Survival and Dissemination of Verticillium in Infected Safflower Seed

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ABSTRACT

Verticillium dahliae was frequently isolated from seeds harvested from inoculated safflower plants grown in the field and greenhouse. Microsclerotia were found on external and internal tissue of the pericarp, and on the testa of some infected seeds at harvest. Hyaline and torulose mycelia were located in sclerenchyma tissue of the pericarp. *Verticillium* grew on agar from mycelia in tissue fragments, and from single microsclerotia taken from infected seeds up to 2 years after harvest. Plants were infected by seedborne *V. dahliae* in autoclaved and nonautoclaved soil. The fungus was isolated from cotyledons, hypocotyls, and occasionally from the roots of 7- to 10-day-old seedlings, and from cotyledons and hypocotyls of germinated embryos. Microsclerotia formed on infected seed pericarps in autoclaved and nonautoclaved soil. Such pericarps proved to be effective inoculum when incorporated in soil. The fungus is not killed by maneb used as a seed treatment for control of seedborne safflower rust.

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In 1959, Sackston and Martens (4) reported transmission of a microsclerotial form of *Verticillium albo-atrum* Reinke & Berth. (*V. dahliae* Kleb.) by sunflower (*Helianthus annuus* L.) seeds. They related their findings to studies conducted by researchers in previous years on the relation of *Verticillium* spp. to the seeds of other host plants.

Since 1959, *Verticillium* has been reported to be seedborne in the weed host *Xanthium pungens* Wallr. (1), and in groundsel (*Senecio vulgaris* L.) (5), safflower (*Carthamus tinctorius* L.) (8, 12), and spinach (*Spinacia oleracea* L.) (9).

Infection of plants by seedborne *Verticillium* has been reported (1, 4, 5, 9).

Verticillium was found in testa or pericarps of *X. pungens,* sunflower, and spinach seeds, and under moist conditions, microsclerotia formed on the seed parts. Although the fungus was recovered from infected groundsel seeds, microsclerotia were not detected on infected seed parts.

Presumably, *Verticillium* can be disseminated by safflower seeds (achenes). Although microsclerotia form on pericarps of infected safflower seeds under moist conditions (2), little is known about the pathogen-seed relationship, or the fate of the fungus in the tissue of safflower seed. This study describes the incidence, survival, and dissemination of *Verticillium* in safflower seeds. A brief report of this work has been published (3).

MATERIALS AND METHODS.—Safflower plants (cultivar Gila) were grown in the field and in autoclaved soil in the greenhouse as a source of infected seeds. Plants were inoculated at 8-10 weeks of age with a cotton-defoliating strain of *V. dahliae* that causes wilt in safflower (6, 7). Conidial suspensions were prepared with sterile distilled water added to 7- to 10-day-old cultures on potato-dextrose agar (PDA) in test tubes. A wound was made at the cotyledonary node with a sterile transfer needle, and each plant was injected with approximately .05-ml of the suspension (4×10^5 conidia/ml) with a hypodermic syringe.

Mature seed receptacles were harvested from plants that showed extensive wilt symptoms before maturity. Seeds were removed from single receptacles with sterile forceps, placed in sterile petri dishes, stored in the laboratory at 23 to 24 C, and later used in this study.

Sodium hypochlorite (1%) was used for surface-sterilizing the seeds, seed parts, or plant parts, which were then thoroughly rinsed in sterile distilled water and placed on moist filter paper, plain agar, or PDA in assays for *Verticillium*.

Single microsclerotia were isolated from surface-sterilized seeds and incubated on PDA in plastic petri dishes at 6-degree intervals from 12 to 30 C. The cultures were examined daily thereafter for 10 days for growth of *Verticillium* colonies.

Thin-sections of pericarps with or without microsclerotia, were cut with a razor blade, stained in cotton-blue lactophenol, and examined with a microscope for *Verticillium* structures.

Seeds showing microsclerotia and healthy appearing seeds were planted one per pot at 21 C in autoclaved loam soil. Two weeks after emergence, the plants were transferred to a greenhouse in which daytime temperatures ranged from 27 to 30 C. The plants were observed for wilt symptoms until maturity.

Seedlings that emerged from infected seeds planted in autoclaved soil were studied for evidence of *Verticillium* infection. Seedlings 7-10 days old were removed from the soil, washed, and surface-sterilized for 2-3 minutes. The cotyledons were cut from the hypocotyl-root portion, and all parts were placed on PDA.

Germinated embryos (24-48 hours old) from surface-sterilized seeds incubated on moist filter paper were assayed for *Verticillium*. Whole embryos were removed and surface-sterilized for 5 minutes, then rinsed and placed on PDA. The pericarps were assayed to determine the number of infected healthy appearing seeds.

Maneb, used as a seed treatment, controlled seedborne safflower rust (*Puccinia carthami* Cda.) spores (10), but its effect on seedborne *Verticillium* is not known. *Verticillium*-infected safflower seeds were treated with maneb in slurry form at a dosage of 56.7 g/45.5 kg. Treated seeds were stored in closed containers for 4-6 weeks then assayed on PDA and moist filter paper.

RESULTS.—*Incidence of Verticillium in seed from infected plants.*—Safflower plants in the greenhouse produced from 20 to 25 seeds per receptacle, of which 50 to 55% yielded *Verticillium* on PDA. Thirty percent of the

seeds were laden with dark, elongated microsclerotia, which were usually present in the hilum area (Fig. 1-A), or had also developed over a large part of the pericarp of some seeds. Dissection of seeds heavily laden with microsclerotia revealed that some lacked an embryo, and that microsclerotia had formed on the inner wall of the seed coat.

Safflower plants in the field yielded 50-60 seeds per receptacle, of which 2% to 36% were infected. Although most of the seeds examined from 272 receptacles appeared to be healthy, microsclerotia were found on pericarps of one-to-three seeds in each of three receptacles. When healthy appearing seeds were surface-sterilized and placed on a moist filter paper or plain agar, microsclerotia formed on pericarps of infected seeds.

Verticillium structures in pericarp tissue.—Microsclerotia were found in inner sclerenchyma tissue of the pericarp (Fig. 1-B), and on the testa of seeds having microsclerotia on the pericarp. Torulose mycelia were found in tissues underlying or near the microsclerotia (Fig. 1-C). In the healthy appearing seeds (Fig. 1-D), segmented, hyaline mycelia were in the sclerenchyma tissue in pericarps of seeds that were infected. The mycelia were dense in the loosely compacted cells of the hilum area (Fig. 1-E), and often extended to the seed coat.

The fungus was viable in the infected seeds for a period of at least two years, during which time it was recovered from fragments of pericarps with or without microsclerotia. New growth developed from 58 single microsclerotia (Fig. 1-F) of the 78 isolated and placed on agar.

Infection of plants by seedborne Verticillium.—Among 144 plants from healthy appearing seeds, 20 (14%) developed wilt symptoms in autoclaved soil. Nine (17%) of 53 plants that originated from seeds with microsclerotia developed symptoms. In subsequent plantings of healthy appearing seeds in nonautoclaved

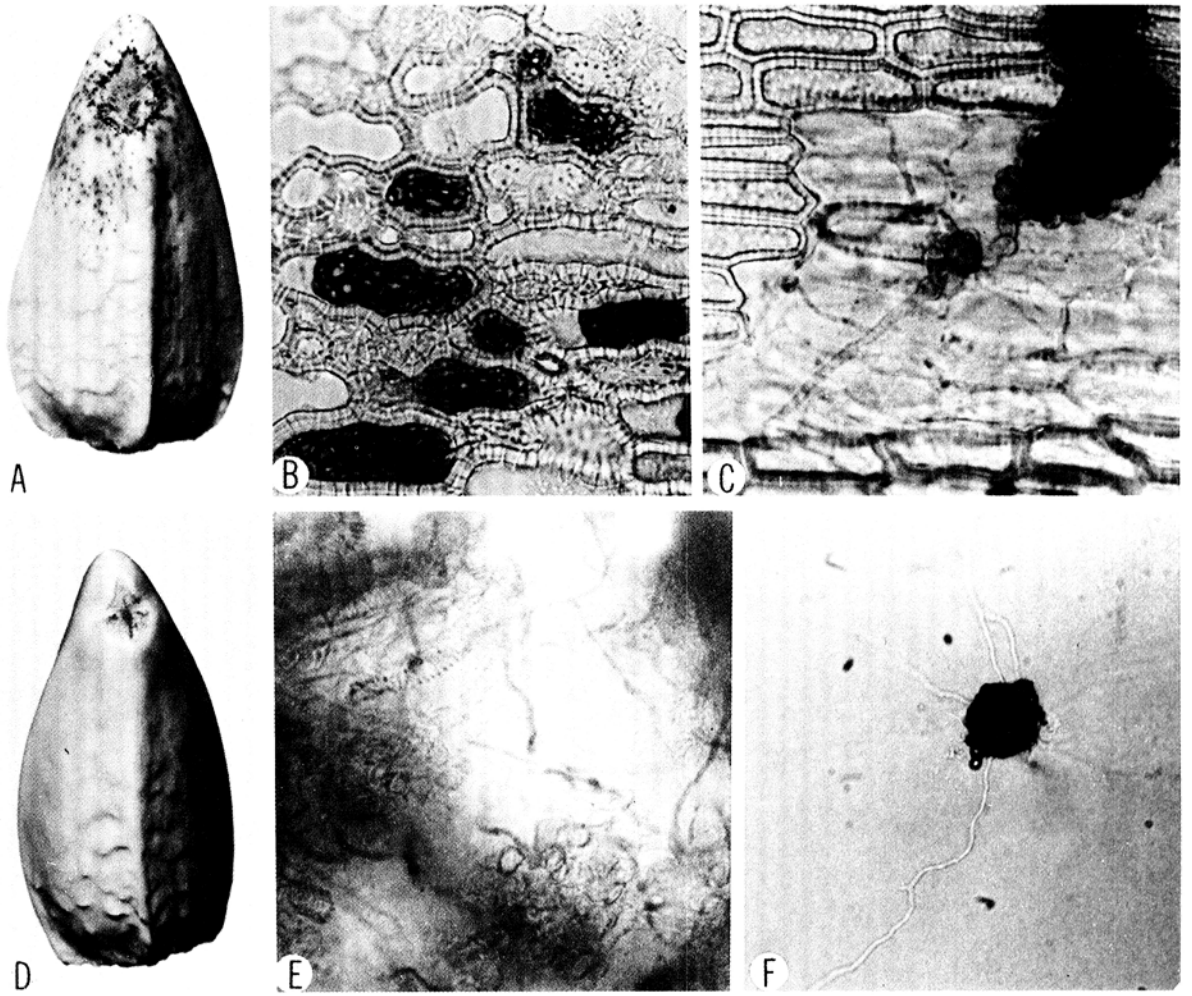

Fig. 1-(A to F). Structures of *Verticillium dahliae* in safflower seed. **A)** Seed with pericarp, showing microsclerotia at harvest; **B)** Microsclerotia formed in sclerenchyma cells; **C)** Torulose hyphae joined to a microsclerotium in pericarp tissue; **D)** Healthy appearing seed; **E)** Hyaline hyphae in hilum sclerenchyma; **F)** Growth of *Verticillium* from a single microsclerotium isolated from pericarp.

soil under similar conditions, 5% or less of the resulting plants developed wilt symptoms. *Verticillium* was isolated from the diseased plants, but not from healthy plants.

Seedling cotyledons that had a small necrotic spot or were noticeably deformed yielded *Verticillium,* however, the fungus also grew from apparently normal cotyledons. *V. dahliae* was isolated from hypocotyls, and from roots just below the hypocotyl, but not as often as from cotyledons.

In assays of germinated embryos, *Verticillium* colonies developed from 3 of 34 (9%) embryos from seeds with microsclerotia, and from 2 of 25 (8%) embryos from infected healthy appearing seeds. Fungus growth originated from embryo cotyledons more often than from hypocotyls. The fungus was not recovered from root radicles. Tufts of hyaline mycelia supported by shallow penetrating hyphae were found on the epidermis of cotyledons stained with cotton-blue lactophenol. It was not determined whether the fungus was in tissues of dormant seed embryos.

Dissemination of pathogen to soil.—During emergence of the safflower seedling, the pericarp usually remains in the soil near the hypocotyl. Pericarps of 21 healthy appearing seeds planted in autoclaved soil, and 22 seeds planted in nonautoclaved soil were recovered and examined under a dissecting microscope. Microsclerotia were found on 38 of 43 pericarps that were recovered from both soils. The structures were more abundant on the seed pieces recovered from the autoclaved soil. Single microsclerotia taken from pericarps that had been recovered from the soil and air-dried for 6 weeks, and surface-sterilized, produced *Verticillium* colonies on PDA. The pericarps that had microsclerotia were incorporated into autoclaved soil in a 15.2-cm diameter (6-inch) pot that was subsequently planted with safflower seeds. The efficacy of infested pericarps as inoculum was shown when two of six plants developed wilt symptoms. *Verticillium* was recovered on PDA from root and stem tissue of the diseased plants.

Effect of maneb on seedborne Verticillium.—In assays of infected seeds treated with maneb, *Verticillium* grew from infected seed pericarps on PDA. Microsclerotia formed on treated pericarps placed on moist filter paper and in the soil, but less than on nontreated seed pericarps.

DISCUSSION.—The incidence of *Verticillium*-infected safflower seeds from wilt-infected plants coincides with that found by Schuster and Nuland (8), and Zimmer (12). Microsclerotia on safflower seeds, as observed in this study, apparently have not been previously observed on seeds of safflower or other hosts (1, 4, 5, 9) at harvest. The few microsclerotia present on safflower seeds produced in the field, and the abundance of microsclerotia formed on seeds from plants in the greenhouse, suggests that humidity and temperature affect their formation on seeds in the seed receptacle.

The presence of the fungus in cotyledons and hypocotyls of young embryos and seedlings, suggests that infection by seedborne *Verticillium* occurs during seed germination. Thus, the number of diseased plants from infected seeds should have been greater than that obtained in this study. Perhaps infected plants result only from seeds in which the embryo was infected during its development. However, it is not known whether dormant seed embryos are infected. Postemergence infection by seedborne *Verticillium* would depend on the proximity of the pericarp in the soil to the hypocotyl or root, and on the activity of the fungus as it is affected by moisture and other factors in the soil. Postemergence infection of roots of safflower plants in the field may also result from naturally occurring soilborne propagules of *Verticillium.*

Dissemination of *V. dahliae* by safflower seed is enhanced by the persistence of the fungus in tissues of the seed pericarp. The infested pericarps that remain in the soil are potential sources of inoculum. Presumably, residue from subsequently diseased plants would increase inoculum. The spread of different strains of *Verticillium* (7) in safflower seed to other areas is a threat to production of safflower and other susceptible crops. Both the exchange of seeds of genetic breeding lines that originate in different areas (11), and the sowing of seeds harvested from diseased fields, increase the probability of establishing new areas of infection. Suspect safflower seed lots suspected of being infected with Verticillium and intended for sowing should be assayed for *Verticillium* (2) to avoid its spread.

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