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MANAGEMENT OF ASPARAGUS ROTS

By

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ABSTRACT

MANAGEMENT OF ASPARAGUS ROTS

By

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Asparagus rots, caused by *Fusarium oxysporum* f. sp. *asparagi* and *Phytophthora asparagi*, greatly impact Michigan asparagus production. Greenhouse and field experiments with asparagus were conducted to investigate the effect of selected herbicides on (i) plant growth and (ii) *Fusarium* crown and root rot incidence. In the greenhouse, *Fusarium* crown and root rot incidence was low, while reduction in crown weight among plants grown in mesotrione-treated soil was observed. In the field, mesotrione caused phytotoxicity, herbicide effects on *Fusarium* crown and root rot were not tested. Current results suggest select herbicide applications can compromise fern growth, which could influence yields in subsequent years. A series of growth chamber studies using detached asparagus spears were conducted to (i) elucidate the influence of temperature on spear infection by *P. asparagi*; (ii) investigate the susceptibility of three regions of the spear to *P. asparagi*, (iii) evaluate susceptibility of asparagus to *P. cactorum, P. capsici, P. citricola, P. drechsleri, P. megasperma* and *P. nicotianae*, (iv) compare the susceptibility of various sized spears to *P. capsici* isolates. *Phytophthora nicotianae* and *P. capsici* incubated at 25°C caused lesions comparable to those caused by *P. asparagi*. *Phytophthora capsici* isolates caused larger lesions in small spears compared to the larger ones.

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LITERATURE REVIEW

ASPARAGUS

Asparagus, described by Linnaeus in 1735, is a monocotyledonous genus in the family Liliaceae, order Asparagales and contains 170 to 300 species (90). *Asparagus officinalis* L., or garden asparagus, is a perennial, dioecious crop widely cultivated for the edible spears. Asparagus is considered native to Europe and Asia. In ancient Europe asparagus was used as a diuretic, sedative and pain killer (18,32,46).

North America and Europe have historically been the largest asparagus producers. Other production areas include South America, especially Peru (29,131) and Chile; Asia, mainly Japan and China (81,117); and Australasia, with Australia and New Zealand representing emerging markets (14). Michigan ranks third in United States asparagus production behind Washington and California (6). In 2009, Michigan produced 12 million kg of asparagus valued at $16.5 million on 4,532 ha, accounting for 26% of the total U.S. production. Harvesting, which is done by hand, begins in May for Michigan and Washington and in February for California. Mechanical harvest has been proposed and the economic advantage has been evaluated (89), but it has not been implemented by asparagus growers in Michigan (30).

Biology and Physiology. The asparagus plant is composed of the crown and the fern. The crown consists of three underground plant parts: rhizomes, adventitious roots and storage roots. The rhizome begins as a cluster of buds with a growing tip, which develop into new lateral buds producing a recurrent pattern of bud clustering. The extensive network of fleshy storage roots store water and nutrients to feed the buds, which develop into spears. The root system, consisting of adventitious and storage roots, can grow more than 1.5 m in length (99). In the crown, lateral roots are responsible for

absorbing water and nutrients; their numbers decrease yearly after crop establishment. As storage roots grow, they create a dense network and cause the upward movement of the crown in the following years (46).

The fern consists of a central stem with scale leaves from which lateral branches arise. Lateral branches produce secondary branches with cladophylls. Cladophylls are modified branches where photosynthesis takes place, resulting in enlarging of roots and production of new bud clusters in the crown (46).

Stems arising from individual buds in the crown, if not harvested as spears, develop into ferns reaching up to 2 m in height. In this stage, female asparagus plants produce yellow or white flowers, which develop into bright red berries with hard black seeds. Wild asparagus plantings are mixtures of male and female plants (dioecious) (46). Growth suppression and apical dominance signals differ between male and female plants; male plants take less time to grow the next bud, have higher carbohydrate content and less fern area. Male hybrids have greater yields, lower mortality rate, and a longer production season than female plants. Also, male plants produce more spears, but female plants produce larger spears (46). Breeders generate male hybrids for their multiple advantages (69). Male hybrids such as 'Jersey Giant' and 'Jersey Knight' are popular asparagus cultivars with increased vigor and yield potential. Currently, growers in Michigan use 'Millennium' and 'Jersey' as their major asparagus varieties.

Cultural practices. Michigan's harvesting season lasts from mid-April through June (23,46), and the spears can be distributed for fresh or processing markets. Processing asparagus spears for canning are harvested when they reach 12 cm in height, whereas fresh market asparagus is picked when the spears are longer (approx. 20 cm) to

allow for trimming and uniformity (89,109). Spear freshness (turgidity), trimming, straightness, lack of damage or decay symptoms, stalk diameter, and green color percentage are important quality measures for fresh market asparagus (99).

The perennial nature of the crop makes it productive for 15 to 20 years under optimal cultural practices and maintenance (46,109). Well-drained sandy or sandy loam soils, free of perennial weeds and grasses, are needed to establish asparagus fields. Acidic soils are not recommended to grow asparagus; the soil pH should range between 6.8 and 7.5, however, Michigan soils average a pH of 5.6 (82,109). Incorporation of green manure crops or livestock manure before planting is beneficial and increases organic matter in the soil.

Asparagus fields can be direct-seeded (25,115), but most Michigan fields are established by transplanting one-year-old crowns from seedling nurseries. Large crowns are highly desired, since root density and activity after establishment will determine the productivity and life span of the asparagus field (83,109). Spacing between plants in the field depends on whether the grower is establishing a nursery or transplanting one-year-old crowns (Table 1). A nursery field has a higher plant density than a production field (Table 1), because less space is required for crown expansion and development (115). Seedling nurseries are established in mid May or when the soil is warm with temperatures ranging from 15 to 27^{o}C. After selecting the desired asparagus cultivar, seeds are sown in a prepared soil bed (25). After one year in the field, the crowns are dug in early spring (March to April) and stored under cool conditions until planting in productions fields.

Asparagus plant development during the production season goes through the following stages: bud break, spear growth, fern expansion, bud development, root

growth, fern senescence, and bud dormancy (46). Bud break and spear growth are controlled by apical dominance and occur at ≥5°C (39,45). Spears are harvested at >20 cm in length, or subsequent spear growth will be delayed. The production of buds and bud clusters depends on adequate fern expansion (46). The size of the plants depends on their age, length of the growing season and any condition that can potentially affect the performance of the crop in subsequent years (85). Spears, the edible part of the plant, are the emerging stems. Inappropriate cultural practices and diseases can affect the longevity of the crop, decreasing its productivity by 5 to 10 years (53,77).

Table 1. Differences between nursery and production asparagus fields.

	Nursery	Production
Row spacing	45 – 60 cm	1.2 – 1.5 m
In row spacing	8 – 10 cm	22 – 30 cm
Depth	Seed: 2.5 – 4 cm	Crown: 15 cm

Weed management. Weeds compete with crop plants for light, space and nutrients, causing quality and yield reduction and crop losses (2,126). Weed management is an integral part of agriculture; weeds can be managed based on weed life cycles (e.g., annuals, biennials or perennials), production climatic conditions and the portion of the crop plant that is harvested (e.g., the stem in asparagus) (84,123). Herbicides have a significant role in weed management in the production of vegetables, ornamentals, fruits, nuts, and other crops in U.S. and global agro ecosystems, having a great impact on the economic value of the crops (26). Herbicide management is widely used in asparagus production. Asparagus does not compete well with weeds during the nursery stage, because the crop canopy is not dense enough, or during the harvest season,

when weeds can interfere with developing spears (123). In Michigan, losses in asparagus in 1992 were 20% when weeds were managed with herbicides and hand weeding, and 70% when best management practices were used but no herbicides were applied (26).

In late spring, herbicides are commonly applied preemergence and incorporated mechanically into the soil. During harvest, herbicides may be applied postemergence and after the final harvest (22). Effective herbicide usage in asparagus production eliminates possible damage to the crown resulting from tillage to control weeds (70,135). Herbicides are applied directly to soil in any combination of three crop stages: preplant, preemergence or postemergence, but not during spear harvesting. Preplant herbicides can be mechanically mixed into the soil, in contrast to the pre and postemergence herbicides which are applied to the soil surface (34). Mechanically incorporated herbicides are immediately effective without moisture, whereas some surface-applied herbicides must be moved into the soil by water before they can become active. Herbicide half-lives range from a few days to months from the application date, and some persist in the soil for more than a year depending of the active ingredient (33,34).

Herbicides can cause weakening of the plant and increased vulnerability to pathogens or saprobes. The effect of herbicides on plant disease can be direct or indirect (1,4). Direct effects include stimulation or delay of pathogen growth, and increasing or decreasing host susceptibility to the pathogen. Indirect effects can be related to soil microflora activity, change in microclimate of the crop, and selection or elimination of microorganisms (136). The increase in host susceptibility can be due to changes, such as a weakening of structural defenses of the host, plant exudation stimulation, pathogen growth augmentation, or inhibition of competing microflora. Changes in host reaction to

a pathogen is primarily due to herbicide-induced morphological and physiological changes, such as reduction of wax formation on leaves, changes in carbohydrate, nitrogen, or glucoside metabolism; or retardation or stimulation of plant growth (1,4).

Herbicide usage appears to have a positive effect in some pathosystems, as in Phytophthora collar rot of various crops (1,4) where the disease is ameliorated. Several well known cases show that herbicide usage can aggravate severity of diseases, as in sudden death syndrome caused by *Fusarium solani* f. sp. *glycines* (116), or in the root rot and damping-off caused by *Rhizoctonia solani* Kuhn in soybean, sugar beet, and cotton (75). From these cases, an herbicide-phytoalexin interaction has been identified; a reduction of phytoalexin production is directly proportional to the concentration of the herbicides applied in the Sclerotinia stem rot and soybean pathosystem (103).

Crop rotation and cover crops. Sustainable agricultural settings, especially in vegetable crops, have been gaining importance in the last decade, mainly to lessen the agrochemical effects on the environment, address public health concerns, and increase interest in organic production (100). Cover crops, in contrast to traditional practices, are an ecological alternative for weed management, causing weed seed decay, breaking disease and pest cycles, and reducing pesticide usage (122). Cover crops can provide greater yield stability of crops (124) with less fertilization, while improving soil characteristics (104). In addition, cover crops could potentially reduce the reliance on agrochemicals in disease management (112).

Perennial and herbicide resistant weeds present in the soil bank are favored and their populations may increase in asparagus production, a monoculture that remains in place for years. Thus, weed control is essential to achieve profitability (138). Soil

improvement is difficult, as tillage is not recommended for asparagus (70,135), and crop rotation is not a possibility (8). However, cover crops represent an opportunity to maintain productivity, soil health and weed control; it has been very useful in other crops (104) as living or dying mulches in the field (122). Currently, cover crops are recommended in organic asparagus production (91) and could be introduced into traditional asparagus cropping systems. Barley, used in Japan as living mulch (8), suppressed weed emergence of lamb's quarters (*Chenopodium album* L.). Extensive research would be needed to assess the effect of cover crops on weeds common to asparagus fields.

ASPARAGUS DISEASES

Diseases can be a limiting factor in asparagus anytime during production of this perennial crop. Diseases can be present in newly-planted seedling fields and in old asparagus fields, when production may be decreased due to a combination of pathogens that can develop in the crop. Grogan and Kimble first described asparagus decline syndrome in 1959 as a "slow decline in the productivity of old asparagus plantings and the inability to reestablish productive plantings" (74). A marked reduction in the size and number of spears and death of the crown is characteristic of decline (53), making harvest unprofitable, and resulting in premature abandonment of the asparagus field (42). The fungal components of asparagus decline syndrome include *Puccinia asparagi* DC. in Lam. & DC., *Stemphylium vesicarium* (Wallr.) E. Simmons (telemorph: *Pleospora herbarum*), *Cercospora asparagi* Sacc., and several *Fusarium* spp., causing rust, purple spot, *Cercospora* blight, and crown and root rot, respectively (53). Three viruses have been associated with decline; asparagus virus I, asparagus virus II, and tobacco streak

virus (56). *Phytophthora* spp. have also been reported as destructive pathogens (60,73,118), but are not currently in the list of pathogens causing decline.

Due to the perennial nature of the crop and the important role of the crown and roots in asparagus, pathogens able to infect these main organs compromise longevity and productivity; thus, crown and root rots are management priorities (38). Fusarium crown and root rot has been widely studied and characterized, but Phytophthora spear and root rot has recently been identified as a threat in Michigan fields (118,119).

Fusarium crown and root rot

***Fusarium* generalities**. The genus *Fusarium* Link include some of the most common and important fungal pathogens, parasites and saprobes widely distributed in soil and organic matter and associated with plants and animals (20). *Fusarium* spp. are primarily soilborne, but some are airborne (27,98). *Fusarium* spp. are well known necrotrophic, vascular wilt pathogens, causing dysfunction of the xylem (1). Identification of *Fusarium* spp. is based on microscopic morphology of asexual structures, and macroscopic colony characteristics. The genus *Fusarium* produces macroconidia in sporodochia and microconidia on primary conidiophores, as well as chlamydospores and occasionally sclerotia (120). Falcate or fusiform macroconidia are the most distinctive feature in genus identification. Although other fungi may have macroconidia with a similar shape, the ontogeny is different (120). Due to the plasticity and instability of *Fusarium* spp. in culture (105), identification based on production of secondary metabolites such as mycotoxins, and genetic sequences (such as elongation factor, β tubulin) has been proposed and recently used (65,129).

Anamorph and teleomorph connections have been established for several *Fusarium* spp. The sexual reproduction, or teleomorphic stage of *Fusarium* spp. are perithecium-forming fungi in the genera *Giberella* and *Nectria* within the order Hypocreales, class Sordariomycetes and phylum Ascomycota. Other related teleomorphs such as *Cosmospora, Calonectria, Hyphomyces* and *Bionectria* have *Fusarium*-like anamorphs, but lack one or more of the microscopic features previously described (120).

***Fusarium* species pathogenic to asparagus.** *Fusarium* can cause an array of symptoms in asparagus that have yielded multiple disease names such as asparagus dwarf, wilt, rot; seedling blight, foot rot, dead stem, and spear spot and rot, in addition to the most common crown and root rot disease (48,51). Fusarium crown and root rot symptoms are evident in seedlings, young plants and mature plants in established fields. Seedling damping-off is common in nurseries. Chlorotic and stunted plants may be present in fields that have been harvested for several years. Spears become shriveled and stunted, which can be very similar to abiotic stresses such as winter injury, drought or soil compaction (121). Fewer and smaller or damaged spears are produced as a consequence of the infection and progression of the disease. When dormant infected crowns grow fern in summer, the infection is noticeable as yellowing of the fern canopy (51). Disease extends up from the base of stem, brown necrosis follows initial chlorosis, and in advanced disease stages, stem and crown death become evident in the field as stand loss (48).

Symptoms are caused predominantly by *F. oxysporum* (Schlecht) f. sp. *asparagi* (S. I. Cohen)(*FoA*) and *F. proliferatum* (Matsushima) Nirenb. (telemorph: *Gibberella fujikuroi* (Sawada)) (51,52). The first report of *Fusarium* spp. infecting asparagus was

made in 1908 in Massachusetts by Stone (127). In 1941, Cohen and Heald described *F. oxysporum* as the rot etiologic agent (35), followed by the addition of the term forma specialis *asparagi* by Grogan and Kimble in 1959. *Fusarium oxysporum* f. sp. *asparagi* has a wide host range, opening the discussion of forma specialis term usage (51). *Fusarium oxysporum* f. sp. *asparagi* is seedborne, soilborne, and can be found in one-year-old crown tissue, implying that crowns used to establish production fields can be a source of inoculum (16). However, isolates can be pathogenic or nonpathogenic. For example, only 15% of *Fusarium* isolates from soil in Michigan fields were pathogenic on asparagus (78).

Isolates of *F. oxysporum* f. sp. *asparagi* are classified in at least 6 different vegetative compatibility groups (VCGs), and phylogenetic analysis has shown multiple evolutionary origins (12). No association between pathogenicity and VCGs has been found, at least in the forma specialis *asparagi*, which is composed of genetically diverse populations (88). Some authors argue in favor of Fusarium crown and root rot as a disease complex caused by *F. oxysporum* f. sp. *asparagi*, *F. proliferatum* and *F. redolens* Wollenw in asparagus (13), but this concept is not widely accepted. Some other species have been reported to cause a variety of symptoms on asparagus (41,52,68).

Among the other species, *F. culmorum* (W.G. Smith) Sacc. has been reported to cause stem chlorosis, death of mature stems (51), and has contributed to crown and root rot predisposition. *Fusarium redolens* causes a postharvest spear spot mainly in Europe, and is characterized by a tissue softening in storage (13). *Fusarium subglutinans* (Wollenw. & Reinking) Nelson Toussoun & Marasas and *F. moniliforme* Sheld. have

been isolated from asparagus and showed pathogenicity as well (41,52), but are not major asparagus pathogens.

Management. Effective management of Fusarium crown and root rot should include a combination of cultural practices, adequate agrochemical programs, and alternatives like biological control. Several asparagus varieties are vigorous and can withstand adverse conditions and suppress crown and root rot (114,125). However, there are no *Fusarium*-resistant asparagus cultivars on the market, despite attempts to identify resistance using somaclonal variation and variety screening (43).

Stress is a factor that can predispose asparagus plants to disease (106). Winter injuries, drought, high weed pressure, and harvesting or overharvesting can increase disease incidence (50,109,110). Soil with pH level <6.0 can enhance disease. With this knowledge, it is critical to maintain the soil pH between 6.8 and 7.5 by application of lime. Careful use of amendments such as nitrogen or sodium chloride (NaCl) may have a positive effect on the crop (54). NaCl has been applied to decrease Fusarium crown and root rot incidence in asparagus crops and increase yield (38,111). NaCl can have limited success as a disease control strategy if the amount and periodicity is not regulated. The mechanisms of *Fusarium* suppression by NaCl are not known, but the plant produces more feeder roots and the colonization of *F. oxysporum* and *F. proliferatum* is reduced. It is possibly related to changes in the microbial soil composition (49).

Fungicides are an important part of disease management and are used at different crop stages (110). Fungicide-treated seeds can be used to start nursery seedlings, one-year-old crowns can be dipped in fungicide before planting, and untreated crowns can be planted into fumigated soil or a combination of these practices used (15,79). Soil

fumigants include 1,3-dichloropropene/chloropicrin (Telone C35), metam sodium (Sectagon 42, Vapam HL), or metam potassium (Sectagon-K54) (15,79).

Alternative strategies to reduce crown and root rot disease using biological controls have been studied including *Trichoderma* (10,109,110), beneficial bacteria (133), mycorhizae (11) and nonpathogenic *F. oxysporum* isolates (109). Biological soil disinfestations using Italian ryegrass has been shown to decrease the population diversity and viability of *Fusarium* spp. in abandoned asparagus fields (17).

Phytophthora spear and root rot

***Phytophthora* generalities.** Among plant pathogenic oomycetes, *Phytophthora* spp. are parasitic on diverse host plants, with broad or narrow host ranges (55). Diseases caused by *Phytophthora* spp. produce rapid and destructive symptoms with important socioeconomic impact (55). Morphological identification of *Phytophthora* spp. is based on antheridium location, sporangial size, length and breadth; sporangial caducous nature, presence of papillae in sporangia, chlamydospore presence and size, oospore size and abundance, cardinal growing temperatures, hyphal swellings, and homo- or heterothallism (55). At the DNA level, *Phytophthora* taxa are differentiated using ribosomal DNA, internal transcribed spacer sequences (ITS), and β tubulin sequences to support morphological classification criteria (37).

***Phytophthora* species pathogenic to asparagus.** *Phytophthora* causes spear and root rot in asparagus. Flooded or overly wet soils are needed for *Phytophthora* zoospore dispersal; therefore, the rot is favored by excessive rainfall and poor soil drainage (60,118,119). Symptoms include distinctive water-soaked lesions on shoots and roots; sometimes symptoms can be confused with herbicide damage (118,119), especially when

lesions elongate and turn brown, causing spears to shrivel and curve, resulting in unmarketable spears (9,118). Early reports of Phytophthora rot were made in 1938 in California (9). *Phytophthora megasperma* and some other *Phytophthora* spp. were identified as the causal agents. Historically, five different species of *Phytophthora* have been reported to cause disease in asparagus: *P. megasperma* Drechsler (60), *P. megasperma* var. *sojae* Hildebrand (58), *P. richardiae* Buisman (55,60) , *P. cryptogea* Pethybridge & Lafferty (55,58) and *P. cactorum* Lebert & Cohn (60) (Table 2). *Phytophthora megasperma* taxa have been a subject of continuous discussion regarding species delimitation and variety names. Due to morphological variability, *P. megasperma* isolates that were divided according to host specificity are now considered different species; *P. megasperma* var. *sojae* (118) is now renamed as *P. sojae* (36).

In Michigan, a homothallic *Phytophthora* sp. was reported in 2004 (119), when a spear and root rot outbreak occurred due to the excessive precipitation and poor drainage in asparagus fields during the harvesting season. This species was characterized based on 131 isolates with ovoid nonpapilate, noncaducous sporangia, amphigynous oospores, and sensitivity to mefenoxam (100 ppm) and named *P. asparagi* (73,119).

Management. Strategies to manage *Phytophthora* in asparagus require knowledge of the species associated with the crop, resistance to agricultural chemicals, and cultural management. Correct irrigation is key to avoid plant stress, since flooding of the field can be conducive for infection by *Phytophthora* spp., including *P. asparagi* (59,73,119). Currently, few Michigan growers irrigate their asparagus spears.

Fungicide application is recommended and may occur before planting as crown soaks prior to planting the crowns in fields. Soil fumigation is recommended only in

fields with high disease pressure, and the combination of crown soak and soil fumigation is recommended in replanting fields (79). In the United Kindom, metalaxyl is considered an acceptable *Phytophthora* spear rot management practice (73), but it could have phytotoxic effects in high doses (>150 ppm) (57). Fungicides such as mefenoxam (Ridomil Gold EC, Ultra Flourish), mono/dibasic sodium salts of phosphorous acid (Phosphite), mono/dibasic sodium, potassium and ammonium phosphites (Phostrol or Prophyt) are labeled to be used to control asparagus Phytophthora spear and root rot in Michigan (15).

Phytophthora-resistant asparagus varieties have been developed using recurrent selection in California (57), but their resistance has not been assessed in Michigan. Other alternative control measures in the *P. asparagi*/asparagus pathosystem need further research.

Table 2. Morphological characteristics of *Phytophthora* described infecting asparagus.

		P. megasperma	*P. sojae*	*P. richardiae*	*P. cryptogea*	*P. cactorum*	*P. asparagi*
Sexual reproduction	Homothallic	Yes	Yes	Yes	No	Yes	Yes
	Heterothallic	No	No	No	Yes	No	No
Sporangia	Amphigynous	Variable	Yes	Amphigynous to paragynous	Yes	Paragynous	Yes
	Papillate	No	No	No	No	Yes	No
	Caducous	No	No	No	No	Yes	No
	Shape	Ovoid to obpyriform	Ovoid to obpyriform	Variable	Ovoid to irregular	Ovoid to obpyriform	Ovoid to obpyriform
	Branching	Unbranched	Unbranched	Unbranched	Unbranched	Single sympodial	Single branched
	Length (μm)	35-60	23-89	40-60	35-63	19-55	42-47
	Breadth (μm)	25-45	17-52	20-40	24-35	16-46	23-40
	Average (μm)	41 x 28	58 x 38	52 x 33	52 x 30	38 x 26	45 x 38
	Proliferation	Yes	Yes	Yes	Yes	No	Yes
Chlamydospores	Production	No	Rare	No	No	Rare	No
	Average diameter	No	Varies	No	No	25 - 40	No
	Hyphal swellings	Yes	Yes	Yes	Yes	No	Rare

CHAPTER I

GREENHOUSE AND FIELD HERBICIDE EVALUATION ON ASPARAGUS PLANTS

Manuscript accepted in Acta Horticulturae

ABSTRACT

Pre and postemergence herbicides are used to control weeds in Michigan asparagus nurseries and production fields. Greenhouse and field experiments were conducted to test the effect of 10 herbicides on (i) growth of asparagus plants and (ii) susceptibility to crown and root rot caused by *Fusarium oxysporum* f. sp. *asparagi.* Crowns were planted in pots containing infested soil (*F. oxysporum* f. sp. *asparagi*) or noninfested soil and sprayed individually with the selected herbicides and grown in the greenhouse. Crown and fern health were evaluated three months after planting. Obvious growth effects of the herbicide treatments on asparagus growth were not commonly observed and incidence of crown and root rot was low. However, a reduction in crown weight was observed among some plants grown in herbicide-treated soil. Another experiment was conducted whereby crowns were planted in a commercial field and treated prior to emergence with one of the following herbicides: glyphosate, halosulfuron, mesotrione, norflurazon, linuron, sulfentrazone, s-metolachlor, metribuzin, diuron, and terbacil. Weed control, asparagus fern stand and height, and overall fern health were assessed. In the field, mesotrione had a negative effect on asparagus fern health, which was also observed in the greenhouse. Current results suggest select herbicide applications can compromise fern growth, which could influence yields.

INTRODUCTION

Herbicides play an important role in asparagus; maximizing crop production for a given amount of land, and minimizing unwanted or harmful non-crop plants that potentially can become established in asparagus fields and compete for space and nutrients (76,84). Due to the perennial nature of the asparagus crop, tillage as a weed control strategy has been reduced or eliminated, since crowns will be exposed to injury and infection by *Fusarium* spp., increasing Fusarium crown and root rot incidence (22). However, absence of tillage promotes perpetuation of the weed bank in the soil when annual and perennial weeds prevail (135), and as a result herbicide application (137) and/or hand weeding are needed .

A considerable number of herbicides are available in the market; they differ in toxicology, action spectrum, activity, crop safety and environmental effects (87). In the United States and Canada a list of 27 herbicide groups (by active site of action), with an average of four chemical families within the group, are available for weed control, accounting for at least 200 active ingredients (97). In asparagus, 18 active ingredients are recommended in Michigan for established fields, seven in seed beds for crown production, and only five active ingredients can be used in newly planted asparagus (137).

Herbicide modes of action target normal physiological processes in weeds, but the same processes can be affected in crop plants. In fact, herbicides such as linuron, diuron, norflurazon and metribuzin can be used to partially control volunteer asparagus while controlling other weeds in the field (22,23). Also, herbicides may have adverse effects

on crop health; ranging from phytotoxicity, harmful effects in plant stand, to inductive stress causing predisposition to disease (4).

Fusarium oxysporum f. sp. *asparagi* causing Fusarium crown and root rot is a concern in any developmental stage of the asparagus crop, contributing to asparagus decline worldwide (51). The characteristic symptoms of the disease such us destruction of feeder roots, fern yellowing and senescence, and crown rot and death, are evidence of the damage cause by this soil inhabitant (53). Management of Fusarium crown and root rot relies on a combination of strategies, such as crown fumigation, planting in fields with no history of decline and adequate cultural practices (15,53). Currently, the effects of herbicides on asparagus plants and their interaction with Fusarium crown and root rot are unknown. The purpose of this study was to determine the effect of herbicides on asparagus plant stands and on Fusarium crown and root rot incidence in one-year-old asparagus crowns.

MATERIALS AND METHODS

Greenhouse herbicide studies. One-year-old, untreated 'Millenium' asparagus crowns from a nonfumigated commercial nursery (Dillinghams Farms in Oceana County, MI) were rinsed of debris and visually screened to ensure absence of disease. Crowns were weighed (between 25 to 45 g) and stored in individual plastic bags overnight. Sandy loam soil was steam-pasteurized 18 h for two consecutive days to decrease microbial load that could interfere with the following soil infestation process.

Inoculum was prepared using a pathogenic *F. oxysporum* f. sp. *asparagi* isolate *FoA* 50 from the culture collection at M. K. Hausbeck's laboratory at Michigan State University (MSU). The isolate was recovered originally from a Michigan asparagus field

by W. Elmer (109). Milk jugs containing 1 lb of millet seed and 150 ml of water were autoclaved for 1 hour on two consecutively days. A millet seed sterility test per container was conducted on potato dextrose agar media (PDA). Eight 0.7-mm plugs of fungal mycelium from five-day-old cultures of *F. oxysporum* f. sp. *asparagi* (*FoA*) were used to inoculate containers with 1 lb of sterile millet, and allowed to grow for two weeks in laboratory environmental conditions at 21 ± 2°C under fluorescent lighting, and shaken occasionally to avoid clumping. Fungal growth was assessed visually and using microscopy. *FoA*-infested millet (400 g) and 40 liter of soil (10 g/liter) were mixed with steamed sandy loam soil in a cement mixer (109,133), yielding 4 x 10^4 colony forming units (cfu) per gram of soil (pooled average for both experiments). Control soil consisted of sterile millet seed mixed with the soil in the same proportion as the infested one.

Of 220 pots, 110 were filled with a mixture of 10 g infected millet seed per liter of sandy loam soil. The remaining 110 pots were filled with a mixture of sterile millet seed and sandy loam soil, as a negative control. Two-gallon pots (Hummert International, Earth City, MO) were filled to 1/4 with the soil and millet mixture (*FoA*infested or noninfested), then the crowns were transferred to the pot, and pots were filled to the top with the respective soil mixture.

Ten herbicides and two soil treatments were tested in a complete randomized design (CRD), and the trial was conducted twice. Twenty plants per herbicide treatment and 10 plants per soil treatment were used. Soil herbicide application treatments (Table 3) were conducted one week after the crowns were transplanted to the pots. Herbicide application was preemergence of weeds and spears, and the rate used followed the MSU extension recommendation and manufacturer instructions. Herbicide rates were chosen

based on a hypothetical high weed pressure in the field when a grower would use the herbicides at higher recommended rates (Table 3). Treatments were applied in the spray chamber of the pesticide application laboratory at MSU, for each herbicide treatment, starting with the control soil pots and followed by the *FoA* infested ones. The nozzle was rinsed three times prior to a new treatment being applied. The nozzle was 33 cm from the soil surface, the nozzle (8001 E) roll at 1 mile per hour, using a 25 psi (172/5KPa) pressure at the sprayer head, simulating 20 gallons per acre (GPA) (187 liter/ha), dispensing 0.077 gallons per minute (0.29 liter/min) from the nozzle.

Table 3. Herbicide treatment rates applied to asparagus in greenhouse and field experiments.

		Greenhouse	Field
Treatment No.	Herbicide active ingredient (AI)	AI kg ha^{-1}	AI kg ha^{-1}
1	Linuron	1.121	1.682
2	Norflurazon	4.484	1.121
3	s-metolachlor	2.130	1.491
4	Sulfentrazone	0.420	0.280
5	Halosulfuron	0.005	0.067
6	Glyphosate	4.484	3.363
7	Diuron	0.897	1.121
8	Mertibuzin	1.121	0.740
9	Flumioxazin	0.215	
10	Mesotrione	0.270	0.213
11	Terbacil		0.280
12	Mixture[z]		1.682

[z] Mixture contains sulfentrazone 0.348 kg ha^{-1}, metribuzin 0.561 kg ha^{-1}, s-metolachlor 1.682 kg ha^{-1}, glyphosate 3.363 kg ha^{-1}.

Irrigation was carefully controlled in the greenhouse; after herbicide application the pots were watered to fulfill the requirements of water-dependent herbicides, but avoiding herbicide leaching. A week after herbicide application, pots were watered as needed every three days. In order to allow complete fern expansion, fertilizer was applied 60 days after planting (DAP), with NH_4NO_3 (42-0-0) and KCl (0-0-62) at rates of 0.07 and 0.04 lb/ (31.7 and 18.1g) per plant, respectively.

Herbicide injury was monitored and recorded, as the first shoots appeared and throughout the duration of the trial. Disease symptom appearance and development in stems were monitored, as well as phytotoxicity or abnormal growth. Numbers of stems and fern length were recorded three times. Plant stand counts (number of stems per plant and total fern length) were recorded during both trials. A destructive measurement was conducted at the end of the trial for variables such as crown fresh weight, percentage of crown weight gain (subtracting final fresh weight from the crown initial fresh weight), presence of crown and root disease symptoms (reddening), fern dry weight, crown fresh and dry weight, and total plant dry weight. Disease was assessed before obtaining the dry weight variables, and consisted of the percentage of roots exhibiting lesions or discoloration (1=0-10%, 2=11-20%; 3=21-30%, 4=31-40 %, 5=>40%). The reisolation of *Fusarium* for 5% of plants was conducted to confirm *FoA* infection (10,133).

Field herbicide studies. One-year-old 'Millennium' crowns were planted in sandy loam soil on a commercial asparagus farm in Oceana County, MI previously planted to cucurbits. Fertilizer, insecticides, and fungicides were applied according to commercial practices. The field was fumigated in fall with sodium methyldithiocarbamate (Sectagon 42, Tessenderlo Kerley, Inc Phoenix AZ) at 40 gal/acre

(378 liters/ha) and planted in the spring with 'Millennium' asparagus crowns grown in a fumigated nursery. Eleven herbicide treatments and an untreated control (Table 3) were arranged in a completely randomized block design with four replications. Each treatment plot was 1 row wide (152 cm) and 6 m long, with crowns spaced 18 cm apart. Eleven herbicide treatments (Table 3) were applied according to the rate commonly used by growers in Michigan, prior to emergence, with a CO_2 backpack sprayer and a 3-nozzle boom equipped with 50-mesh screens and 8003 XR nozzles (Teejet technologies Wheaton, IL) spaced 48.3 cm apart, calibrated to deliver 50 GPA (407 L/ha). The numbers of healthy bleached ferns or ferns with epinasty were counted 45 DAP. The efficacy of the herbicides for control of grass and broadleaf weeds were evaluated 25 and 45 DAP by counting the number of each kind of weeds: grasses or narrowleaf that were presented together and; broadleaf (i.e. pigweed, lambsquarters, eastern black nightshade) in plots and compared with the control plots an expressed as the percentage. Phytotoxic effects on the fern were evaluated 30 DAP using a 1 to 5 rating scale (1 = healthy, 2 = minor chlorosis, 3 = moderate chlorosis, 4 = major chlorosis, 5 = fern death). The numbers of healthy ferns were recorded and fern height was measured 60, 90 and 120 DAP.

Statistical analysis. Descriptive statistics were performed for seven continuous variables and four categorical variables. Normal distribution and equality of variances were checked, and if not fulfilled, appropriate transformation or grouping was conducted when needed. When normal distribution and equal variance assumption were fulfilled; analysis of variance (ANOVA) was conducted using PROC MIXED and PROC GLIMMIX procedure (SAS institute Inc., Cary, NC), treatment means were compared

using Fisher's protected least significant difference (LSD) and were considered significant using α=0.05.

RESULTS

Greenhouse herbicide studies. After soil pasteurization, assessment of the microbial community was conducted using dilution plating, resulting in high numbers of miscellaneous bacteria and fungi (2×10^4 cfu/g soil). Prevalent organisms were tested in vitro to determine whether they inhibit *F. oxysporum* f. sp. *asparagi* (*FoA)* growth on PDA, with no apparent in vitro inhibition. The infested soil had 3×10^4 and 5×10^4 *FoA* cfu per gram of soil for the first and second trial respectively.

During both trials fern-yellowing incidence was low among herbicide treatments. Fusarium crown and root rot disease incidence in crowns and roots was low; 78% of the crowns showed minor or no reddening symptoms (disease scale rating=1), and only 4% showed symptoms rating between 3 and 4 (from 20 to 40% lesion and discoloration coverage) when the crowns were grown in *FoA* infested soil. From crowns planted in control soil, 17.9% of the crowns rated as 2 and 3, and 82% of the crowns showed ≤10% lesion coverage. Five crowns were dead (no spear emergence).

Phytophthora asparagi symptoms were observed when the crowns were rated for Fusarium crown and root rot. The lesions were brown and water soaked but the incidence was low. For both soil types, 68% of crowns showed ≤10% lesion coverage for Phytophthora rot symptoms, 27% of the crowns showed from 20 to 30% lesion coverage, while only 5% showed from 30 to 40% crown lesion coverage. Disease severity for both asparagus rots were tested using Chi square or PROC GLIMMIX, as a

result no significant differences were found for herbicide or soil factors, or for their interaction.

Phytotoxicity was documented as abnormal growth (Table 4), but only 13% of the total plants showed distorted growth, stunting or a combination of the two. Damage was observed in the first emerging spear/fern but the following ferns did not show damage. In pots sprayed with sulfentrazone, 20% of the plants showed phytotoxicity, independent of the presence of the pathogen (infested and noninfested soil). Plants growing in sterile millet (20%) and growing in *FoA* infested soil (15%) showed phytotoxicity in the metolachlor treatment.

Table 4. Number of plants showing phytotoxicity in uninfested field soil or soil infested with *Fusarium oxysporum* f. sp. *asparagi* following treatment with herbicides.

	Soil treatment		
Herbicide treatment	Sterile millet	*FoA* infested soil	Total
Linuron	3 (20)[z]	4 (20)	7 (40)
Norflurazon	3 (20)	3 (20)	6 (40)
s-Metolachlor	4 (20)	3 (20)	7 (40)
Sulfentrazone	4 (20)	4 (20)	8 (40)
Halosulfuron	2 (20)	0 (20)	2 (40)
Glyphosate	2 (20)	4 (20)	6 (40)
Diuron	3 (20)	2 (20)	5 (40)
Metribuzin	1 (20)	2 (20)	3 (40)
Flumioxazin	2 (20)	4 (20)	6 (40)
Mesotrione	3 (20)	0 (20)	3 (40)
Control	2 (20)	2 (20)	4 (40)
Total	29 (220)	28 (220)	57 (440)

[z] Number in parenthesis indicates total number of plants.

From seven variables (presence of crown and root disease symptoms, tallest fern, fern dry weight, percentage of crown weight gain, crown dry weight, and total plant dry weight), only tallest fern, fern dry weight, crown dry weight, and total plant dry weight showed significant differences within soil treatments, and three (crown weight gain and crown dry weight, and total plant dry weight) showed significant differences among herbicide treatments ($P<0.05$). None of the variables showed significant interaction between soil and herbicide treatments.

When taking into account only the tallest fern of every crown (plant), asparagus ferns growing in soil infested with *FoA* were significantly smaller ($P=0.02$) than ferns growing in the control soil (Figure 1).

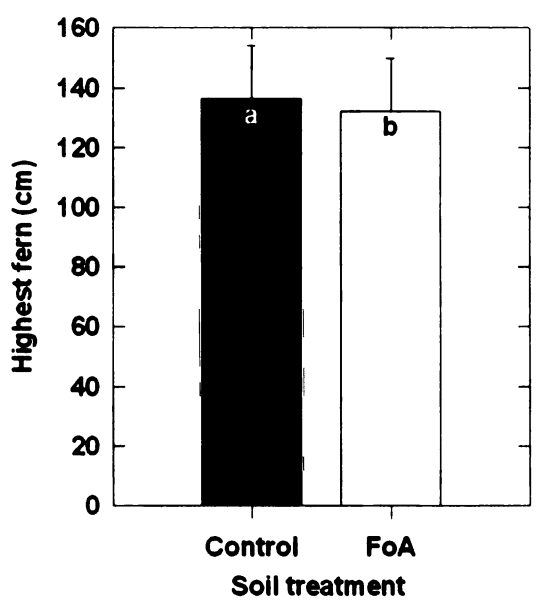

Figure 1. Effect of soil treatment on asparagus fern length (tallest fern only) in greenhouse experiments with asparagus plants grown in soil mixed with sterile millet seed (solid bar) and in soil infested with *F. oxysporum* f. sp. *asparagi* (*FoA* white bar) across herbicide treatments. Bars with common letters are not significantly different, LSD $P=0.05$.

Fern, crown and total dry weight showed significant differences between soil treatments; asparagus plants grown in soil infested with *FoA* had a higher average weight compared to the plants growing in soil mixed with sterile millet (Figures 2, 3A and 5A).

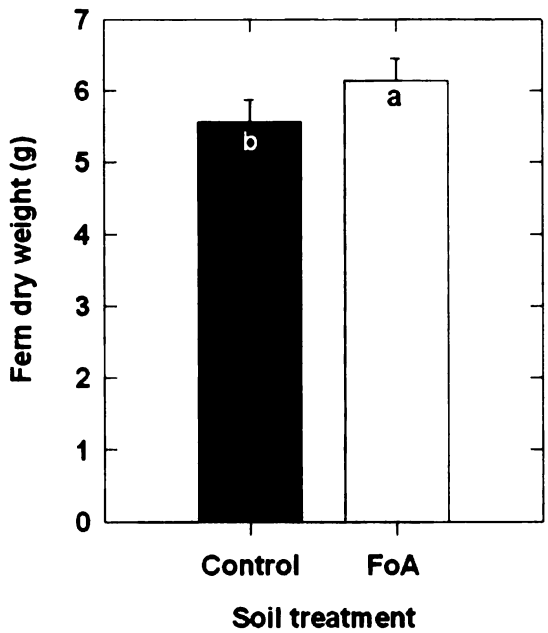

Figure 2. Effect of soil treatment on asparagus fern dry weight in greenhouse experiments with asparagus plants grown in soil mixed with sterile millet seed (solid bar) and in soil infested with *F. oxysporum* f. sp. *asparagi* (*FoA* white bar) across herbicide treatments. Bars with common letters are not significantly different, LSD P=0.05.

The effect of herbicide was significant for three variables: crown weight gain, crown dry weight, and total dry weight. When making comparisons among herbicides across soils, crowns growing in pots treated with mesotrione showed the smallest weight gain. Plants growing in pots sprayed with diuron, linuron, glyphosate and flumioxazin were significantly different form the ones in mesotrione but no with the control plants. Norflurazon, s-metolachlor, sulfentrazone, halosulfuron and metribuzin showed homogeneous averages not significantly different form mesotrione or the control (Figure 4).

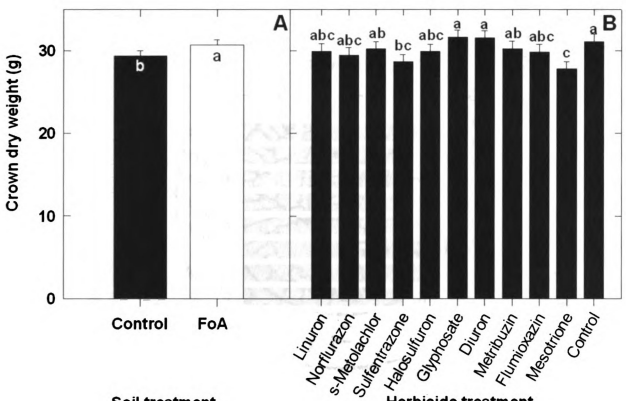

Figure 3. A, Effect of soil treatment on crown dry weight in greenhouse experiments with asparagus plants grown in soil mixed with sterile millet seed (solid bar) and in soil infested with *F. oxysporum* f. sp. *asparagi* (*FoA* white bar) across herbicide treatments. **B,** Effect of soil treatment on crown dry weight in greenhouse experiments with asparagus plants grown across soil treatments. Bars with common letters are not significantly different, LSD P=0.05.

Asparagus plants growing in pots sprayed with mesotrione and sulfentrazone showed the lowest crown dry weight average and were significantly different from the control (Figure 3B), whereas crown dry weight averages for plants sprayed with glyphosate, diuron, s-metolachlor, metribuzin, linuron, norflurazon, halosulfuron and flumioxazin had averages similar to the control (Figure 3B); linuron, norflurazon, halosulfuron and flumioxazin were not significantly different from mesotrione or sulfentrazone treatments.

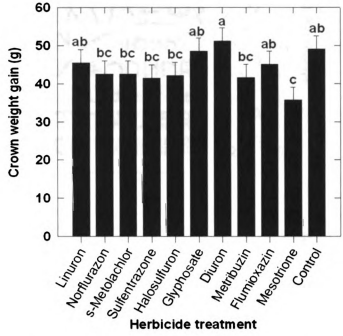

Figure 4. Effect of herbicide treatment on asparagus crown weight gain in greenhouse experiments with asparagus plants grown across soil treatments. Bars with common letters are not significantly different, LSD P=0.05.

Results for asparagus plant total dry weight (Figure 5) were similar to crown dry weight (Figure 3). Plants growing in pots sprayed with mesotrione showed significantly smaller total dry weight than the control (Figure 5B).

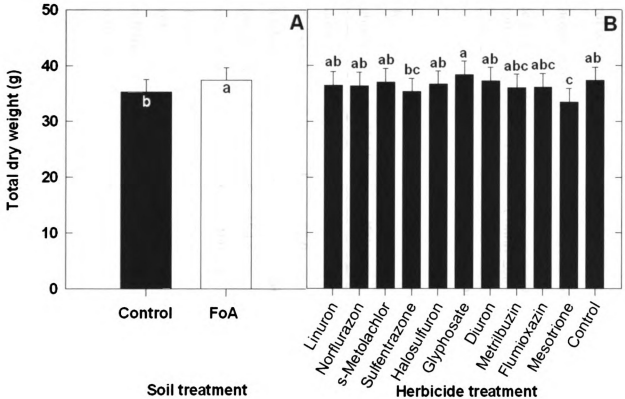

Figure 5. A, Effect of soil treatment on asparagus total dry weight in greenhouse experiments with asparagus plants grown in soil infested with *F. oxysporum* f. sp. *asparagi* (solid bar) or in soil mixed with sterile millet seed (line bar) across herbicide treatments. **B,** Effect of herbicide treatment on asparagus total dry weight in greenhouse experiments with asparagus plants grown across soil treatments. Bars with common letters are not significantly different, LSD P=0.05.

Field herbicide studies. Herbicide control efficacy was evaluated for grasses and three main broadleaf weeds: *Chenopodium album* L. (lambsquarters), *Amaranthus retroflexus* L. (pigweed) and *Solanum ptycanthum* Dunn. (eastern black nightshade). Many of the herbicides tested showed effective weed control (≥80%) 25 DAP. Grass control was significantly different ($P<0.0001$) among herbicide treatments (Table 5), with glyphosate showing the highest percentage of weed control (95.7%).

Table 5. Effect of herbicide treatments on weeds counts (compared with the untreated control) in asparagus field, Oceana County 2009.

	Weed control efficacy (%)[z]			
		Broadleaf weeds[y]		
Herbicide Treatment	Grass weeds[x]	PigweedF[w]	Lambsquarters[v]	Eastern black nightshade[u]
Linuron	51.3 de[t]	83.8 abc	89.4 ns[s]	100.0 ns[s]
Norflurazon	43.8 de	95.0 ab	100.0	100.0
s-Metolachlor	51.3 de	57.5 cd	100.0	100.0
Sulfentrazone	15.0 ef	100.0 a	95.3	100.0
Halosulfuron	6.3 ef	100 a	100.0	86.2
Glyphosate	95.7 a	38.8 d	100.0	95.8
Diuron	53.8 cd	85.0 abc	82.5	100.0
Mertibuzin	87.5 b	67.5 bcd	100.0	94.2
Mesotrione	31.3 def	97.5 ab	94.2	100.0
Terbacil	71.3 abc	45.0 d	90.9	90.9
Mixture	99.0 abc	96.3 ab	100.0	92.1

[z] Percentage based on number of weeds at the control plot

[y] Data collected 45 DAP. [x] Data collected 25 DAP

[w] *Amaranthus retroflexus.* [v] *Chenopodium album.* [u] *Solanum ptycanthum*

[t] Columns with common letters are not significantly different, LSD $P=0.05$.

[s] not significant ($P>0.05$)

Phytotoxicity was observed as chlorosis in the asparagus spears with some degree of epinasty and curving. Mesotrione showed the highest rate of phytotoxicity with an average of 4.5, and significantly differed from the remaining herbicide treatments ($P<0.0001$). The remaining herbicide treatments did not show significant phytotoxic effects with averages ranging from 1 to 1.75. Stand counts significantly differed among treatments when comparing the number of healthy spears to spears with phytotoxicity (curving and bleaching). Only 4.8% of the spears in plots sprayed with mesotrione exhibited bleaching while 10% of spears in plots sprayed with glyphosate showed curving as previously described (3).

The percentage of change was estimated by comparing the initial numbers of spears at 30 DAP to the number of spears and new fern development at 60 DAP. Although not significantly different from the untreated, plots treated with mesotrione and halosulfuron showed fewer new spears and young fern growth. Terbacil, glyphosate and the herbicide mixture had more spears per plot compared to the control (Figure 6).

Additional stand counts and fern height measurements were taken at 60, 90 and 120 DAP to determine negative herbicide residual effects and weed competition that limit asparagus plant expansion. Herbicide treatments showed significant differences among asparagus plant heights ($P<0.0001$). Linuron and diuron consistently had taller plants than the control at 60 and 120 DAP, while plots sprayed with terbacil had the highest fern number for all DAP measurements (Table 6). The smallest fern height averages were obtained from plots sprayed with mesotrione and sulfentrazone (Table 6). At 60 DAP, fern heights on plots sprayed with mesotrione and the herbicide mixture were comparable to the untreated control. However, the herbicide mixture had a significantly greater

number of ferns than the control and mesotrione (Table 6). At 90 and 120 DAP, mesotrione-treated plots produced average fern heights that were significantly less than the untreated control and all other treatments, with the exception of sulfentrazone.

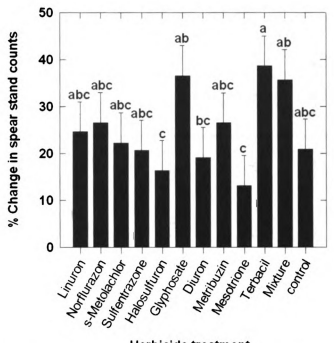

Figure 6. Effect of herbicide treatment on asparagus spear counts 30 and 60 days after planting (DAP) in the field. Bars with common letters are not significantly different, LSD P=0.05.

The number of ferns at 60, 90 and 120 DAP in plots sprayed with sulfentrazone were not significantly different from the control or the plots sprayed with mesotrione. Nonetheless, plots treated with sulfentrazone showed the smallest fern counts and heights of all the herbicide treatments at 60 and 90 DAP. At 120 DAP, plots sprayed with sulfentrazone showed fern heights that were similar to mesotrione; both were significantly smaller than the control ferns (Table 6).

Table 6. Effect of herbicide treatments on numbers of asparagus ferns and fern height at 60, 90 and 120 days after planting (DAP).

	60 DAP		90 DAP		120 DAP	
Herbicide treatment	Number healthy fern[z]	Fern height[z] (cm)	Number healthy fern	Fern height (cm)	Number healthy fern	Fern height (cm)
Control	47 cd[y]	59 d	46 bc	66 bcd	83 bc	64 cd
Linuron	61 abc	66 ab	53 b	69 abc	103 ab	68 b
Norflurazon	55 abcd	61 cd	59 ab	64 de	95 abc	68 bc
s-Metolachlor	51 bcd	65 ab	52 b	67 bc	80 bc	73 a
Sulfentrazone	39 d	57 e	35 c	56 f	73 c	61 ef
Halosulfuron	50 bcd	60 cd	46 bc	66 bcd	78 bc	65 bcd
Glyphosate	61 abc	67 a	49 bc	72 a	85 bc	64 cd
Diuron	48 cd	65 ab	46 bc	69 ab	80 bc	72 a
Metribuzin	65 ab	63 bcd	60 a	65 cde	103 ab	64 cd
Mesotrione	47 cd	58 d	48 bc	61 e	81 bc	58 f
Terbacil	70 a	63 bc	71 a	65 cde	124 a	68 b
Mixture[x]	70 a	60 d	58 ab	66 bcd	108 ab	63 de

[z] Average across plots.

[y] Data in columns with common letters are not significantly different, LSD P=0.05.

[x] Mixture contains sulfentrazone 0.179kg ha^{-1}, metribuzin 0.425kg ha^{-1}, s-metolachlor 1.599kg ha^{-1}, glyphosate 1.678kg ha^{-1}

DISCUSSION

Asparagus production relies on herbicide usage for weed management (137). However, herbicide selection poses challenges in terms of selectivity to decrease the potential adverse effects over the crop health. The effects of herbicides on Fusarium crown and root rot in asparagus were inconclusive in the greenhouse experiments due to low disease incidence. Low disease incidence could be attributed to the presence of (i) innocuous crown inhabitants and/or (ii) microbial community activity in the rhizosphere—both of which may compete with *FoA* establishment (134). It is also possible that the environment did not favor disease development. Even though herbicides have been shown to have different effects on soil microbial community composition and/or activity (4,95), we did not include these interactions in our original objectives.

Phytotoxicity was low but observed among the herbicide treatments and in *FoA*-infested and noninfested soil, significant difference among herbicides treatments or herbicide-soil interaction was not observed. The majority of the herbicide tested had some plants showing mild phytotoxic effects in the greenhouse. However abnormalities were observed in the first emerging spears only. Subsequent spears and ferns were normal, suggesting a contact rather than residual effect.

Plants sprayed with mesotrione showed the smallest crown weight gain in both soils (*FoA*-infested and noninfested) compared with the control, but crowns were smaller in noninfested soil. This pattern was observed also for crown, fern and total dry weight, suggesting mesotrione can have a detrimental effect on asparagus crown health.

Sulfentrazone showed the second smallest crown and total dry weight averages after mesotrione. Despite the warnings on flumioxazin and s-metolachlor as injurious to

asparagus, neither of them caused significant phytotoxicity or decrease in plant growth when applied on the higher recommended rates (137) in the greenhouse experiments. Glyphosate has been reported as causing spear deformation (3), but it did not show any phytotoxicity in these experiments.

The greenhouse experiment addressed application of individual herbicide active ingredients at the higher recommended rate (137), whereas in the field individual and a mixture of herbicides (an additional treatment) were evaluated (Table 3) using rates half the maximum recommended rate (137). In the field experiment, the herbicide mixture covers a broad spectrum of weeds grasses and broadleaf weeds, which is a common grower practice. Herbicide treatments were effective in controlling *S. ptycanthum* and *C. album*, and no significant differences were observed. Glyphosate, terbacil, and s-metolachlor, were the least effective in controlling *A. retroflexus* (Table 5). Ineffective grass control (Table 5) and, more importantly, phytotoxicity were observed in the plots sprayed with mesotrione, which could explain the significantly lower fern heights observed later in the growing season.

When herbicide phytotoxicity in the greenhouse and the field was observed, only the first-emerging spears were initially affected, but over time the secondary ferns and spears appeared normal. In the field, extreme phytotoxic symptoms were observed in plots sprayed with mesotrione. Crop injury has also been reported on potato, carrot, broccoli, cucumber, and onion plots treated with mesotrione (24,113).

The application of a single herbicide appeared to be less effective in controlling weeds than the herbicide mixture used in this study. Despite minor growth abnormalities witnessed with the lone sulfentrazone greenhouse treatment, no noticeable phytotoxicity

was observed in the field. Its lack of control against grasses may have contributed to the smaller number of spears and reduced fern height. Our data indicate that a mixture of products provides effective weed control, higher fern numbers and greater fern height than any of the individual herbicide tested.

During asparagus plant development, the ferns expand and store carbohydrates in the crown for the following season's growth (46). Consequently, crowns increase in size and weight. Crown fresh weight gain and crown dry weight represent the degree of expansion and growth. Enlargement of the crown is highly desirable since it will result in more buds, increased carbohydrate storage, and fern biomass production. Any condition that limits fern growth or crown expansion may impact plant performance in the subsequent growing seasons (46,53,121).

Asparagus growers must take into consideration a broad spectrum of weed control (grasses and broadleaf), but also avoid using herbicides that can cause detrimental effects on asparagus plants. Care should be taken when using herbicides such as mesotrione, since our experiments showed a detrimental effect on asparagus plant health.

CHAPTER II

PHYTOPHTHORA ASPARAGI VIRULENCE IN DETACHED ASPARAGUS SPEARS AND SUSCEPTIBILITY TO OTHER *PHYTOPHTHORA* SPECIES

ABSTRACT

Phytophthora spear and crown rot, caused by *P. asparagi*, was recently identified in Michigan as a major limiting disease of asparagus. Although different species of *Phytophthora* have been reported in asparagus production areas worldwide, only *P. asparagi* has been found infecting asparagus in Michigan. Growth chamber studies were conducted to (i) elucidate the influence of temperature on spear infection by *P. asparagi*; (ii) investigate the susceptibility of three regions of the spear to *P. asparagi*, (iii) evaluate susceptibility of asparagus to *P. cactorum, P. capsici, P. citricola, P. drechsleri, P. megasperma* and *P. nicotianae*, (iv) compare the susceptibility of various sized spears to *P. capsici* isolates, and (v) test *P. asparagi* virulence and *P. capsici* pathogenicity in asparagus crowns. When detached spears were wounded and inoculated at 2, 9 or 16 cm from the asparagus tip and incubated at 20°C, similar-sized lesions developed at all inoculated regions of supermarket spears. When 'Millennium' spears were inoculated, lesions at the spear base were larger than the other inoculated regions. *Phytophthora nicotianae* and *P. capsici* incubated at 25°C caused lesions comparable to those caused by *P. asparagi*. The pathogenicity among *P. capsici* isolates was investigated in larger spears (>0.8 cm in diameter) and small spears (≤0.8 cm in diameter). Small spears developed larger lesions compared to larger spears.

INTRODUCTION

Michigan ranks third in U.S. asparagus production, after California and Washington. In 2009, asparagus producing counties in Michigan planted 4,532 ha, producing 12 million kg of asparagus spears valued at $16.5 millions (6). Diseases can be a limiting factor in asparagus at any point during production of this perennial crop. Historically, the most important soilborne disease affecting asparagus has been *Fusarium* crown, root and stem rot. The causal agents *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* reside in the soil, crop debris, or may be introduced on asparagus crowns used to establish production fields (48,51). When infection by *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* occurs, disease symptoms include fern yellowing and crown death (51) .

Phytophthora is a major genus of plant pathogens, consisting of at least 60 species (55). *Phytophthora* spp. can infect a broad range of hosts including asparagus, and five species have been reported to infect this perennial crop (7,58,119,132). *Phytophthora* was not a concern in Michigan asparagus fields until 2004, when Saude et. al. (2005) reported *P. asparagi* infecting spears and roots during the harvest season. Disease symptoms on spears included brown, water-soaked lesions at the base, shriveling, and twisting (119). On the crown, water-soaked lesions and shriveling of the storage roots predominates (119). Symptoms are most common following excessive precipitation and typically occur in areas of the field with poor drainage (119).

In addition to *P. asparagi*, other *Phytophthora* spp. in Michigan may pose a threat to asparagus production. *Phytophthora megasperma* Dreshler infects a broad range of hosts and various plant parts (55). *Phytophthora megasperma* taxa are a broad group that

had been divided based on morphological variability and complexity (55,62). *Phytophthora megasperma* var. *sojae, P. megasperma* var. *medicaginis* and *P. megasperma* var. *trifoli* were previously considered to belong to *P. megasperma* taxa but are now considered separate species: *P. sojae* Kaufman & Gerderman, *P. medicaginis* Hansen & Maxwell and *P. trifoli* Hansen & Maxwell, respectively (55). In the late 80s, *P. megasperma* and *P. sojae* (formerly *P. megasperma* var. *sojae*) were reported infecting asparagus in New Zealand (19,55,58,60), and California (9,55,58,60). In 2003, *P. megasperma* Drechsler was reported on asparagus in Canada (132). In California, *P. sojae* Pethybridge & Lafferty predominated among asparagus fields and occasionally *P. cryptogea* Pethybridge & Lafferty was isolated from asparagus spears with mild disease symptoms (60). *Phytophthora nicotianae* Galindo & Holt infects roots, stems, trunks, leaves, and fruits of a wide host range including 255 genera in 90 families (55). In 2008, Aragon-Caballero described a heterothallic *Phytophthora* sp. (Mating Type -MT: A2) inciting asparagus root rot in Peru (7) that was identified as *P. nicotianae* based on morphological and molecular characteristics.

Management of diseases caused by *Phytophthora* spp. includes effective fungicides application, appropriate crop rotation, and a variety of cultural practices (i.e. raised beds, plastic mulch and drip irrigation) (80). The fungicide metalaxyl provided disease control and improved asparagus yields in California (58). Control of *Phytophthora* spp. using chemical control is hampered by the presence of fungicide resistance in pathogen populations (28,55,107,130). *Phytophthora asparagi* isolates form Michigan were sensitive to mefenoxan (119). The efficacy of crop rotation as a disease management strategy is limited by the broad host range of some *Phytophthora* spp.

(55,61,93). The ability of *P. asparagi* to infect crops commonly growing in asparagus production areas was investigated by Saude et. al. (2008). *Phytophthora asparagi* caused lesions on cucurbit fruit and was reisolated from the roots of asymptomatic red clover, alfalfa, and soybean plants (119). Studies to evaluate the susceptibility of asparagus to *Phytophthora* spp. will help determine if this perennial crop could be used in rotational scheme to plant in soils where other crops have failed or were abandoned due to a history of *Phytophthora* infestation (i.e. *P. capsici* Leonian) (80,108).

The objectives of this study were to (i) elucidate the influence of temperature on spear infection by *P. asparagi*; (ii) investigate the susceptibility of regions of the spear to *P. asparagi*, (iii) evaluate susceptibility of asparagus to *P. cactorum, P. capsici, P. citricola, P. drechsleri, P. megasperma* and *P. nicotianae*, (iv) compare the susceptibility of various sized spears to *P. capsici* isolates, and (v) test *P. asparagi* virulence and *P. capsici* pathogenicity in asparagus crowns.

MATERIALS AND METHODS

***Phytophthora* spp. selection and culture.** Isolates of *P. cactorum, P. capsici, P. citricola, P. drechsleri, P. megasperma* and *P. nicotianae* were obtained from the *Phytophthora* spp. culture collection maintained in M. K. Hausbeck's laboratory at Michigan State University. Isolates were transferred from long-term stock cultures onto AR-V8 (100 ppm of ampicillin and 30 ppm of rifampicin amended unclarified V8 juice agar) (72) and maintained at room temperature (21±2°C) under continuous fluorescent lighting. Agar plugs (4-mm in diameter) from actively growing colonies were used to inoculate spears.

Phytophthora asparagi isolates C013, SP319 and SP3236 were recovered from asparagus crown (C013) or spear (SP319 and SP3236) tissue from northwest Michigan in 2005 (119) and determined to be sensitive to mefenoxan. Other *Phytophthora* spp. were included: *P. nicotianae* (isolate 13048) from snapdragon, *P. cactorum* (isolate 4001) from ginseng, *P. drechsleri* (isolate 4355) from poinsettia, *P. megasperma* (isolate 3100) from an unknown host, *P. citricola* (isolate 3200) from an unknown host and *P. capsici* (isolate OP97) from pickling cucumber. To compare *P. capsici* isolates for virulence in asparagus, a variety of isolates in terms of mating type (A1 or A2) and sensitivity to the fungicide mefenoxam (S: sensitive, or I: insensitive) were included as follows: *P. capsici* OP97 (A1 S) from pickling cucumber, SP98 (A2 S) from pumpkin, 12889 (A1 I) from pepper, SFF3 (A2 I) from pickling cucumber and 13351 from eggplant (A2 S).

Spear inoculation and incubation. Commercial grade spears (>0.8 cm in diameter) were visually screened for disease and mechanical damage, selected and surface disinfested with 5% sodium hypochlorite for 5 seconds, rinsed three times in distilled water for 5 seconds and air-dried at room temperature. After drying, spears were trimmed to 18 cm, and surface wounded with a flame-sterilized needle prior to inoculation (119). A 0.4 cm mycelial plug was placed over the wound and secured with parafilm. Humid chambers consisted of rectangular polystyrene containers (23x10x32 cm, Potomac display, Hampstead, MD) filled with 1liter of tap water. A WatchDog (Spectrum Technologies, Inc., Plainfield, IL) was placed inside the humid chamber to record temperature and relative humidity during the incubation time. The spears were placed on a clear acrylic platform (0.8 cm thick, 26x21 cm x H=2.5 cm) inside the humid chambers and incubated in growth chambers (Conviron: model CMP3244, Pembina, ND)

with a 16-hour photoperiod for 5 days at 15, 20 or 25°C. After incubation, the length and width of each lesion was measured and presence of mycelia noted. The lesion area was calculated based on the ellipse area ($\pi^* r_1 * r_2$, where r_1 was length/2 and r_2 was width/2). Approximately 10% of the spears where sampled to re-isolate the pathogen from the spear tissue.

Susceptibility of spear regions to *P. asparagi*. A flame-sterilized needle was used to wound each spear at 2, 9, and 16 cm from the apical end (also referred to as tip, middle and base spear regions, respectively). All wounds were covered with a mycelial plug (as described above) of *P. asparagi* isolates C013, SP319 or SP3236. Uninfested AR-V8 media plugs were used as a negative control. Five spears per isolate were used. Spears were placed in a humid chamber and incubated in a growth chamber. Experiments were replicated six times; four times using supermarket spears and twice with 'Millennium' spears.

Susceptibility of asparagus to *Phytophthora* spp. The pathogenicity of six *Phytophthora* spp.: *P. capsici, P. cactorum, P. citricola, P. drechsleri, P. megasperma* and *P. nicotianae* were compared to *P. asparagi* isolate (SP319). Uninfested AR-V8 media plugs were used as a negative control. Six spears were inoculated per isolate. Spear inoculation and incubation were performed as described above. Incubation temperatures of 15, 20 and 25°C were used. Experiments were replicated three times using 'Jersey Knight' spears. Jersey Knight' spears were inoculated at 9 cm (middle) from the tip because bacterial spear tip rot was common on supermarket spears regardless of incubation temperature.

Susceptibility of various-sized asparagus spears to *P. capsici* isolates. Five isolates of *P. capsici* (13351, SFF3, 12889, SP98, OP97) were evaluated in two experiments and compared to *P. asparagi* isolate SP319 and a negative control using uninfested AR-V8 media plugs. The first experiment used spear grades that were 0.8 to 1.3 cm in diameter and was conducted twice with 'Millennium' spears using the middle inoculation point. Five spears were used per isolate.

The second experiment used smaller asparagus spears (<0.8 cm in diameter) that were not of a marketable grade. Small spears were trimmed at 14 cm from the apical end, and inoculated in the middle (7 cm from the tip). The experiment was conducted four times using 'Millennium' spears and twice with 'Jersey Knight' spears; there were two replicate spears inoculated for each *P. capsici* isolate. Incubation temperatures of 15, 20 and 25°C were used for the two experiments conducted using *P. capsici*.

Crown inoculation experiments. One-year-old 'Tiessen' dormant crowns obtained from a commercial nursery (Malburg Farm in Oceana County, MI), were washed and visually screened for disease, mechanical damage and size homogeneity, and stored in a cold room (4°C) until they were used. Crown roots were measured and roots ≥0.3 cm in width and ≥14 cm in length were selected. *Phytophthora asparagi* C013 and *P. capsici* 13351 were used to inoculate the crowns. Crowns were wounded with a flame-sterilized needle and 0.2-cm mycelial plugs were attached with parafilm to the roots. Inoculated and non-inoculated crowns were placed in the humid chambers and incubated in a growth chamber at 15, 20 and 25°C as described above for the spears. Uninfested AR-V8 agar plugs were used as a negative control. Crowns in humid chamber were incubated for five days in darkness (Diurnal growth chamber 3740,

Thermo Scientific: formerly Forma Scientific, Waltham, MA). These experiments were conducted four times and six crowns per isolate were included.

Statistical analysis. A split-split plot design with three fixed factors was used as follows: experimental replicate as the blocking factor, temperature as the whole-plot factor within each block (trial), and isolate as the sub-plot factor within each temperature. For the experiments investigating the susceptibility of spear regions to *P. asparagi,* an additional factor was included: the sub-sub-plot factor spear regions within each isolate. The PROC MIXED and PROC GLIMMIX were used as implemented in SAS statistical analysis software (SAS Institute Inc., Cary, NC). Data were tested for equal variances and normal distribution. A square root transformation was conducted to fulfill normal distribution assumptions when necessary. ANOVA was evaluated using type 3 effects output ($P<0.05$). Significant differences were determined using Fisher's protected least significant difference (LSD) for investigated factor (i.e spear regions, isolates and temperatures) and factor interaction for all the experiments. Differences were considered significant with $\alpha=0.05$.

RESULTS

Susceptibility of spear regions to *P. asparagi*. Infection and lesion development occurred when spears were inoculated with *P. asparagi* and incubated at 15, 20 and 25^{o}C. The three *P. asparagi* isolates caused lesions that were similar in appearance. Dark brown water-soaked lesions and spear shriveling were characteristic. When supermarket spears were inoculated, there were no significant differences in lesion size among *P. asparagi* isolates (Figure 7A). Isolate SP319 caused significantly smaller lesions ($P=0.03$) on 'Millennium' asparagus spears (Figure 7B) compared to isolates

SP3236 and C013. Neither sporulation nor mycelia were observed within the lesions. All *P. asparagi* isolates were successfully reisolated from the lesions.

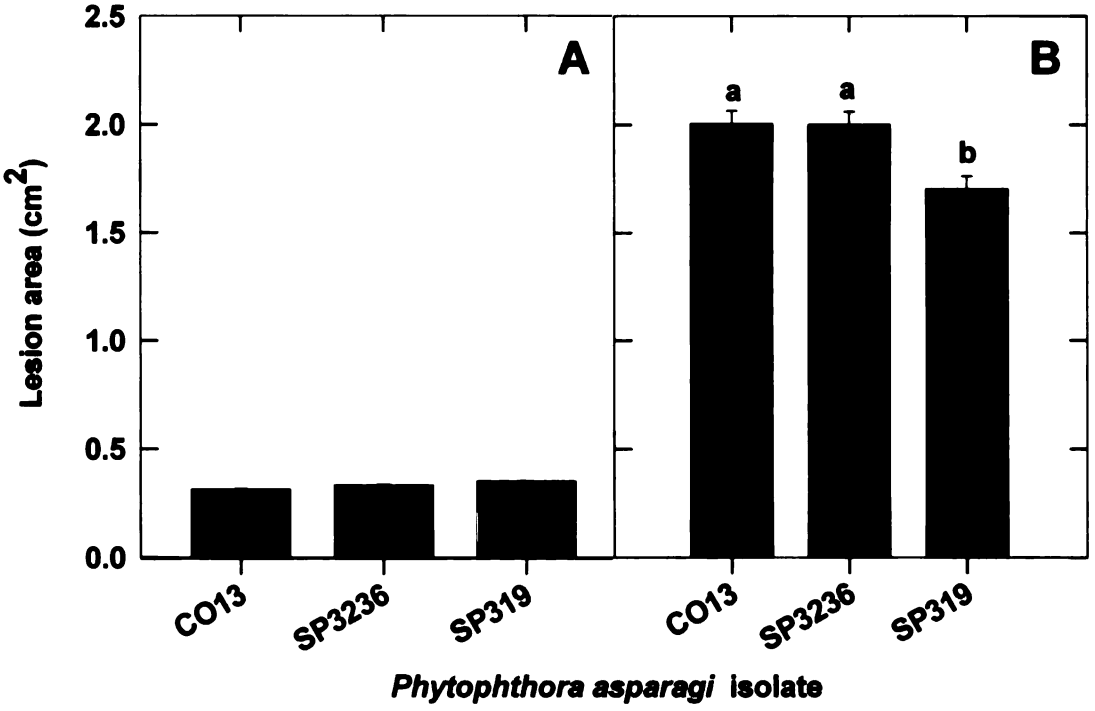

Figure 7. Mean lesion area on detached asparagus spears 5 days after inoculation with *P. asparagi* isolates C013, SP3236 and SP319 on **A,** supermarket spears and **B,** 'Millennium' spears. Bars with common letters are not significantly different, LSD *P*=0.05.

Lesion area caused by *P. asparagi* differed significantly among incubation temperatures. At 20°C, *P. asparagi* isolates caused the largest lesions in both 'Millennium' and supermarket spears (Figure 8A and B). When supermarket asparagus spears were used, the smallest lesions occurred at 25°C (Figure 8A). When pooling means by spear region inoculation on supermarket spears, there were no significant differences in lesion size (*P*=0.701) (Figure 9A). However, lesions caused by *P. asparagi* in the spear tip when incubated at 15 and 25°C were significantly different from lesions at the middle and base of the spear at the same temperatures. Similarly, lesions caused by the pathogen at the tip when incubated at 15°C were significantly larger than

those developed at the tip incubated at 25°C (Figure 10B). Lesions on 'Millennium' spears were similar at 15 and 25°C but significantly smaller than those at 20°C when lesions were pooled across inoculation region and across isolates (Figure 8B).

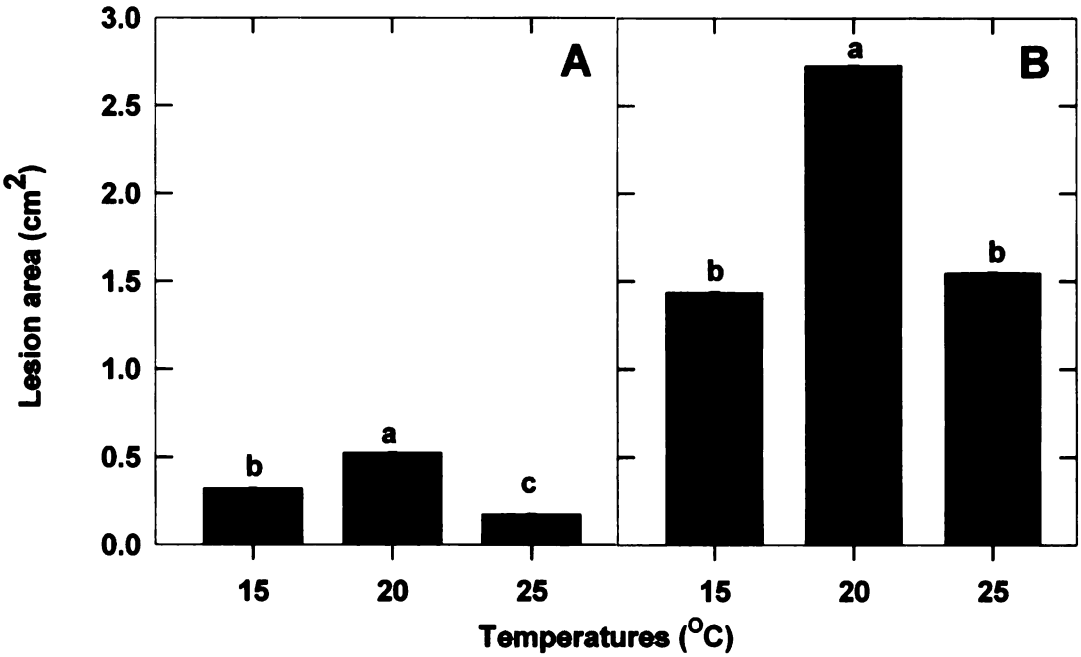

Figure 8. Effect of temperature on mean lesion area on detached asparagus spears 5 days after inoculation with *P. asparagi* on **A**, supermarket spears, and **B**, 'Millennium' spears. Bars with common letters are not significantly different, LSD P=0.05.

Significant differences among spear regions were found when the experiments were conducted using 'Millennium' spears ($P<0.001$, Figure 9B); lesion means were larger compared with those observed in supermarket spears.

A significant interaction between spear regions and temperature was observed, although interaction patterns differed between experiments using supermarket or 'Millennium' spears (Figure 10A and B). In general, lesions on spears incubated at 20°C were larger than for other temperatures tested, regardless of the spear region inoculated.

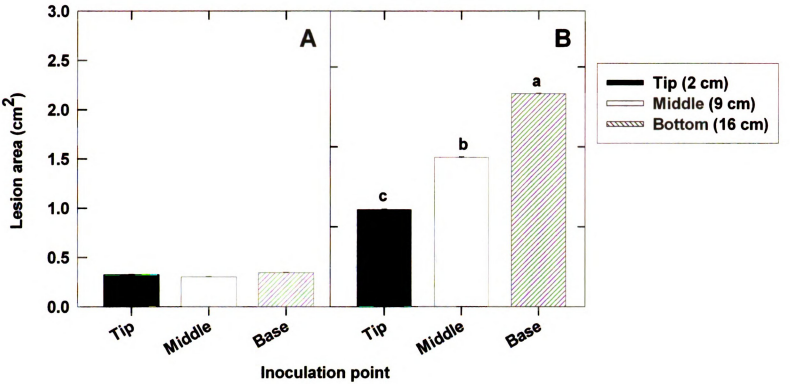

Figure 9. Effect of spear region on mean lesion area on detached asparagus spears 5 days after inoculation with *P. asparagi* on **A**, supermarket spears and **B**, 'Millennium' spears. Bars with common letters are not significantly different, LSD P=0.05.

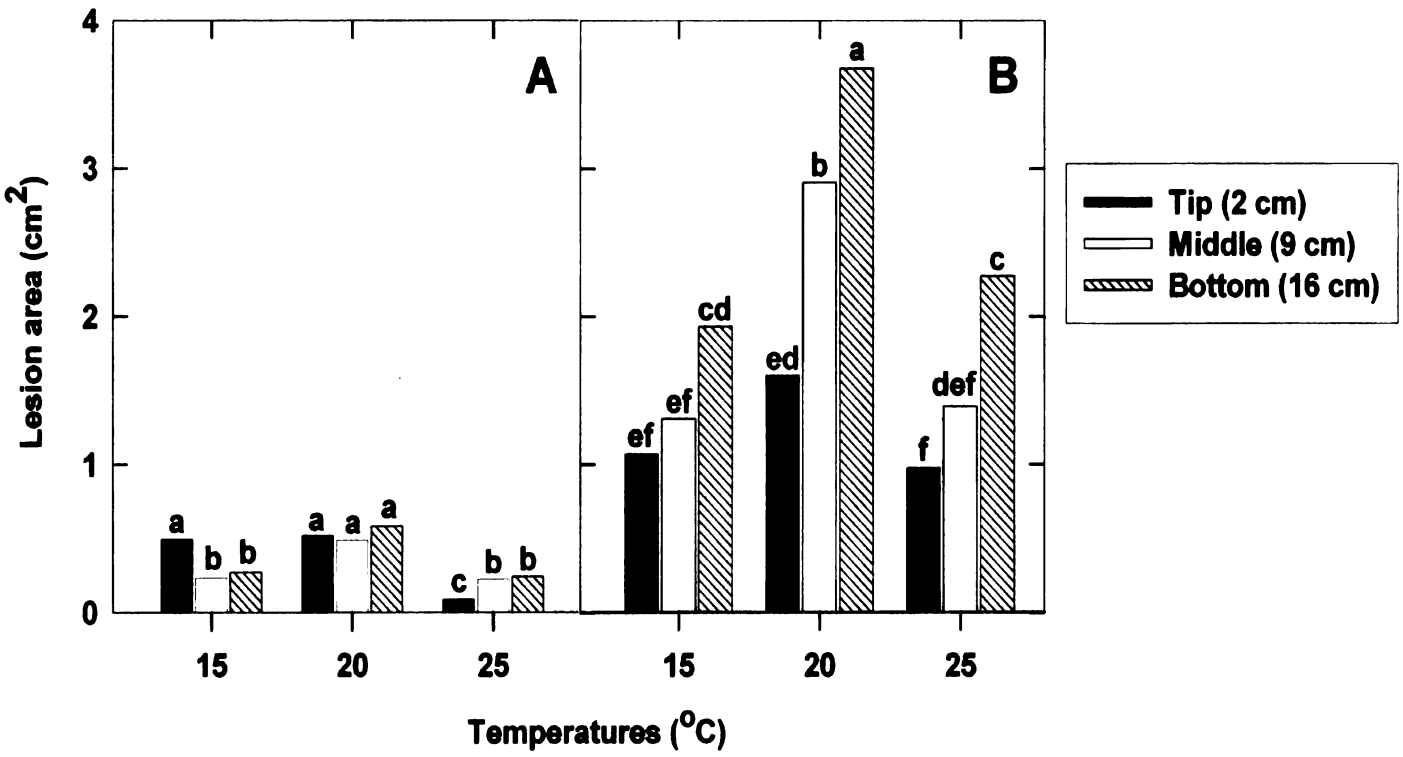

Figure 10. Effect of temperature and spear regions on mean lesion area on detached asparagus spears 5 days after inoculation with *P. asparagi* on **A,** supermarket spears and **B,** 'Millennium' spears. Bars with common letters are not significantly different, LSD *P*=0.05.

In addition, lesions at the base of the spear were commonly larger than those at the middle; lesions at the tip of the spear were the smallest (Figure 10B). At all temperatures tested, lesions at the base of the spear were larger than at the tip or middle of the spear.

Susceptibility of asparagus to *Phytophthora* spp. Spear lesions caused by *P. asparagi* were larger than those caused by all other *Phytophthora* spp., when means were pooled among temperatures tested (Figure 11A). Lesions caused by *P. nicotianae* and *P. capsici* were larger (0.56 and 0.37 cm^2 respectively) than those caused by *P. cactorum*, *P. citricola*, *P. drechsleri*, and *P. megasperma*. *Phytophthora asparagi* was recovered from spear tissue readily (92%), while *P. nicotianae* and *P. capsici* were isolated less frequently (22% and 11%, respectively). *Phytophthora cactorum*, *P. citricola*, *P. drechsleri*, and *P. megasperma* were not successfully recovered from the spear tissue. When average lesion sizes were pooled among *Phytophthora* spp. lesions were significantly larger ($P<0.0001$) when spears were incubated at 25°C compared to 20°C and 15°C (Figure 11B). The smallest lesions developed when the incubation temperature was 15°C (Figure 11B).

Average lesion size caused by *P. asparagi* 319 at 25°C was not significantly different from any of the other *Phytophthora* spp.-temperature combinations, except for *P. capsici* OP97 and *P. nicotianae* 13048. Lesions caused by *P. nicotianae* at 20 and 25°C, and by *P. capsici* at 25°C were not significantly different from lesions caused by *P. asparagi* at 15 and 20°C (Figure 11C)

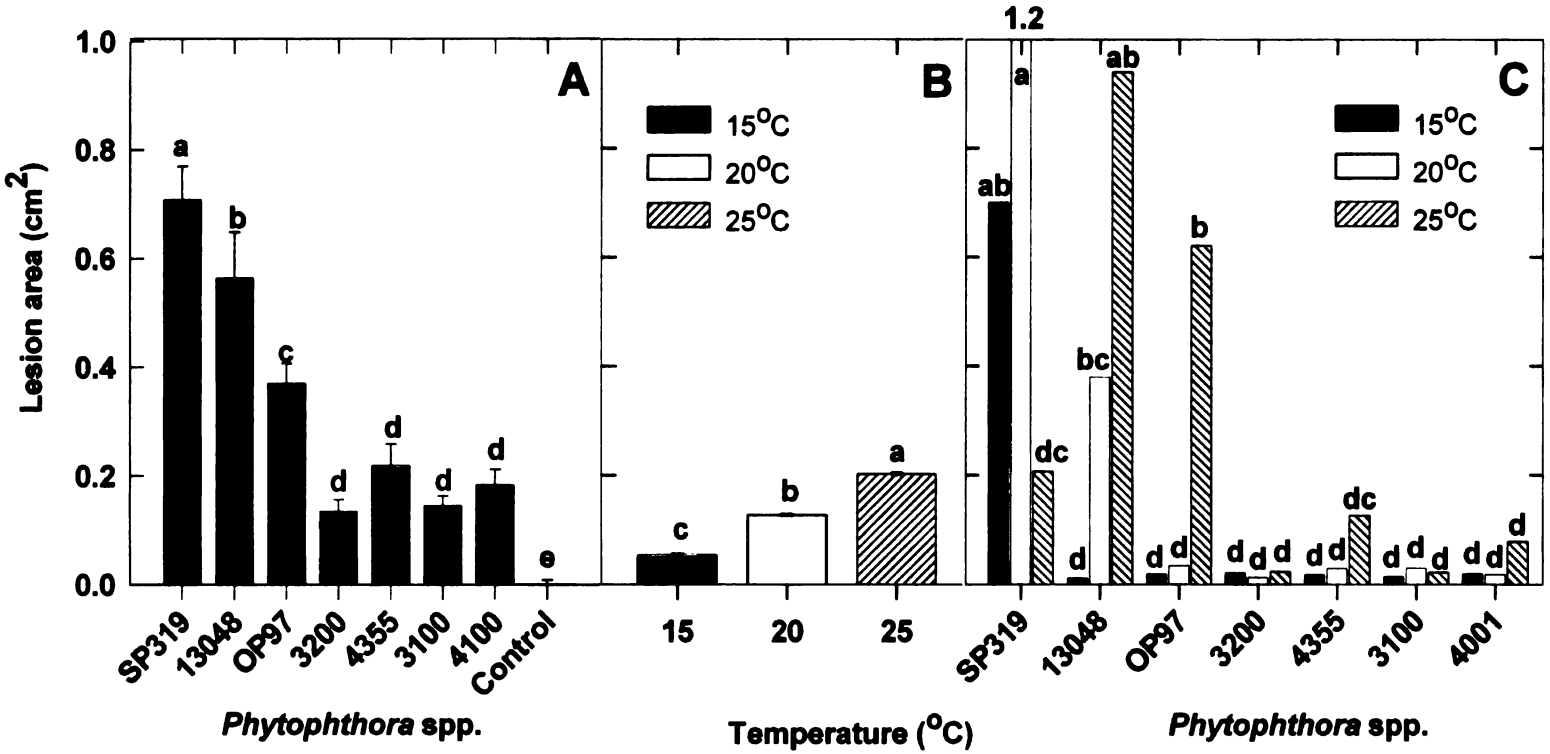

Figure 11. **A,** Effect of *Phytophthora* spp. (*P. asparagi* SP319, *P. nicotianae* 13048, *P. capsici* OP97, *P. citricola* 3200, *P. drechsleri* 4335, *P. megasperma* 3100 and *P. cactorum* 4001) averaged across temperatures (15, 20 and 25°C) on mean lesion area 5 days after inoculation (0.07 was subtracted from the lesion average to account for the effect of wounding). **B,** Effect of temperature averaged across *Phytophthora* spp. on mean lesion area 5 days after inoculation. **C,** *Phytophthora* spp. and temperatures interaction on mean lesion area 5 days after inoculation. 'Jersey Knight' detached asparagus spears were used in this study. Bars with common letters are not significantly different, LSD P=0.05.

Susceptibility of various sized spear asparagus to *P. capsici.* When larger 'Millennium' asparagus spears were inoculated with *P. capsici,* lesions were significantly smaller compared to lesions resulting from inoculation with *P. asparagi* (P=0.0001, Figure 12A) regardless of the incubation temperature (Figure 12C). Lesion size by temperature showed the same pattern (i.e. lesions were favored at 20°C) observed in previous *P. asparagi* experiments (Figure 12B). Reisolation of *P. capsici* from asparagus tissue was achieved for isolate 13351 (25%) and less frequently for SFF3 (5%) when spears were incubated at 25°C. Recovery was not possible at 15 or 20°C, for isolates 13351 and SFF3. Reisolation from the spear tissue was not successful for *P. capsici* isolates OP97, SP98 and 12889.

P. asparagi caused lesions on small spears, that were comparable in size to lesions formed on larger spears. In small spears, virulence varied among *P. capsici* isolates (Figure 13A). When incubated at 25°C, *P. capsici* 13351 caused lesions similar in size to those caused by *P. asparagi* at all temperatures. Similarly *P. capsici* SFF3 caused lesions similar in size to those caused by *P. asparagi* at 15 and 25°C (Figure 13C).

Crown experiments. *Phytophthora asparagi* caused restricted light brown water-soaked lesions on the roots of crowns. *Phytophthora capsici* caused lesions that were barely discernible, without characteristic color, although the inner tissue had turned reddish brown. Lesions caused by *P. asparagi* were significantly bigger than those caused by *P. capsici* 13351 (Figure 14A).

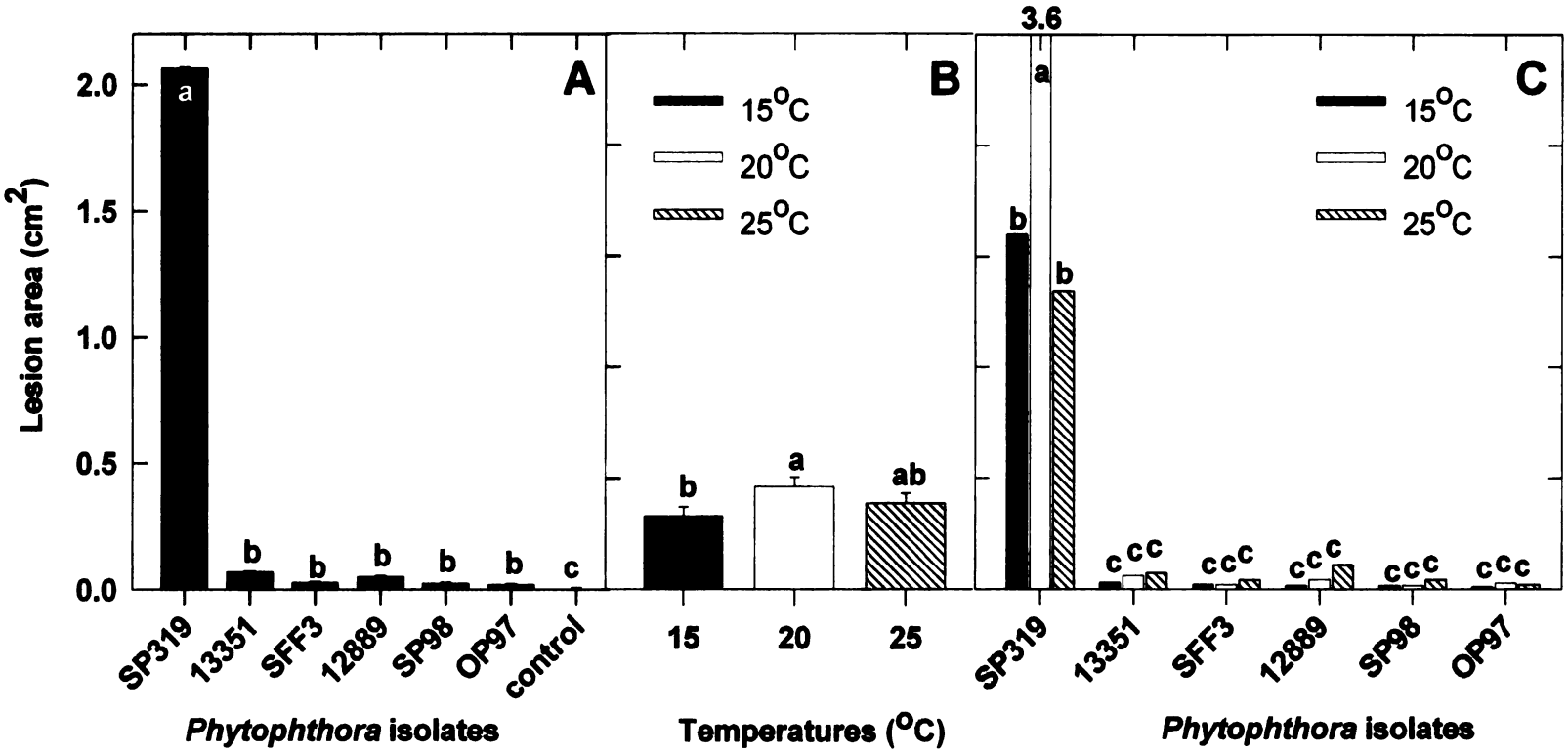

Figure 12. **A,** Effects of *P. asparagi* (isolate SP319) *P. capsici* (isolates 13351, SFF3, 12889, SP98, OP97) averaged across temperatures (15, 20 and 25°C) on mean lesion area 5 days after inoculation. **B**, Effect of temperature averaged across *Phytophthora* spp. (*P. capsici* and *P. asparagi*) on mean lesion area 5 days after inoculation. **C,** *Phytophthora* spp. and temperatures interaction on mean lesion area 5 days after inoculation. All conducted on 'Millennium' detached asparagus spears. Bars with common letters are not significantly different, LSD P=0.05.

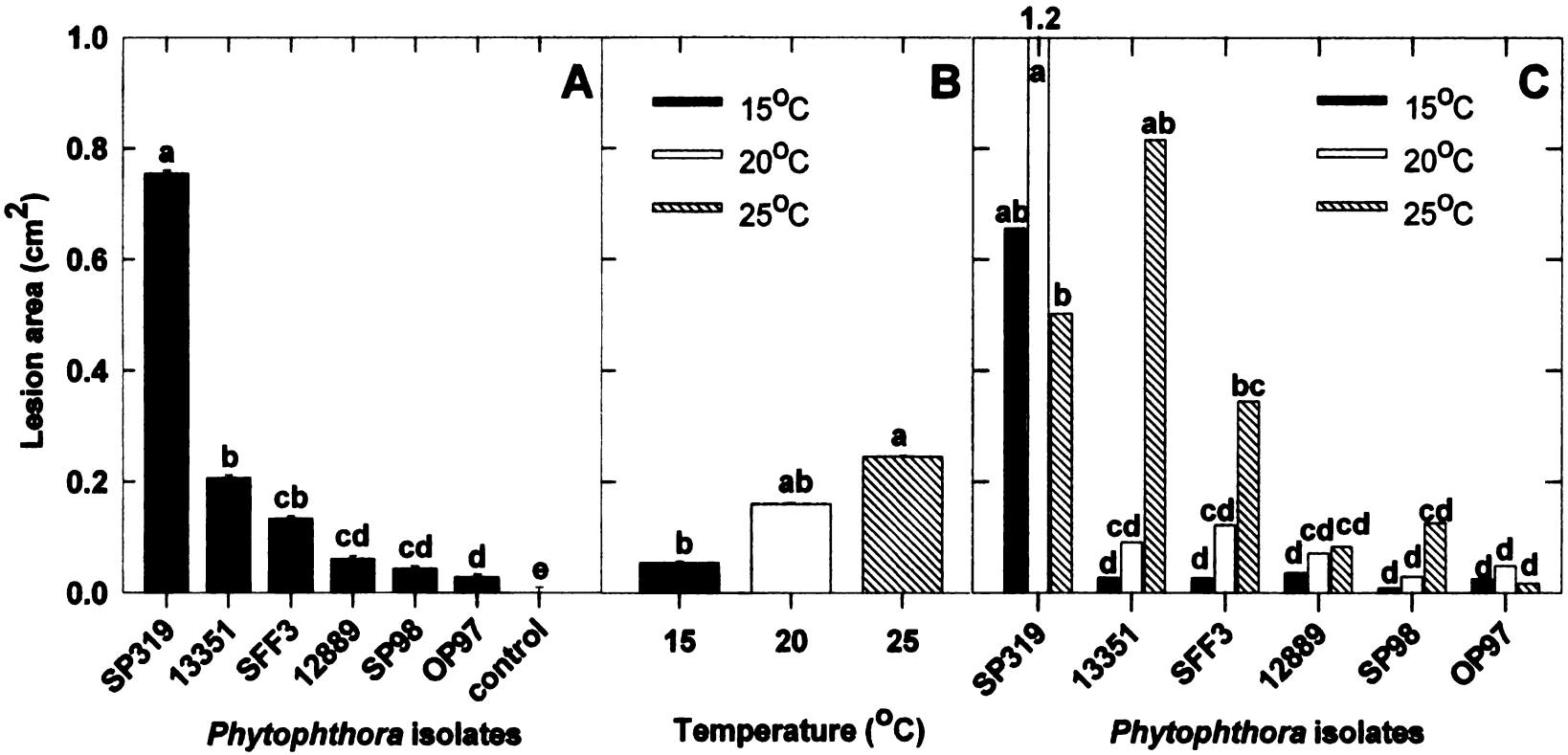

Figure 13. **A,** Effects of *P. asparagi* (isolate SP319) and *P. capsici* (isolates 13351, SFF3, 12889, SP98, OP97) averaged across temperatures (15, 20 and 25°C) on mean lesion area 5 days after inoculation. **B,** Effect of temperature averaged across *Phytophthora* spp. (*P. capsici* and *P. asparagi*) on mean lesion area 5 days after inoculation. **C,** *Phytophthora* spp. and temperatures interaction on mean lesion area 5 days after inoculation conducted on 'Millennium' and 'Jersey Knight' detached small asparagus spear. Bars with common letters are not significantly different LSD P=0.05.

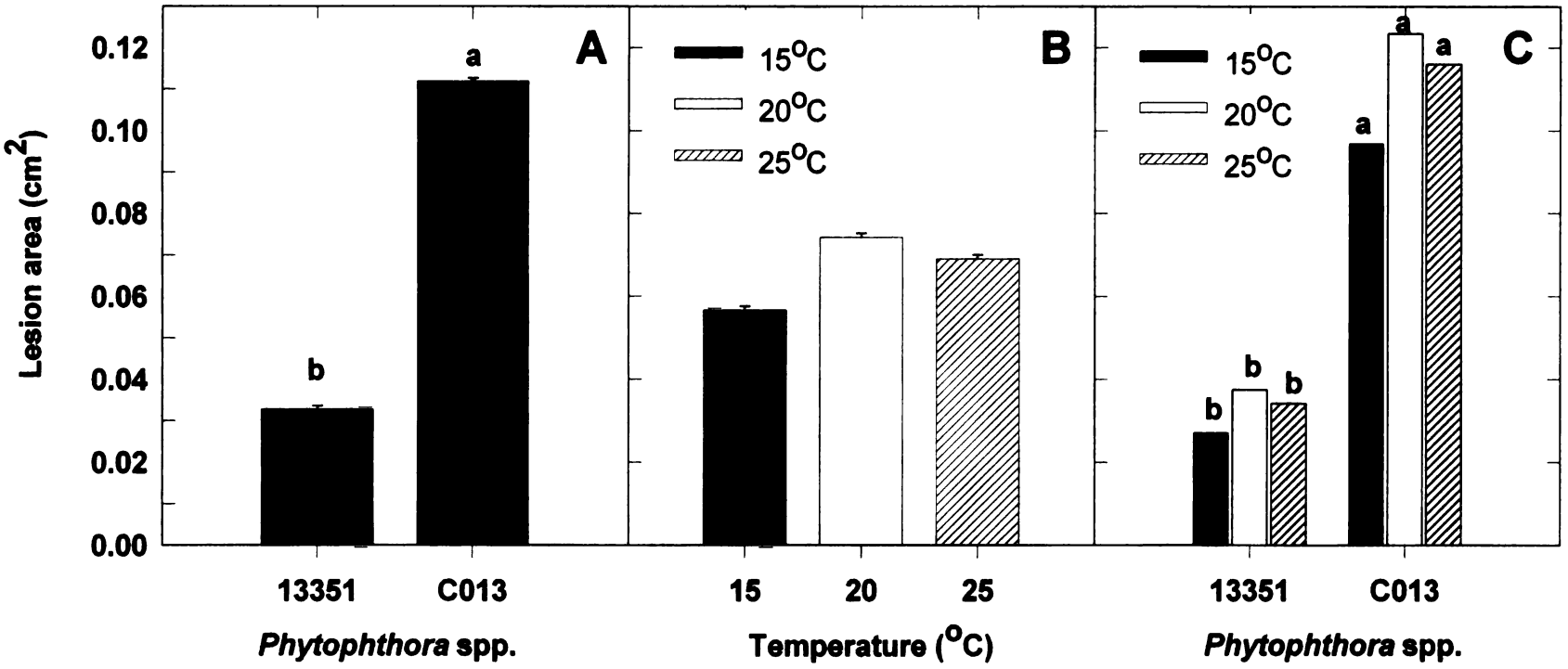

Figure 14. **A**, Effect of *P. asparagi* C013 and *P. capsici* 13351 averaged across temperatures (15, 20 and 25°C) on mean lesion area 5 days after inoculation on asparagus crowns ('Tiessen'). **B**, Effect of temperature averaged across *Phytophthora* spp. on mean lesion area 5 days after crowns inoculation. **C**, *Phytophthora* spp. and temperatures interaction on mean lesion area 5 days after crowns inoculation. Bars with common letters are not significantly different, LSD P=0.05.

There were no significant differences among temperatures (Figure 14B) or in the interaction between temperature and *Phytophthora* spp. (Figure 14C). However, *P. capsici* and *P. asparagi* showed a common pattern where the larger lesions occurred at 20°C and the smallest lesions were observed at 15°C (Figure 14B). Reisolation from the crown tissue was not successful for *P. capsici* and low (<5%) for *P. asparagi.*

DISCUSSION

When asparagus spear were inoculated with *P. asparagi, P. nicotianae*, and *P. capsici,* lesions developed and the pathogens were recovered from the symptomatic tissue. Lesions were necrotic and water-soaked in appearance in agreement with previous descriptions of *P. asparagi* (119) and *P. nicotianae* (7) symptoms in asparagus. Conversely, no mycelia were observed within the lesions or in adjacent tissue as commonly reported for *P. capsici* (66). *Phytophthora asparagi* was more virulent on asparagus spears than the other *Phytophthora* spp. tested in our experiments. The lesions that formed on spears inoculated with *P. citricola*, *P. drechsleri*, *P. megasperma*, and *P. cactorum* were significantly larger than the lesions on control spears. However, since none of these species could be recovered from the spears, the lesions may be due to a wounding response or perhaps these *Phytophthora* spp. are only weakly pathogenic to asparagus spear. *Phytophthora megasperma, P. sojae, P. cryptogea, P. cactorum, P. richardiae, P. nicotianae* and *P. asparagi* have been previously reported as pathogens to asparagus either in the field or post-harvest (55,59-61). The results of this study agree with previous reports of *P. asparagi* (118,119) and *P. nicotianae* (7) as pathogens of asparagus. The isolates of *P. cactorum* and *P. megasperma* used in this study did not cause disease despite previous reports of pathogenicity (58). Only a single isolate of each

species was used in our studies; further experiments with additional isolates of *P. cactorum* and *P. megasperma* may be necessary.

Lesions were largest on spears inoculated with *P. asparagi* and incubated at 15 or 20°C and on spears inoculated with *P. nicotianae* and incubated at 25°C. Lesions were also observed on spears inoculated with *P. nicotianae* and incubated at 20°C and on spears inoculated with *P. capsici* and incubated at 25°C. *Phytophthora* spp. growth on culture media varies among species. *P. cactorum* and *P. megasperma* have similar minimum, optimum, and maximum temperatures (2°C, 25°C, and 31°C, respectively). Although while *P. nicotianae* and *P. capsici* have optimum growth temperatures from 27-32°C and can grow in media up to 35°C, these temperatures can vary depending on the isolates (101). Since larger lesions caused by *P. nicotianae* and *P. capsici* occurred at the higher temperatures used in our study (20°C and 25°C), it would be interesting to determine the infective capability of these pathogens at higher temperatures (above 25°C).

In Michigan, air temperatures the harvest season would be expected to range between 13 to 27°C (http://climate.geo.msu.edu/index.html). Lesions caused by *P. asparagi* in our experiments were favored at 20°C but also occurred at 15 and 25°C, which is not surprising given that the minimum, optimum, and maximum temperatures for growth of *P. asparagi* are 10, 25, and <30°C, respectively (119). Asparagus spear emergence occurs when soil temperatures range from 12.7 to 17.7°C one day before emergence. According to our results, *P. asparagi* can grow and infect spears in the same

range of temperatures required for spear emergence. It is possible that in the field, *P. asparagi* infects emerging spears only under a narrow range of environmental conditions but additional studies are needed to investigate this further.

The largest mean lesion size was observed when the base of 'Millennium' spears was inoculated with *P. asparagi* and incubated at 20°C. The base of the spear has higher fiber and sugar content, and lower protein and metabolic activity (i.e. respiration rate) (39,86,96) compared with the middle and tip regions. These characteristics of the spear base could be more conducive to *P. asparagi* disease compared to the middle or tip of the spear. However, to the best of our knowledge, no studies have been published exploring the effect of sugar, fiber, and protein content and metabolic activity on *Phytophthora* infection. Larger lesions were observed on 'Millennium' spears, suggesting they were more susceptible to *Phytophthora asparagi* than the supermarket or 'Jersey Knight" spears. A lack of freshness or differences in cultivar may have influenced susceptibility of supermarket spears. Structural changes within the spear occur as soon as 1 day after harvest (31,96). Supermarket spears originated from California, Mexico and Peru. The main cultivars grown in these areas differ from those grown in Michigan (5,102,115,128). In California production areas, the main varieties planted are 'UC157', 'Grande, Apolo', 'Brock imperial', 'Ida Lea', and 'Atlas' 'Mary Washington', 'Palmeto', 'Argentenil', 'UC157' and 'UC 72' are grown in Peru and Mexico among others. Whereas in Michigan growers favor 'Jersey', 'Millennium' and 'Tiessen'.

Phytophthora capsici is widespread in Michigan fields used for production of susceptible vegetables such as cucurbit and solanaceous crops (55,63,80). Due to the importance and prevalence *P. capsici* in Michigan studies were conducted, to determine

whether asparagus is susceptible to this pathogen. In larger 'Millennium' asparagus spears of commercial grade, *P. capsici* isolates did not cause significant infection and lesions were not comparable in size with those caused by *P. asparagi*. Homogeneity of lesion size was observed among *P. capsici* isolates infecting larger spears. Conversely, when small spears that were not of commercial grade were inoculated with *P. capsici,* virulence was variable among the isolates. Small spears inoculated with *P. capsici* isolates from eggplant (13351) and pickling cumber (SFF3) and incubated at 25°C developed lesions comparable in size to those caused by *P. asparagi* regardless of temperature. Crown inoculation needs to be refined, since the conditions used in our experiments were not conducive for *P. asparagi* infection and characteristics lesion formation observed in naturally infected crowns from field nurseries (119).

Based on our studies, we do not consider *P. capsici* a significant threat to "Millennium' asparagus spears. However, during warm harvest seasons (approximately 25°C) infection of small spears and perhaps asparagus seedlings by *P. capsici* could occur. Field studies are required to establish whether *P. capsici* can infect asparagus under natural field conditions and the pathosystem needs to be studied further to determine the impact on crop rotation strategies. Over a dozen of different asparagus varieties are grown in the US (47,128). Future research could compare *P. asparagi, P. nicotianae* and *P. capsici* virulence on a broad range of asparagus cultivars using more and diverse isolates, since cultivar resistance is a preferred strategy to manage Phytophthora spear and root rot in asparagus fields.

Phytophthora spp. have been found in rivers, ponds, ditches and irrigation systems (67,92), and are dynamic pathogens capable of infecting a broad range of hosts

(55,61). In soil, *Phytophthora* spp. may be present as mycelia (55) or oospores (21,44,64) that can persist in fields for many years serving as primary inocula and limiting grower's ability to plant susceptible crops (40). The inbreeding in homothallic species such as *P. asparagi,* limits genetic variability (71). In species such *P. nicotianae* and *P. capsici* (heterothallic) sexual reproduction is a source of genetic variation (94), which gives these pathogens an increased probability of gaining traits such as increased virulence and/or pathogenicity on additional hosts. Further studies could investigate whether these three *Phytophthora* spp. are present in Michigan asparagus fields and determine the risk of disease

APPENDIX

FUNGICIDE EFFICACY ON PHYTOPHTHORA ROT IN ASPARAGUS SEEDLINGS IN THE GREENHOUSE

ASPARAGUS (*Asparagus officinalis*) 'Millenium'
Phytophthora spear and root rot;
Phytophthora asparagi

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Evaluation of fungicides to control Phytophthora spear and root rot on asparagus seedlings, 2009.

This study was conducted in the greenhouse using *Phytophthora asparagi* inoculum, prepared by growing the pathogen in flasks containing sterile millet for 3 weeks. Inoculum was mixed with soilless media (8 oz/ft^3) and distributed into 2 x 3 cell trays, where untreated asparagus 'Millenium' seeds were planted on 10 Aug. Treatments were replicated six times in randomized complete design. Fungicides were applied as a drench, using drench bottles, starting 10 Aug and ending on 24 Sep. The numbers of emerged seedlings and diseased seedlings were counted and a disease severity rating was taken (1 to 5; 1=no disease, 2=slight seedling curving, 3=moderate curving/water-soaking, 4= extensive water-soaking, 5=seedling death) were recorded on 25 and 27 Aug, and 1, 3, 8, 10, 15, 17 and 24 Sep, and used to calculate the area under the disease progress curve (AUDPC).

Disease severity for the untreated control was 73.69, with 26 infected seedlings out of the 39 germinated. Based on the ANOVA, treatments had a significant effect on AUDPC, and AUDPC differed among treatments with the untreated control. All treatments decrease disease severity compare with the untreated control. In ascending order, Revus, Acrobat, Gavel, Aliette, and Ranman were especially effective and had the lower AUDPC values. Although fungicide treatments reduced seedling infection by *P. asparagi,* different numbers of infected seedlings were observed among fungicides.

Kocide and Curzate had the highest probability of infection with the disease ratings ranking among 2 or 3, while Revus and Acrobat had the smallest probability of infection in 31 days.

Table 7. Effect of fungicides on the area under the disease progress curve (AUDPC) values for the disease incidence on asparagus seedlings caused by *Phytophthora asparagi.*

	Severity AUDPC[z]		
Treatment and rate/50 gal	Mean		std error[y]
Untreated.....	73.69	c[x]	9.62
Presidio SC 0.25 pt.....	35.78	ab	2.52
Revus SC 0.5 pt.....	29.77	a	1.03
Previcur Flex SC 1.2 pt.....	43.61	ab	4.87
Manzate DF 3 lb.....	38.85	ab	2.68
Tanos DF 0.5 lb.....	40.08	ab	3.87
Ranman SC 0.17 pt.....	32.46	a	1.81
Bravo Weather Stik SC 2 pt	41.19	ab	5.57
Gavel DF 2 lb.....	31.41	a	2.64
Aliette WG 5 lb.....	31.46	a	1.15
Ridomil Gold EC 1 pt.....	42.58	ab	3.72
Kocide 2000 DF 2 lb.....	49.31	b	8.50
Acrobat WP 0.4 lb.....	30.62	a	0.56
Curzate DF 0.31 lb.....	49.19	b	9.20

[z]AUDPC values for *P. asparagi* disease incidence on asparagus seedlings. Calculated using nine dates of disease severity ratings. AUDPC minimum=30, maximum=150.
[y]Treatments have unequal variances (shown in standard error)
[x]Columns with common letters are not significantly different, LSD P=0.05.

Table 8. Effect of fungicides on probability of disease on asparagus seedlings caused by *Phytophthora asparagi.*

Treatment and rate/50 gal	Total emerged seedlings[z]	No. infected seedlings[y]	Disease probability[x]	Overall probability of diseased seedling[w]
Untreated.....	39	26	0.6000 f[v]	0.6000 g
Presidio SC 0.25 pt.....	33	4	0.1212 cb	0.0910 cd
Revus SC 0.5 pt.....	36	2	0.0555 b	0.0213 a
Previcur flex SC 1.2 pt.....	31	9	0.2903 cde	0.1813 e
Manzate DF 3 lb.....	24	4	0.1667 bcde	0.1195 de
Tanos DF 0.5 lb.....	29	5	0.1724 bcde	0.1400 e
Ranman SC 0.17 pt.....	28	10	0.3571 de	0.0722 cd
Bravo weather Stik SC 2 pt	33	8	0.2424 cde	0.1507 e
Gavel DF 2 lb.....	33	4	0.1212 cd	0.0530 ef
Aliette WG 5 lb.....	30	4	0.1333 bcd	0.0570 c
Ridomil Gold EC 1 pt.....	28	20	0.7143 e	0.3000 f
Kocide 2000 DF 2 lb.....	38	13	0.3421 de	0.2604 f
Acrobat WP 0.4 lb.....	31	0	<0.010 a	0.0240 ab
Curzate DF 0.31 lb.....	25	10	0.4000 e	0.2798 f

[z]One seed planted in a cell tray can give rise to one or more seedlings. Counted 31 days after planting (24 Sep).

[y]Infected out of the germinated seedlings, when disease rating was greater than 2. Counted 31 days after planting (24 Sep).

[x]Probability of seedlings getting disease, out of the total germinated seedlings. Calculated 31 days after planting (24 Sep).

[w]Probability of seedlings getting disease, out of the total germinated seedlings, across time. Calculated using all evaluation times (25-27 Aug, 1, 3, 8, 10, 15, 17 and 24 Sep). [v]Columns with common letters are not significantly different, LSD, P=0.05.

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