Contamination of Asparagus Seed by *Fusarium oxysporum* f. sp. *asparagi* and *Fusarium moniliforme*

DEBRA ANN INGLIS, Graduate Research Assistant, Department of Plant Pathology, Washington State University, Pullman, 99164

ABSTRACT

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Assays of Washington-produced asparagus seed revealed both *Fusarium oxysporum* f. sp. *asparagi* and *F. moniliforme*. A small percentage of *F. oxysporum* f. sp. *asparagi* may infect seeds internally, but the pathogen is primarily an external seed contaminant. *F. moniliforme* is present externally in the natural crevices of the seed coat or in the cavities of seed damaged by asparagus beetles. Infestation occurs when the seed is extracted from the berries. Wash water contaminated by diseased host tissue may contain several thousand conidia of *F. moniliforme* per milliliter. The surface of an asparagus seed is rough, and fungal spores are trapped in natural crevices or insect tunnels.

Additional key words: scanning electron microscopy

Fusarium oxysporum Schlecht. f. sp. *asparagi* Cohen and *F. moniliforme* Sheldon are seedborne pathogens of asparagus (*Asparagus officinalis* L.) (5,6) causing a vascular wilt (4) and crown and stem rot (9), respectively. Seed lots from many geographic locations (1,7,11) are contaminated with these pathogens, which may be an important means of introducing them into noninfested areas. Treatments for elimination of seedborne inoculum have been developed (7), but more timely or simplified control measures might be used if the mechanisms of seed contamination were known. In this study with Washington-produced asparagus seed, attention was given to the possible mechanisms of internal infection and external infestation by *F. oxysporum* f. sp. *asparagi* and *F. moniliforme*, respectively.

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MATERIALS AND METHODS

Grove (7) found that surface disinfestation with 1% sodium hypochlorite in 50% ethanol for 60 min reduced levels of *F. oxysporum* f. sp. *asparagi* in asparagus seed from 9.3 to 1% or less. He therefore presumed that the fungus was borne internally in a low percentage of seed. Grogan and Kimble (6) concluded from their work that asparagus seeds were surface-contaminated with *F. oxysporum* f. sp. *asparagi*. Therefore, a histological study was undertaken to ascertain whether *F. oxysporum* f. sp. *asparagi* infects asparagus seed internally. Seeds for the study were selected randomly from seed lots with known high percentages of contamination. Seeds from berries of diseased plants and seeds yielding colonies of *F. oxysporum* upon germination also were collected. All seeds were cut in half and placed in formalin-acetic acid-alcohol (8). They were dehydrated by the tertiary butyl alcohol method (8), sectioned at 8 μm, and stained with safranin and fast green (12).

To determine if *F. oxysporum* f. sp.

asparagi invades seeds by systemic spread in the vascular system, isolations were made from portions of mature plants with Fusarium wilt. Small pieces from stems and branches were dipped in a solution of 1% sodium hypochlorite in 95% ethanol for 30 sec and flamed. Strips of the vascular tissue were pulled away with a sterile forceps and placed onto Komada's medium (10). If the stem section under examination had produced berries, the berries were placed in the hypochlorite-alcohol solution for 15 sec and set on clean paper towels to drain. Flesh from the inside of the berry was scraped away with a sterile knife and placed on the medium. Seeds and sections of each berry pedicel were transferred individually to the medium.

Samples of Washington asparagus seed also were assayed by a blotter technique for the presence of *F. oxysporum*. Seeds were soaked for 24 hr in tap water, drained, and placed on moist blotter paper in sterile metal trays 2.5 cm deep. The trays were covered with Saran Wrap and kept in the laboratory at approximately 22 C. After 2 wk, seeds or seedlings positive for *F. oxysporum* were counted and single spores were transferred to potato-dextrose agar to produce isolates for identification. Some seed lots were contaminated with both *F. moniliforme* and *F. oxysporum*. The following procedures were used to determine if *F. moniliforme* was an external contaminant: seeds were wrapped in a cheesecloth bag and tied to a water faucet to gently wash in a stream of water for 24 hr, or they were soaked first in tap water for 24 hr, then in a solution of 1% sodium hypochlorite in 95% ethanol with 0.5 ml of Tween 20 for 1 hr, and finally rinsed 10 times in tap water (7). The blotter technique was then used to assay for seedborne fungi. Commercial seed extraction operations in Washington State also were observed to discern whether harvesting procedures contribute to external contamination by *F. moniliforme*. Isolations were made from samples of berries, seed, wash water, and extraneous host debris to detect *F. moniliforme*.

The scanning electron microscope was used to observe the morphology of the seed coat and the presence of *Fusarium* spores or other propagules. Seeds observed in this way were normal or damaged by asparagus beetles. Specimens, fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, were dehydrated by a standard ethanol series and prepared for critical point drying using TF Freon as a transition fluid (3). They were coated with 45 nm of gold and observed at an accelerating voltage of 20 kv. A Polaroid camera was used for photographs.

RESULTS AND DISCUSSION

Paraffin sections of seeds from lots with known high percentages of *F. oxysporum* showed no evidence of internal fungal infection; furthermore, seeds from diseased plants and seeds colonized by *F. oxysporum* on blotter paper before fixation apparently were not internally infected. Grove (7) concluded that *F. oxysporum* f. sp. *asparagi* was internally seedborne because surface sterilization failed to eliminate the fungus. Failure to detect internal infection histologically may be due to the low percentage of internally infected seeds. *F. oxysporum* was recovered from diseased asparagus tissue as high as 60 cm above the soil line; it was recovered from side branches in a few instances but never from berries, seeds, or berry pedicels. These negative results do not preclude the possibility of occasional invasion of fruit and seed by *F. oxysporum*, but such invasion must be rare at least.

The blotter technique disclosed that up to 4.7% of tested seeds were infested with *F. moniliforme*. Some seed lots had numerous seeds with large cavities in the hilum region, apparently due to feeding by asparagus beetles. The cavities ranged from 1–3.5 mm wide, 1.5–4 mm long, and 0.25–1 mm deep. As many as 90% of the beetle-damaged seeds yielded colonies of *F. moniliforme*. In all cases, mycelium originated directly from the cavity and not from splits in the seed coat.

Rinses with running tap water reduced infestation by *F. moniliforme* in intact seeds from 2.3 to 0.33%. Surface treatments with 1% sodium hypochlorite in 95% ethanol reduced *F. moniliforme* in beetle-damaged seeds from 90 to 0.02%. When 3 g of beetle-damaged seeds was added to 300 ml of sterile water, assays of the water after 24 hr gave propagule counts of *F. moniliforme* as high as 16,500 spores/g of seed. When seeds treated with hypochlorite and ethanol were placed in the same water for 30 min, 12.5% of the soaked seeds subsequently assayed were colonized by *F. moniliforme*. Surface treatments eliminated external infestation, a suspension of spores was obtained by merely soaking infested seeds in water, and the suspension was able to contaminate noninfested seeds. *F. moniliforme* therefore appears to be borne externally on asparagus seeds.

An asparagus seed has a rugged surface. The epidermal cells or tubercles (13) of the seed coat are tubular in shape

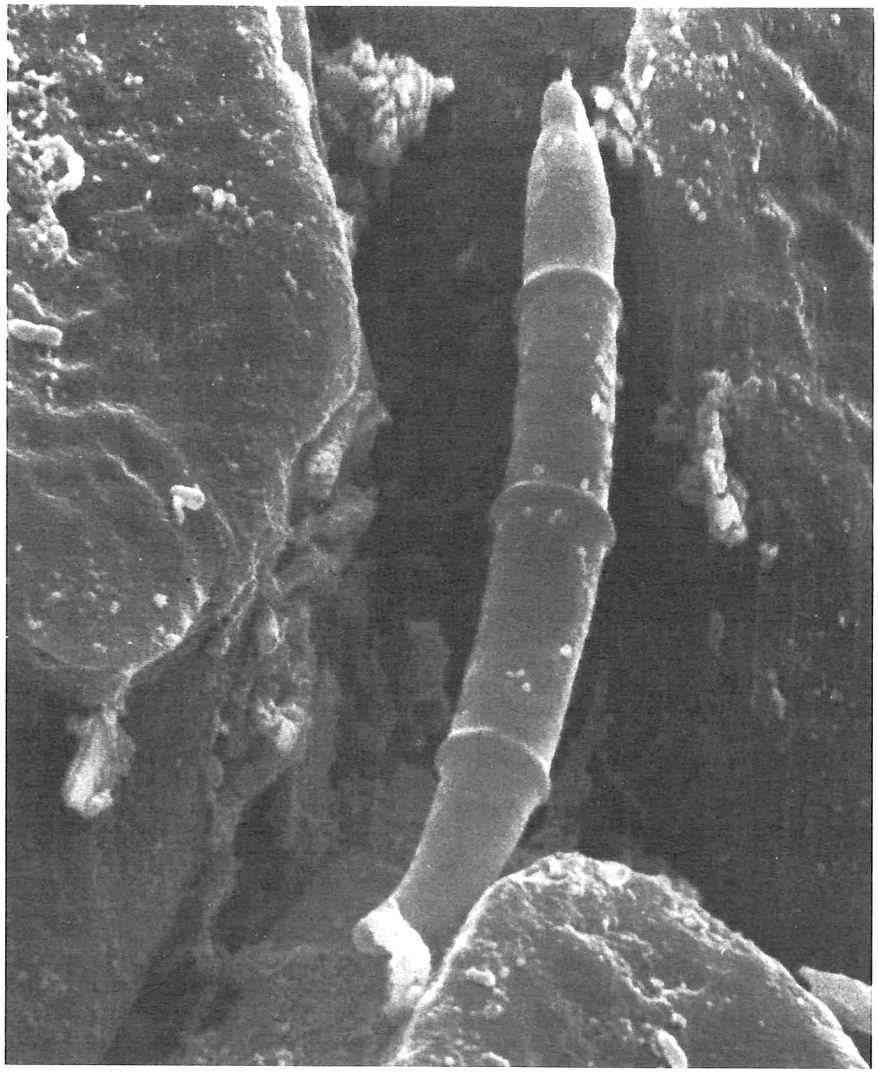

Fig. 1. A macroconidium presumed to be *Fusarium moniliforme* lodged in a crevice of an asparagus seed coat. (×5300).

and are separated from one another by crevices with almost vertical sides that range up to 10 μm across and are effective for trapping fungal spores. *Fusarium* macroconidia were observed lodged in seed coat crevices. At least one such conidium (Fig. 1) was *F. moniliforme*, because a colony of *F. moniliforme* was cultured from a small weft of hyphae emanating from this same seed before its fixation and the spore measurements fit the dimensions given by Booth (2) for this species (23–36 × 2.5–3.5 μm).

Asparagus plants are prolific berry producers. Each berry contains five or six hard, shiny black seeds. Seeds are not ready for harvest until mid-October when they have "hardened off." Although still fleshy at this time, the berries must be harvested before they drop from the plants. The fern is cut at the soil line, loaded onto a truck bed, and transported to a mechanical thresher or to an area where the berries can be threshed by hand. They are placed in a Waring Blendor or apricot pulper, water is added, and a slurry of berries and seeds is produced. Often the berries are contaminated with asparagus stems, debris, and soil. Sometimes it is impractical to process the berries immediately, and they are placed in cold storage for up to several weeks. Once the berries are added to the blender or pulper, seed may be processed immediately or remain in the slurry for varying times. They are then stirred in water to separate the light seed and air-dried on wire racks.

Dilutions made of berry slurry collected from two commercial seed extraction operations yielded 50,000–125,000 propagules of *F. moniliforme* per milliliter of slurry. Infected berries and asparagus debris appear to be the source of this inoculum, since pathogenic isolates of *F. moniliforme* were obtained from this material. As the seeds remain in the slurry, cavities and natural crevices provide ideal places for spores to lodge. The seed apparently becomes infected with the fungus during extraction. The fact that seeds remain in the slurry for varying times may explain the variation in infestation levels of different seed lots. Also, the large percentage of *F. moniliforme* in seeds with cavities may be due to the likelihood of spores lodging in large cavities rather than relatively narrow crevices of the seed coat. A similar mechanism probably contributes to surface contamination of asparagus seed by *F. oxysporum* f. sp. *asparagi*. Despite the earlier suggestion that a small amount of internal infection occurs, virtually all *F. moniliforme* and *F. oxysporum* f. sp. *asparagi* contamination of seed apparently is on the surface but well protected in crevices and cavities. Measures for controlling contamination of *Fusarium* propagules during the seed extraction process should be developed.

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