*. Lewis and Shoemaker (1964a) sampled seeds from New Jersey, Massachusetts, Ontario, and Michigan, and found that seeds from all the samples were infected with *F. oxysporum* f. sp. *asparagi*. Inglis (1980) found that many seed lots were contaminated with both *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*, and though seeds were not infected internally, deep crevices and beetle feeding sites provided ideal places for spores to lodge and escape disinfestation. Gilbertson and Manning

(1983) reported that seed grown in Massachusetts had a *F. proliferatum* contamination rate of up to 10%. Damicone et al. (1981) found *Fusarium* contamination on every seed lot they tested, and determined that the only way to completely disinfect seeds was to soak them in 25,000 ppm benomyl in acetone for 24 hours. Blok (1996b) reported that Dutch seed lots were consistently infected with *F. oxysporum* f. sp. *asparagi*, and showed that infestation usually occurs during the harvesting process when infested soil adheres to fallen berries.

Another important source of inoculum is infected crowns from seedling nurseries. According to Gilbertson et al. (1981), *Fusarium* infections of one-year-old crowns used to plant asparagus fields can exceed 50%. Blok (1996b) similarly reported that 75% of one-year-old Dutch crowns were infected with *F. oxysporum* f. sp. *asparagi*. Fantino (1990) reported that in Italy, *Fusarium* contamination is common in seedling nurseries throughout the country.

Asparagus miners can be an important disease vector. Gilbertson et al. (1985) reported that larval feeding by asparagus miners resulted in increased stem rot caused by *F. proliferatum*, and that asparagus miners can carry both *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. Damicone et al. (1987) found that insecticide treatment could reduce crown rot incidence by 30%. Asparagus miner control is not currently practiced in Michigan, and the insects may be an important factor in disease spread.

The ubiquity of *Fusarium* isolates pathogenic to asparagus in nature has led researchers to question the biological relationships between isolates of these fungi. To this end, several researchers have investigated vegetative compatibility groups in an

attempt to understand the diversity and relatedness of *Fusarium* spp. in the field. Elmer and Stephens (1989) investigated 97 strains of pathogenic *F. oxysporum* from Michigan fields, and world collections. Their research identified 43 distinct vegetative compatibility groups of *F. oxysporum* that could infect asparagus, some members of which were isolated from other crops. LaMondia and Elmer (1989) tested isolates from three plants in a single plot in Connecticut and found that eight of 18 *F. oxysporum* isolates belonged to unique vegetative compatibility groups. Blok and Bollen (1997) similarly found that 24 Dutch isolates of *F. oxysporum* f. sp. *asparagi* belonged to 18 different vegetative compatibility groups. Because vegetative compatibility groups are presumed to be genetically isolated from one another (Correll et al., 1987), this study suggests that the ability to cause disease on asparagus may have evolved independently in a number of different strains. Although this kind of diversity has also been observed in *F. oxysporum* f. sp. *lycopersici* (Elias and Schneider, 1991), most pathogenic forms of *F. oxysporum* are comprised of one or only a few vegetative compatibility groups (Burgess, 1981; Jacobson and Gordon, 1990). With *F. proliferatum*, Elmer (1990c) found 20 vegetative compatibility groups among 110 isolates from four fields in Connecticut, though most isolates fell into six groups that were found in all the fields. LaMondia and Elmer (1989) found 13 vegetative compatibility groups in 97 isolates from three plants in a single test plot. Thus, the virulence trait is present among a number of diverse isolates, though some may predominate in asparagus fields. The significance of these results cannot be overstated. The fact that many different groups of the pathogens may be able to infect asparagus has important implications on disease control strategies, and may preclude the

development of robust gene for gene resistance through breeding efforts.

It is also interesting to note the range of virulence between different isolates of the pathogens. Grogan and Kimble (1959) noted a wide range of pathogenicity in both *F. oxysporum* and *F. proliferatum*, from those that were nonpathogenic, to those that killed 100% of inoculated seedlings. Elmer and Ferrandino (1992) described a wide range of pathogenicity for isolates in section *Liseola* (*F. proliferatum*, and *F. moniliforme*). Similarly, Damicone and Manning (1985) observed a wide range of pathogenicity with both *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*, and LaMondia and Elmer (1989) noted differences in mean virulence ratings of isolates belonging to different vegetative compatibility groups of *F. proliferatum*. Different kinds of infections (cortical, vascular) have been reported for *F. oxysporum* f. sp. *asparagi* (Smith and Peterson, 1983; Grogan and Kimble, 1959; Graham, 1955; Cohen and Heald, 1941), and it is possible that different strains of the fungus could exhibit different types of infection, though this idea is largely speculative.

The host range of the *Fusarium* pathogens involved in crown and root rot of asparagus have been investigated by several researchers. Cohen and Heald (1941) tested *F. oxysporum* f. sp. *asparagi* against a range of crops. In these experiments, the pathogen did not cause disease on potatoes, tomatoes, or carnations, and did not rot potato tubers or onion bulbs held in a moist chamber. It did cause disease on ornamental asparagus, and caused a slight wilt of Alaska peas. Graham (1955) also tested *F. oxysporum* f. sp. *asparagi* against onions, spinach, lucerne, gladiolus, oats, barley, rye, and corn. Of these, only gladiolus developed lesions from which the pathogen was re-isolated. Armstrong

and Armstrong (1969) found that *F. oxysporum* f. sp. *asparagi* did not cause disease on celery, lupine, vegetable sponge, Maltese cross, cotton, chrysanthemum, eggplant, alfalfa, Chinese lantern plant, Mexican sunflower, garden pea, safflower, tobacco, tomato, or carnation. Blok and Bollen (1997) tested a number of different plants with their Dutch isolates, and found that only asparagus, and occasionally pea and lupine were susceptible. While the host range of *F. proliferatum* has been investigated less extensively, it is important to note that cross-pathogenicity has been observed between asparagus and corn (Damicone et al., 1988), a finding that has important implications for crop rotation.

A number of different factors contribute to the severity of *Fusarium* infection, and thus contribute to asparagus decline. Nigh (1990) states that asparagus serves as a host to *Fusarium* spp. without disease symptoms until one or a combination of factors causes plant stress. Once these conditions are met, the plant becomes susceptible and the fungus is able to cause root rot and wilt. Nigh's research indicates that any factor causing plant stress was found to increase the incidence and severity of the disease. In his studies on moisture level, infection was increased when water was limiting. It is likely that water is less often limiting in Michigan and other production regions than it is in Yuma, AZ where these studies were conducted, but farmers do correlate dry years with increased death and seedling blight (N. Myers, County Extension Agent, personal communication). Wilcox-Lee (1987), similarly, found decreases in fern height, number of shoots per plant, fern dry weight, and root dry weight with decreasing soil matric potentials. Although Wacker (1988) found significantly increased disease severity and higher populations of *F. oxysporum* f. sp. *asparagi* at higher soil moisture levels (0 MPa vs. -1.5 MPa),

experiments by Elmer and LaMondia (1997) have shown the opposite effect with both *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. Graham (1955) also found that low levels of soil moisture were more conducive to seedling damping off than high levels.

Hodupp (1983), Welker and Brogdon (1972), and Damicone et al. (1987) have all shown decreased yield or injury from herbicide applications (terbacil, simazine, diuron, metribuzin) indicating that herbicide use may be an important stress factor. Weed competition, however, can also cause additional plant stress. Shelton (1978) showed increased crown survival and yield in both *Fusarium*-infected, and non-infected plots with herbicide use, as compared to hand-weeded plots which he was not able to keep weed free throughout the season. Shelton (1978) also showed that over-harvesting can weaken asparagus plants by reducing the level of carbohydrates stored in the roots. Evans and Stephens (1989) demonstrated that asparagus infected with the viruses AV-I or AV-II developed significantly more disease from *F. oxysporum* f. sp. *asparagi* than did virus-free plants, and correlated the presence of the virus with increased root exudation and susceptibility of root tissue to infection. Hodupp (1983) has shown that low soil pH can inhibit asparagus growth. Though these experiments never correlated low pH with *Fusarium* disease, acidic soil may contribute to plant stress and increase disease susceptibility. Hodupp (1983) also correlated more soil compaction with decreased asparagus vigor, suggesting that this may be an important stress factor as well.

The stress factor that has been studied most extensively is the effect of autotoxic root exudates from asparagus plants. Beginning with the earliest disease reports, researchers have observed that decline is most severe in fields that have previously grown

asparagus, and that the problem may persist for many years (Elmer et al., 1996). A number of allelopathic compounds have been identified from asparagus, including caffeic, ferulic, malic, citric, methylenedioxycinnamic, asparagusic and chlorogenic acids, saponins, and glycosides (Keulder, 1997). Yang (1982) found that extracts from both field-grown asparagus plants, and plants grown in sterile culture delayed the germination of asparagus seed and inhibited root and shoot development. Because activated charcoal and autoclaving did not reduce the toxicity of the extracts, Yang suggested that the toxic substances were very stable and could persist in fields. Hartung and Stephens (1983) looked at the effects of asparagus toxins on *Fusarium* populations and on the susceptibility of roots to infection. These investigations revealed that disease caused by *Fusarium* spp. was much more severe in the presence of asparagus tissue, but asparagus tissue did not enhance the growth of the pathogens. Pierce and Miller (1990) confirmed this synergistic relationship, and showed that soluble solids from asparagus extract enhanced the severity of disease caused by *Fusarium*, though there was also an autotoxin effect independent of this. Their research also indicated that the activity of asparagus autotoxin dissipated over time. Hartung et al. (1989) showed that respiration and peroxidase activity decreased, and electrolyte efflux increased in the presence of asparagus extracts, suggesting that the chemicals may have direct physiological and biochemical effects on asparagus plants that make them more susceptible to disease. Blok and Bollen (1993) found inhibitory effects of root extracts up to 10 years after asparagus fields had been taken out in laboratory experiments, but suggest that the amount of root biomass present in the field after termination of the crop in the previous

season is not high enough to have a direct growth inhibition effect. It is likely, they conclude, that the effect of root residues is of secondary importance to the buildup of pathogen populations in the soil which have been shown to correlate directly with the severity of replant disease (Blok and Bollen, 1996a). However, as Blok and Bollen suggest (1993), the toxic residues may contribute to the problem by affecting the populations of soil microflora and predisposing roots to *Fusarium* infection. Further investigations by these researchers (Blok and Bollen, 1996c) confirm, in fact, that root extracts from asparagus residues retard the growth of a wide variety of fungi including *Trichoderma* and *Gliocladium* spp., but do not affect *Fusarium* spp. In the same study, however, the researchers did not find any increase in disease severity in soils containing plant residues in the Netherlands.

A significant amount of research has been dedicated to developing strategies for the control of *Fusarium* crown and root rot. Cohen and Heald (1941) determined that nutrition and pH are ineffective as controls, and stated that the only real control for the disease would be development of plant resistance. Lewis and Shoemaker (1964b) tested a number of different species in the genus *Asparagus* for resistance to *Fusarium* infections. They found only one species that was resistant to the disease and speculated that it was too distantly related to commercial asparagus to be useful in breeding. Stephens et al. (1989) tested a number of different varieties and species for disease resistance and found slightly less susceptibility in some all-male hybrids as well as *A. densiflorus*, but no true disease resistance. The original publications on these varieties also report increased tolerance to disease, but not disease resistance (Ellison, 1985;

Ellison et al., 1990; Ellison and Kinelski, 1986). Despite the development of an in vitro assay to evaluate resistance (Stephens and Elmer, 1988), and considerable interest on the part of growers for resistant varieties, this type of disease control does not appear to be forthcoming. Only one of the more recent cultivar trials specifically addresses *Fusarium* crown and root rot resistance (Elmer et al., 1997). In one field trial, moderate resistance to disease was found with the varieties Emerald and Rutgers Syn #2 which had consistently high yields and lost less than 40% of their crowns over seven years, compared to an average loss of over 60%. In another trial, cultivars Lucullus and Jersey General lost only 8% of crowns over seven years compared to a loss of almost half of the crowns in susceptible cultivar Mary Washington (Elmer et al., 1997). Caution should be exercised in extrapolating these results to Michigan conditions since cultivars that exhibit a high productivity in one region do not always do so in another (Elmer et al., 1997).

Because of the ubiquitousness of the pathogens, and the perennial growth habit of the crop, chemical control strategies have thus far demonstrated, at best, only marginal success. Damicone et al. (1981) have shown that the difficulty of disinfesting seeds can be overcome by soaking them in benomyl (25,000 ppm) in acetone. Knaflewski and Sadowski (1990) showed that although seed treatment with benomyl in acetone did not exclude *Fusarium* infection, disease severity in one-year-old crowns was reduced slightly with this treatment, and no other chemical treatment completely eliminated *Fusarium* contamination on seeds. Di Lenna and Fotello (1990), in contrast, found no advantage to seed disinfestation with benomyl in acetone for disease reduction, suggesting that seed contamination may not be important in all cases. Manning and Vardaro (1977) reported

that soil fumigation (methyl isothiocyanate/chlorinated C₃ hydrocarbons) in addition to fungicide crown soaks (benomyl or captafol) of *Fusarium*-free crowns (grown in a greenhouse) resulted in increased vigor in plots infested with *Fusarium* spp. Damicone et al. (1987) also found increased yield when disease-free crowns were transplanted into fumigated (methyl isothiocyanate/chlorinated C₃ hydrocarbons) soil. Lacy (1979), in contrast, found that fungicide soaks of crowns grown in naturally infested nursery soils did not result in better survival or spear production in subsequent years. This study also showed that crowns grown in infected nurseries did not show higher yields in fumigated (DD-MENCS) plots than in non-fumigated plots. Lacy (1979) did show, however, that crowns grown in fumigated soil and transplanted to fumigated soil had higher yields than those grown in non-fumigated soil, and transplanted into fumigated soil. These studies seem to indicate that chemical treatment is ineffective once seedlings have been infected in the nursery. Molot et al. (1985) found that crowns grown in fumigated (methyl bromide) nursery soils were healthier than those grown in non-fumigated soils, but that subsequent growth was unexpectedly much better for infected crowns than for healthy ones, and that the healthy crowns from the fumigated field actually became more susceptible to infection than unhealthy crowns. These researchers suggested that the lack of mycorrhizal colonization in the fumigated soils limited the ability of these crowns to thrive, and made them more susceptible to disease. This study also found little advantage to pre-plant crown treatments (bleach, carbendazim/captafol, “Ceretal”/bleach). DiLenna and Foltello (1990) reported that the location from which crowns originated was much better correlated to disease reduction than chemical soil disinfestation, noting that

nurseries in regions where decline was less problematic produced much healthier crowns than those in regions where decline is severe. These researchers suggested biological soil suppressiveness as the reason for this geographical difference in disease incidence. Faloon and Fauser-Kevern (1996) demonstrated *Fusarium* disease control in New Zealand with thiabendazole crown soaks. Though it was not clear whether these experiments were conducted with disease-free or infested crowns, the question may be moot for Michigan growers because the manufacturer (Novartis Crop Protection, Inc. Greensboro, NC) has indicated that they have no plans to register this fungicide for use on asparagus in the United States (A. Talley, Fungicide Development Director, personal communication).

The role of nutrition in disease development has been studied in a number of different host-pathogen systems. Many different macro- and micronutrients have been implicated in both increased disease susceptibility and increased control (Huber, 1990). Disease suppression with mineral nutrition can be due to effects on the nutrition of the plant, effects on the nutrition of the pathogen, effects on other organisms in the system, or a combination of factors (Woltz and Jones, 1981). Because of the complexity of interactions between plants, pathogens, other microbes, and the soil, mechanisms for disease control with nutrients are poorly understood. A significant amount of research has focused on the effects of nutrients on plant disease because nutrition can be managed easily and cheaply. Though little research specifically addresses the asparagus-*Fusarium* system, the role of nutrition in *Fusarium* control has been studied in a number of crops.

Although not technically a plant nutrient, lime can be extremely important in the

nutritional regime of the plant because it affects nutrient availability and contains calcium, which is often implicated in disease suppression. Contrary to published reports (Stephens and Elmer, 1988), increased pH has never been specifically linked with decreased Fusarium disease in asparagus. Increased pH has been shown to increase yields (Hodupp, 1983), but the mechanism by which this increase occurs has never been studied. Fusarium wilt of cotton caused by *F. oxysporum* f. sp. *vasinfectum* has been associated with acidic soils (Bell, 1989). Increased pH has been linked with the suppression of Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* (Jones et al., 1989). This may simply be a calcium effect, since it has been suggested that calcium inhibits the activity of polygalacturonase produced by *Fusarium* (Corden, 1965). Calcium is also important in carbohydrate removal, neutralization of cell acids, cell wall deposition, and the formation of pectates in the middle lamella, which suggests that it may have a role in forming physical barriers to plant invasion (Huber, 1990; Huber, 1989). However, other researchers have shown that high tissue calcium was not important in the reduction of the Fusarium wilt of tomato, so lime may be affecting disease by some other mechanism (Jones et al., 1989).

Both the form and the amount of nitrogen supplied to plants have been implicated as important factors in disease development. High levels of nitrogen have been found to encourage a number of plant diseases, including Fusarium corm rot of gladiolus caused by *F. oxysporum* f. sp. *gladioli* (Jones et al., 1989). A number of researchers have also studied how the form of nitrogen (ammonium vs. nitrate) affects the development of Fusarium disease. Most studies have found that the nitrate form of nitrogen decreases

Fusarium disease as compared to ammonium, especially when combined with high soil pH (Jones et al., 1989). This phenomenon has been reported for tomato, watermelon, chrysanthemum, muskmelon, cucumber, radish, lime, cotton, celery, and other crops (Jones et al., 1989; Schneider, 1985). Elmer (1989) has tested nitrogen form on Fusarium crown and root of asparagus and found, similarly, that weights of seedlings were increased with nitrate-nitrogen in the presence of *F. oxysporum* f. sp. *asparagi* or *F. proliferatum*.

The effects of other nutrients on the development of Fusarium disease have been less widely studied. Several researchers have found decreased disease with increased levels of potassium (Jones et al., 1989). Potassium chloride has been shown to decrease Fusarium yellows of celery caused by *F. oxysporum* f. sp. *apii*, though this researcher attributed the effect to the ratio of potassium to chloride in the plant, rather than to either ion alone (Schneider, 1985). Several studies have shown that high rates of phosphorus can increase disease severity. Levels of phosphorus above what is needed for plant growth favored Fusarium disease in tomato, muskmelon, and cotton (Jones et al., 1989). Micronutrient nutrition may also be important in Fusarium disease development. Studies with *F. oxysporum* f. sp. *lycopersici* have demonstrated repeatedly the relatively high requirement of this organism for copper, iron, manganese, and molybdenum, and that increasing the amounts of the nutrients was beneficial to the growth and sporulation of the pathogen (Jones et al., 1989). This, in fact, may be the reason that lime is effective in suppressing disease (Jones et al., 1989). Most micronutrients are much less available as pH increases, and may therefore be limiting to *Fusarium* growth.

Another interesting factor in Fusarium disease development is the suppressive effect of chloride salt. For many years, applying salt to asparagus plantings was a common practice. Asparagus is one of the most salt tolerant crops grown commercially, and researchers working in the early part of this century showed empirically that salt applications can increase yields (Walker, 1905; Rudolfs, 1921), though the mechanism is not clearly understood. More recently, research has focused on the role of salt in the control of Fusarium crown and root rot. Elmer (1989) first identified the potential suppressiveness of salt on Fusarium disease while exploring the effect of different nutrient formulations on disease development. In these experiments, NaCl reduced disease development and root colonization on asparagus seedlings. KCl was also effective in disease control, especially when the amount of potassium otherwise supplied was limited, leading to the conclusion that disease suppression was due to a chloride effect, and that the ratio of potassium to chloride may be the most important factor in disease suppression. Another interesting observation in this study was that fresh weights of seedlings increased with chloride applications, while dry weights did not always increase, indicating that chloride applications may have affected osmotic regulation in the plants.

Further research (Elmer, 1992) confirmed that NaCl applications were suppressive to Fusarium crown and root rot in both greenhouse and field plots. NaCl was also more effective in controlling disease in these plots than KCl, and plants were shown to actually accumulate some Na⁺ (up to 5.5 mg/g of tissue). Fern concentrations of sodium were also correlated with increased plant weights and decreased the fraction of diseased roots,

leading to the speculation that not just chloride, but also sodium was having an effect on disease development. Since applications of NaCl did not reduce populations of *Fusarium* in the soil, and did not control weeds in this experiment, it was speculated that applications affected the susceptibility of the host to the disease. Elmer (1992) proposed that the increase in Cl^- concentration in the plant balanced the influx of cations (NH_4^+ , K^+ , Ca^{2+} , etc.) that may otherwise be offset by the synthesis of organic acids such as malate, and thereby reduce the exudation of organic substrates into the rhizosphere, which could play a role in reducing *Fusarium* colonization and infection. Other experiments have shown that NaCl applications can effect carbohydrate and malate production in the roots of asparagus plants (Elmer, 1990a).

Other investigations into the mechanism by which NaCl suppresses crown and root rot have focused on plant water relations. A number of researchers have explored the effect of water relations on *Fusarium* disease (Cook, 1973; Cook, 1981; Cook and Papendick, 1972; Papendick, 1974; Schneider, 1993; Schneider and Pendery, 1983). In general, *Fusarium* diseases are most severe in dry years, with water stress predisposing plants to disease (Cook, 1973; Cook, 1981; Schneider and Pendery, 1983). Cultural practices, and the levels of different nutrients in the plant are known to affect water potentials and can increase the rate of *Fusarium* disease development (Papendick, 1974). In a study by Elmer and LaMondia (1997), NaCl applications resulted in lower fern water potential, and increased turgor pressure. It was hypothesized that the uptake of chloride was responsible for the maintenance of lower osmotic potential in the plant, thereby increasing turgidity and decreasing plant stress during periods of drought. Other

researchers have also suggested that lowered water potentials can limit pathogen invasion (Cook and Papendick, 1972).

Salt may be eliciting not simply a plant effect, but one that involves other aspects of the soil community. Elmer (1995) has investigated the effect of NaCl applications on the density and composition of root colonizing bacteria. This study showed a significant increase in the fraction of manganese-reducing bacteria on the roots of NaCl treated plants, and an increase in the level of manganese in the roots of these plants compared to the control. In addition, several strains of these manganese-reducing bacterial were able to decrease disease incidence in greenhouse trials.

In contrast to other studies on NaCl applications to asparagus (Elmer, 1989; Elmer, 1992; Elmer, 1995; Elmer and LaMondia, 1997; Rudolfs, 1921; Walker, 1905), Francois (1987) showed decreasing yields with increasing salinity levels. In these experiments, salt was applied in irrigation water every 12 days, rather than in a single yearly application to the soil. In light of the investigations by Elmer (1989, 1992, 1995, 1997) it is possible that the constant salinity in the solution supplied to the plants in this experiment never allowed for an osmotic potential differential to be developed, and thereby negated the mechanism by which salt aids the growth of plants. It is plausible that this differential in osmoticum between soil and root allows for differences in either nutrient uptake or root exudation which may result from applications of NaCl to asparagus. Similarly, a study by Porter et al. (1917) showed increased yield with a single application of NaCl (1120 kg/ha), and decreased yield with multiple applications (280 kg/ha four times). An alternate explanation for these differences could be simply that the

yield increases seen with NaCl are simply the result of decreases in *Fusarium* disease, which offset decreases in plant yield with salt applications. Elmer (1989, 1992, 1995, 1997) clearly showed that NaCl applications reduce *Fusarium* disease and increase yield, and it is possible that the experiments of Walker (1905) and Rudolphs (1921), which showed increased yield with salt applications, were done in a system infected with *Fusarium* pathogens. Nor is this explanation necessarily inconsistent with the results obtained by Porter et al. (1917), who showed increased yields with a single salt application and not with multiple salt applications, but applied their treatments to different parts of the field, and thus may have had different levels of *Fusarium* infection in each treatment.

The mechanism by which salt affects disease may involve a number of factors and is not fully understood, yet it is clear that NaCl has the potential to decrease the severity of *Fusarium* disease and increase yields in asparagus. Because studies with other crops have actually shown increases in the severity of *Fusarium* disease with the application of NaCl (Standaert et al., 1978) it is likely that the benefit of salt applications may be related to the status of asparagus as a salt tolerant plant. Furthermore, it is possible that the mechanism of salt tolerance will lend some insight to the question of why NaCl decreases disease in asparagus. Salt tolerance in asparagus is an organismal trait, not present in undifferentiated tissue to the extent that it is in the whole plant (Mills, 1988). Hence, it is supposed that there are physiological and anatomical mechanisms at work that allow the plant its halophytic characteristics. Asparagus, like other halophytes, has developed a mechanism by which it is able to take up Cl^- while excluding Na^+ (Elmer, 1992; Mills,

1988). All halophytic plants seem to respond positively to NaCl applications (Flowers et al., 1977). Salt applications commonly result in increased fresh weight in halophytes, indicating a rise in water content (Flowers et al., 1977), and the osmotic effects of high salt concentrations can be quite dramatic (Waisel, 1972). Other research has also shown an increase in lignin and cellulose in halophytes when salt was applied (Flowers et al., 1977), factors that have often been implicated in disease resistance (Agrios, 1997).

Another strategy that may have a place in the control of *Fusarium* crown and root rot is the use of biological control organisms. The suppressiveness of some soils to *Fusarium* diseases has led researchers to the conclusion that microbial activity may be an important factor in disease inhibition (Louvet et al., 1981). Several different organisms have been identified as having potential for the biological control of *Fusarium* diseases in asparagus and other crops. The potential of non-pathogenic strains of *F. oxysporum* to protect plants against disease caused by pathogenic strains was first recognized in the 1960's (Davis, 1967; Davis, 1968). Since this time cross-protection, or biological control with non-pathogens, has been demonstrated for a number of crops including tomato (Fuchs et al., 1997), chickpea (Hervas et al., 1995), watermelon (Larkin et al., 1996), cucumber (Mandeel and Baker, 1991), pea (Oyarzun et al., 1994), carnation (Postma and Rattink, 1992), and asparagus (Blok et al., 1997; Damicone and Manning, 1981; Damicone and Manning, 1982; Tu et al., 1990). Damicone and Manning (1981; 1982) demonstrated significant reduction in disease symptoms in greenhouse trials with asparagus. Blok et al. (1997) reported more than a 50% decrease in root rot severity with each of 13 nonpathogenic isolates from asparagus roots or field soils. Both studies

reported differences in the ability of individual isolates to reduce disease, where some were recognized as particularly useful while others were not. The mechanism of disease suppression in asparagus is not clearly understood, though studies with both watermelon and tomato have suggested that non-pathogenic isolates induce resistance to pathogenic isolates in these plants (Fuchs et al., 1997; Larkin et al., 1996). Field experiments using non-pathogenic *Fusarium* isolates have shown the potential for use on young plants, but haven't been effective on older plantings already infected with the disease (Damicone and Manning, 1981; Damicone and Manning, 1982; Tu et al., 1990; Blok et. al, 1997).

Another organism with the potential for biological control is the fungus *Trichoderma*. *Trichoderma* spp. can be antagonistic to pathogenic fungi by competition, antibiosis, or the production of lytic enzymes, and are able to grow on hyphal cell walls or living mycelium (Benhamou and Chet, 1993; Elan et al., 1983; Sivan and Chet, 1986). *Trichoderma* has been shown to attach its self to other fungi either by appressoria or hyphal coils (Elad et al., 1981), and can even inhibit the germination of *F. oxysporum* chlamydospores (Sivan and Chet, 1989). Gennari et al. (1990) and Nipoti et al. (1990) have shown increased germination of asparagus seedlings with soil and seed applications of *T. harzianum*. Arriola (1997) has also shown decreased disease and increased plant weights when *T. harzianum* was applied to peat infested with *F. oxysporum* f. sp. *asparagi*.

Vesicular-arbuscular mycorrhizae are common inhabitants in soil, and commonly form symbiotic associations with a number of plant species (Wacker, 1988). These organisms are capable of increasing growth and yield in crop plants by increasing nutrient

uptake and resistance to salinity and drought (Menge, 1983; Nelsen and Safir, 1982). Mycorrhizal associations have also been shown to affect the incidence and development of plant diseases (Dehne, 1982; Schenck, 1981). Several studies have emphasized the importance of mycorrhizal colonization of asparagus roots (Burrows et al., 1990; Hussey et al., 1984; Pederson et al., 1991). Especially interesting is a study by Pederson et al. (1991) who showed increases in asparagus dry weight and bud formation when mycorrhizae were applied to plants grown in fumigated field soil. Other researchers have observed decreases in the severity of *Fusarium* crown and root rot with mycorrhizal inoculations (Arriola, 1997; Wacker et al., 1990a), underscoring the importance of these relationships in disease development. Applications of mycorrhizal fungi show potential for use in disease suppression, but because different mycorrhizal fungi predominate in asparagus of different ages (Wacker et al., 1990b), the species introduced may be an important consideration.

Elson (1993; Elson et al., 1994) isolated a species of *Streptomyces* from asparagus soil that was found to be antagonistic to *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. Chemical extraction yielded an inhibitory compound which reduced the populations of pathogenic *Fusarium* in the soil but also reduced root length. Cells of the bacteria colonized asparagus roots, but not in sufficient concentrations to control *Fusarium* disease. Elmer (1995) also identified bacterial species (*Pseudomonas corrugata*, *Serratia* spp.) capable of reducing disease without negative effects on plant growth. Further study is needed to identify the potential of these organisms for biological control.

Since the causal organisms were first discovered, investigators have struggled to find a solution to the problem of *Fusarium* crown and root rot. In Michigan, no specific control strategies are practiced other than a modest amount of crop rotation, and the use of high yielding varieties with which a certain amount of loss can be tolerated. It is clear from decades of research that a single conventional strategy (chemical control, avoidance, resistance breeding) for effective disease control is not forthcoming. There are, however, a number of factors that, when employed together, may constitute an effective strategy for reducing disease to an acceptable level. Several researchers have investigated integrated strategies for *Fusarium* disease control in different systems (Beale and Pitt, 1990; Chakravarty et al., 1990; Garibaldi and Lodovica-Gullino, 1984; Minuto et al., 1995; Sivan and Chet, 1993). Damicone and Manning (1985; Damicone et al., 1987) attempted to develop an integrated strategy for *Fusarium* disease control in asparagus that did not, it seems, save asparagus production in Massachusetts. However, many advances in disease control have been made since that time.

Clearly, more research is needed on the potential for integrating various disease control strategies into a modern asparagus production system. However, the following components warrant consideration for inclusion in an integrated disease management system:

- 1) Selection of a vigorous, high yielding, and moderately resistant variety of asparagus suited to the particular region in which it will be planted (Elmer et al., 1997).
- 2) Selection of a seedling nursery field with low concentrations of *Fusarium* pathogens (Didelot et al., 1996) have developed a prediction test for root rot that could be easily

modified for the Michigan industry), or fields that have been out of asparagus production for as long as possible, and have not been rotated with corn (Damicone et al., 1988), peas (Cohen and Heald, 1941), or their relatives.

3) Treatment of asparagus seed prior to planting with benomyl in acetone (Damicone et al., 1981; Knaflowski and Sadowski, 1990), or *Trichoderma* spp. (Gennari et al., 1990; Nipoti et al., 1990).

4) Fumigation of asparagus seedling nurseries (Lacy, 1979; Molot et al., 1985), followed by reintroduction of mycorrhizal fungi (Molot et al., 1985; Wacker, 1988).

5) Fumigation or chemical treatment of asparagus fields into which healthy asparagus crowns are planted (Damicone et al., 1987; Lacy, 1979; Manning and Vardaro, 1977).

6) Application of biological control agents at the time of crown planting (Arriola, 1997; Blok *et al.*, 1997; Damicone and Manning, 1982; Tu et al., 1990; Wacker, 1988).

7) Maintenance of proper pH for healthy asparagus growth (Hodupp, 1983).

8) Use of the nitrate form of nitrogen on fields with a high potential for decline, or fields that have begun to decline (Elmer, 1989).

9) Judicious use of NaCl on declining fields if it can be established that this does not pose a risk to the nutrient status of the crop, soil structure, rotation crops, or groundwater quality (Elmer, 1992).

10) Irrigation of asparagus plantings, especially crown nurseries, in times of severe water stress (Nigh, 1990).

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SECTION I

EFFECT OF SODIUM CHLORIDE AND LIME APPLICATIONS ON COMMERCIAL
ASPARAGUS AND THE EFFECT OF ALTERNATE FORMS OF CHLORIDE SALT
ON FUSARIUM CROWN AND ROOT ROT

ABSTRACT

Experiments with NaCl (high and low rates) and lime applications were conducted in asparagus fields in commercial production (one healthy and one declining), and an experiment with NaCl was conducted in a badly declined research field at Michigan State University from 1998 to 2000 to test the ability of these treatments to control *Fusarium* crown and root rot of asparagus. Growth chamber and greenhouse studies were conducted to test the ability of alternative forms of chloride salt to reduce disease caused by *Fusarium oxysporum* f. sp. *asparagi* and *Fusarium proliferatum*. NaCl applications led to increased yield and fern health in the research plot, but no treatment effects were observed in commercial fields. Soil tests in these fields indicated that NaCl did not decrease levels of calcium, magnesium, or potassium, and did not affect pH. In growth chamber studies with asparagus seedlings in Hoagland's agar test tubes, and in greenhouse studies with asparagus seedlings in soil, no forms of chloride salt tested were more effective than NaCl in controlling *Fusarium* crown and root rot.

INTRODUCTION

Fusarium crown and root rot, caused by *Fusarium proliferatum* (T. Matsushima) Nirenberg and *Fusarium oxysporum* (Schlect.) f.sp. *asparagi* (Cohen & Heald) is the major cause of asparagus early decline, an economically important disease worldwide. Symptoms of the disease include yellowing and senescence of ferns, destruction of feeder roots, collapse of storage roots, and death of crowns (Elmer et al., 1996). In a perennial crop such as asparagus, the lack of effective chemical controls (Lacy, 1979) and the limited resistance of available cultivars (Stephens et al., 1989), makes Fusarium crown and root rot an especially difficult disease to control.

A disease management strategy that has shown promise is the use of sodium chloride (NaCl) salt as a soil amendment. Asparagus is an extremely salt tolerant crop, and salt applications were recommended by gardening experts until the early part of this century (Bennett, 1910; Burr, 1865; Harris, 1883). Elmer (1989;1992) demonstrated a reduction in Fusarium crown and root rot with NaCl applications in the greenhouse and in small research field plots, and some commercial asparagus growers have adopted the practice.

Salt applications on field soils can potentially cause problems such as loss of soil structure, soil acidification, nutrient displacement, groundwater contamination, and damage to sensitive rotation crops (Brady, 1990). Although the mechanism by which NaCl suppresses disease is not clearly understood, it is likely that the chloride molecule is playing a role (Elmer, 1989; Elmer, 1992). Research has suggested that potassium chloride (KCl) is not as effective as NaCl in disease suppression (Elmer, 1992), but the

effects of other forms of chloride salt on disease, have never been studied.

A high pH has been shown to decrease the severity of *Fusarium* diseases in some crops including cotton (Bell, 1989), and tomato (Jones et al., 1989). Hodupp (1983) has demonstrated an increase in asparagus yields at high pH, and soil pH can affect the availability of nutrients to the plant, which can often be a factor in *Fusarium* disease suppression (Huber, 1989; Huber, 1990).

The purpose of this study was to determine the effects of yearly lime and NaCl applications on the yield and fern vigor of commercial asparagus plantings in Michigan, both declining and healthy; to determine the effect of these treatments on pH, and the quantity of sodium, magnesium, potassium, and calcium in the soil; and to determine whether alternative forms of chloride salt could be as effective as NaCl in disease suppression.

MATERIALS AND METHODS

Growth chamber studies with chloride salts. Growth chamber studies were conducted to test the ability of different forms of chloride salt to suppress disease caused by *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. In tests with *F. oxysporum* f. sp. *asparagi*, treatments consisted of the following: *i*) a control (no salt added), *ii*) NaCl (17.1 mM), *iii*) NaCl (34.2 mM), *iv*) CaCl₂ (17.1 mM), *v*) NH₄Cl (34.2 mM). For tests with *F. proliferatum*, treatments consisted of *i*) a control, *ii*) NaCl (34.2 mM), *iii*) CaCl₂ (17.1 mM), *iv*) MnCl₂ (8.55 mM), *v*) NH₄Cl (34.2 mM). Hoagland's solution agar (Damicone and Manning, 1982; Davis, 1967) was prepared and chloride salt added to

provide an equal amount of chloride to each treatment except the control, and the MnCl_2 treatment, which was toxic to the plants when too much was applied. All treatments were standardized to a pH of 6.0 using sodium hydroxide (NaOH). Agar (12-16 g/L) was added to the control and each treatment to achieve a uniform consistency. Each solution was autoclaved and added aseptically to sterile glass test tubes (16x150 mm).

Asparagus seeds (cv. 'Mary Washington', Rupp Seeds, Wauseon, OH) were sterilized according to the method of Damicone et al. (1981) by spinning on a magnetic stir plate for 24 hours in a mixture of 100 ml acetone and 2.5 g benomyl (5 g Benlate 50 DF, E. I. du Pont de Nemours & Co. Inc., Wilmington, DE), rinsing them in acetone (3x) and in distilled water (3x), then spinning for 1 hour in 1.05% sodium hypochlorite (20% household bleach), and rinsing again in sterile distilled water (3x). Seeds were air dried, and placed on water agar under sterile conditions. Once hypocotyls began to emerge, healthy, uniform seedlings were transferred to the test tubes under sterile conditions. Tubes were capped, and seedlings were grown for 10 days. Twenty and 40 seedlings were used for each treatment in tests with *F. oxysporum* f.sp. *asparagi* and *F. proliferatum*, respectively.

F. oxysporum f. sp. *asparagi* and *F. proliferatum* were isolated from diseased asparagus crowns and stems in 3 commercial asparagus fields in Oceana County, Michigan, and a research plot at the Michigan State University Botany and Plant Pathology Farm. Four hundred and fifty nine isolates were tested for pathogenicity by inoculating seedlings growing in test tubes filled with Hoagland's Solution agar in the method described by Stephens and Elmer (1988). Isolates judged to be highly virulent on

asparagus seedlings were put into storage on silica gel according to the procedure described by Windels et al. (1988). Highly virulent isolates of *F. oxysporum* f. sp. *asparagi* (isolate 2-7) and *F. proliferatum* (isolate P-67), were grown on carnation leaf agar for two weeks under fluorescent lights. The surface of the plates were flooded, scraped with a scalpel to loosen spores, filtered through three layers of sterile cheesecloth, and diluted with sterile water to 1×10^6 conidia/ml using a hemacytometer. A spore suspension (0.5 ml) was added to each seedling tube, and plants were grown for 4 weeks in a 25°/20° C (day/night) growth chamber under fluorescent plant lights, with a 16 hour photoperiod.

Seedlings were assessed for disease based on the percentage of the root system that exhibited lesions or had collapsed. Dead plants were considered to be 100% infected. Each experiment was conducted twice.

Greenhouse studies with chloride salts. ‘Mary Washington’ asparagus seeds were sterilized as previously described and germinated in the dark on sterile water agar petri plates. Seedlings were then planted into 64 cc plug trays filled with High Porosity Professional Planting Mix (Michigan Peat Co., Houston, TX) as soon as hypocotyls emerged. Plants were grown for 14 days, then clean, uniform seedlings were transferred to 512 cc plastic pots.

Inoculum was prepared using a modification of the method described by Stephens and Elmer (1988). Distilled water (100ml) was added to breathable autoclave bags (Field & Forest Products Inc., Peshtigo, WI) containing 200g of pearl millet seed (Rupp Seeds, Wauseon, OH). Bags were autoclaved for 1 hour on consecutive days, and allowed to

cool. Several pieces of silica gel infested with the isolates were added to separate bags and allowed to grow for 2 weeks. Bags were shaken occasionally to prevent clumping. Millet seed was dried in autoclaved paper bags for 7 days, then ground in a Thomas Mill (Arthur H. Thomas Co. Philadelphia, PA). Ground millet (150 g) infested with a mixture of *F. osysporum* f. sp. *asparagi* (isolates 2-7 and FOA-50) was combined with ground millet (150g) infested with a mixture of *F. proliferatum* (isolates P-67 and M-6374), and added to 40 L of a 4:1 mixture of steamed sandy loam to Bacto High Porosity Professional Planting Mix (Michigan Peat Co., Houston, TX), to yield an inoculum density of 320,000 CFUs per gram of soil. Isolate FOA-50 originating from a Michigan asparagus field and isolate M-6374 from an asparagus field in Connecticut were obtained from W. Elmer, Connecticut Agricultural Experiment Station. Soil for the uninfested control treatments consisted of a 4:1 mixture of steamed sandy loam to Bacto High Porosity Professional Planting Mix which no pathogen was added.

Seedlings were washed free of soil, and inspected for lesions or discoloration, and uniform, healthy crowns were planted into either infested or uninfested soil in 512 cc plastic pots. The following treatments were applied to each of 16 plants in infested soil in amounts adjusted to give equivalent Cl⁻ content in each pot: *i*) no salt (control), *ii*) NaCl (0.32 g/pot), *iii*) CaCl₂*2H₂O (0.40 g/pot), *iv*) NH₄Cl (0.29 g/pot), *v*) KCl (0.40 g/pot), and *vi*) Mn₂Cl*4H₂O (0.53 g/pot). An uninfested control was also included in this experiment. Plants were allowed to grow for 3 weeks, and were fertilized twice weekly with 15 ml of Hoagland's solution. Plants were watered as needed with distilled water. Treatments were arranged in a completely randomized design, and the experiment was

conducted twice. Fresh weights of roots and shoots were taken, and the percentage of the root system exhibiting lesions was estimated.

NaCl and lime applications in established asparagus fields. A single research plot (688 m²) was established at the Botany and Plant Pathology Research farm in East Lansing, Michigan in a 17-year-old asparagus field (cv. 'Mary Washington') with severe symptoms of *Fusarium* related decline including thin stands, missing crowns, and poor fern growth. Rows were spaced 1.52 m apart, and the soil was a sandy loam. Treatments consisted of an untreated control, and rock salt (NaCl) (PureMelt Ice and Snow Melting Salt, ICM Kalium Co., Bannockburn, IL) applications (1 120 kg/ha). Salt was applied on 17 April (1998), 30 April (1999), and 25 April (2000), using a Gandy drop spreader (Gandy Co., Owatonna, MN).

Treatments were arranged in a randomized complete block design with 5 blocks, each separated by an untreated buffer row. Treatment plots consisted of a 6.1 m section of a single row. Pelletized lime (Nutralime, Mineral Processing Co., Carey, OH) was applied to all treatments in the spring of each year at a rate of 6719 kg/ha, and fertilizer (12-12-12, Northern Star Minerals, E. Lansing, MI) was applied at 336 kg/ha. Pesticide sprays were applied as needed for the control of insects, weeds, and foliar diseases. Spears greater than 12.71 cm in height were harvested by hand snapping between 5 and 26 May in 1998 (10 harvests), between 10 May and 9 June 1999 (17 harvests), and between 2 May and 6 June in 2000 (14 harvests).

Two research plots were established in Oceana County, Michigan in loamy-sand asparagus fields (cv. 'Syn-456') in commercial production, that had been previously

cropped in asparagus. One plot was established in a healthy-appearing 2.02 ha field of 11-year-old asparagus. A second plot was established nearby in a declining (thin, and unproductive) 3.44 ha field of 10-year-old asparagus. In each plot, crowns were planted approximately 25 cm apart in rows spaced 1.52 m apart. Treatments consisted of the following: *i*) an untreated control, *ii*) a low rate of salt (560 kg/ha), *iii*) a high rate of salt (1120 kg/ha), *iv*) a pelletized lime application (6719 kg/ha), *v*) a low rate of salt and pelletized lime, *vi*) and a high rate of salt and pelletized lime. All treatments were applied using a Gandy drop spreader on 10 April (1998), 27 April (1999), and 19 April (2000).

Treatments were arranged in a randomized complete block design with 6 blocks. Treatment plots consisted of a 6.1 m section of 3 parallel rows. Two buffer rows separated each treatment and 3.05 m separated each block within the rows. Fertilizer and pesticide sprays, with the exception of lime were applied to these plots as needed by the growers.

All spears greater than 12.70 cm in height were harvested by hand snapping between 2 May and 15 June (26 harvests) in 1998, 3 May and 12 June in 1999 (25 and 24 harvests for the declining and healthy fields, respectively), and 2 May and 13 June and 30 April and 13 June for the declining and healthy fields respectively (22 harvests each) in 2000. All plots were evaluated for fern health by counting the number of stalks (excluding volunteer seedlings) greater or less than 0.79 cm in diameter 12.70 cm above the soil surface approximately 5 weeks after the termination of harvest.

Soil samples were taken from each treatment plot in both the experimental and commercial fields in April (before treatments were applied), July, and October 1998, in

April and October 1999, and in April 2000. Samples consisted of eight 30.48 cm soil cores split into the top 15.24 cm and bottom 15.24 cm and homogenized. All soil samples were analyzed at the Michigan State University Soil Testing Laboratory for pH, and concentrations of calcium, potassium, magnesium, and sodium.

Statistical analysis. The Proc Mixed procedure was used with SAS statistical analysis software (SAS Institute Inc., Cary, NC) for all analyses. Data from all tests were evaluated by comparing Bonferroni adjusted Least Squared means for all treatments. In soil tests, data from treatments in spring 2000 (after two treatment applications) were compared using spring 1998 data (before any treatments were applied) as a covariate. Differences in Na levels between fall 1998 and fall 1999 were compared to determine whether Na had built up in the soil between these years. Harvest data from the three years were evaluated as repeated measures. Percentage data (root system covered with lesions) for growth chamber trials with the pathogen *F. proliferatum* were transformed using the arcsine transformation to normalize data. Because of the heterogeneous variance in trial by treatment combinations in percentage data (root system covered with lesions) for greenhouse trials, variance component estimation was carried out by using the Repeated statement with Group Option in SAS. L. S. means differences were considered significant at $P=0.05$, and data for the two replications of each experiment is presented together unless trial by treatment interactions were significant.

RESULTS

Growth chamber studies with chloride salts. A large fraction (84.4%) of the

root system of control plants exhibited lesions or collapse caused by *F. proliferatum*, while plants treated with NaCl (57.0%) or CaCl₂ (69.6%) exhibited significantly less root rot (Figure 1). MnCl₂ did not significantly limit root rot compared to the control, but was not significantly different than the NaCl and CaCl₂ treatments. NH₄Cl treatments were not effective and resulted in significantly more disease than the control.

Root rot caused by *F. oxysporum* f. sp. *asparagi* was limited significantly by a high rate (32.4 mM) of NaCl (44.4%) compared to the control (77.5%) and all other treatments with the exception of the low rate (17.1 mM) of NaCl (62.3%) (Figure 2). Neither CaCl₂ or the low rate of NaCl were significantly more effective than the control in reducing disease caused by this pathogen. Plants treated with NH₄Cl were significantly more diseased than the control.

Greenhouse studies with chloride salts. In experiment 2, only NaCl-treated plants exhibited significantly less root rot (18.6%) than the infested control (33.9%), but were not significantly different from the other treatments (Figure 3). In both experiments, the uninfested control remained virtually disease free (< 1%).

In experiment 1, NaCl treated plants had significantly greater fresh root weight (1.50 g) than the infested control (0.95 g) and all other treatments except KCl (1.13 g) (Figure 4). Significant differences in fresh root weight were not observed among the treatments in experiment 2, although NaCl had a higher mean weight (1.30 g) than the infested control (0.89 g). The uninfested control had significantly greater fresh root weights than all other treatments in both experiments.

There were no significant differences in fresh shoot weight when treatments were

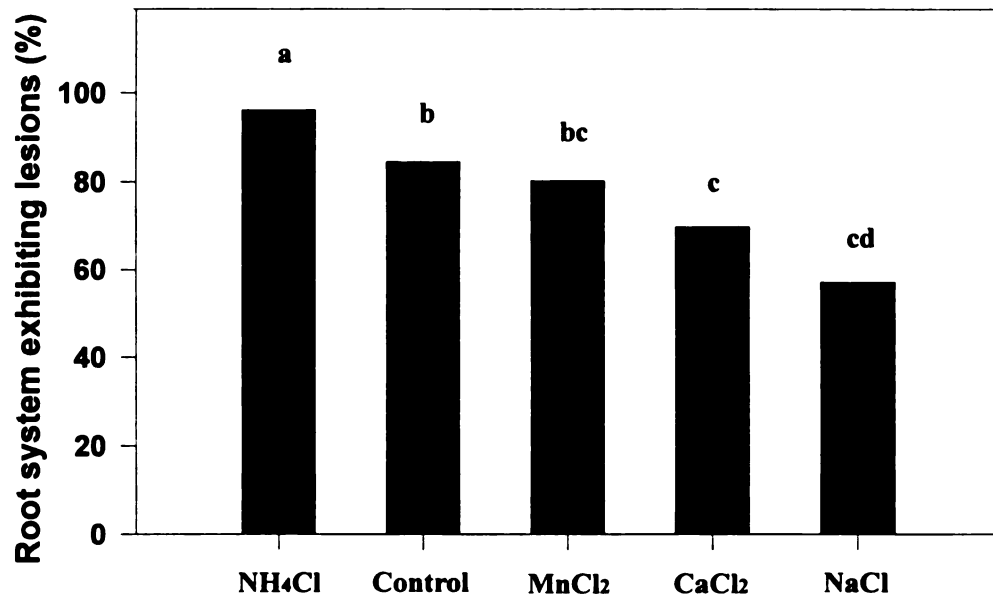


Figure 1. Effect of different forms of chloride salt on root rot (%) in two experiments with asparagus seedlings grown in Hoagland's agar in test tubes and infested with *F. proliferatum*. Different letter notation indicates significant differences in L S means comparisons.

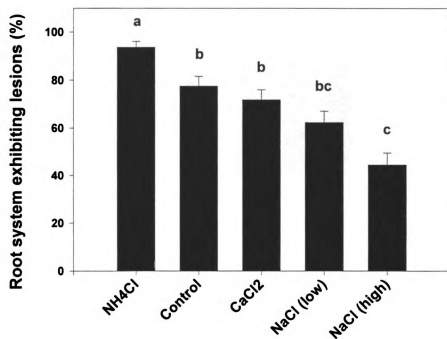


Figure 2. Effect of different forms of chloride salt on root rot (%) in two experiments with asparagus seedlings grown in Hoagland's agar in test tubes, and infested with *F. oxysporum* f. sp. *asparagi*. Error bars represent standard errors, and different letter notation indicates significant differences in L S means comparisons.

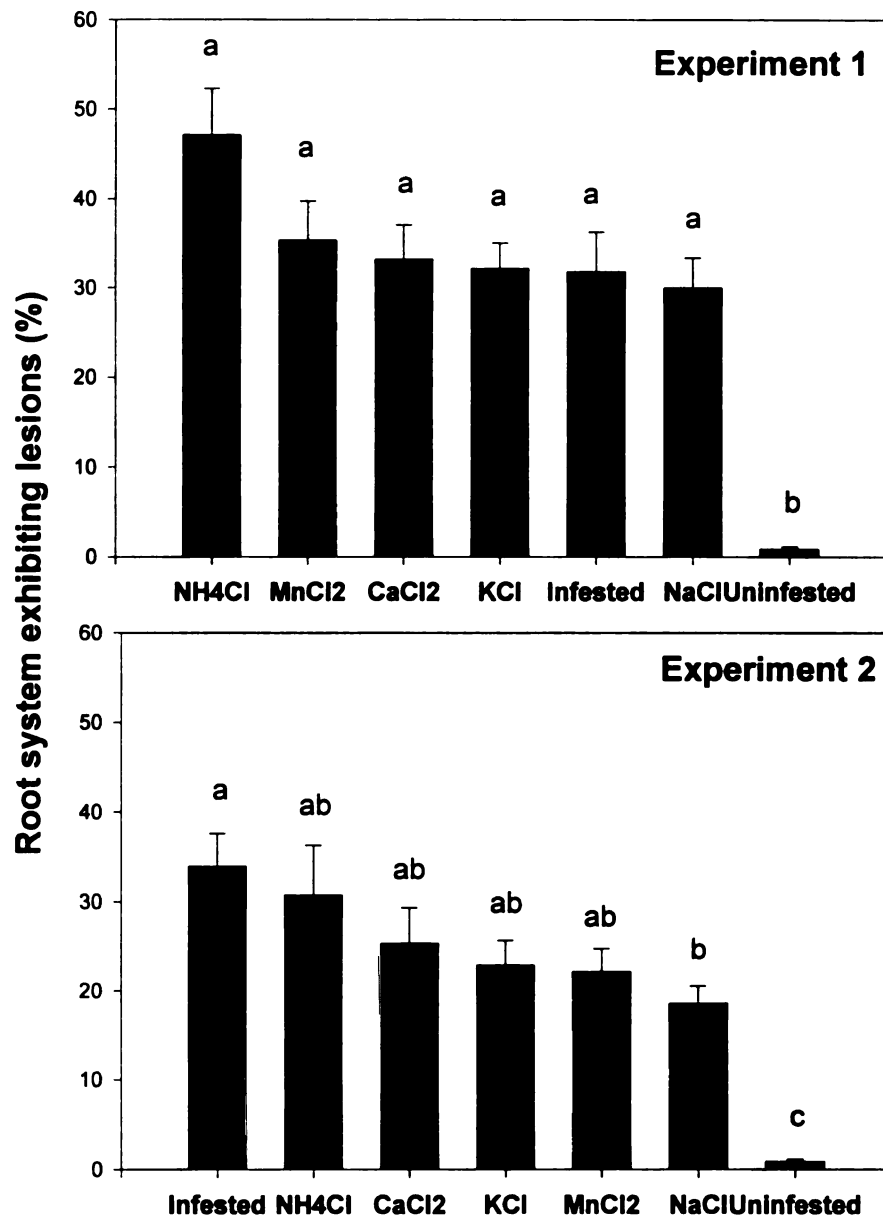


Figure 3. Effect of different forms of chloride salt on root rot (%) in two greenhouse experiments with asparagus plants grown in soil infested with *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. Error bars represent standard error, and different letter notation indicates significant differences in L S means comparisons.

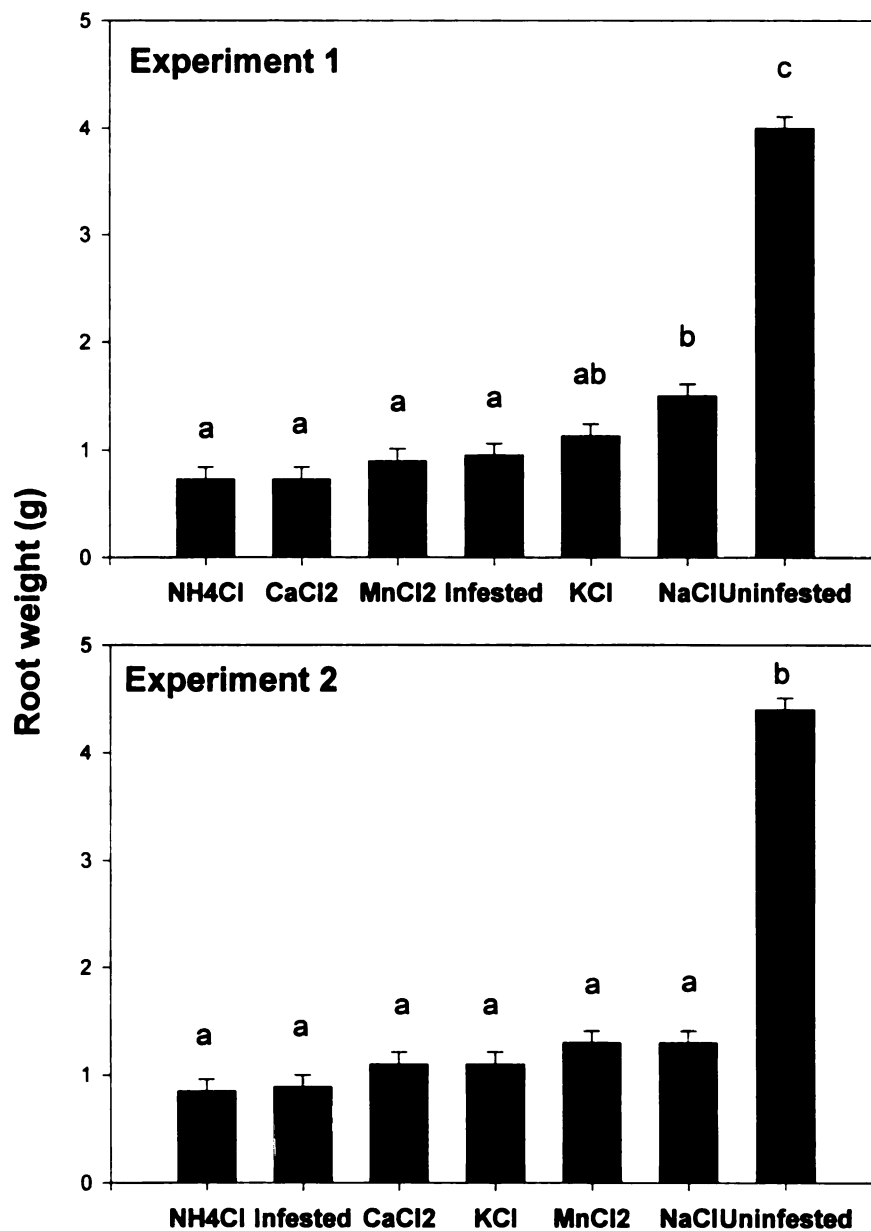


Figure 4. Effect of different forms of chloride salt on root weight (g) in two greenhouse experiments with asparagus plants grown in soil infested with *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. Error bars represent standard error, and different letter notation indicates significant differences in L S means comparisons.

compared to the control (Figure 5). However, NaCl was superior to CaCl_2 , MnCl_2 , and NH_4Cl treatments. The uninfested control had significantly greater shoot weight than all treatments.

Field trials with NaCl and lime applications. No significant treatment or year by treatment differences were found in asparagus yield in the commercial fields (Figure 6). Significant differences between years were observed, and were due to differences in the number of harvests taken each year, as well as environmental conditions. Although significant treatment differences were found between the control and 1120 kg NaCl treatments in the diseased field in 1999 ($P=0.020$) no overall treatment or year by treatment effect was found in the percentage of stalks larger than 0.79 cm in diameter (Figure 7).

Significant differences ($P=0.0420$) in year by treatment effect were found in the comparison of yield between salt treated and untreated plots in the research field at the Michigan State University Botany and Plant Pathology Farm in 2000, but not in 1998 or 1999 (Figure 8). Significant differences were found in the percentage of stalks larger than 0.79 cm in diameter in 2000 ($P=0.0049$), but not in 1998 or 1999 (Figure 9).

Soil tests. NaCl applications did not significantly affect levels of pH, potassium, magnesium, or calcium after two treatments in the research plot at the MSU Botany and Plant Pathology Farm (see appendix). Sodium levels were significantly higher in salt-treated plots (90 ppm) than in untreated plots (21 ppm) in the top 15 cm of soil. Similarly, in the 15-30 cm soil level sodium levels were higher in the treated plots (94.6 ppm) than in untreated plots (26.6 ppm).

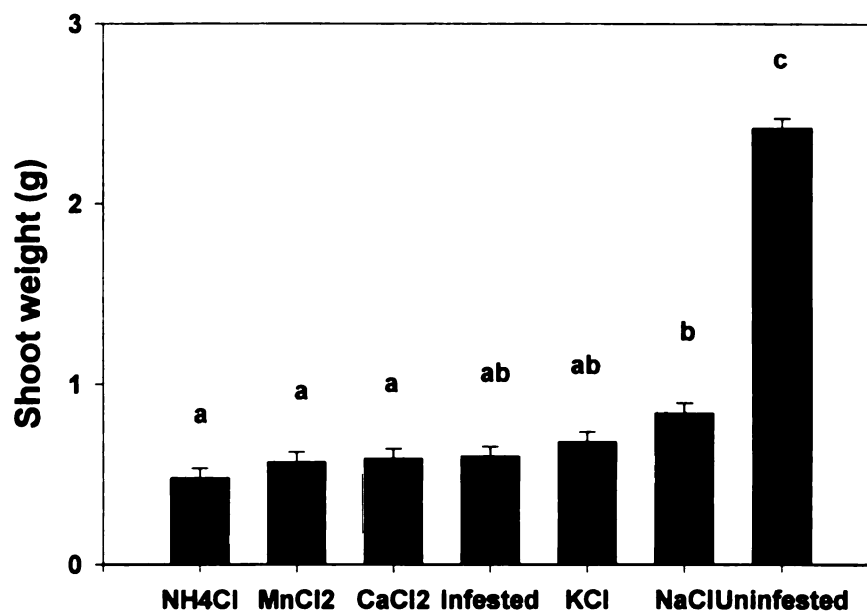


Figure 5. Effect of different forms of chloride salt on shoot weight (g) in two greenhouse experiments with asparagus plants grown in soil infested with *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. Error bars represent standard error, and different letter notation indicates significant differences in L S means comparisons.

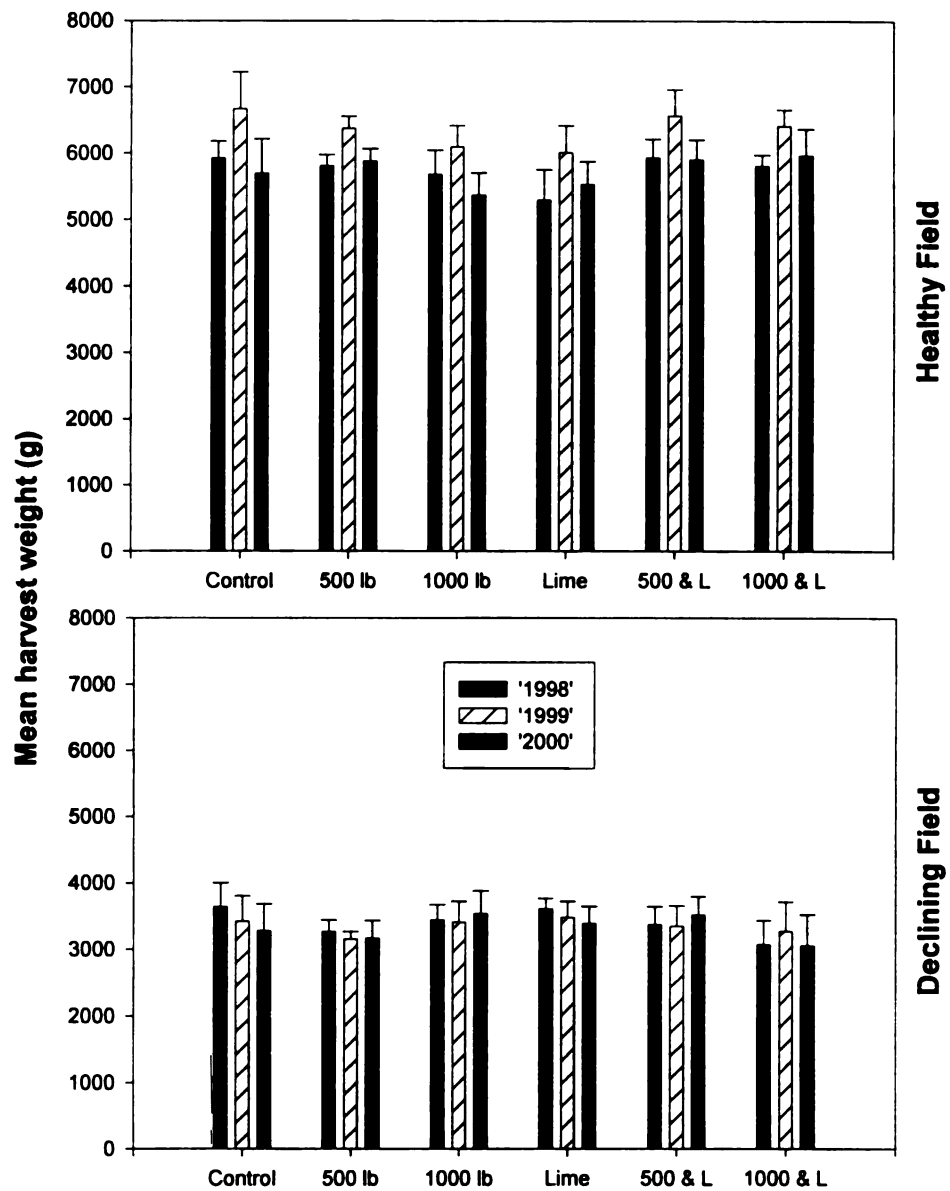


Figure 6. Mean harvest weight of asparagus spears from commercial field plots in Oceana County, MI 1998-2000 during 1998 to 2000. Error bars represent standard error.

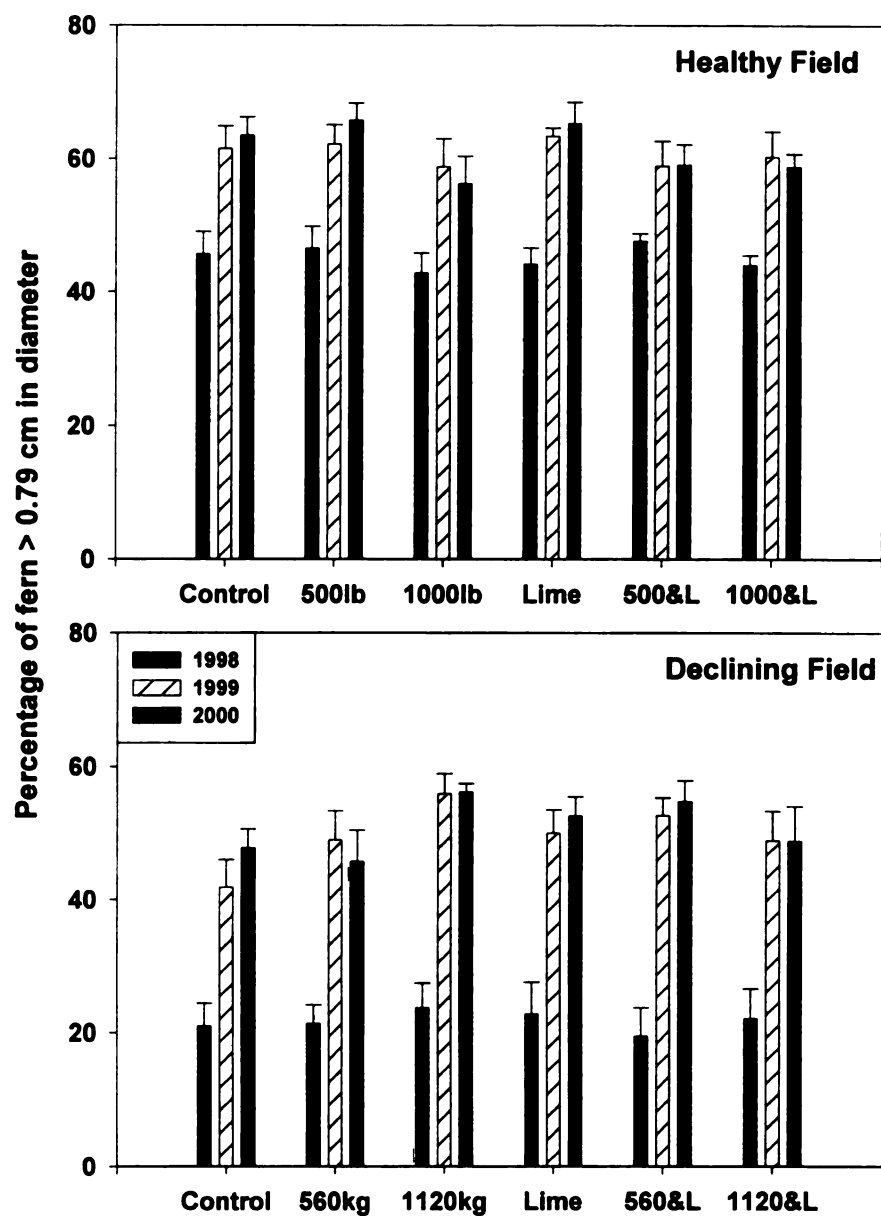


Figure 7. Percentage of asparagus stalks > 0.79 cm in diameter in commercial field plots in Oceana County, MI 1998 to 2000. Error bars represent standard error.

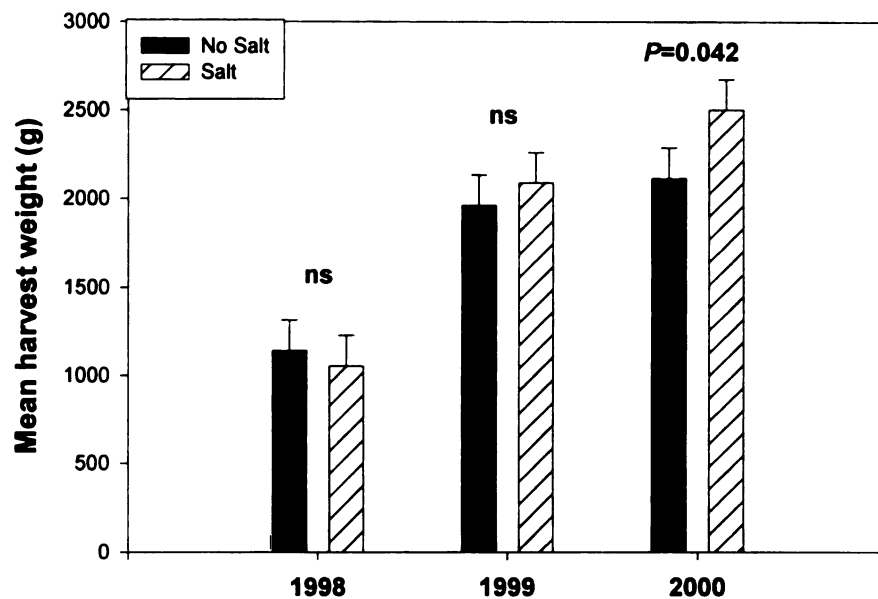


Figure 8. Mean harvest weight of asparagus from research plot at MSU Botany and Plant Pathology Farm 1998 to 2000. Error bars represent standard error.

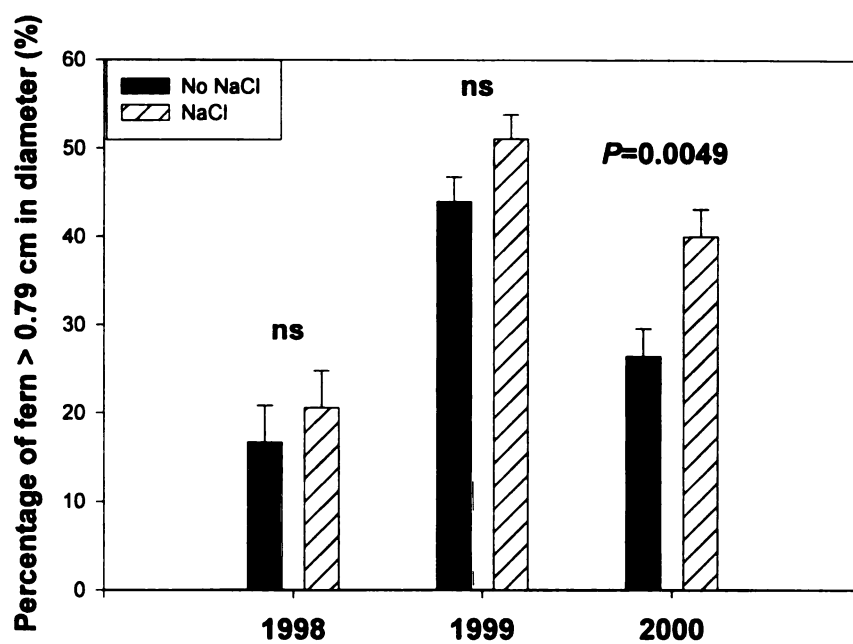


Figure 9. Percentage of asparagus stalks > 0.79 cm in diameter in research plot at M.S.U. Botany and Plant Pathology Farm 1998-2000. Error bars represent standard error.

In the commercial field plots in Oceana County, no significant differences were found in sodium levels in the top 15 cm of soil (see appendix). Significantly higher sodium levels were found in the 1120 kg NaCl (51 ppm) and 1120 kg NaCl + lime treatments (56 ppm) than in any other treatment in the 15-30 cm soil level. In addition, the 560 kg NaCl treatment showed significantly higher sodium levels (36 ppm) than control (24 ppm), lime (24 ppm) or 560 kg NaCl + lime (29 ppm) treatments. In general, increasing levels of salt application led to increased sodium levels in the soil both with and without lime and at both soil levels. Analysis of sodium levels at the two fall samplings (1998 and 1999) did not show significant increases in sodium levels in either of the commercial fields at either soil level in any treatment (data not shown). Significant increases in sodium levels (+35 ppm) were shown from these sampling dates from the 15-30 cm level in the heavier soil at the MSU Botany and Plant Pathology Farm. pH, magnesium and calcium levels were significantly affected by lime applications in the commercial fields. Significantly higher pH levels were found in the 1120 kg NaCl + lime (6.50), lime (6.55), and 560 kg NaCl + lime (6.58) treatments than in the 560 kg NaCl treatment (6.19) and significantly higher levels were found in the lime treatment, than in the 1120 kg NaCl treatment (6.27) in the top 15 cm of soil in combined results from both fields. Combined results also show that in the treatment that received 1120 kg NaCl + lime had significantly higher pH levels (6.26) in the 15-30 cm soil level than either the control treatment (5.87) or the 560 kg NaCl treatment (6.00). Differences among treatments in potassium levels were not significant in either the top 15 cm or 15-30 cm level of soil. Significantly greater levels of calcium were found in the top 15 cm of soil in

the 560 kg NaCl + lime (970 ppm) and the 1120 kg NaCl + lime (979 ppm) treatments than in the 560 kg NaCl treatment (755 ppm). No significant differences in calcium level were found in the 15-30 cm soil level. Significantly higher magnesium levels were found in the top 15 cm of soil in the lime (168 ppm) and 560 kg NaCl + lime (162 ppm) treatments than in the untreated control (80 ppm) in the declining field, but no significant differences were found in the healthy field at this soil level. Significantly higher levels of magnesium were also found in the lime treatment (154 ppm) than in the 560 kg NaCl treatment (116 ppm) at the 15-30 cm soil level.

DISCUSSION

Fusarium crown and root rot has become limiting to asparagus production in Michigan as growers are forced to rely more on fields that had been previously cropped to asparagus. In our study, NaCl treatments suppressed disease in a research plot at the MSU Botany and Plant Pathology Farm verifying earlier reports (Elmer, 1989; Elmer, 1992). However, NaCl treatments did not significantly affect disease in commercial replant fields.

Yield increases with NaCl applications are closely related to its ability to suppress Fusarium disease (Elmer, 1990b; Elmer, 1992; Elmer, 1995; Elmer and LaMondia, 1997). The effectiveness of this treatment may be correlated to the amount of disease pressure in the field. Experiments conducted with NaCl and its effect on asparagus yield have been conducted in Connecticut, where asparagus production was abandoned because Fusarium decline was so severe (Elmer, 1992). Our own study showing the effectiveness of salt

was conducted in a severely declined plot at the Botany and Plant Pathology Farm at MSU. Yield increases have yet to be demonstrated in commercial fields, which are generally taken out of production once yield becomes limited by disease. It is possible that salt treatments will show significant yield effects with continued applications as commercial field plots decline further. Similarly, lime applications, which have been shown to decrease Fusarium disease on other crops (Bell, 1989; Jones et al., 1989) did not significantly affect yield or stalk diameter.

In the commercial fields, treatment areas were separated by at least 3.05 m on all sides. However, sodium levels and pH levels increased over time in plots not treated with NaCl or lime. Increases in pH may be partially explained by the fact that each of the fields was accidentally treated with a single lime application during the experiment by the growers. There was some movement of sodium from treated into untreated plots over time, especially in the lower strata of soil. Although data from soil tests seems to suggest that much of the movement occurred while the crowns were dormant, the fact that there was some salt movement into untreated plots may have had an effect on the results of this study. This may partially explain why yield differences in these plots have not been shown, and suggests that in future tests, treated areas should be separated by a greater distance.

The effect of NaCl on the nutrient content and pH of soils, on rotation crops and groundwater, and the potential development of soil sodicity are all concerns that must be carefully considered. Our results did not show decreases in the levels of magnesium, potassium, or calcium with NaCl applications, and salt does not appear to be affecting

pH. Our analyses also suggest that salt moves through the soil profile quickly in Michigan asparagus fields, and we did not show significant buildup of sodium in soils in commercial fields between October 1998 and October 1999. Mechanical analysis have shown that these fields can have a sand content of 85% or more, and NaCl may leach easily through the profile with rainwater. Because there is little structure in these soils to begin with, sodicity buildup is less of a concern than with other soil types. Furthermore, no changes in bulk density of soil have been detected in previous experiments with NaCl at these levels (Elmer, 1992). Because NaCl does not appear to build up in soils, and because asparagus fields are often left fallow for a season after being taken out of production, the affect of salt on rotation crops may not be of great concern in this region. Furthermore, no weed control has been observed with direct salt applications at these levels (Elmer, 1992) suggesting that the affect of salt applications on other plants grown in these fields a year or more later may be relatively benign. Caution must be exercised, however, in drawing these conclusions based on less than three years of soil data, and observations with particular weeds. We have also shown, with soil tests in the sandy loam in the research plots at M.S.U., that salt buildup is more of a concern in heavier soils. It may be wise for asparagus growers to test their soils for sodium levels after repeated salt applications, and to avoid salt-sensitive crops such as carrots or fruit trees in their rotations for several years. Salt buildup, if it does become a problem, can be treated with applications of gypsum or by irrigating the affected soils (Brady, 1990).

Because of the potential problems with NaCl applications to commercial field soils, we also compared the potential of alternative forms of chloride salt, that could also

supply nutrients necessary for plant health, to that of NaCl in disease suppression. The effectiveness of NaCl in suppressing disease in our studies using agar filled test tubes confirms earlier reports (Elmer, 1989, Elmer, 1992). NaCl was more effective than other forms of chloride salt in controlling disease caused by *F. proliferatum*, and plants treated with NaCl had lower levels of disease caused by *F. oxysporum* f. sp. *asparagi* than any other treatment. These results suggest that disease control is not simply the result of increased chloride nutrition.

Calcium has been shown to be important in disease resistance in some plant-disease systems by protecting pectate from maceration, and ameliorating the effects of toxins (Huber, 1990). The increased disease control with CaCl_2 shown in tests against the pathogen *F. proliferatum* may be due to the effect of increased calcium or to an unknown disease control mechanism involving chloride. Manganese has been implicated as an important element in disease control in a number of different plant systems (Huber, 1990). Its lack of effectiveness in these tests may be due to its immobility at the relatively high pH of the agar (6.0). The increased chloride nutrition provided did not appear to affect disease in this treatment. It is interesting to note that NH_4Cl -treated plants were more diseased than control plants in tests with both pathogens. In other systems, it has been suggested that increased nitrogen can make plants more susceptible to disease by increasing succulence, and decreasing physical barriers to invasion (Huber, 1990). Elmer (1989) has also shown that plants fertilized with ammonium nitrogen were more susceptible to disease than those fertilized with nitrate nitrogen. It appears that increased ammonium levels increased the plants susceptibility to the pathogens in these studies.

The results of greenhouse experiments with different forms of chloride salt generally confirm the results of the test tube studies. No other form of chloride salt tested was more effective in controlling disease or showed greater fresh weight or roots or shoots than NaCl when equivalent levels of chloride were applied. In these experiments, NH_4Cl again seemed to favor disease.

Although we did not find significant increases in plant health with NaCl applications in commercial fields, our research has confirmed the potential of NaCl and other chloride salts for use in disease control. Future studies on the long term effects of salt applications to commercial fields, both on plant health, and soil could add significantly to our present understanding of its usefulness. Despite considerable effort (Elmer, 1990a; Elmer, 1995; Elmer and LaMondia, 1997), the mechanism by which NaCl suppresses disease is not yet clearly understood, and continued research in this area could be beneficial to the effort to find an effective control for *Fusarium* crown and root rot of asparagus. It is also possible that higher levels of KCl or CaCl_2 could have a greater effect on disease suppression, and since the potentially negative effects of NaCl are not a concern with these salts, further experimentation with these salts should not be discounted, since some disease suppression was detected with each.

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SECTION II

THE USE OF FUNGICIDES AND BIOLOGICAL CONTROLS IN THE SUPPRESSION OF FUSARIUM CROWN AND ROOT ROT OF ASPARAGUS

ABSTRACT

Growth chamber, greenhouse, and field experiments were conducted with fungicides and biological controls including experimental non-pathogenic isolates of *Fusarium oxysporum* to test their ability to control disease caused by *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. In greenhouse studies with asparagus seedlings in soil, *Trichoderma harzianum*, benomyl, and fludioxonil treatments increased root weight and decreased root disease compared to the infested control when a low level of *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* was used. The fungicide fludioxonil limited plant death caused by *Fusarium* spp. at high inoculum levels, while *T. harzianum* was not effective. Non-pathogenic *F. oxysporum* isolates were effective in limiting *Fusarium* disease on asparagus seedlings in culture tubes, although isolates differed in their ability to control disease caused by *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. In greenhouse studies no significant differences in plant death were found between asparagus plants growing in media infested with *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* and left untreated, and those treated with non-pathogenic *F. oxysporum*. Our attempt to test fungicides and biological control products under commercial field conditions was not successful due to low disease pressure.

INTRODUCTION

The fungal pathogens *Fusarium proliferatum* (T. Matsushima) Nirenberg and *Fusarium oxysporum* (Schlect.) f. sp. *asparagi* (Cohen & Heald) cause Fusarium crown and root rot, the primary factor in asparagus early decline which is an economically important disease in Michigan and throughout the world (Blok et al., 1997; Elmer et al., 1996; Faloon and Fauser-Kevern, 1996; Fantino, 1990; Grogan and Kimble; Tu et al., 1990). Efforts to manage the disease through the development of resistant cultivars have met with limited success (Elmer et al., 1997; Stephens et al., 1989). Although fungicides have not provided adequate disease suppression (Di Linnea and Foletto, 1990; Lacy, 1979), the chemical 2-(4-Thiazolyl)benzimidazole (Novartis Crop Protection Inc., Greensboro, NC) was effective in New Zealand study (Faloon and Fauser-Kevern, 1996) but is not registered for use on asparagus in the U.S. Currently, fungicides are not used to manage the disease in Michigan.

Asparagus is a deep-rooted perennial, making application of chemical or biological controls difficult once the crop is established. Commercial asparagus is seeded and grown in specialized seedling nurseries for one year before crowns are dug out and planted into production fields. Infection of seedlings while in the nursery predisposes a crop to severe decline (Di Lenna and Foletto, 1990; Lacy, 1979) making the seedling stage a critical time to apply control measures for Fusarium crown and root rot.

Researchers have investigated the potential of biological control agents to suppress Fusarium disease on a number of crops (Beale and Pitt, 1990; Cassini et al., 1985; Davis, 1967; Hervas et al., 1995; Larkin et al., 1996; Mandeel and Baker, 1991;

Marois, 1993; Oyarzun et al., 1994) The fungus *Trichoderma harzianum* has been shown to be effective against *F. oxysporum* f. sp. *asparagi* on asparagus seedlings in greenhouse trials (Arriola, 1997). Nonpathogenic strains of *F. oxysporum* have also consistently shown effectiveness against *Fusarium oxysporum* f. sp. *asparagi* in growth chamber and greenhouse studies, and were shown to be effective in one field study, but not in others (Blok et al., 1997; Damicone and Manning, 1982; Tu et al., 1990).

The objective of this study was to determine whether fungicides and biological controls including experimental non-pathogenic isolates of *F. oxysporum* could control disease caused by *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* in growth chamber, greenhouse, and field experiments.

MATERIALS AND METHODS

Greenhouse experiments with fungicides and a biological control product.

Asparagus seeds were sterilized by spinning on a magnetic stir plate for 24 hours in a mixture of 100 ml acetone and 2.5 g benomyl (5 g Benlate 50 DF, E. I. du Pont de Nemours & Co. Inc., Wilmington, DE), rinsing them 3 times each in acetone then distilled water, then spinning for 1 hour in 1.05% sodium hypochlorite (20% household bleach), rinsing 5 times with sterile distilled water and blotting on a sterile paper towel (Damicone et al., 1981; Elmer and Stephens, 1989). Seeds were germinated in the dark on sterile water agar petri plates, and planted into 96 cc plug trays filled with Bacto High Porosity Professional Planting Mix (Michigan Peat Co., Houston, TX) as soon as hypocotyls emerged.

Plants were grown for 14 days, then clean, uniform seedlings were transferred to 512 cc plastic pots filled with steam sterilized sandy loam soil. Soil for treatments containing *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* was infested by growing cultures of the pathogens on sterile millet seed for two weeks, drying the seed in sterile paper bags, grinding the millet, and mixing equal parts infested with each pathogen into steam sterilized soil in a cement mixer for 4 hours (Wacker, 1988). Separate experiments were performed with a high (200,000 cfu/g soil) and low concentration (12,600 cfu/g soil) of pathogen inoculum. Each experiment was conducted twice.

Treatments included an uninfested control (untreated) mixed with sterile ground millet seed, an infested control, and infested soil drenched with either benomyl (Benlate 50 DF at 0.6g/L), 2-(4-Thiazolyl)benzimidazole (Mertect 340-F at 2.34 ml/L), fludioxonil (Maxim 1.56 ml/L), or *T. harzianum* (Root Shield at 8.98 g/L). The fungicide 2-(4-Thiazolyl)benzimidazole was excluded from the experiment with low pathogen concentrations because the manufacturer does not plan to label this product for use on asparagus (personal communication, Novartis Crop Protection Inc.). All drenches were applied two days after planting.

Effectiveness of the treatments in the experiments with a high inoculum level was evaluated by counting the number of dead plants weekly, and effectiveness of the treatments in the experiment with a low inoculum level was evaluated by measuring the fresh weight of roots and shoots, and visually estimating the percentage of the root system covered with lesions.

Evaluation of nonpathogenic *F. oxysporum* isolates for control of Fusarium

Crown and Root Rot of asparagus. Four commercial asparagus fields in Oceana Co., Michigan, and a research field on the campus at the Michigan State University (M.S.U.) Botany and Plant Pathology farm were sampled for *Fusarium* spp. isolates between October 1987 and April 1988. Isolates were collected from asparagus crown and root tissue (17 isolates), the vascular tissue at the base of asparagus ferns (97 isolates), and soil from asparagus fields (224 isolates). All plant tissue was rinsed in 20% household bleach for 30-60 seconds, rinsed in sterile distilled water, and placed on Komada's media (Komada, 1975) which is selective for *Fusarium* spp. Cultures were obtained by diluting the soil onto Komada's media and incubating for 5-7 days at 25°C. Fungal cultures exhibiting vigorous fluffy mycelial growth were transferred to carnation leaf agar (CLA) and allowed to grow for 10-14 days under fluorescent plant lights. CLA plates were flooded with sterile distilled water, scraped with a scalpel to dislodge spores, and poured into sterile 100 ml Erlenmeyer flasks through three layers of sterile cheesecloth. Spore solutions were diluted to approximately 1×10^5 spores per ml, and five drops of each solution were placed on tilted water agar plates and allowed to run to the bottom. Water agar plates were incubated for 8 to 12 hours at room temperature, then single germinated macroconidia were picked from plates with a flattened sterile dissecting needle in a laminar flow hood with the use of a dissecting microscope and transferred to CLA. After 10 to 14 days, colonies identified as *F. oxysporum* according to the criteria of Nelson et al. (1983) (227 isolates) were tested for pathogenicity in a method adapted from Stephens and Elmer (1988). Spore solutions (1×10^6 spores/ml) were made from CLA plates, and 0.5 ml of spore solution from each isolate was added to each of five sterile, capped, 16 x

150 mm test tubes containing two-week-old asparagus seedlings grown in 6.5 ml of Hoagland's solution agar. Asparagus seeds (cv. 'Mary Washington', Rupp Seeds, Wauseon, OH) were sterilized according to the methods of Damicone et al. (1981), and germinated on water agar plates, then aseptically placed in the culture tubes once their radicals had emerged. Cultures were allowed to infest the seedling tubes for 28 days in a growth chamber under fluorescent lights (16 hours of light/day) at temperatures of 25°C (day), and 20°C (night). Disease symptoms were evaluated by visually estimating the percentage of the root system exhibiting lesions or collapse. Cultures in which each of the five seedlings exhibited disease symptoms on less than 5% of their roots were considered non-pathogenic, and were put into long term storage on silica gel according to the method described by Windels et al. (1988).

Forty-one fungal cultures isolated from Michigan asparagus fields and identified as nonpathogenic forms of the fungus *F. oxysporum* were tested for their potential to control Fusarium crown and root. Two additional *F. oxysporum* isolates found to be nonpathogenic on asparagus, but pathogenic on celery and cyclamen, respectively, were also included in this screen. Each of the isolates was added to 8 two-week-old 'Mary Washington' asparagus seedlings grown in Hoagland's solution agar in the manner described previously. Cultures were allowed to colonize the tubes for 7 days, then a 0.5 ml of spore solution (1×10^6 spores/ml) of a virulent, pathogenic isolate of *F. oxysporum* f. sp. *asparagi* isolated from a diseased asparagus crown at the M.S.U. Botany and Plant Pathology Farm was applied to four tubes colonized by each isolate, and 0.5 ml of a spore solution (1×10^6 spores/ml) of a virulent, pathogenic isolate of *F. proliferatum* isolated

from the base of a diseased asparagus stalk from a commercial asparagus production field in Oceana Co., MI was applied to the other four tubes. The pathogens were allowed to infest the plants for 28 days, after which time the seedlings were evaluated for root rot severity by a visual estimation of the percentage of the root system exhibiting lesions or collapse.

Five isolates were chosen from this test for further study based on their potential to control both *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. Each had an average root rot rating of 25% or below when challenged with each *Fusarium* pathogen, while most isolates allowed a high level of disease in the presence of either one or both pathogens. These isolates, designated D-1, S-13, E-12, F-21, and 2-5 were then evaluated in a more rigorous experiment conducted in the growth chamber. Each isolate was added to 15 two-week-old seedlings in tubes as 0.5 ml of a 1×10^6 spores/ml solution in the manner previously described, allowed to colonize for 7 days, then challenged with the virulent isolate of *F. oxysporum* f. sp. *asparagi*. A control was included in the experiment by adding 0.5 ml of sterile distilled water to one set of tubes, instead of a spore solution from a non-pathogenic isolate. The experiment was arranged in a completely randomized design, and conducted twice in a growth chamber with fluorescent lights on a 16-hour cycle, and with temperatures of 25°C (day) and 20°C (night). The experiment was conducted in the same manner for the pathogen *F. proliferatum*. Results of all experiments were evaluated by visually estimating the percentage of the root system that was discolored or collapsed. Dead plants were considered to be 100% infected.

Greenhouse experiments were performed to test the effectiveness of non-pathogenic *F. oxysporum* isolates in a potting mixture. Seedlings of cultivar Mary Washington (Rupp Seeds, Wauseon, OH) were disinfested by the method previously described and planted into 96 cc plug trays filled with Bacto High Porosity Professional Planting Mix (Michigan Peat Co., Houston, TX). Plants were grown for three weeks, then clean, uniform seedlings were transferred to 512 cc plastic pots filled with steam sterilized sandy loam soil and Bacto High Porosity Professional Planting Mix in a 5:1 ratio either infested with *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*, infested with these pathogens and non-pathogenic isolates of *F. oxysporum*, or left uninfested. *Fusarium* spp. were added to the soil by mixing infested ground millet seed into the potting mixture in a cement mixer for two hours. Pathogens were added to the potting mixture as *F. oxysporum* f. sp. *asparagi* isolate 2-7 and *F. proliferatum* isolate P-67, both from Michigan asparagus fields at 1 g/L of soil each. Non-pathogenic *F. oxysporum* isolates (D-1, F-21) were added to pathogen-infested potting mixture. Eighteen plants were used for each treatment and pots were arranged in a completely randomized design. The plants were fertilized twice weekly with Peters 20-20-20 fertilizer (Grace Sierra Horticultural Products Company, Milpitas, CA) and were grown for six weeks before the number of dead plants in each treatment was counted. The experiment was conducted twice.

Field experiments with fungicides and biological controls. Plots were established in Oceana County, Michigan in two non-adjacent commercial fields previously cropped to asparagus, and having a history of *Fusarium* seedling blight.

Asparagus seeds (Jersey variety) were sown into 1.22 m beds in three rows spaced 45.72 cm apart at a rate of one seed every 3.18 cm (8.97 kg seed/ha) using a Gaspardo vacuum planter (Gaspardo Co., Morsano, Italy). Treatment plots consisted of a 3.05 m section of the center row on each bed. Five feet of buffer were left between each treatment within a row, and data were taken from a 2.13 m section in the center of each plot. Treatments were arranged in a randomized complete block design with six blocks.

Treatments were applied as drench applications made to a 22.86 cm wide section of the seedling row (except where noted), applied immediately following planting, and consisted of the following: *i*) an untreated control (water only applied), *ii*) the fungicide fludioxonil at a high rate (42.06 l/ha Maxim 4FS, Novartis Crop Protection Inc., Greensboro, NC), *iii*) fludioxonil at a low rate (2.03 kg/ha Medallion, Novartis Crop Protection Inc., Greensboro, NC), *iv*) fludioxonil at a high rate (42.06 l/ha Maxim 4FS) applied every 30 days until harvest, *v*) the fungicide fludioxonil at a low rate (2.03 kg/ha Medallion) applied every 30 days until harvest, *vi*) the biological control fungus *T. harzianum* (24.64 kg/ha RootShield, BioWorks Inc., Geneva, NY), *vii*) the fungicide benomyl (1.17 l/ha Benlate 50 DF, E. I. du Pont de Nemours & Co. Inc., Wilmington, DE), *viii*) non-pathogenic isolates of the fungus *F. oxysporum* (infested, ground pearl millet seed 429.25 kg/ha) broadcast over the treatment row and worked into the soil 20 cm deep, *ix*) uninfested ground pearl millet seed (429.25 kg/ha) broadcast over the treatment row and worked into the soil, *x*) and fludioxonil (0.11 ml/kg Maxim 4FS) applied as a seed treatment.

At the end of the season (7 September, field 1; 15 September, field 2), plants were

dug, roots and shoots weighed, plant height measured, the number of shoots per plant counted, and the root system visually evaluated for the total root area (%) exhibiting lesions.

Statistical Analysis. All analyses were conducted using the Mixed Model procedures of SAS statistical analysis software (SAS Institute Inc., Cary, NC), except for those involving the number of dead plants, which were analyzed using the Exact procedure of LogXact statistical analysis software (Cytel Software Corporation, Cambridge, MA). Before the analyses were carried out, data on the percentage of root system exhibiting lesions (growth chamber and greenhouse experiments) and fresh weight of shoots (greenhouse experiments) were transformed using the arcsine square root and log transformations respectively (Sokal and Rohlf, 1969). Differences between treatments were determined by using Least Squared Means comparisons with Bonferroni adjustment factor ($P < 0.05$). Data from the two replications of each experiment were presented together unless significant experiment by treatment interactions were found.

RESULTS

Greenhouse experiments with fungicides and a biological control product. A high level of pathogen inoculum resulted in plant death in all treatments except for the uninfested control (no treatment) (Figure 10). By week five, all plants were dead in both the infested control and *T. harzianum* biological control treatments, significantly more than in any other treatment. An average of two plants (20%) died over the five weeks in the fludioxonil treatment which was comparable to uninfested control and 2-(4-

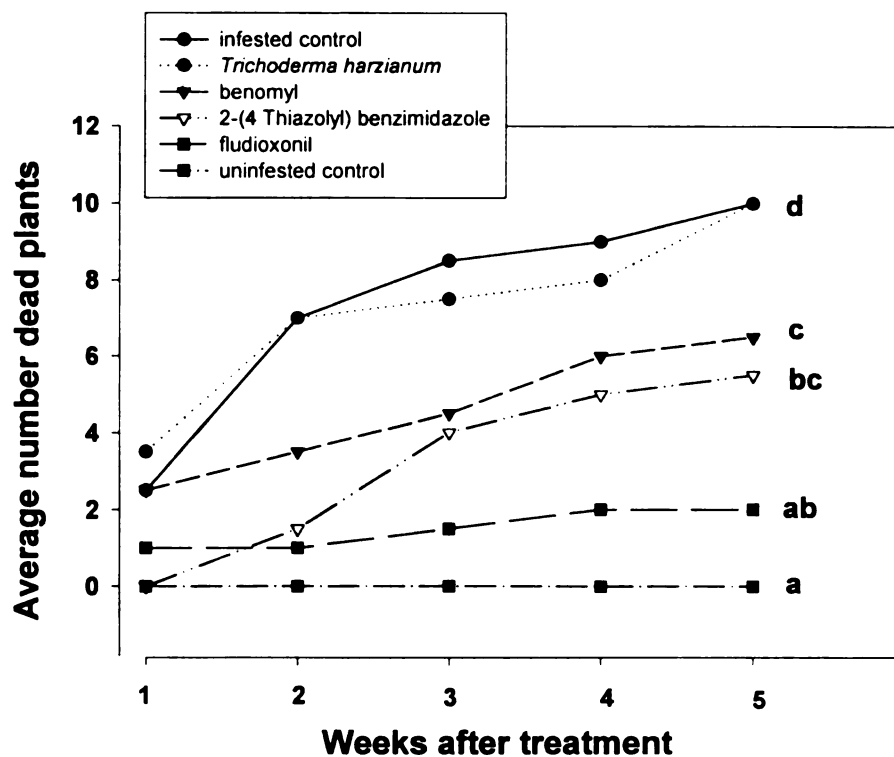


Figure 10. Average number of asparagus plants killed in two greenhouse experiments when grown in soil infested with a high level of *F. proliferatum* and *F. oxysporum* f. sp. *asparagi* and treated with a chemical or biological control product. Letter notation indicates significant differences between treatments.

Thiazolyl)benzimidazole treatments. An average of 6.5 (65%) plants were dead after five weeks with the benomyl treatment, significantly more than either the fludioxonil treatment or the uninfested control.

When the lower pathogen inoculum level was used, all plants survived, regardless of treatment. Fresh shoot weights for the uninfested control varied between the two low inoculum experiments, resulting in a significant experiment by treatment interaction. In the first experiment, the only significant difference observed was in the infested control, which exhibited lower mean shoot weights than the uninfested control (Figure 11). Fresh shoot weight of the treatments did not differ significantly from either control. In the second low inoculum experiment, the shoot weight of the uninfested control was significantly greater than that of the fludioxonil treatment and the infested control. Significant differences among other treatments were not noted. Because the analysis of root weight and root system exhibiting lesions (%) showed no significant experiment by treatment interaction, the data for each of these parameters were combined. Root weight was significantly greater for the uninfested control than for any other treatment or the infested control (Figure 12). Root weights for all treatments were significantly greater than the infested control, but were not significantly different from each other. The uninfested control plants had a lower percentage of root system with lesions than any other treatment (Figure 13). All treatments resulted in reduction of root lesions compared to the infested control, but did not differ significantly from one another.

Growth chamber and greenhouse studies with non-pathogenic forms of *F. oxysporum*. All five of the selected non-pathogenic *F. oxysporum* isolates significantly

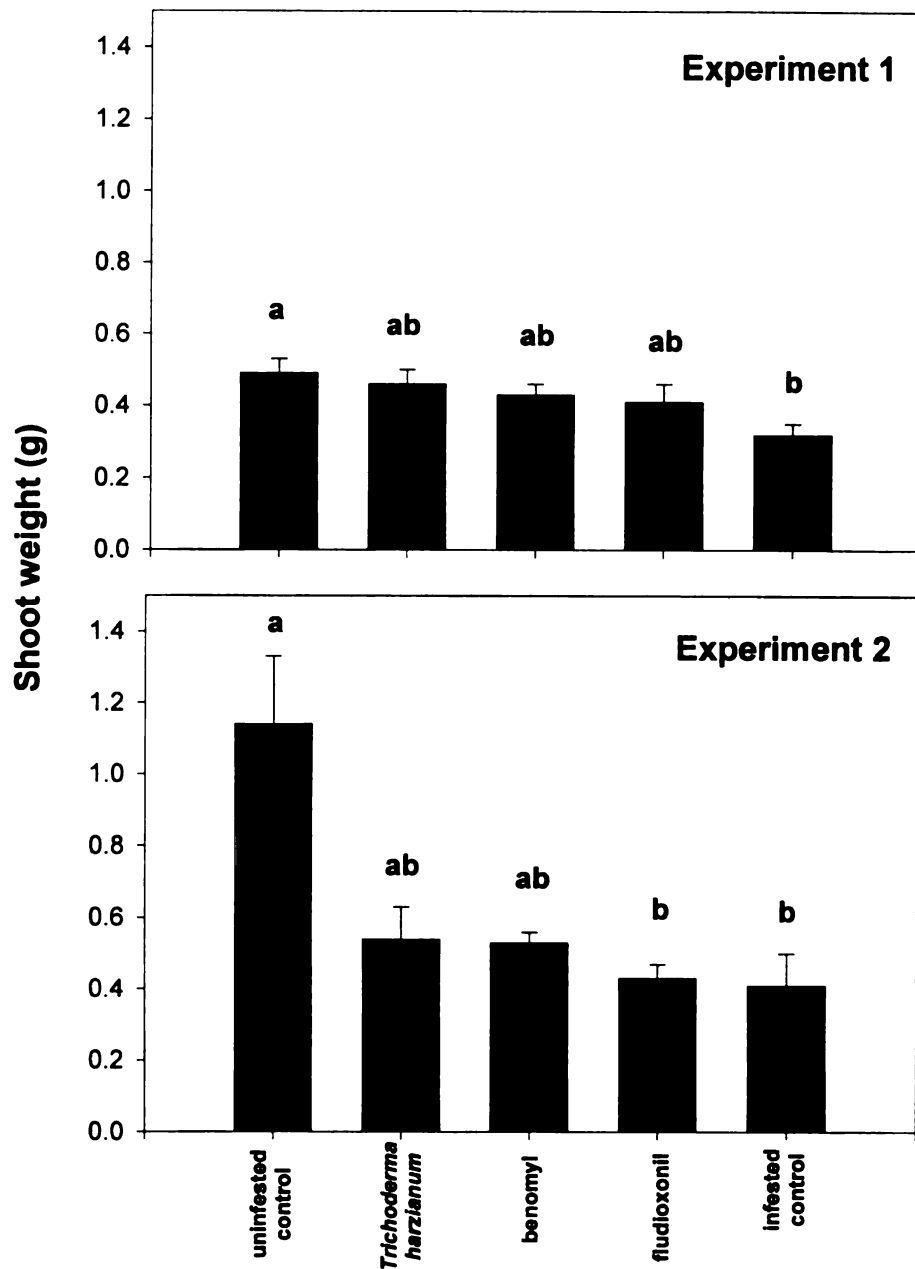


Figure 11. Shoot weight of asparagus seedlings from two greenhouse experiments when grown in soil infested with a low level of *F. proliferatum* and *F. oxysporum* f. sp. *asparagi* and treated with a chemical or biological control product. Error bars represent standard error, and letter notation represents significant differences in L. S. means comparisons.

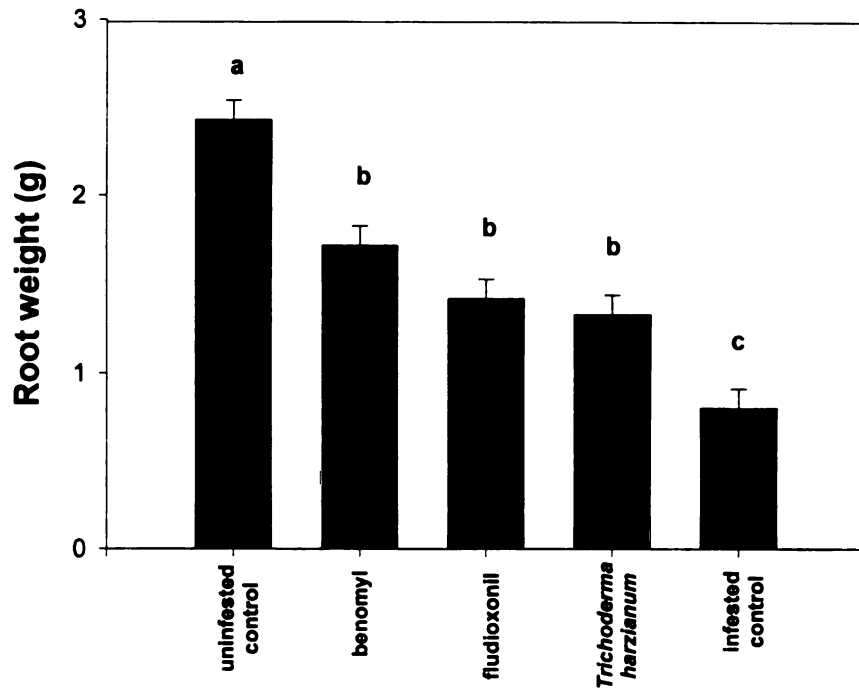


Figure 12. Root weight of asparagus seedlings from two greenhouse experiments when grown in soil infested with a low level of *F. proliferatum* and *F. oxysporum* f. sp. *asparagi* and treated with a chemical or biological control product. Error bars represent standard error, and letter notation indicates significant differences in L. S. means comparisons.

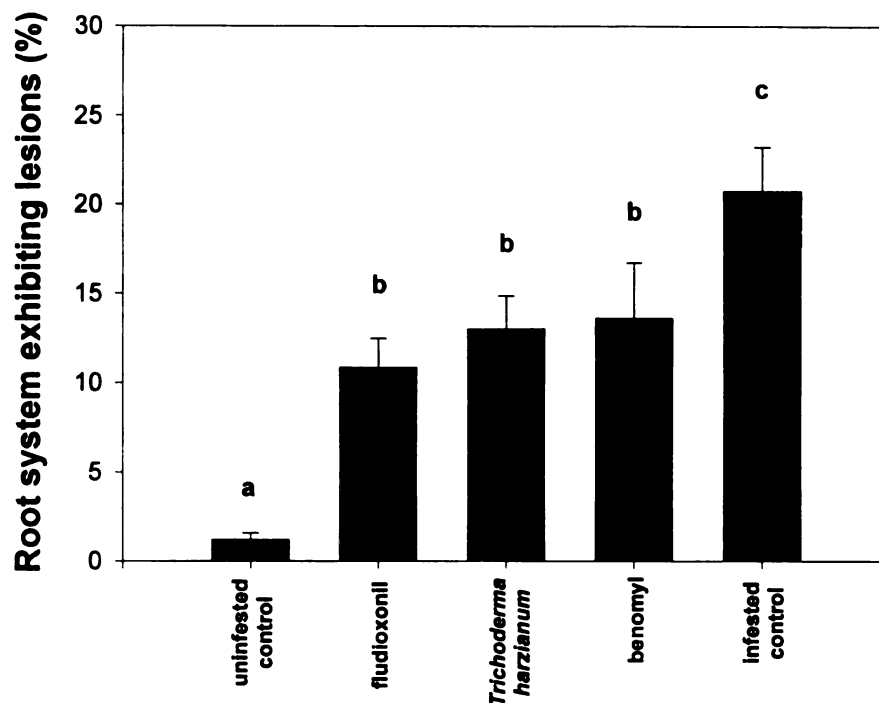


Figure 13. Roots of asparagus seedlings exhibiting lesions (%) in two greenhouse experiments when grown in soil infested with a low level of *F. proliferatum* and *F. oxysporum* f. sp. *asparagi* and treated with a chemical or biological control product. Error bars represent standard error, and letter notation indicates significant differences in L. S. means comparisons.

reduced root disease compared to the infested control in two growth chamber experiments with *F. oxysporum* f. sp. *asparagi* (Figure 14). Isolate D-1 was significantly more effective at reducing root lesions than all other isolates in experiment 1, and significantly more effective than all isolates except for S-13 in experiment 2. Isolate S-13 was significantly more effective than isolates 2-5 and E-12 in experiment 2 only.

No significant treatment by experiment interaction occurred in trials with the pathogen *F. proliferatum*, thus the data from these experiments were combined (Figure 15). All isolates showed significant disease reduction compared with the infested control, although isolate D-1 was significantly less effective than the other isolates. Isolate F-21 showed the lowest mean disease rating of all the isolates.

In both greenhouse studies no significant differences in plant death were found between asparagus plants growing in media infested with *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* and left untreated, and those infested with pathogenic *Fusarium* spp. as well as non-pathogenic isolates. In experiment 1, 9 plants (50%) were killed in both the infested control and the non-pathogen treatment, and in experiment 2, 12 plants (66.7%) were killed in the infested control, while 13 (72.2%) were killed in the non-pathogen treatment. No plants were killed in the uninfested control in either experiment.

Field trials using fungicides and biological controls. Although the trials were conducted in seedling fields considered to be highly infested with pathogenic *Fusarium* spp., mean root lesions did not exceed 3% in the untreated control or the treatment plots in either field. Root infection (%) was not significantly different among treatments, and no consistent trends among the measured parameters were observed between the two

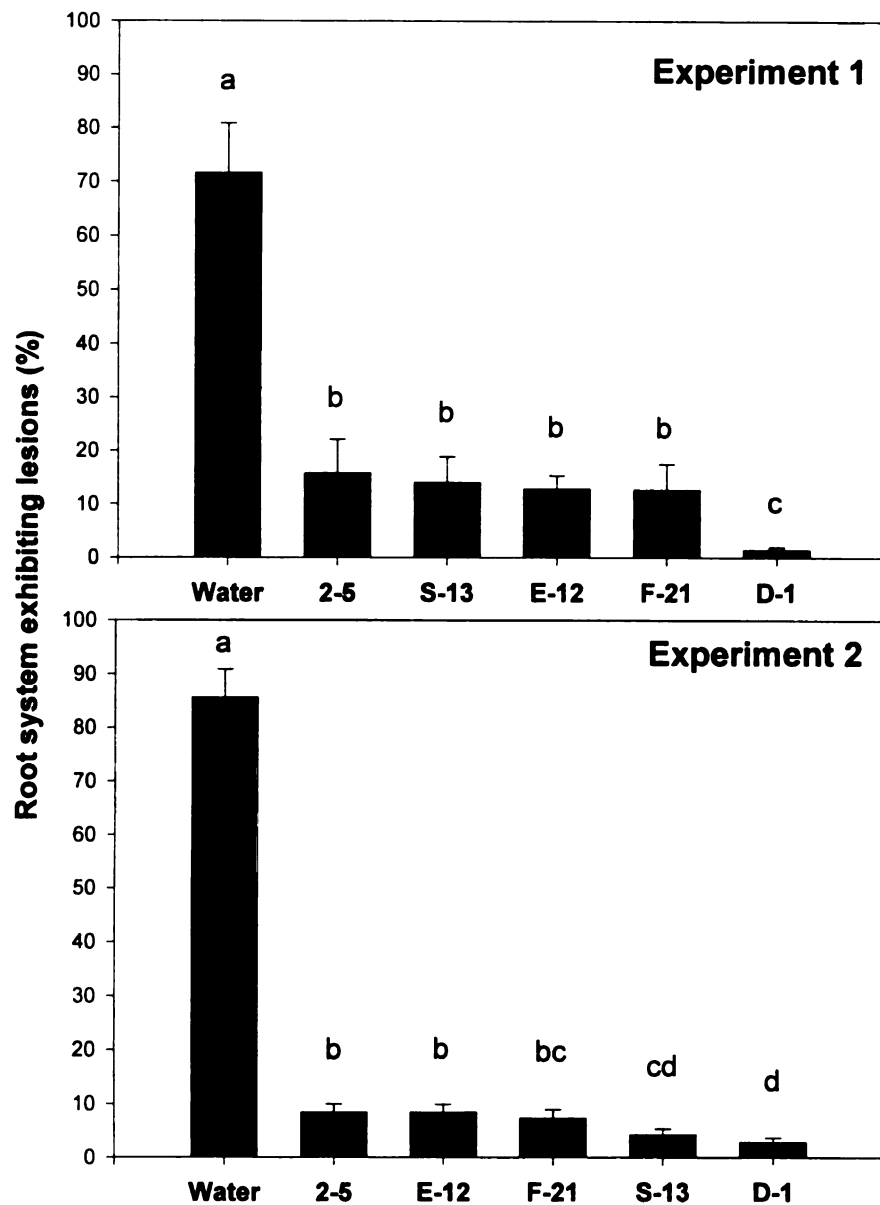


Figure 14. Root system of asparagus seedlings exhibiting lesions (%) in two growth chamber experiments when grown in culture tubes infested with *F. oxysporum* f. sp. *asparagi* and treated with non-pathogenic *F. oxysporum*. Error bars represent standard error, and different letter notation indicates significant differences in L. S. Means comparisons.

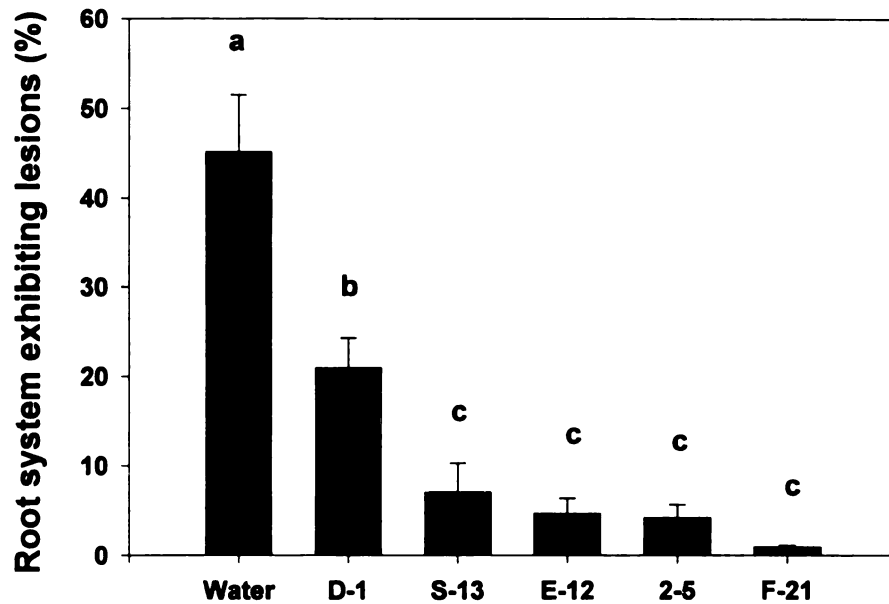


Figure 15. Root system of asparagus seedlings exhibiting lesions (%) in two growth chamber experiments when grown in culture tubes infested with *F. proliferatum* and treated with non-pathogenic *F. oxysporum*. Error bars represent standard error, and different letter notation indicates significant differences in L. S. means comparisons.

replicate fields (see appendix).

DISCUSSION

Fusarium spp. pathogenic to asparagus are found in all Michigan soils, and *Fusarium* decline has become a serious production limitation as pathogen populations have built up in fields previously planted in the crop (Elmer et al., 1996; Hartung et al., 1990). Our goal to develop an effective management strategy utilizing a commercially available biological control product (*T. harzianum*), non-pathogenic isolates of *F. oxysporum*, and fungicides has met with limited success.

Non-pathogenic *F. oxysporum* isolates were effective in limiting *Fusarium* disease on asparagus seedlings grown in culture tubes, although isolates differed in their ability to control disease caused by *F. proliferatum* and *F. oxysporum* f. sp. *asparagi*. Isolate D-1 was the most effective in controlling disease caused by *F. oxysporum* f. sp. *asparagi*, but was the least effective isolate when tested against *F. proliferatum*. Vegetative compatibility testing has shown that *F. oxysporum* f. sp. *asparagi* is a diverse group. In one experiment, 26 strains of this pathogen were placed into 8 vegetative compatibility groups, while 34 strains appeared to belong to unique compatibility groups (Elmer and Stephens, 1989). This kind of diversity is rare among pathogenic *Fusarium* spp., and non-pathogenic isolates may have varied activity against different isolates in a single pathogenic formae.

When non-pathogenic isolates which effectively limited both *F. proliferatum* and *F. oxysporum* f. sp. *asparagi* in culture tube tests were mixed, and tested in greenhouse

studies with asparagus seedlings grown in soil, they were not effective in controlling disease. It is likely that the high level of pathogen inoculum (180,000 cfu/g soil) used in this experiment overwhelmed and out-competed the non-pathogens. Hartung et. al. (1990) found that levels of *Fusarium* in asparagus field soils in Michigan were generally much lower than this (between 0 and 60,000 cfu of *Fusarium* spp. per gram of sieved soil). Furthermore, Hartung et. al.'s (1990) numbers represent both pathogenic, and non-pathogenic *Fusarium* spp. found in soil, although *F. proliferatum* isolates were likely underestimated because they are found more consistently on plant residue than in soil. It is also possible that because the non-pathogenic isolates did not have a week to colonize the asparagus seedlings, as in the growth chamber tubes, they could not provide adequate protection. The added complexity of the soil system, or the method of inoculation could have accounted for the failure of these isolates to control disease in the greenhouse system as well.

Additional greenhouse studies with asparagus seedlings using fungicides and a biological control product indicated that *T. harzianum*, benomyl, and fludioxonil increased root weight and decreased root disease compared to the infested control when a low level of *F. proliferatum* and *F. oxysporum* f. sp. *asparagi* was used. Although shoot weights did not differ significantly among these treatments, the trends were similar to those for the root data. The fungicide fludioxonil limited disease caused by *Fusarium* spp. at both low and high inoculum levels. *T. harzianum*, however, was not effective when a high rate of inoculum was used, indicating that the effectiveness of this product may be dependent on the organism not being overwhelmed by the pathogen. Benomyl is

not currently labeled for use on asparagus in Michigan, and experiments by Lacy (1979) indicated that it was not effective in reducing disease when applied as a dip to one-year-old crowns from seedling nurseries.

Our attempt to test fungicides and biological control products under commercial field conditions was not successful. The low level of seedling infection in the fields where the treatments were tested may be attributed to environmental conditions, and may have been responsible for the absence of significant or consistent differences between the treatments. Previous research has suggested that dry conditions favor disease development in seedlings (Graham, 1955; Elmer et al., 1997). In addition, Michigan growers have observed that the critical time for seedling infection by *Fusarium* is during establishment (Jon Bakker, Director, Oceana County Asparagus Research Farm, Personal Communication). Rainfall during the early part of the season was 13.35 cm (27 April through 12 June), and total rainfall was 41.28 cm (27 April through 6 October).

Biocontrol activity of non-pathogenic *F. oxysporum* isolates has been recognized by others (Blok et al., 1997; Damicone and Manning, 1982; Tu et al., 1990). However, only Damicone and Manning (1982) were successful in demonstrating disease control in the field using uninfested, greenhouse grown seedlings dipped in 0.4% methyl cellulose containing non-pathogenic *F. oxysporum* spores ($4\text{--}6 \times 10^6$ spores/ml). Both Blok et. al. and Tu et. al. failed to find disease suppression in fields amended with soil containing non-pathogenic *Fusarium* spp., and an effective disease control product has not been developed for the field. Since studies by other researchers (Di Lenna and Foletto, 1990; Lacy, 1979) have suggested that *Fusarium* infection in seedling nurseries may be the most important factor in predisposing asparagus plantings to decline, and have not

demonstrated disease control with fungicides when crowns were already infected, this may be the best place to initiate root colonization by non-pathogenic species.

Future investigations on the use of non-pathogenic *F. oxysporum* should consider the optimal stage of plant development for non-pathogen colonization, different methods for infesting plants with non-pathogenic isolates, and the level of inoculum needed to obtain effective disease control. Using a mixture of avirulent strains may provide enhanced disease suppression against a range of pathogenic *Fusarium* isolates, and using native isolates may insure that inoculum is able to survive and proliferate in the conditions under which it may be used. New chemical and biological control products should be tested further for their effectiveness against *Fusarium* spp. pathogenic to asparagus, and integrated strategies for disease control, such as those developed for other *Fusarium* pathogens (Beale and Pitt, 1990; Chakravarty et al., 1990; Sivan and Chet, 1993) may be explored as well.

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APPENDIX

SECTION I

<u>Measured Parameter</u>	<u>Year</u>	<u>Treatment</u>					
		<u>Control</u>	<u>560 kg</u>	<u>1120 kg</u>	<u>Lime</u>	<u>560 kg & Lime</u>	<u>1120 kg & Lime</u>
pH Top	1998	5.59	5.66	5.53	5.75	5.78	5.69
	2000	6.28 abc	6.19 a	6.27 ab	6.55 c	6.58 bc	6.51 bc
pH Bottom	1998	5.54	5.68	5.58	5.58	5.57	5.53
	2000	5.87 a	6.00 a	6.11 ab	6.12 ab	6.16 ab	6.26 b
Potassium Top	1998	258	268	264	272	245	264
	2000	375 ns	374 ns	384 ns	343 ns	373 ns	391 ns
Potassium Bottom	1998	223	229	233	232	254	233
	2000	301 ns	306 ns	315 ns	290 ns	298 ns	293 ns
Calcium Top	1998	723	843	715	782	817	800
	2000	799 ab	755 a	826 ab	914 ab	970 b	979 b
Calcium Bottom	1998	711	798	744	730	671	711
	2000	587 ns	639 ns	641 ns	631 ns	601 ns	750 ns
Magnesium Top (Healthy)	1998	182	193	166	216	162	208
	2000	239 ns	196 ns	213 ns	228 ns	245 ns	234 ns
Magnesium Top (Declined)	1998	52	60	50	65	69	66
	2000	80 a	93 ab	85 ab	168 b	162 b	151 ab
Magnesium Bottom	1998	78	93	83	89	85	92
	2000	127ab	116 a	109 ab	154 b	141 ab	134 ab
Sodium Top	1998	3.8	4.8	5.8	8.8	7.5	7.8
	2000	26.9 ns	29.0 ns	35.7 ns	26.9 ns	27.4 ns	37.7 ns
Sodium Bottom	1998	9.1	9.0	6.6	3.7	6.0	6.3
	2000	24.1 a	35.7 b	51.4 c	23.8 a	29.3 a	55.6 c

Table 1. Mean data for soil samples from 4/98 (before any treatments were applied) and 5/00 (after two years of treatment) in Oceana County field plots, tables show combined data for both fields unless significant field by treatment effects were found, letter notation indicates significant differences between treatment means from 2000 with 1998 data used as a covariate.

<u>Year</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>
<u>pH Top</u>		<u>pH Bottom</u>		
1998	5.38	5.34	5.74	5.62
2000	5.98 ns	5.72 ns	6.20 ns	5.84 ns
<u>Year</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>
<u>Potassium Top</u>		<u>Potassium Bottom</u>		
1998	175	202	95	91
2000	230 ns	242 ns	110 ns	224 ns
<u>Year</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>
<u>Calcium Top</u>		<u>Calcium Bottom</u>		
1998	2618	2455	2800	2609
2000	2609 ns	2590 ns	2819 ns	2724 ns
<u>Year</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>
<u>Magnesium Top</u>		<u>Magnesium Bottom</u>		
1998	250	220	254	207
2000	249 ns	290 ns	240 ns	269 ns
<u>Year</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>	<u>NaCl Applied</u>	<u>No NaCl Applied</u>
<u>Sodium Top</u>		<u>Sodium Bottom</u>		
1998	10.2	10.0	6.0	5.8
2000	90.0 b	21.0 a	94.6 b	26.6 a

Table 2. Mean data for soil samples from 4/98 (before any treatments were applied) and 5/00 (after two years of treatment) at MSU Botany and Plant Pathology Farm, letter notation indicates significant differences between treatment means from 2000 with 1998 data used as a covariate for top and bottom data separately.

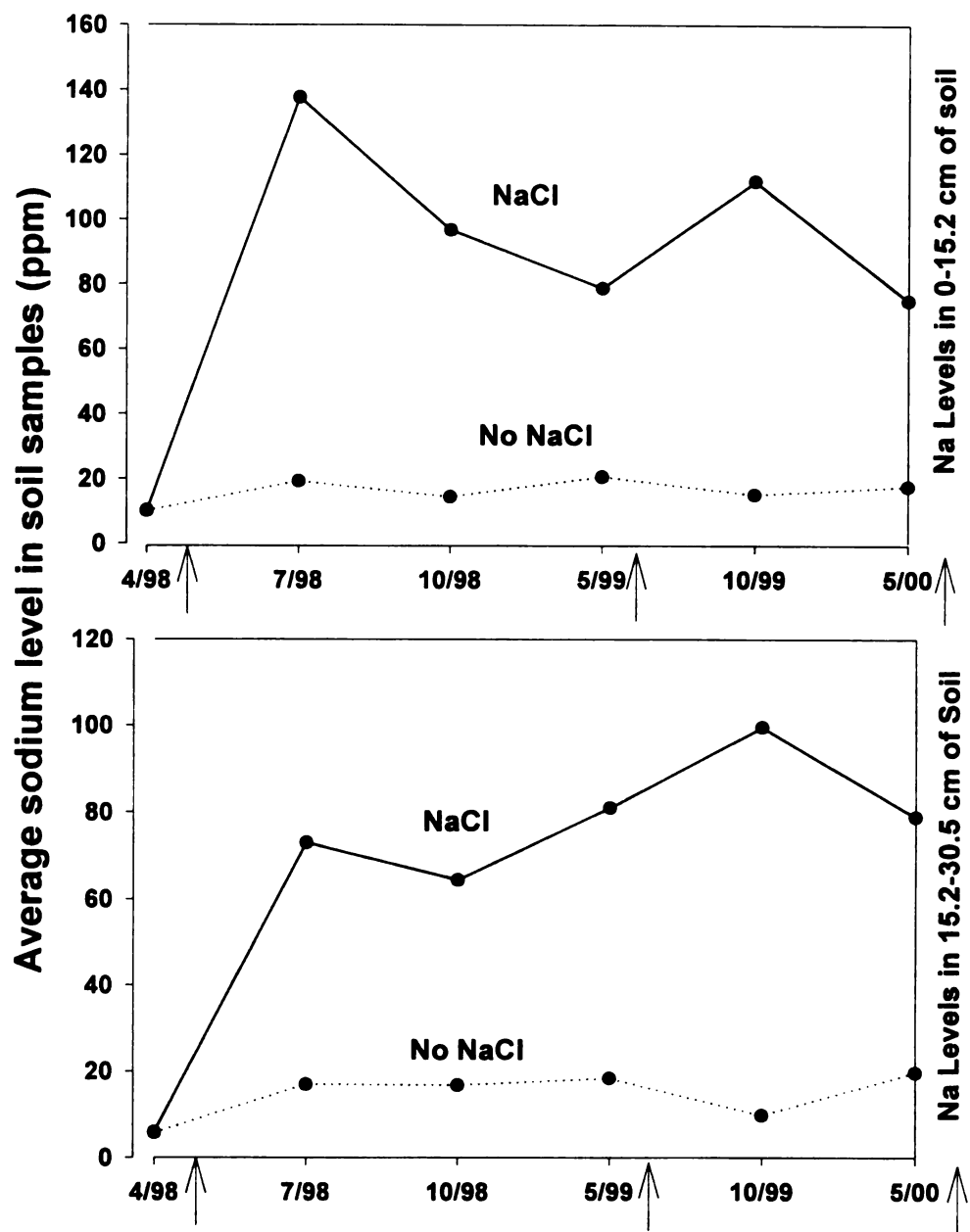


Figure 16. Mean sodium level in soil samples from MSU Botany and Plant Pathology Farm field 4/98-5/00 in parts per million, arrows indicate treatment application times.

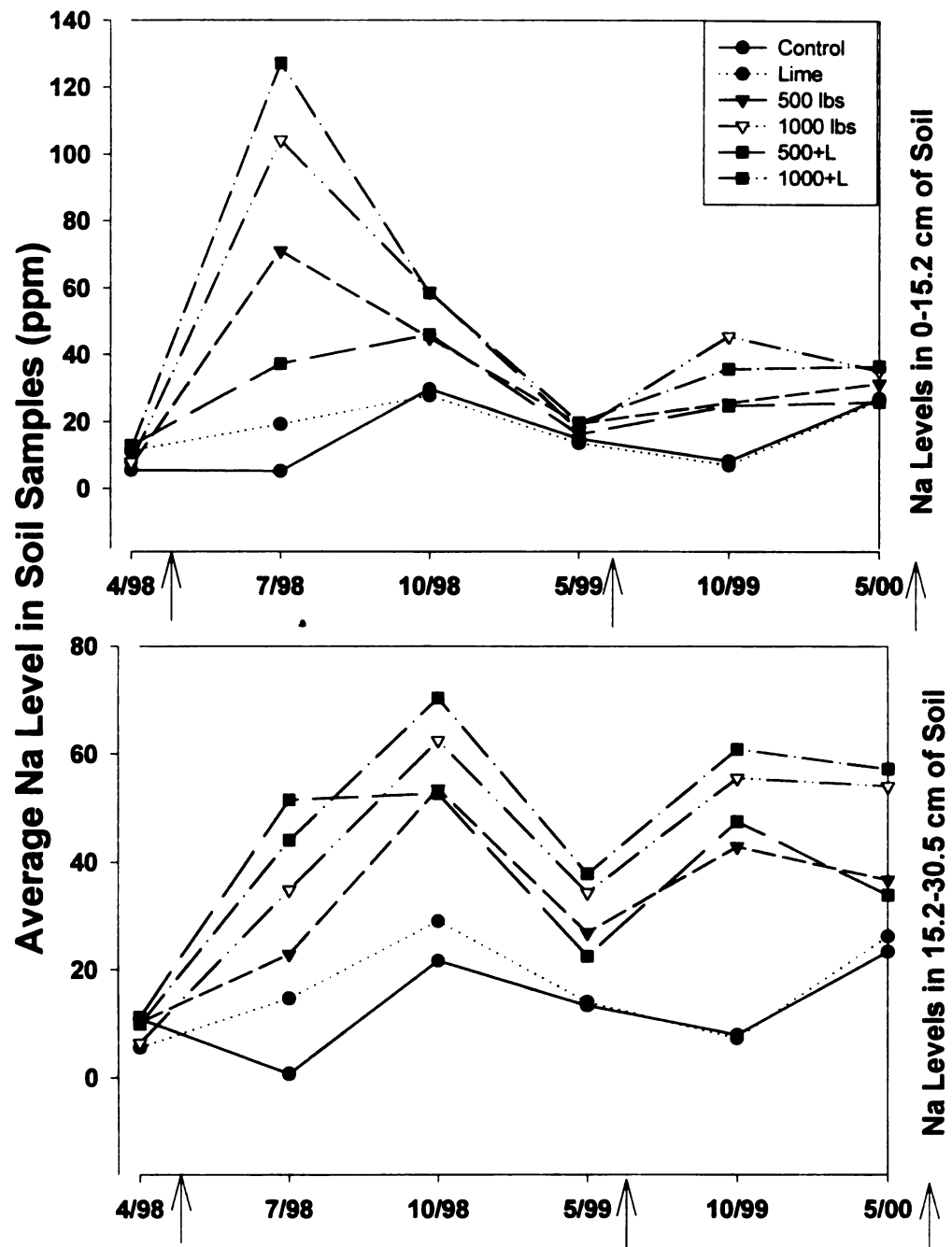


Figure 17. Mean sodium level in soil samples from healthy field in Oceana County between 4/98 and 5/00 in parts per million, arrows indicate treatment application times.

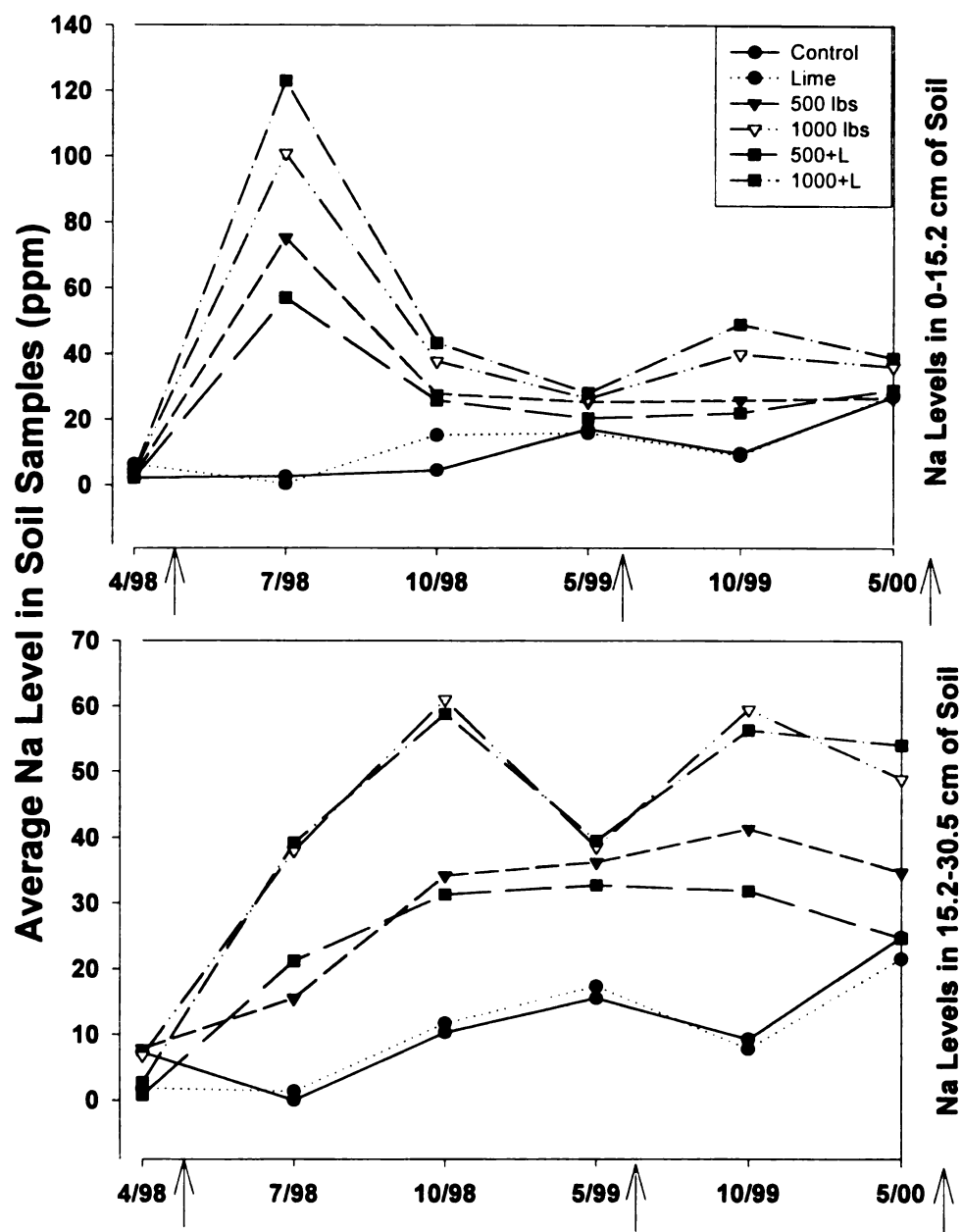


Figure 18. Mean sodium level in soil samples from declined field in Oceana County between 4/98 and 5/00 in parts per million, arrows indicate treatment application times.

APPENDIX

SECTION II

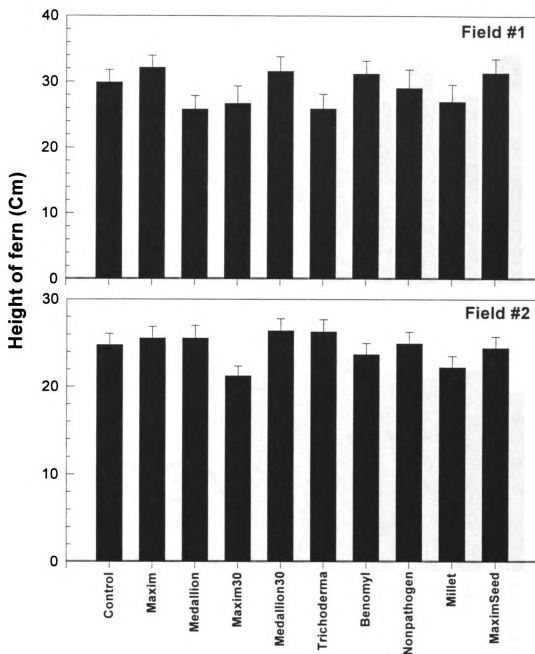


Figure 19. Mean fern height in two seedling nursery trials with chemical and biological controls. Error bars represent standard error.

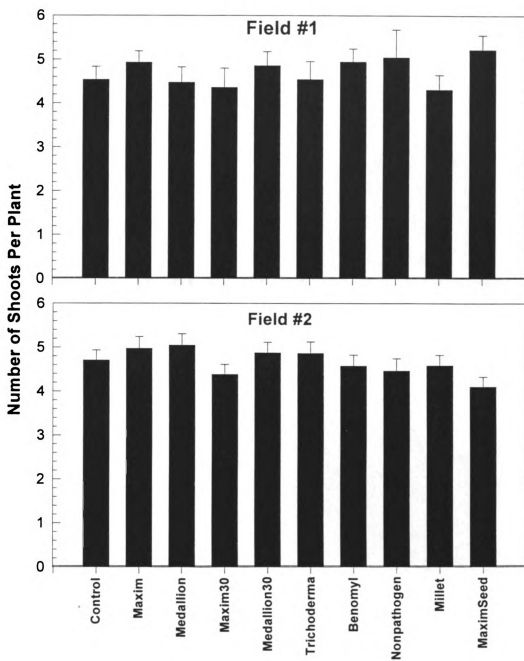


Figure 20. Number of shoots per plant in two seedling nursery trials with chemical and biological controls. Error bars represent standard error.

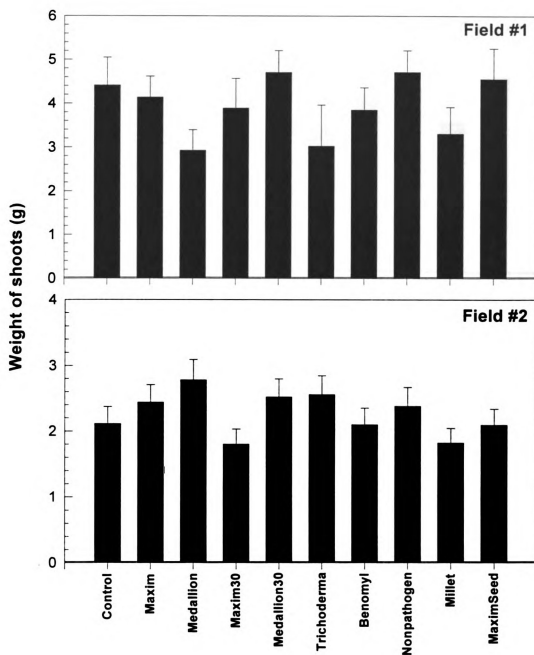


Figure 21. Fresh weight of shoots in two seedling nursery trials with chemical and biological controls. Error bars represent standard error.

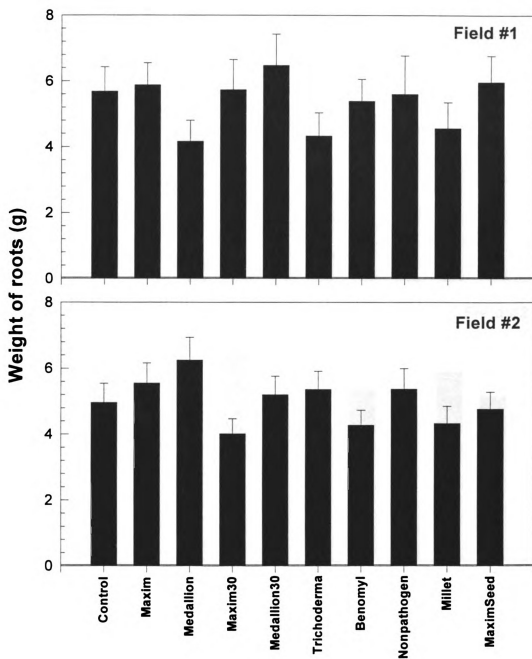


Figure 22. Fresh weight of roots in two seedling nursery trials with chemical and biological controls. Error bars represent standard error.

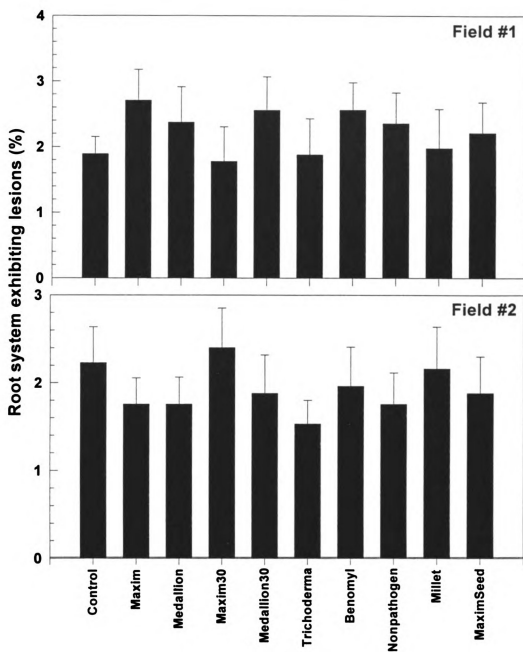


Figure 23. Percentage of root system exhibiting lesions in two seedling nursery trials with chemical and biological controls. Error bars represent standard error.

