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# IDENTIFICATION AND ETIOLOGY OF *FUSARIUM SPP.* ASSOCIATED WITH ASPARAGUS CROWN DISEASE IN SOUTHERN CALIFORNIA AND NORTHERN MEXICO

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Jose Cosme Guerrero-Ruiz

entitled Identification and Etiology of Fusarium spp. Associated with Asparagus Crown Disease in Southern California and Northern Mexico.

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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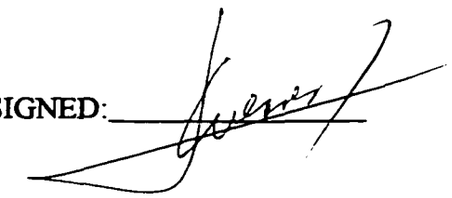
SIGNED: 

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## ABSTRACT

Considering the economic importance of asparagus as a crop and the historical association of *Fusarium spp.* as a principal cause of stand decline of this crop, a study was conducted from 1995-1997 in Southern California and Northern Mexico. The main objectives were to determine the causal agent of asparagus crown rot and the study the etiology of the causal agents which affects asparagus spears in these two important growing regions.

Asparagus crowns exhibiting symptoms of crown decay were selected from each of the above production regions and processed in the laboratory. Based on morphological characteristics, *F. proliferatum* and *F. oxysporum* were the dominant species isolated from crowns. *F. proliferatum* produced mono and polyphialides and conidia in long chains. *F. oxysporum* was distinguished by the production of chlamydospore and conidia not produced in chains. Both species were recovered from marketable spears with a incidence ranged from 20-90%. Pathogenicity test on asparagus seedlings with isolates of *F. proliferatum* and *F. oxysporum* obtained from spears were positive. To determine the source of spear infection in commercial asparagus plantings, crowns and spears were collected from two fields in the Imperial Valley of California. Both *F. proliferatum* and *F. oxysporum* were recovered from crown tissues and from spears. However, *F. proliferatum* was the most prevalent species of *Fusarium* isolated from both spears and crowns. Evaluation of the influence of *Fusarium* species on quality characters of

marketable asparagus was also studied. The quality of marketable spears infected with F. proliferatum and F. oxysporum was found to decrease significantly as the length of storage increased from five to ten days and as temperature of storage was increased from 5 C to 26 C.

Since some species of *Fusarium* are known to produce fumonisins (a mycotoxin), and investigation of the possible presence of fumonisins in commercial asparagus spears was conducted. Spears were obtained from two different geographic regions of Mexico and two in California. Spear samples naturally colonized by *Fusarium spp.* were analyzed for fumonisin B1, B2 and B3. No detectable levels of fumonisin, regardless of geographic location of samples, were founded.

# CHAPTER I

# INTRODUCTION AND LITERATURE REVIEW

## Economic importance of asparagus

Asparagus ( *Asparagus officinalis* L. ) is an important vegetable crop worldwide. Production trends reflect increase in the world demand for asparagus, particularly in the northern hemisphere . The consumption of fresh asparagus is increasing compared to processed asparagus (Nichols, 1990).

This crop is considered one of the most important perennial vegetable grown in the United States. Today, 95% of asparagus in the U.S.A. is produced in California, Michigan and Washington. In 1992 more than 35,000 has. were harvested in the United States with a production of 103,500 Tons, worth approximately 162 million US dollars (Elmer et al, 1996).

California is the leader in fresh market asparagus production for fresh market, followed by Washington and Michigan. It provides most of the supply during February through April, and Michigan and Washington produce during May and June (Cantaluppi and Precheur, 1993). In California during 1974, 44,000 acres of asparagus were under cultivation. Production was 128 million pounds of marketable asparagus valued at more than $36 million. Of the total produced, 60.8 million pounds were marketed as fresh green asparagus and 67.1 million pounds were processed (Takatori et al, 1977).

Asparagus is planted in Michigan on approximately 6,000 Has., and are concentrated in Southwestern and Central Western Michigan (Harsh et al, 1970).

In 1982 the value of the asparagus crop to the economy of Washington state was about $75,000,000., approximately 9,500 Has are concentrated in south central Washington. Most of the asparagus grown in this state is for processing (Thornton et al, 1982).

In Mexico, there are 3 major asparagus producing areas: Caborca, Guanajuato and Mexicali. Approximately 8,000 has. are planted ( Nigh,1996).

## Origin of Asparagus species

The genus *Asparagus*, is a member of the Asparagaceae family (Zomlefer, 1994). The origin is reported near the mediterranean sea and was considered a very fine vegetable by the Greeks. Growing methods were described in 200 B.C. This genus has at least 150 species native to Africa, Asia and Europe. Some species are herbaceous, others are woody, and both erect and climbing forms are known. In addition to the common edible asparagus, the genus contains several species used for ornamental purposes, including *Asparagus sprengeri* and *A. plumosus*. Common edible asparagus, *A. officinalis*, is a herbaceous perennial developed from the wild *A. officinalis* of Europe and Asia.

## Botanical Characteristics of Asparagus

The asparagus plant develops both fleshy and fibrous roots. The surface of young fleshy roots is covered with functioning root hairs. Asparagus plants develop widespread root systems. The rhizome is an underground stem. The aerial shoots arise from the rootstock. If the shoot is allowed to grow, it develops branches, leaves, and flowers. The needle-like stems of asparagus are known as "cladophylls" and are the principal food

manufacturing part of the plant, although the main stem and branches manufacture food also. The true leaves of the asparagus plant called bracts are thin, scale-like structures that always arise at the nodes. They constitute a small portion of the plant surface and, while they contain chlorophyll for a time, they soon become dry and are, therefore, of little importance in food manufacture. Asparagus is dioecious, that is, it has two types of flowers: staminate and pistillate, but generally only one type is formed on a plant. The flowers are small, bell-shaped, and yellowish green in color. The staminate flower is larger and longer than the pistillate flower and contains 6 well-developed stamens and a rudimentary pistil. The pistillate flower has 6 rudimentary stamens and a three-lobed, well developed ovary. The young fruit is green in color, becoming red as it matures. It is nearly spherical in shape and usually has three seed cavities. The seeds are 32 mm. or less in diameter, black in color, rounded on the back and somewhat flattened on the opposite side. ( Thompson, 1942).

Cultivated asparagus stands usually remain productive for time periods in excess of 15 years. However, growers in many parts of the world encounter a disorder known as stand decline which reduces the longevity to 8-10 years. Another name for this disorder is asparagus decline syndrome. Symptoms of stand decline, which are commonly observed in the 3rd to 4th year of production, include a progressive decrease in quantity and quality of spears, and later crown death.

Different factors contribute to asparagus decline, including abiotic and biotic factors. Among the biotic factors, several fungal pathogens acting individually or together have been associated with decline: *Fusarium oxysporum* Schlechtend. f. sp.

*asparagi* S.I. Cohen & Heald, *F. proliferatum* (T. Matsushima Nirenberg), *Puccinia asparagi* De Candolle, *Pleospora herbarum* (Pers.: Fr) Rabenh., *Cercospora asparagi* Sacc., *Phytophthora megasperma* Drechs var. sojae Hildebrand. Three viruses maybe involved in the decline: asparagus virus I, asparagus virus II, and tobacco streak virus (Elmer et al, 1996; Falloon et al, 1991.)

The genus *Fusarium* spp. is considered to be the main cause of asparagus decline and several species are reportedly associated with the disorder. They are listed in Table 1.

Table 1. Chronological report of the suspected causal agents associated with crown and root rot of asparagus

| COUNTRY | NAMES | REFERENCES |
|---|---|---|
| 1. U.S.A., Washington | Wilt and root rot, *F. oxysporum* | Cohen and Heald (1941) |
| 2. MEXICO, Mexicali | Crown rot<br>*F. moniliforme*<br>*F. oxysporum* | Nigh (1978) |
| 3. U.S.A., Massachusetts | Root, stem and crown rot of asparagus<br>*F. moniliforme*<br>*F. oxysporum* | Damicone, et al (1981) |
| 4. SPAIN | Fusariosis, *Fusarium spp.* | Tello (1985) |
| 5. U.S.A., Michigan | Fusarium wilt and root rot<br>*F. oxysporum*<br>Crown rot, *F. moniliforme* | Smith, et al (1990) |
| 6. U.S.A., Connecticut | Fusarium crown and root rot<br>*F. proliferatum* | Elmer (1990) |
| 7. ITALY | Asparagus decline<br>*F. oxysporum*<br>*F. moniliforme* | Fantino (1990) |

*Fusarium spp.* as a causal agent of Asparagus Decline

*Fusarium oxysporum* was first reported causing a wilt and root rot of asparagus fields in Washington, U.S.A. (Cohen and Heald, 1941).

Asparagus seedling blight caused by *F. oxysporum* Snyder & Hansen was reported in Ontario, Canada in 1955. Under field conditions symptoms on older seedlings included stunting, yellowing and wilting. The pathogen penetrates directly into the embryonic area of the root tip (or through stomata on the hypocotyl) colonizing the host intercellulary (Graham, 1955).

Asparagus decline was reported in California in 1958. Symptoms included poor plant growth, yellowing of ferns and stunting. Below ground, symptoms consisted of sunken lesions and rusty flecks at the base of stalks. The vascular system often showed a reddish-brown discoloration, and roots were discolored area or were completely dead. *F. oxysporum* f.sp.*asparagi* was isolated from symptomatic plants (Grogan and Kimble, 1959).

In 1971, Endo and Burkholder reported that *F. moniliforme* was isolated from 93% of the asparagus crowns and *F. oxysporum* from 22% in southern California. The main symptoms were: reduction of vegetative spears in number and size, yellowing of stalks, stunting and reduced vigor. Brown crown rot was also observed. These study, indicated that *F. moniliforme*, for the first time was an important pathogenic component of the crown rot complex.

One of the principal factors involved in reduced asparagus acreage in New Jersey is asparagus decline. This disease caused a reduction in area from 32,500 acres in 1957 to

2,300 acres in 1977 (New Jersey Crop Reporting Service, 1977). *Fusarium moniliforme* was isolated in stem and crown lesions, while *F. oxysporum* f. sp. *asparagi* was detected consistently in discolored vascular root tissue, cortical root lesions, and sometimes in stems and crowns in New Jersey by Johnston et al . The proposed name for the first causal agent is *Fusarium* stem and crown rot, and *Fusarium* wilt and root rot, when caused by *F. oxysporum* f.sp. *asparagi*. Both species are reported to cause asparagus decline (Johnston et al, 1979).

Studies by (Damicone and Manning, 1980) indicated that asparagus seedlings (cv. Mary Washington) developed root and stem lesions and crown infections two weeks after inoculation with *F. moniliforme* var. *subglutinans*. This pathogen also colonizes sweet corn, and serves as a reservoir of inoculum for this fungus.

*Fusarium moniliforme* has been isolated from flowers, fruit, and seeds of plants growing in commercial asparagus fields in western Massachusetts. This study also showed that the source of contamination can be from airborne spores (Gilbertson and Manning, 1983).

*Fusarium* isolation were made from crown tissues in Mary Washington and Jersey Centennial asparagus transplants. *F. moniliforme* was isolated in 57.6% and *F. oxysporum* in 30.4% of the samples. *F. solani* was also detected in very low population (Damicone and Manning, 1985).

Studies by Evans and Stephens, during 1989, demonstrated that asparagus seedlings infected with virus AV-1 and AV-II were more susceptible to the effect of *F. oxysporum* f.sp. *asparagi*. The increased levels of *Fusarium* crown and root rot in

asparagus infected with any of these viruses, were correlated with an increase in root exudation and also an increase in susceptibility of root tissues to infection by the pathogen.

During 1990, a pathogenicity study of *F. proliferatum* on asparagus was reported in Connecticut. Transplants of cv. Mary Washington, developed discolored stems, crowns and root rots ten weeks after inoculation (Elmer, 1990).

According to Elmer and Ferrandino, *F. moniliforme* Sheldon produces chains and false heads of microconidia only on monophialides while *F. proliferatum* (Matsushima) produces microconidia in chains and false heads on both monophialides and polyphialides (Elmer and Ferrandino, 1992). Based on this information the two species were separated and it was concluded that all previous reports of *F. moniliforme* in asparagus were, in reality, *F. proliferatum.*

Elmer (1995) reported that a single mating population of *Giberella fujikuroi* assigned to "D" population, which the anamorph type is *F. proliferatum*, predominates in asparagus fields in Connecticut, Massachusetts and Michigan.

The identification of *Fusarium spp.* and the study of the etiology of this disease affecting crowns is important. Implementation of control measures are dependent upon the correct identification of species associated with stand decline.

## Objectives

Nigh (1978) reported that asparagus production in northern Mexico was initiated in 1960, and that stand decline problems were noticed by growers beginning in 1963. The cause of this disorder had not been identified but was tentatively attributed to

*Fusarium spp*. He suggested that a similar scenario in asparagus plantings in southern California.

The objectives of the studies reported herein were: (1) to determine if pathogenic agents were associated with the declining asparagus fields in northern Mexico and southern California asparagus production areas, (2) identify the suspected causal agents associated with asparagus crown rot in each of the above areas, and if recovered (3) study the etiology of the disease.

# CHAPTER 2

# INCIDENCE OF *FUSARIUM SPP.* IN ASPARAGUS FIELDS IN MEXICO AND SOUTHERN CALIFORNIA

## Introduction

The genus *Fusarium* is regarded throughout the world as the most limiting factor in the production of asparagus. Diseases of asparagus attributed to species of *Fusarium* include seedling blight, crown and root rot, wilt, stem and crown rot, and stand decline (Cohen and Heald, 1941; Graham, 1955; Endo and Burkholder, 1971; Johnston et al, 1979).

Disease symptoms in asparagus include a reduction in the number and size of spears, yellowing of stalks, stunting, reduction in vigor, and crown rot. Stress factors have been implicated as predisposing asparagus to infection by *Fusarium*. These factors involve, drought, insect and diseases damage to roots and foliage, excess soil moisture, root injury during harvest, overcutting, and herbicide damage (Nigh, 1978).

In Mexico, species of *Fusarium* have been implicated as causal agents of stand decline. Nigh (1978), showed that *Fusarium oxysporum* and *F. moniliforme* were isolated from asparagus crowns (exhibiting discoloration and decay) from the state of Baja California Norte, Caborca, Sonora and the state of Guanajuato, Mexico. Both species were proven virulent and pathogenic. *Fusarium moniliforme*, (probably *F. proliferatum*) was also isolated by Nigh from the root systems of regional vegetation that included arrow weed, palo verde (*Parkinsonia sp.*) and mesquite (*Prosopis sp.*).

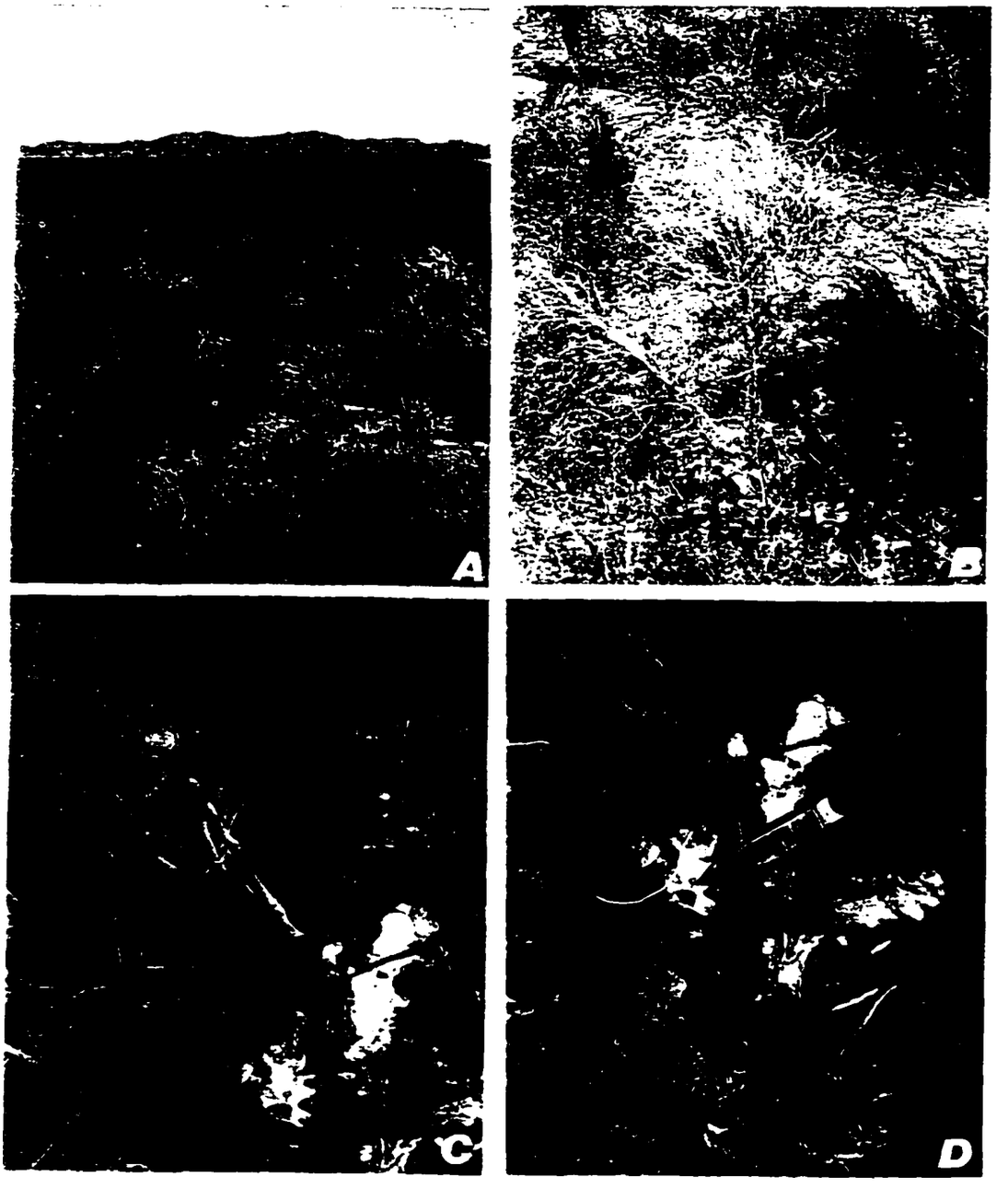


Figure 1. Stand decline and crown rot of asparagus caused by *Fusarium proliferatum*. A & B: Dessicated ferns 6 months after harvest of spears. C & D: Necrotic tissues in the crowns of asparagus typical of advanced stages of decay.

In Southern California, *Fusarium spp.* have been associated with stand decline of asparagus. *Fusarium moniliforme* has been isolated from 93% of crowns from asparagus plants exhibiting symptoms of stand decline. *F. oxysporum* was also isolated from crowns of 22% of asparagus plants exhibiting these symptoms. *F. moniliforme*, however, was considered to be the most important component of the crown rot complex (Endo and Burkholder, 1971).

Endo (1978) reported *F. oxysporum* to be the cause of asparagus crown rot in California, and indicated a variation in pathogenicity between isolates of *F. oxysporum.* In this study, he indicates that *F. oxysporum* and *F. moniliforme* show differences in levels of virulence. Also, he report that *F. moniliforme* primarily infects feeder roots.

More recently, Elmer et al (1996) indicates that *F. proliferatum* is the cause of crown rot but that the fungus was misidentified as *F. moniliforme.* Therefore, objectives of this study were to identify the species of *Fusarium* associated with asparagus crown rot in two important production areas (i.e. Southern California and Northern Mexico).

## Material and Methods

Asparagus plants growing in fields with a history of stand decline in the Imperial Valley of California, and Caborca, Sonora, Mexico were sampled. Crowns with attached roots and soil were excavated from fields and transported to the laboratory for the isolation of suspected causal agents of crown rot.

Three asparagus fields planted with the asparagus variety UC-157, ranging in age from 5-9 years old, were sampled in Caborca, Sonora., Mexico. Crown samples were collected from these fields in 1995 and 1996 (Tables 1,2). Ten crowns were taken from

each field and isolation were made for the presence of *Fusarium spp*. Additionally, five fields (Table 3) in the Brawley, California area were sampled on September 29, 1995. Ten crowns exhibiting symptoms of crown decay were selected from each of these fields and processed in the laboratory. The age of the asparagus plants in the fields varied from 5 to 9 years in age (Table 3).

Another six fields were sampled from the Holtville area of California from February through April 1996. The fields had been planted to asparagus between 8-9 years (Table 4).

Isolation Protocol of *Fusarium* Species from

Asparagus Crowns

Crowns were washed in running tap water for 5 minutes. Four 2 mm internal tissue sections showing discoloration from the margin of lesions were excised. Crown tissue was surface-disinfected with 0.53% (10% household bleach) sodium hypochlorite for 5 minutes, rinsed 3 times with sterile distilled water(SDW), and blotted dry. Surface disinfected tissue was aseptically transferred to Komada's media, which is selective for *Fusarium spp*. (Singleton et al, 1992) contained in 9 cm. diameter sterile plastic petri dishes. Dishes were incubated at 24 C for 7 days. After incubation for 4 days, sporulating colonies originating from the infected tissue were transferred to test tubes containing 9 ml of sterile distilled water (SDW). The tubes were shaken for 10-15 seconds. Twenty ul of aqueous suspension from the test tube with the addition of 300 ul of SDW were spread onto PDA plates. The plates were inverted and incubated at room temperature (20-22 C) for 2 days. Following incubation, hyphal tips from a selected representative colony were

transferred to fresh PDA. Single spore cultures obtained and transferred to Carnation Leaf Agar (CLA) and incubated for 3 days at 24 C. Cultures were then identified to species.

## Identification of Fusarium Species

Speciation in *F. proliferatum* is determined by the presence of long chains of conidia originating on either mono or poliphyalides. *F. oxysporum* is identified by the absence of conidial chains and the presence of chlamydospore.

## Pathogenicity Tests

Asparagus seeds (cultivar UC-157) were surface disinfected in 1.5% sodium hypochlorite solution for 5 min., rinsed with SDW 3 times, and allowed to air dry for 5 minutes under sterile conditions. Seeds were placed on 2% water agar and incubated at 28 C in the dark for 7 days. A single germinated seed was then aseptically transferred from the agar to an individual test tube (2.5X15cm.) containing approximately 20 ml. modified Hoagland's No. 2 solution (Conger, 1964) in 0.6% Difco Agar. The tubes were sealed with plastic caps, and incubated at 28 C under fluorescent lighting for a 16 hr. photoperiod. After 14 days, asparagus seedlings developed secondary roots in the agar tubes. A spore suspension (0.5ml) containing 1X10 conidia/ml of each Fusarium specie was placed on the agar surface in 5 replicated tubes and incubated as above for a period of 4 weeks. Spore suspensions were produced on PDA from single spore from cultures of *F. proliferatum* and *F. oxysporum*. Seedlings were rated for disease development by examining the root system at the end of 4 wk using a scale of 1-5, where 1= no disease, 2= lesions present on 0-25% of the root system, 3= lesions 25-50% of the root system, 4=

lesions on 50-75% of the root system and 5= lesions on 75-100% of the root system. Pathogenicity tests for each isolate were repeated twice.

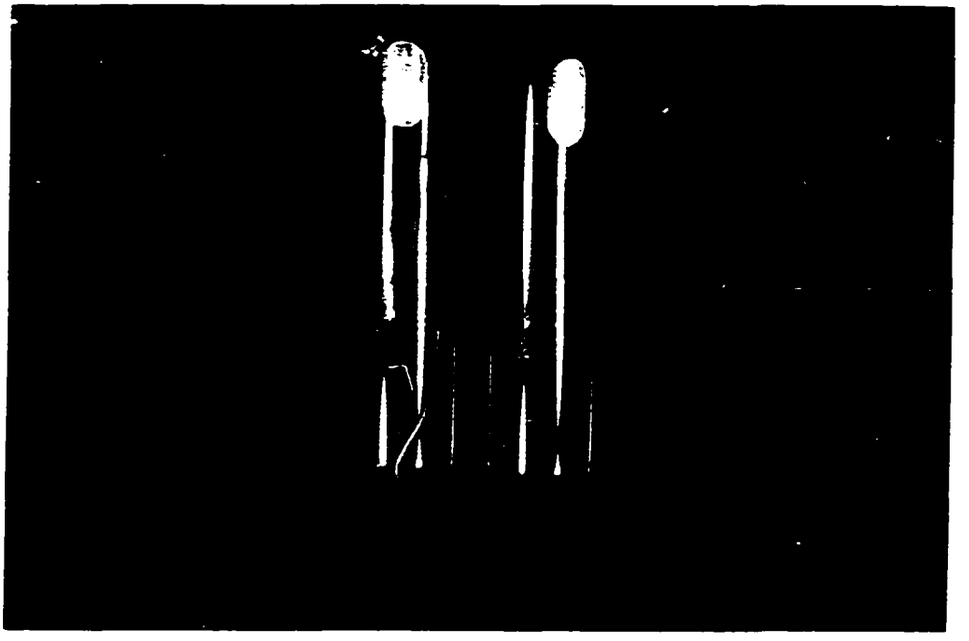

Figure 2. Test tube method of evaluation of the virulence of *Fusarium spp.* to asparagus seedlings. Left = check, right = seedling death following inoculation with *F. proliferatum.*

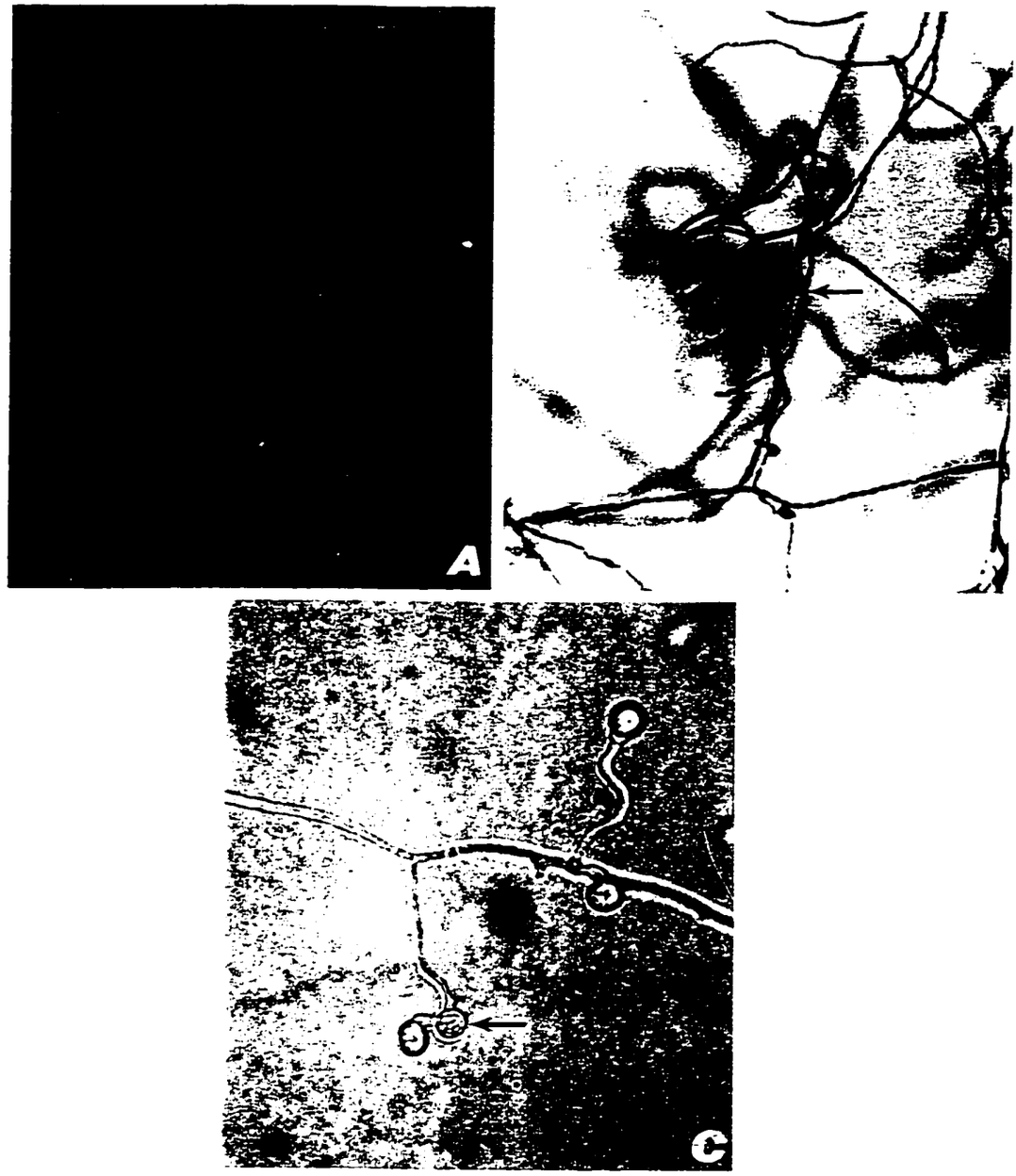


Figure 3. Morphological characteristics of *Fusarium spp.* from cultures. A; Mono (MP) and Polyphialids (PP) of *F. proliferatum* on CLA medium. B; Chains of microconidia of *F. proliferatum*. C; Chlamydospores of *F. oxysporum* produced on carnation leaf agar (CLA), no microconidia produced.

## Results and Discussion

Based on the morphological characteristics, *F. proliferatum* and *F. oxysporum* were the dominant *Fusarium* species isolated from diseased asparagus crowns from each of the sampled areas. *F. proliferatum* produced mono and polyphialides and conidia in long chains. *F. oxysporum* was distinguished by production of chlamydospore and no conidia in chains. During the course of this investigation, a discovered that *F. proliferatum* could be identified simply by plating infected tissue directly in water agar medium. Polyphialides and chain of conidia were produced on the infected tissues and in the agar approximately 4 days after incubation at 24 C. These results significantly reduced not only the time necessary for identification to species but also obviated the need for growth of the fungus on Komada's and CLA media.

One week after inoculation, asparagus seedlings began to exhibit black lesions on the roots, and growth of secondary roots was inhibited. Fungal sporulation appeared at the base of the seedlings and after 3 weeks, plant mortality could be observed. The results demonstrate that *F. proliferatum* and *F. oxysporum*, isolated from crowns, were pathogenic to the asparagus seedlings.

From three fields sampled in Caborca, Sonora, Mexico in 1995, between 80-100% of individual crowns were infected with *Fusarium spp.*: 60-80% of the recovered isolates were identified as *F. proliferatum*, and 15-20% as *F. oxysporum* (Table 2).

Table 2. *Fusarium spp.* isolated from asparagus crowns (Caborca, Sonora, Mexico fields)

| Field Name | Plant age | *F. proliferatum* | *F. oxysporum* |
|---|---|---|---|
| 1. Mexicanos I | 5 yrs. | 60% | 20% |
| 2. Mexicanos II | 5 yrs. | 65% | 15% |
| 3. R. Caborca | 8 yrs. | 80% | 20% |

a) Percent infected from 10 crowns sampled/field

*Fusarium* isolation made from the same fields on September 1996, indicate that *F. proliferatum* was the most prevalent species, with a range of 80-90%, and *F. oxysporum* was isolated between 5-10% (Table 2).

Table 3. *Fusarium spp.* isolated from asparagus crowns (Caborca, Sonora, Mexico fields)

| Field Name | Plant age | *F. proliferatum* | *F. oxysporum* |
|---|---|---|---|
| 1. Mexicanos I | 6 yrs. | 85% (a) | 5% |
| 2. Mexicanos II | 6 yrs. | 80% | 10% |
| 3. R. Caborca | 9 yrs. | 90% | 10% |

a) Percent infected out of 10 crowns sampled/field.

From the five fields sampled in Imperial Valley, California in 1995; between 70-100% of the individual crowns were infected with *Fusarium spp.*: 70-90% of the isolates identified as *F. proliferatum*, and 0-10% as *F. oxysporum* (Table 4).

Table 4. *Fusarium spp.* isolated from asparagus crowns (Brawley, California fields)

| Field Location | Plant age | *F. proliferatum* | *F. oxysporum* |
|---|---|---|---|
| 1. Spruce 45-A | 5.5 yrs. | 80% (a) | 10% |
| 2. Spruce 45-B | 5.5 yrs. | 90% | 10% |
| 3. Sandal 171 | 9.0 yrs. | 80% | 5% |
| 4. Eucalyptus 154 | 5.0 yrs. | 70% | 0% |
| 5. West Side Main | 9.0 yrs. | 80% | 5% |

a) Percent infected from 10 crowns sampled/field

Imperial Valley fields, sampled during February and April 1996, were also found to be infected predominately by *F. proliferatum*, ranging from 60-80% of the isolates from all crowns. *F. oxysporum* was detected from 5-20% of the total crowns sampled (Table 5).

Table 5. *Fusarium spp.* isolated from asparagus crowns (Imperial Valley, California fields 1996)

| Field Location | Age | *F. proliferatum* | *F. oxysporum* |
|---|---|---|---|
| 1. Niez, North | 8 yrs. | 70% (a) | 10% |
| 2. Mcgrew | 9 yrs. | 80% | 5% |
| 3. Tony Abatta | 8 yrs. | 70% | 5% |
| 4. Niez, South | 8 yrs. | 70% | 10% |
| 5. Ash 192 | 8 yrs. | 80% | 0% |
| 6. J.B. Field* | 7 yrs. | 60% | 20% |

Although *F. oxysporum* was detected in both Caborca, Sonora., Mexico and Imperial Valley, California, our study shows that *F. proliferatum* is the predominant species associated with asparagus crowns in declining stands Southern California and Northern Mexico. *F. oxysporum* and *F. moniliforme* have previously been reported infecting asparagus in the Imperial valley of Southern California. For example, *F. moniliforme* (probably *F. proliferatum*) was reportedly isolated from 93% of the crowns (Endo and Burkholder, 1971). Nigh (1978) and Endo (1978), also reported both *Fusarium* species causing crown rot in asparagus in Mexico and California, which is confirmed by data obtained in our study.

This study shows that *F. proliferatum* is the primary species associated with declining stands. This study concluded that *F. proliferatum* and *F. oxysporum* is capable of causing asparagus crown rot in Northern Mexico and Southern California, with *F. proliferatum* being the more prevalent species.

# CHAPTER 3

## INCIDENCE OF *FUSARIUM spp.* IN ASPARAGUS SPEARS

### Introduction

In addition to causing asparagus decline species of *Fusarium* have also been associated with decay of asparagus spears during storage and transit of marketable spears.

Although the precise mechanism of the origin of *Fusarium* spear infection is unknown, rotting of asparagus spears is present especially when high temperatures occur during the postharvest period. According to Montealegre and Palma (1994), *F. moniliforme* is considered one of the main causal agents of postharvest rot of asparagus spears. Warunnee et al (1990) reported that *Fusarium spp.* cause spear soft rot, affecting the quality strongly. *F. moniliforme* has been isolated in California from female flowers, fruit and seed of asparagus plants growing in commercial fields. Airborne spores of this fungus specie appear to be the major source of inoculum (Gilbertson and Manning,1983).

*F. moniliforme* and *F. oxysporum* do not produce a systemic type of infection in asparagus (Johnston et al, 1979; Gilbertson, 1981).

Although *F. oxysporum* has not been shown to produce a systemic infection in asparagus, the specie does initiate systemic infections on other plants species. Gamliel et al (1996) report that *F. oxysporum f. sp. basilici* cause a wilt, and a crown root rot of sweet basil (*Ocimum basilicum* L.) in all regions of Israel. *Fusarium oxysporum f. sp. basilici* was isolated from *Ocimum basilicum* L. from roots and from all above ground

portions of interior parts of the diseased plants. Macroconidias were commonly present on stems of diseased plants and Gamliel et al, consider this pathogen to be soilborne but possessed an aerial type of dissemination.

Spores of *F. oxysporum* have been obtained from air inside greenhouses used for production of tomatoes (Rowe et al, 1977). The sources of these airborne microconidia were presumed to be infected tomato stems, straw mulch and decomposing tomato vines from dump areas outside the greenhouse (Rowe et al, 1977).

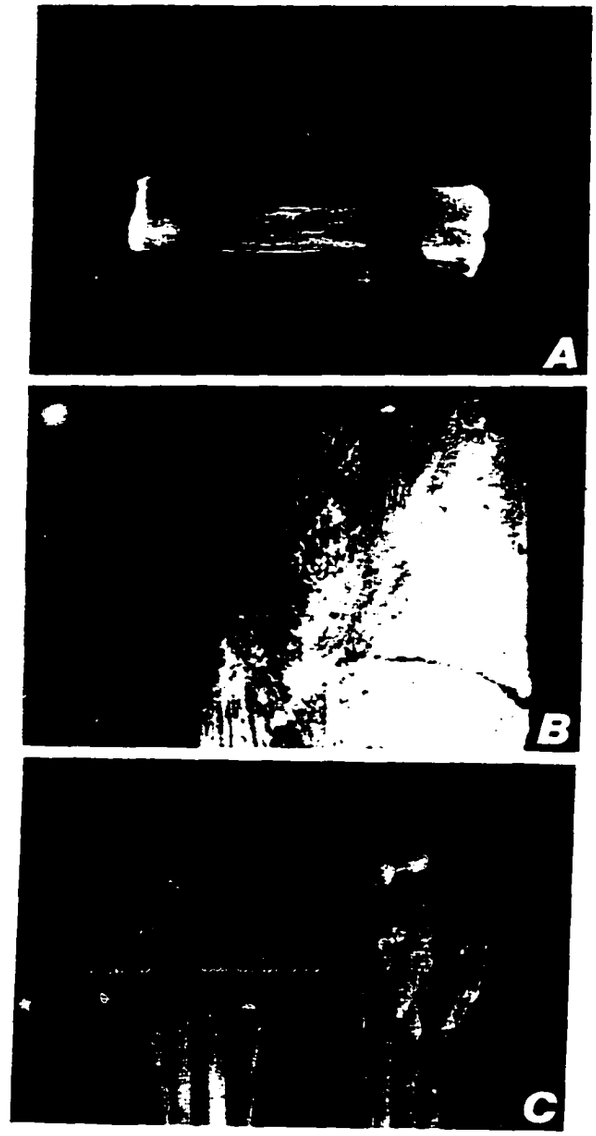


Fig. 4. Signs of *Fusarium spp.* infecting asparagus spears. A. Growth of *Fusarium sp.* on a spear section, five days incubation at 22°C and 80% relative humidity. B. Sporulation of *F. proliferatum* at the base of an asparagus spear. C. Effect of storage conditions on the decay of asparagus spears by *F. proliferatum*: left; asparagus spears stored at 5°C, right; complete destruction of spears at 22°C.

Limited information exists concerning the role that *Fusarium spp.* play in the decay of marketable asparagus spears. The objectives of this study were to determine the species of *Fusarium* involved in marketable asparagus spears and evaluate the percentage of spear colonization by this pathogen.

## Material and Methods

During the cropping years 1995-1997, 10 to 200 asparagus spears were randomly collected from asymptomatic plants in fields and packing houses and assayed for *Fusarium spp.* Spear collections in three localities of Mexico were taken directly from the packing shed. In California, some spears were taken directly in the fields and others after the packing process. Spears from Peru were assayed in storage facilities. To isolate *Fusarium spp.* from spears, basal portion (5-6 cm.) were dissected and disinfected with 0.015% A.I. sodium hypochlorite solution, and rinsed 3 times with sterile distilled water. The portions were split in half and blotted dry and plated on PDA under aseptic conditions. Plates were incubated at 24 C dark. Identification of species was determined on Carnation Leaf Agar (CLA), previously described. Ten *Fusarium* isolates from each one of the samples were challenged on asparagus seedlings to demonstrate pathogenicity, using the same procedure that was described in chapter 2.

## Results and Discussion

*Fusarium proliferatum* and *Fusarium oxysporum* were recovered from all spear samples from all geographical locations. Incidence ranged from 20-90%. Isolates of both *Fusarium* species were found to be pathogenic when challenged to asparagus seedlings. *F. proliferatum* predominated in samples from Northern Mexico and Southern California,

and Peru (Tables 6,7,8,11). Percentages of occurrence of this species in these areas was from 12-81%, and *F. oxysporum* was obtained from 8-14% of the samples. *F. oxysporum* predominated in a bigger range in Northern California (20-58%) (Tables 9,10).

*F. proliferatum* predominate in spears obtained from Caborca, Sonora, Mexico ranging from 40-81%, and *F. oxysporum* was detected in a range of 9-20%. Samples taken in January had less presence of *F. proliferatum* and more presence of *F. oxysporum*. This maybe due to temperature conditions (Table 6).

Table 6. *Fusarium spp.* Isolated from marketable asparagus spears (Caborca, Mexico)

| Sampling Date | No. of Spears | Spears W/*Fusarium* | *Fusarium proliferatum* | *Fusarium oxysporum* |
|---|---|---|---|---|
| Jan. 1996 | 20 | 60% | 40% | 20% |
| Mar. 1996 | 10 | 70% | 56% | 14% |
| Feb. 1997 | 90 | 90% | 81% | 9% |

Spears taken from Mexicali, Mexico area, had low incidence of *F. proliferatum* during January, but samples taken during March were detected with higher incidence of *F. proliferatum*. The same pattern of incidence was detected in *F. oxysporum* (Table 7).

Table 7. *Fusarium spp.* Isolated from marketable asparagus spears (Mexicali, Mexico)

| Date of Sampling | No. of Spears | Spears W/*Fusarium* | *Fusarium proliferatum* | *Fusarium oxysporum* |
|---|---|---|---|---|
| Jan. 1996 | 40 | 20% | 12% | 8% |
| Mar. 1996 | 10 | 50% | 33% | 17% |

The incidence of *F. proliferatum* from spears collected from a commercial packings at Imperial Valley, California was 19%, with *F. oxysporum* detected in 11% of the samples (Table 8).

Table 8. *Fusarium spp.* Isolated from marketable asparagus spears (Imperial Valley, California)

| Date of Sampling | No. of Spears | Spears W/ *Fusarium* | *Fusarium proliferatum* | *Fusarium oxysporum* |
|---|---|---|---|---|
| Mar. 1996 | 10 | 30% | 19% | 11% |

During the years 1995, 1996 and 1997 spears collected in Salinas, California had lower incidence of *F. proliferatum* (6-21%) comparing with *F. oxysporum* (24-58%). This data maybe have been affected by moving climatic conditions between each year (table 9).

Table 9. *Fusarium spp.* Isolated from marketable asparagus spears (Salinas, California)

| Date of Sampling | No. of Spears | Spears W/Fusarium | *Fusarium proliferatum* | *Fusarium oxysporum* |
|---|---|---|---|---|
| Mar. 1995 | 10 | 30% | 6% | 24% |
| Mar. 1996 | 10 | 70% | 21% | 49% |
| Apr. 1996 | 15 | 33% | 7% | 26% |
| Feb. 1997 | 90 | 65% | 7% | 58% |
| Apr. 1997 | 200 | 40% | 16% | 34% |

Analysis of spears collected from Stockton,California, indicates that *F. oxysporum* is more prevalent (20-39%), than *F. proliferatum*, which was founded in 10-14% of the spears. These results are similar to those from Salinas, California (Table 10).

Table 10. *Fusarium spp.* Isolated from marketable asparagus spears (Stockton, California)

| Date of Sampling | No. of Spears | Spears W/*Fusarium* | *Fusarium proliferatum* | *Fusarium oxysporum* |
|---|---|---|---|---|
| Mar. 1996 | 10 | 30% | 10% | 20% |
| Apr. 1996 | 15 | 53% | 14% | 39% |
| Jun. 1996 | 20 | 45% | 15% | 30% |

Asparagus spears collected from process packed cartons in packing facilities in Peru, were found to have infection by *F. proliferatum* more frequently (50% approximately) and *F. oxysporum* between 27-37%. These data suggest that *F.*

*proliferatum* is more frequently isolated and more distributed in spears from the warmer desert areas (Table 11).

Table 11. *Fusarium spp.* Isolated from marketable asparagus spears (Peru)

| Date of Sampling | No. of Spears | Spears W/*Fusarium* | *Fusarium proliferatum* | *Fusarium oxysporum* |
|---|---|---|---|---|
| Nov. 1995 | 15 | 87% | 50% | 37% |
| Dec. 1995 | 60 | 78% | 51% | 27% |

# CHAPTER 4

# ORIGIN OF *FUSARIUM PROLIFERATUM* AND *F. OXYSPORUM* INFECTION OF COMMERCIAL ASPARAGUS SPEARS

## Introduction

*Fusarium proliferatum* and *F. oxysporum* which are associated with diseases known as *Fusarium* crown rot and root rot of asparagus, are common pathogens in most soils (Damicone and Manning, 1985; Elmer,1990). Initial infection of asparagus by these fungi occurs on the roots and crowns. They invade the feeder and storage roots, cause vascular discoloration of the crown and stems, produce chlorosis, wilt and eventual death of the plan (Graham, 1955).

Gilbertson and Manning (1983) reported that the pattern of isolation of *Fusarium moniliforme* from flowers suggest that contamination was due to the airborne spores, and not via vascular elements, because this fungus was not detected in branchlet bearing contaminated flowers and fruits. Some species of *Fusarium* are also capable of causing soft rot of asparagus spears. At higher temperatures (>22 C), soft rot decay caused by *Fusarium spp.* can be destructive to spears during the postharvest period (Warunne et al, 1990). Guerrero et al (1997) showed that *F. proliferatum* and *F. oxysporum* are two species frequently recovered from commercial spears are capable of initiating spear soft rot of asparagus.

Montealegre and Palma (1994) showed that *Fusarium moniliforme* and *Penicillium hirsutum* were causal agents of postharvest rots. They invaded the bracts and the basal end of cut spears.

Although *Fusarium* species have been documented as causal agents of soft rot decay of asparagus spears, the exact epidemiological mechanism by which they colonize asparagus spears is unknown. Colonization could occur in the packing shed during the processing procedure, from contaminated knives or soil during the harvesting procedure, or from infected crowns in the field prior to the harvesting and packing operation. The objective of this study was to determine the source of spear infection in commercial asparagus fields.

## Materials and Methods

Diseased crown tissue with emerging spears were sampled from two declining asparagus fields in the Imperial Valley of California. In the first field planted with cultivar U.C. 157, 40 asparagus crowns were excavated from the field and 40 spears in the field were collected. Snapping another 40 spears were obtained directly from the packing facility after the packing process. In the second field planted with the cultivar Meloland Select, 70 crowns were sampled. A total of 90 spears from the same field were collected, 40 by hand snapping, 30 cut with knife, 10 from the hydrocooler in the packing facility and 10 from a commercially packaged carton from the same field on the same date.

### *Fusarium* Isolation from Crowns

Isolation of *Fusarium spp.* was performed by cutting discolored crown tissues of infected plants, disinfected with 0.53% (10% household bleach) sodium hypochlorite solution for 3 minutes, rinsing 3 times in sterile distilled water(SDW), then dried by blotting. Pieces of crown tissue approximately 4 mm square, were placed on Komada's

selective media in 9 cm diameter petri. plates. Plates were incubated at 24 C for 6 days in the dark. Identification of species was made using Carnation Leaf Agar (CLA). Once the colony had grown from the tissue onto Komada's medium, a 5 mm. section of emerging hyphae was transferred to a test tube containing 9 ml SDW, agitated for 15 seconds. Twenty $\mu$l of aqueous suspension from the test tube together with 300 $\mu$l of SDW were spread onto the PDA plates. Petri plates were inverted and incubated at room temperature for two days. Fungal colonies were purified by transferring single spore colonies onto the fresh PDA, then transferred onto CLA medium, incubated at 24 C. After 4 days incubation, fungal structures microscopically examined and identified to species.

*Fusarium* Isolation from Spears

In order to isolate *Fusarium spp.* from spears, basal portions of spears (4-5 cm.) were removed and disinfected with 1.5% sodium hypochlorite solution for 3 min., and rinsed 3 times with sterile distilled water. The sections were cut in half, and were dry and plated on PDA aseptically. Plates were incubated in the dark at 24 C for 7 days. Species identification was accomplished were done on CLA, using the procedures described earlier.

Differentiation of *F. oxysporum* and *F. proliferatum* was based on the presence or absence of long conidia chains supported in mono or poliphyalides, present in *F. proliferatum*. After 2 weeks, plates were reexamined to determine the presence or absence of chlamydospores. *F. oxysporum* produces the structure whereas *F. proliferatum* does not.

To determine if *Fusarium* can infect asparagus spears in hydrocooler operation, samples were taken directly from a hydrocooler in a California packing shed.

Chlorine concentration was measured and 40 $\mu$l of this water was applied directly into each of 5 Komada's media petri dishes and spread uniformly. In a second test procedure, ten ml of water was taken from the hydrocooler and passed through 0.22 micron millipore filter. The filter was pressed onto the surface of Komada's selective media. The plates were then incubated in the dark at 24 C. There were 5 replicates in each experiment.

## Results and Discussion

The asparagus crowns from the two sampled fields contained two species of Fusarium: *F. proliferatum* and *F. oxysporum*. In field number 1 of the Imperial Valley, 62% of the crowns were infected with *F. proliferatum* and 37% with *F. oxysporum*. In the field number 2 of the same valley, 63% of the crowns were infested with *F. proliferatum*, and 35% with *F. oxysporum*, indicating that *F. proliferatum was* more prevalent in both fields (Table 12). This agrees with the results previously reported by Damicone and Manning (1985) and Johnston et al (1979), who reported that *F. proliferatum* (formerly *F. moniliforme*) as the dominant species infecting asparagus crowns. In the present study we found that isolates were concentrated only in parts of the crowns. This suggests that the presence of *F. proliferatum* is more commonly recovered in the homestead spears, since *F. oxysporum* is commonly reported in young roots that cause more damage during the seedling stage of asparagus (Cohen and Heald, 1941; Graham, 1955).

Table 12. *Fusarium Spp.* Isolated from crowns of two fields in Imperial Valley, California (April 1996)

| Field | No. of crowns sampled | *F. proliferatum* | *F. oxysporum* |
|---|---|---|---|
| 1. J.B. | 70 | 43 (62%) | 26 (37%) |
| 2. S.C. | 40 | 25 (63%) | 14 (35%) |

Sixty two percent of the spears (J.B. farm) were infected with *F. proliferatum* and 38% by *F. oxysporum* from the J.B. field in spear samples snapped by hand (Table 12). Similar results were obtained when a knife was used to harvest the selected spears from the field.

Spear samples removed directly from cartons that had passed through the hydrocooler, and from normally harvested spears before the packing process, had greater incidence of *F. proliferatum* infection than *F. oxysporum* (Table 13).

Table 13. *Fusarium* isolated from spear samples J.B. farm (Imperial Valley California, April 1996)

| Description | No. of spears | % spears infected | *Fusarium proliferatum* | *Fusarium oxysporum* |
|---|---|---|---|---|
| Snapped by hand | 40 | 100 | 25 (62%) | 15 (38%) |
| Hydrocooled | 10 | 80 | 5 (50%) | 3 (30%) |
| Field Harvested | 10 | 70 | 4 (40%) | 3 (30%) |
| Cut with Knife | 30 | 93 | 18 (60%) | 10 (33%) |

Spears harvested with a knife in the S.C. field showed that 60% of the spears contain *F. proliferatum* and 40% *F. oxysporum*. Isolation from the crowns, corresponding to the 40 spears snapped by hand, showed similar percentage of isolation of *F. proliferatum* and *F. oxysporum* (Table 12). Commercial spear samples taken from the packing shed that had been field harvested by hand and had not been hydrocooled were 65% infested with *F. proliferatum* and 30% with *F. oxysporum* (Table 14).

Table 14. *Fusarium spp.* spears isolated from S.C. Field (Imperial Valley,California, April 1996).

| Description | No. of spears | % spears infected | *Fusarium prolieratum* | *Fusarium oxysporum* |
|---|---|---|---|---|
| Snapped by hand | 40 | 100 | 24 (60%) | 16 (40%) |
| Dir. from pack. | 40 | 93 | 26 (65%) | 12 (30%) |

The percentages of both Fusarium species from the spears were similar to those obtained from the crowns in both fields, with *F. proliferatum* the predominant species. These results agree with the hypothesis and support the conclusion that infection originates directly from crowns. (Guerrero et al, 1997) reported similar results that *F. proliferatum* is the dominant specie on asparagus spears in the two areas sampled having a mean of 70%. *F. oxysporum* was recovered in 48% of spears from commercial packing houses from 6 production areas.

*Fusarium spp.* were not detected in water samples from the hydrocooler, probably because of the presence of high concentration of chlorine (280 ppm). Also no other

microorganisms were detected. This study would indicate that *Fusarium* infection is highly unlikely in the packing process.

In this study we found both *Fusarium* species can be recovered from asparagus crowns and spears. *F. proliferatum* was more prevalent in crowns and spears.

# CHAPTER 5

# EVALUATION OF ASPARAGUS SPEARS INFECTED WITH *FUSARIUM* SPECIES ON SHELF-LIFE AND QUALITY

## Introduction

Marketable asparagus spears have been previously reported to be infected with *Fusarium* (Snowden, 1992). The fungus grows from the infected crowns into the emerging spears. Both *F. proliferatum* and *F. oxysporum* have been isolated from such spears (Guerrero, 1997). In the Salinas Valley, California, the predominant species recovered from marketable spears is *F. oxysporum*, while in the warmer desert regions of Northern Mexico, *F. proliferatum* may be isolated from up to 98% of sampled spears taken directly from packed commercial five kilo cartons.

Although *Fusarium* has been reported previously as a market disease of asparagus (Smith, et al., 1966), there has been no detailed evaluation of the percent of infected spears and what influence infection may have on the shelf-life or overall quality of such spears. As the harvest season progresses, there is reduction in levels of carbohydrates and temperatures normally begin to rise during this time of year. These conditions would be expected to influence the fungus growth within the spear, utilizing sugars, and contributing to increased temperatures. Such effects would be expected to have a detrimental affect on the shelf-life and quality of the spear.

The purpose of this investigation was to evaluate if *Fusarium* species influences quality characters of marketable asparagus spears and to determine if the fungus has any tendency to grow more rapidly in the cut spear as the harvest season progresses.

## Methods and Materials

Quality and shelf-life of *Fusarium* infected marketable asparagus spears were evaluated in comparison with noninfected spears harvested from the same field at the same time. Two fields in different geographical growing areas, Caborca, Sonora, Mexico and Salinas Valley, California, USA were chosen as sample sites for the investigation. The Mexican field was nine years of age and planted with variety Meloland Select while the Salinas field was also approximately nine years and planted with the asparagus hybrid UC 157.

A five kilo commercially packed carton of extra large grade (California quality standards) asparagus spears was selected from each field in March 1997. This represented samples taken approximately two and one-half months into the harvest season for the Caborca field and slightly more than one month (38 days) for the Salinas field.

One hundred spears were randomly selected from each of the cartons. The basal portion (5-6mm) of each spear was removed, sterilized, and split longitudinally; and then, placed on PDA medium in plastic Petri plates. Each spear was tagged with a number and the severed piece and the plate were given the same identical number thus providing a method of identifying the spear with the plate. After an incubation period of five days in a darkened incubator held constantly at 30°C., plates were removed and those with *Fusarium* colonies were matched with the same numbered spears. Spears were grouped into bunches of fifteen each for temperature and storage period studies. The noninfected spears were likewise identified and handled in the same manner as those with *Fusarium* and used as control treatments.

After the appropriate five-or-ten-day time interval at the selected temperature, spears were removed from the temperature control cabinets and the quality visually established. Spears were then held at 10°C. and observed daily to determine the shelf-life as established by the average percent salability for each spear group.

Spear samples were taken from the two fields in different geographic areas of Mexico and California to evaluate if the period of harvest would influence the incidence of *Fusarium* infection.

## Results

Marketable asparagus spears infected with *Fusarium* spp. were found to have lessor quality as the length of storage increased and as temperatures were increased from 5°C. to 26°C. and from five-to-ten-days storage.

Infected spears compared to noninfected spears when retained at 5°C. for five days had no discernible quality differences regardless of area sampled. However, after ten days at the same temperature, quality was reduced by 18% in Caborca samples compared to 9% in the Salinas samples. The remaining shelf-life was reduced by 21% and 15% respectively. Shelf-life reduction would be expected to be higher in this case, as there was an additional five days added to storage that resulted from the initial incubation period involved in identifying infected spears. Storage for ten days at 5°C resulted in a reduction of quality by only 8% of the initial quality in the noninfected spears. Shelf-life was also reduced by 8%. The product was still considered salable and had a remaining shelf-life of approximately 2 to 2-1/4 days. Infected spears however, had a dramatic decrease in quality by 12%, with shelf-life reduced to 1-1/4 days as compared to

noninfected spears from the Caborca area. Results from California samples were proportionate in both quality and shelf-life to that from Caborca.

Storage at five days at 26°C. for the infected spears reduced quality by 32% and 21% from Mexico and California, respectively, when compared to noninfected spears that had a surprising 11% and 9 % reduction in quality. Infected and noninfected samples stored for ten days at 26°C., regardless of geographic sampled area, were deteriorated beyond point of salvage and were discarded.

## Discussion

This investigation has helped answer one of the perpetual questions as to why there is a decrease in asparagus spear quality in a certain percent of spears following periods of storage and varying temperatures while the others, harvested under the identical conditions and sources, retain their quality. Why are some spears affected and others are not affected? One would think, if one is bad, all would be bad proportionately in similar condition. Such differences have normally been attributed to genetic differences or variation in carbohydrate levels. While these factors may contribute to quality differences between spears, data from this investigation indicate that the major contributing factor results from infection of spears by *Fusarium*. Infected spears rapidly decrease in quality and shelf-life compared to noninfected spears. This has been dramatically illustrated by isolating from poor quality spears following prolonged periods of storage (15+ days) and finding greater than 90% of the total infected with *Fusarium* spp. This implicates the fungus(i) as probably the major culprit in asparagus quality and shelf-life.

No conclusions could be drawn from the influence of the harvest season on the incidence of *Fusarium* infected spears due to the many variables that existed between the two areas.

Table 15. Influence of *Fusarium* infected spears on the quality of asparagus

| Collection Area | Asparagus Quality[1] Five Days[2] 5°C. | 26°C. | Ten Days[2] 5°C. | 26°C. |
|---|---|---|---|---|
| Caborca, Mexico | | | | |
| Infected | 10 | 5 | 7 | 0[3] |
| Non-infected | 10 | 8 | 8 | 0 |
| Salinas, CA | | | | |
| Infected | 9 | 6 | 8 | 0 |
| Non-infected | 10 | 8 | 9 | 0 |

1)Quality based on 10=maximum; 0=nonsalable.
2)Length of time in storage.
3)All samples and spears nonsalable

Table 16. Influence of *Fusarium* infected spears on the shelf-life of asparagus

| Collection Area | Shelf-life in Days[1] Five Days[2] 5°C. | 26°C. | Ten Days[2] 5°C. | 26 C |
|---|---|---|---|---|
| Caborca, Mexico | | | | |
| Infected | 3.2 | 1.25 | 0.5 | -[3] |
| Non-infected | 4.0 | 2.15 | 1.15 | - |
| Salinas, CA | | | | |
| Infected | 3.5 | 2.0 | 1.15 | - |
| Non-infected | 4.0 | 2.8 | 2.25 | - |

1) Shelf-life expressed in days with four days maximum.
2) Length of time in storage.
3) All samples and spears nonsalable.

# CHAPTER 6

# EVALUATION OF *FUSARIUM* INFECTED ASPARAGUS SPEARS FOR FUMONISIN MYCOTOXINS

## Introduction

*Fusarium* species have been reported worldwide on a variety of commercially important plants (Leslie, et al., 1992). Some strains produce significant quantities of secondary metabolites that may cause significant physiological and pathological responses in plants as well as animals (Nelson, et al., 1993). The production of compounds such as mycotoxins, phytotoxins, antibiotics, and pigments vary between hosts and the species of *Fusarium* involved.

The mycotoxin producing fungi are important to human health since they infect corn, rice, millet, and sorghum that are staple dietary food items in many parts of the world (Marasas, et al., 1984). Species and strains of *Fusarium* produce a group of toxins, the fumonisins, that are associated with these crops. To date, only three species, *F. moniliforme, F. proliferatum*, and *F. nygamae,* have been found to produce varying levels of the toxin fumonisin B1, one of four analogs of the mycotoxin. Of thirty-one stains isolated from corn, seventeen were high producers (Nelson, et al., 1991). Strains that produce <50 ug/g of fumonisin B1 are considered low producers, 50-500 ug/g are intermediate producers, and >500 ug/g as high producers (Leslie, 1992). Preliminary surveys in the United States of various corn-based human food products determined that 74% had detectable levels of fumonisins (Sydenham, et al., 1991). Studies are in progress to determine the prevalence of the mycotoxins and establish human toxic levels.

*F. proliferatum*, formally identified as *F. moniliforme*, has been reported to infect both established asparagus plants and the commercial spears harvested from them (Guerrero, et al., 1997a). Recent studies have demonstrated that *Fusarium* species infect a far greater percentage of commercial spears than previously imagined (Guerrero, et al., 1997b). The significance of spear infection has received scant attention as a post harvest disease problem. In some of the warmer desert areas where asparagus is grown, infection of spears has been found to exceed 90% of the total harvested crop.

Since the incidence of spear infection has been detected throughout most of the asparagus growing areas of the United States, Mexico, Peru, and Australia, investigations have been established to evaluate the possible presence of fumonisins in commercial asparagus spears.

Two different geographic regions of Mexico and two in California, USA, with climate variability, varietal differences, and different periods of harvest were selected in an effort to obtain as wide of a range in the specific species and strains that may exist in each of the areas. All asparagus was collected from commercially harvested and packed material. Included in the studies was the influence of time and temperature on the possible production of mycotoxin.

## Materials and Methods

In November of 1996, the first asparagus samples were obtained from the Constitucion area of Baja California Sur, Mexico. A commercially harvested and packed five kilo carton of asparagus was obtained two days following harvest. The product was from an eight-year-old field of asparagus planted with the cultivar Meloland Select. From

the sixteen bunches of extra large size spears, 100 were randomly selected. From the basal end of each, approximately 4 mm. were excised, and the pieces were individually sterilized by emersion in a sodium hydroxide (10%) bath for a minimum of three minutes. After rinsing three times in sterile distilled water, the spears were numbered and the corresponding piece placed in 4 cm. plastic Petri plates that had the same identifying number as the spear. Culture medium used was potato-dextrose in agar (PDA). Plates were then placed for five days at 30°C. in an incubator without light.

Following five days incubation in the dark at 30°C., all dishes were visibly examined for *Fusarium* colonies. Single spore isolates were made from each dish for positive *Fusarium* identified. The spores were transferred to Komada medium and incubated for five days, at which time species were identified using the Tousson-Nelson system of identifying *Fusarium* in the Liseola section (Tousson, Nelson, 1976). Spears infected with *F. proliferatum* were separated from noninfected spears, or those with *F. oxysporum*. Twenty-five spears were selected from each species lot and from the noninfected group, the latter serving as the control. These were then retained at 5°C. and 26°C. for five and ten days to determine the influence of temperature and incubation on growth of the *Fusarium* and on the possible production of fumonisin.

Plates were removed and by visual examination of the spear pieces and/or surrounding medium *Fusarium* species were tentatively identified. Samples found infected were then identified with the appropriate numbered spear and the *Fusarium* infected spears were then grouped together. Noninfected spears were also placed together in bunches of fifteen spears each. These grouped samples were then frozen until ready

for shipment to the USDA, National Veterinary Services Laboratory, Ames, Iowa, where they were analyzed for fumonisin $B_1$, $B_2$ and $B_3$ by the high pressure liquid chromatography.

The objective of the second investigation was to determine if *Fusarium* infection and possible production of fumonisin was influenced by the longevity of the harvest. To accomplish this, spear samples were removed from commercially harvested and packed five kilo cartons obtained from the same field in Caborca, Mexico, during January, February, and March at approximately thirty day intervals.

Isolations were made and percent of *Fusarium* infected spears were established by the same procedures used in the first investigation. No attempt was made to identify *Fusarium* species involved, only total infected spears.

Fifty *Fusarium* infected spears and the same number of noninfected spears were retained for five days at 26°C. From each lot, ten spears were randomly selected and cut into basal, mid-stem, and tip sections that were placed on PDA in plastic Petri dishes and allowed to incubate for five days. This procedure established the location of the fungus in the spear approximately three days after harvest. Following the incubation at 26°C., an additional five spears were cut into the three sections, isolated on medium, and the *Fusarium* colonies were compared to the first samples. This establishes the finding of continued growth of *Fusarium* in the spears following harvest

The third investigation was established to evaluate the *Fusarium* infection of commercial spears from two distinct geographic areas with varying climate, soil, varieties, and cultural practices. It could also determine if strain variation existed and

influenced fumonisin production. The areas selected were fields in the Salinas Valley and Imperial Valley, California (USA), and Caborca, Sonora (Mexico). Using the same procedures for spear number selection and isolation as in the previous investigations, *Fusarium* infected spears were analyzed for fumonisin production after incubation periods of five and ten days, each with temperatures of 5°C. and 26°C. Infected spears retained for ten days at 26°C were completely rotted and were not included in the evaluation.

The fourth investigation compared possible fumonisin production from different fields in the same geographic area. Samples were taken from commercial cartons of five kilos produced in two fields in the Salinas Valley, California, in late April. Both fields were planted with the variety UC157. Age of plantings were estimated to be eight and nine years old. Following the same sampling, isolation and extraction procedures as in the previous investigations, the incidence of *Fusarium* infection was established.

Twenty-five *F. proliferatum* spears were grouped together as a sample with two samples established from each field. Treatments consisted of storage periods of five and ten days at two temperatures of 5°C. and 26°C. to evaluate if increased temperatures and time in storage would increase growth of the fungus in the spears and have some influence on fumonisin production. Samples of equal numbers of spears that were noninfected served as controls for each of the treatments. After appropriate exposure of time and temperature, the samples were immediately prepared for fumonisin analysis.

## Results

Results from each of the four individual trials were determined to have nondetectible levels of fumonisin metabolites, regardless of geographic location of sampling, time of harvest season when samples were taken, soil temperatures, and/or varieties of asparagus planted. Neither length of time in storage nor storage temperature had any concernable influence on the production of fumonisin (Tables 1-4). *F. proliferatum* was the predominate species of *Fusarium* recovered from the packed commercial spears taken from the warmer desert regions of Northern Mexico and Southern California. *F. oxysporum* was also recovered but in all samples never exceeding more than 12% of total spear infection. The opposite was observed in the isolations of spears removed from the sampled field of the Salinas Valley region of California that required more spear selection for isolation to obtain the required number of 25 spears infected with *F. proliferatum* than were used for all fumonisin analysis.

Investigations are in progress to establish vegetative mating types of *F. proliferatum* isolates from samples from the four investigations. This is expected to establish if strain differences exist from the various geographical areas involved.

## Discussion

The high rate of infection by *F. proliferatum* in commercial asparagus spears did not influence fumonisin production under any conditions encountered during the periods these studies were in progress. This would tend to indicate that the chemical composition and physiological processes of asparagus does not provide the fungus with the necessary constituents to manufacture fumonisin metabolites.

Table 17. Fumonisin Produced by *Fusarium* Infected Asparagus Spears[1] (Constitucion, B.C., Mexico)[2]

| Sample Type | Percent Fumonisin No. Spears | $B_1$ | $B_2$ | $B_3$ |
|---|---|---|---|---|
| *F. proliferatum* | 25 | 0 | 0 | 0 |
| *F. proliferatum* + *F. oxysporum* | 15 + 10 | 0 | 0 | 0 |
| Noninfected | 25 | 0 | 0 | 0 |

1) 100% spears infected (98% *F. proliferatum*).
2) Field 7 years old, sampled November

Table 18. Influence of Harvest Date on Incidence of Fumonisin Production by *Fusarium* Infected Spears

| Sample Date | F. Proliferatum [1]Infected Spears[2] | PPM Fumonisin $B_1$ | $B_2$ | $B_3$ |
|---|---|---|---|---|
| January 15 | 63 % | 0 | 0 | 0 |
| February 17 | 54 % | 0 | 0 | 0 |
| March 14 | 70 % | 0 | 0 | 0 |

1) Same field of 8 year old Meloland Select in Caborca, Sonora, Mexico, sampled each date.
2) Each fumonisin analyzed sample contained 25 spears infected with *F. proliferatum* and 25 noninfected spears for control.

Table 19. Influence of Temperature and Storage on Fumonisin Production by *Fusarium* Infected Spears.

| Collection Area[3] | % Spear Infection | PPM Fumonisin Ten Days 5°C. | 26°C. | Five Days 5°C. | 26°C. |
|---|---|---|---|---|---|
| Caborca, Mexico[1] | 82 % | 0 | 0 | 0 | 0 |
| Control | noninfected | 0 | 0 | 0 | 0 |
| Imperial Valley, CA[2] | 63 % | 0 | 0 | 0 | 0 |
| Control | noninfected | 0 | 0 | 0 | 0 |

1) Field 9 years old, Meloland Select variety.
2) Field 6 years old, Meloland Select variety.
3) Sampled 17-18 February 1997.

Table 20. Influence of *Fusarium* spp. Infected Spears on Production of Fumonisin

| Collection Area[1] | Spear Infection | PPM Fumonisin Five Days 5°C. | 26°C. | Ten Days 5C. | 26°C. |
|---|---|---|---|---|---|
| 1) Field One[2] | | | | | |
| Sample #1[3] | 31 % | 0 | 0 | 0 | - |
| Sample #2 | 43 % | 0 | 0 | 0 | - |
| Sample #3 | Control | 0 | 0 | 0 | - |
| 2) Field Two[2] | | | | | |
| Sample #1[3] | 52 % | 0 | 0 | 0 | - |
| Sample #2 | 27 % | 0 | 0 | 0 | - |
| Sample #3 | Control | 0 | 0 | 0 | - |

1) Collected in Salinas Valley, CA.
2) First field @ 8 years in age; second field @ 9 years in age; both UC-157 variety.
3) Twenty-five spears in each sample.

## General Discussion

*Fusarium spp.* causing crown rot of asparagus it has been reported in different productive areas (Nigh, 1978; Endo, 1978; Damicone, 1981; Evans and Stephen, 1991; Elmer, 1990). This study report for the first time the occurrence of *F. proliferatum* causing crown rot in the areas of Northern Mexico and Southern California. Also we found *F. oxysporum*, as another specie involved in this disease. Although others researchers have been reported different species of this genus causing crown rot (Endo and Burkholder, 1971; Damicone and Manning, 1985), during the present study only two pathogenic species were founded (*F. proliferatum* and *F. oxysporum*), even other species of *Fusarium* were isolated such as *F. solani*, but this was not pathogenic.

According to the morphological characteristics, *F. proliferatum* and *F. oxysporum* were the dominant species isolated from asparagus crowns. This finding is agree with report of Elmer et al, (1990), which indicate that *F. proliferatum* is the main cause of asparagus crown rot in Connecticut, and that the fungus was misidentified as *F. moniliforme*, which was not founded in this study also. The isolation of *F. proliferatum* and *F. oxysporum* was identified simply by plating infected tissue directly in water agar media and significantly reduced not only the time necessary for species identification, but also avoid the use of selective media, comparing with Komada's method recommended by Singleton et al (1992).

*Fusarium proliferatum* and *F. oxysporum* were isolated from marketable asparagus spears, and also were pathogenic to asparagus seedlings, this report agrees with Montealegre and Palma (1994), Gilbertson and Manning, 1983; Johnston et al; and

Gilbertson, 1981; with the only difference that they report *F. moniliforme*, instead of *F. proliferatum*, which may be misidentified on infected spears.

Both species of *Fusarium* reported inthis study have been found as common pathogens in most soil (Damicone and Manning, 1985; Elmer, 1990). Our results indicate that *F. proliferatum* in sampled areas, which agree with the report of Johnston et al (1970). *F. proliferatum* was commonly concentrated only on crowns and also more recovered from spears, because *F. oxysporum* is more often founded in young roots. Isolation percentages of both *Fusarium* species from the spears were similar to those obtained from the crowns. This is the first report of the origin of *Fusarium spp.* in asparagus spears.

*Fusarium spp.* have been reported affecting asparagus spears and considered as a market disease of asparagus (Snowden, 1992; Smith et al, 1966). Our results indicate that Fusarium spp. affect the quality of spears, depending of the length of storage and temperature. Storage for 5 days at 26 C reduce significantly the shelf life of this vegetable and there is no report on this effect with *Fusarium* species.

It has been reported that some strains of *Fusarium moniliforme, F. proliferatum* and *F. nygame* produce secondary metabolites than can cause health problems(Nelson et al, 1993), and considering recent studies by Guerrero et al (1997a) where indicate that *F. proliferatum* is present in asparagus spears, the study of the presence of fumonisins is very important. In this study no levels of Fumonisins were founded in asparagus spears infected by *F. proliferatum.*

## Summary

1. *F. proliferatum* and *F. oxysporum* are capable of causing crown rot of asparagus in Northern Mexico and Southern California.

2. *F. proliferatum* was the most prevalent specie founded in crown rot and asparagus spears in warmer productive areas.

3. *Fusarium spp.* infection of spears is originated directly from the asparagus crown.

4. Asparagus spears infected with *Fusarium spp.*, have less quality as the length of storage and temperature increase.

5. Asparagus spears infected by *F. proliferatum* did not influence fumonisins production.

# REFERENCES

Cantaluppi, C.J. and R.J. Precheur. 1993. Asparagus Production Management and Marketing. Bulletin 826. The Ohio State University Extension.

Cohen, S.I., and Heald, F.D. 1941. A wilt and root rot of asparagus caused by *Fusarium oxysporum* (Schlecht). Plant Dis. Rep. 25:503-509.

Conger, A. 1964. A simple liquid-culture method of growing plants. Proc. Florida State Horticultural Society 77.

Damicone, J.P., Cookey, D.R. and Manning, W.J. 1980. Elimination of *Fusaria* from asparagus seeds. Plant Disease 70(5):461 (Abstr.).

Damicone, J.P., and Manning, W.J. 1985. Frequency and pathogenicity of *Fusarium spp.* isolated from first-year asparagus grown from transplants. Plant Disease 69:413-416.

Damicone, J.P., and Manning, W.J. 1980. *Fusarium moniliforme* var. *subglutinans* pathogenic on corn and asparagus. Plant Disease 70(5): 461 (Abstr.).

Elmer, W.H. 1990. *Fusarium proliferatum* as a causal agent in *Fusarium* crown and root rot of asparagus. Plant Disease 74(11):938 (Abstr.).

Elmer, W.H., and Ferrandino, F.I. 1992. Pathogenicity of *Fusarium* species (Section Liseola) to asparagus. Mycologia 84(2):253-257.

Elmer, W.H., D.A. Johnson, and G.I. Mink. 1996. Epidemiology and management of the diseases causal to asparagus decline. Plant Dis. 80:117-125.

Elmer, W.H. 1990. *Fusarium proliferatum* as a causal agent in *Fusarium* crown and root rot of asparagus. Plant Disease 74(11):938 (Abstr.).

Elmer, W.H. 1995. A single mating population of *Giberella fujikuroi* (*Fusarium proliferatum*) predominates in asparagus fields in Connecticut, Massachusetts, and Michigan. Mycologia 87(1):68-71.

Endo, R. 1978. Asparagus Workshop Proceedings. University of California, Riverside. pp. 156.

Endo, R.M., and Burkholder, E.C. 1971. The association of *Fusarium moniliforme* with the crown rot complex of asparagus Phytopathology 61:891 (Abstr.).

Evans, T.A., and Stephens, C.T. 1989. Increased susceptibility to *Fusarium* crown root rot in virus infected asparagus. Phytopathology 79:253-258.

Fallon, P.G., Greathead, A.S., Mullen, R.J., Benson, B.L., and Grogan, R.G. 1991. Individual and combined effects of flooding, *Phytophthora* rot, and metalaxyl on asparagus establishment. Plant Dis. 75:514-518.

Fantino, M.G. 1990. Research on asparagus decline in Italy. Acta Horticuturae No. 271:291-298.

Gilbertson, R.L., and Manning, W.J. 1983. Contamination of asparagus flowers and fruit by airborne spores of *Fusarium moniliforme*. Plant Disease 67:1003-1004.

Graham, K.M. 1955. Seedling blight, a fusarial disease of asparagus. Can. J. Bot. 33:374-400.

Grogran, R.G. and Kimble, K.A. 1959. Association of *Fusarium* wilt with the asparagus decline and replant problem in California. Phytopath. (3):122-125.

Grove, M.D. 1976. Pathogenicity of *Fusarium* species associated with asparagus decline in Washington, P.h.D. dissertation, Washington State University, Pullman. 96 pp.

Guerrero, C., Nigh, Jr., E.L., and Stanghellini, M.E. 1997. Incidence of *Fusarium* spp. in asparagus spears. In Eucarpia Proc. 9th International Asparagus Symposium, Pasco, WA USA.

Guerrero, J.C., Nigh, Jr., E.L., and Stanghellini, M.E. 1997. Incidence of *Fusarium spp*. on asparagus spears. Plant Dis. 81:957.

Guerrero, C., Nigh, Jr., E.L., and Stanghellini, M.E. 1997b. Incidence of *Fusarium* spp. in asparagus spears. In Eucarpia Proc. 9th International Asparagus Symposium, Pasco, WA USA. July, 1997. In Press.

Guerrero, C., Nigh, Jr., E.L., Stanghellini, M.E. 1997a. Incidence of *Fusarium* spp. in asparagus fields in Mexico and Southern California. In Eucarpia Proc. 9th International Asparagus Symposium, Pasco, WA USA. July 1997. In press.

Harsh, S., Kelsy, S., and Neibauer, J. 1970. Economics of Asparagus production in Western Michigan. Report No. 163. Dept. of Agricultural Economics, Michigan State University.

Johnston, S.A., Springer, J.K., and Lewis, G.D. 1979. *Fusarium moniliforme* as a cause of stem and crown rot of asparagus, and its association with asparagus decline. Phytopathology 69:778-780.

Johnston, S.A., Springer, J.K., and Lewis, G.D. 1979. *Fusarium moniliforme* as a cause of stem and crown rot of asparagus, and its association with asparagus decline. Phytopathology 69:778-780.

Leslie, John, et al. 1991. Fumonisin B. production by strains from different mating populations of *Gibberella* fujikuroic. Phytopathology 82:341-345.

Lewis, G.D., and Shoemaker, P.B. 1964. Presence of *Fusarium oxysporum f. asparagi* on asparagus seed and *Fusarium* resistance in plant introduction lines of asparagus. Phytopathology 54:128 (Abstr.).

Marasas, W.F.O. and Tousson, T.A. 1984. Toxigenic *Fusarium* species: Identity and mycotoxicology. Univ. Park: The Penn State Univ. Press. 328 pp.

Montealegre, J.R., and Palm, M.J. 1994. Etiology of the main postharvest fungal rots in green asparagus in Chile. Fitopatologia 29:102-105.

Nelson, P.E., et al. 1991. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. Appl. Environ Microbiology 57:2410-12.

Nelson, P.E., et al. 1993. Fumonisins mycotoxin produced by *Fusarium* species. Biology, Chemistry, and Significance. Ann. Rev. Phytopatholgoy. 32:233-52.

New Jersey Crop Reporting Service. 1977. N.J. Dept. of Agric. in Cooperation with U.S. Dept. of Agriculture (Compilers). New Jersey Agricultural Statistics 57 pp.

Nigh, E.L. 1978. Asparagus Workshop Proceedings. University of California, Riverside. pp. 156.

Singleton, L.L., Mihail, J.D., and Ch.M. Rush. 1992. Methods for research on soilborne phytopathogenic fungi. APS press pp. 250.

Smith, M.A. et al. 1966. Market disease of asparagus, onion, beans, peas, carrots, celery and related vegetables. U.S.D.A. HANDBOOK 303.

Snowden, A.L. 1992. Postharvest diseases and disorders of fruit and vegetables. Vol. 2, Ann Arbor, CRC Press. 213 pp.

Sydenhham, E.W., et al. 1991. Fumonisin contamination of commercial corn-based human foodstuffs. J. Agric. Food Chemistry. 39:2014-18.

Takatori, F.H., Souther, F.D., and Stillman, J.I. 1977. asparagus production in California. Bulletin 1882. University of California, Division of Agricultural Sciences.

Tello, J., Gonzalez, M.L., and Lacasa, A. 1985. The "Fusariosis" (Diseases produced by *Fusarium spp.*) of asparagus in Spain. Proc. 6th Int. Asparagus Symposium pp. 126-135.

Thompson, H.C. 1942. Asparagus Production. Orange Judd Publishing Company. New York. 124 pp.

Thornton, R., Ford, W., Dyck, R., Cone, W., and Parker, R. 1982. Washington Asparagus production guide. EB 0997. Cooperative Extension, College of Agriculture. Washington State University, Pullman, Washington.

Tousson, T.A., and Nelson, P.E. 1976. A pictorial guide to the identification of *Fusarium* species. Univ. Park: The Penn State Univ. Press 2nd Ed. 42 pp.

Tu, C.C., and Chang, K.F. 1977. Studies on fusarial wilt and root diseases complexes of asparagus in Taiwan. J. Agric. Res. China 26:216-233.

Warunnee, P., Dhirabhava, W., and Bhudhasamai, K. 1990. Effect of contact icing and storage method of decay, on the quality and storage life of fresh asparagus. Proceedings of National Vegetable Conference p. E-8:1-19, Thailand.

Zomlefer, W.B. 1994. Guide to Flowering Plant Families. pp. 280-282.