

Mycosphaerella melonis on Greenhouse Cucumber

M. W. OLSEN, Research Assistant, and M. E. STANGHELLINI, Professor, Department of Plant Pathology, University of Arizona, Tucson 85721

ABSTRACT

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Mycosphaerella melonis, causal agent of gummy stem blight on cucurbits, infected seedlings, mature plants, and fruit of *Cucumis sativus* 'Toska' in experimental and commercial greenhouses in Tucson, Arizona. The pycnidial stage of the fungus, *Phyllosticta* sp., caused a severe seedling disease. Because attempts to isolate the fungus from seed were unsuccessful, a *Phyllosticta* sp., originating from naturally infected *Opuntia* sp., was implicated as a possible source of indigenous inoculum. Morphological characteristics of pycnidia and pycnidiospores from infected *Opuntia* and infected cucumber were identical. Reciprocal inoculations between isolates from *Opuntia* and cucumber were successful.

In October 1974, 8,000 2-wk-old seedlings of *Cucumis sativus* L. 'Toska' were transplanted into greenhouses at the Environmental Research Laboratory of the University of Arizona in Tucson. The plants were grown in sand and irrigated with complete nutrient solution from a drip system.

Some 1,700 seedlings were observed in various stages of decline and collapse 2-4 days after they were transplanted.

Isolations from lesions at cotyledonary nodes consistently yielded *Phyllosticta* sp. The disease was restricted to seedlings. Attempts to isolate the fungus from seed were unsuccessful.

Another planting was made in November 1975. No seedling disease occurred. One month after transplantation, lesions containing pycnidia were observed on the uppermost leaves of cucumber plants. Leaves immediately below infected leaves exhibited lesions in various stages of development. Perithecia were observed on dried pruning wounds and aborted fruit. The causal organism

was identified as *Mycosphaerella melonis* (Passer.) Chiu & Walker (3,10).

During October 1978, infections occurred at a nearby commercial greenhouse. Leaf and stem lesions containing pycnidia and perithecia were observed on mature plants. Fruit abortion and decay occurred on vines (Fig. 1). Infection progressed from the flower end of fruit. Decay also developed on fruit stored at 52 C.

Disease symptoms developed only between October and January when free moisture occurred on leaf surfaces. Humidity in the greenhouse was difficult to control as plant foliage increased and outside temperatures fell below those in the greenhouse. As a result, water droplets from condensation accumulated on greenhouse roofs, then dripped down the trellised cucumber plants.

An indigenous source of inoculum was suspected, because the fungus was never isolated from seed. *Phyllosticta* pad spot (Fig. 2), a common disease of *Opuntia* sp. in southern Arizona (6), is characterized by the development of pycnidia-covered lesions on cactus pads during cool, wet periods.

Studies were conducted to determine the mechanism of fungus spread within the greenhouse, the environmental factors that favor disease development, and the source of primary inoculum. A preliminary report has been published (9).

MATERIALS AND METHODS

Single pycnidiospore cultures were obtained from pycnidia taken from infected leaf tissue. Single ascospore cultures were obtained from ascospores discharged onto water agar from perithecia on infected stems. All cultures were maintained on 2% water agar supplemented with crushed sterile pea straw. Cultures were incubated in the dark at 23 C. Sporulating cultures, derived from single ascospores and single pycnidiospores, were examined microscopically and compared morphologically with spores produced on naturally infected plant tissue.

Airborne spore dispersal. Petri plates containing straw agar were placed randomly in the naturally infested greenhouse 0–2 m above the soil surface and 0.1–3 m away from plants. Five plates were exposed for 48 hr and nine plates for 72 hr. Plates were then returned to the laboratory, incubated at 23 C, and observed for fungal development.

To determine if ascospores were forcibly discharged, naturally infected plant tissue containing perithecia was placed in the bottom of a petri dish. Petri dish lids containing a thin layer of agar were then placed at various distances above the infected plant tissue and

incubated at room temperature for 48 hr. Lids were then removed and microscopically examined for ascospores.

Pathogenicity. Leaves on 2-wk-old cucumber plants were gently rubbed with a sterile cotton swab saturated with a pycnidiospore suspension from single spore cultures. Leaves rubbed with sterile water served as controls. Stems were inoculated with agar disks from straw agar cultures on wounded and unwounded stem sections. Stems were wounded by puncturing with a sterile dissecting needle. Sterile agar disks served as controls. Inoculated plants were put into mist chambers at 23 C. Seven plants were inoculated in each treatment, and tests were repeated twice.

Cucumber fruit was inoculated by placing colonized agar disks into a hole cut aseptically into surface-sterilized fruit. Sterile agar disks were used in controls. Inoculated fruit was incubated in plastic bags at 23 C. Three inoculations were made in each of two fruits, and tests were repeated three times.

The duration of free moisture required for the formation of fruiting bodies on leaves was determined by removing three inoculated plants from the mist chamber 3, 6, 11, 24, 46, and 96 hr after inoculation. Three uninoculated plants left in the mist chamber served as controls. Plants removed from the mist chamber were covered with polyethylene bags to prevent drying.

Inoculum source. Isolations were made from *Opuntia* lesions (Fig. 2) from two locations in the Tucson area. Pycnidia and pycnidiospores from cactus were compared morphologically with those from cucumber. Single pycnidiospore cultures from naturally infected *Opuntia* and from cultures of the cucumber isolate were used to inoculate *Opuntia* pads. Pads were punctured with a sterile needle and a drop of a pycnidiospore suspension was placed on the wound site. Pads were kept in the mist chamber 7 days.

The *Opuntia* isolate was also tested for pathogenicity on cucumber plants and fruit. Inoculations were done as described for the cucumber isolate. Seven plants were used in each test, and tests were repeated twice.

RESULTS

Microscopic examination of spores obtained from naturally infected cucumber leaves revealed two types of pycnidiospores: an oval, unicellular micropycnidiospore averaging $9.9 \times 6.6 \mu\text{m}$, and a uniseptate macropycnidiospore averaging $13.2 \times 6.6 \mu\text{m}$. This observation corresponds to previous reports (3,10). Straw agar cultures from single macro- or micro-pycnidiospores produced pycnidia containing only micropycnidiospores that averaged $9.9 \times 6.6 \mu\text{m}$.

Perithecia on pruning wounds and aborted fruit contained ascospores morphologically identical to those

previously described for *Mycosphaerella melonis* on cucurbits (2,3,10). Perithecia and ascospores, identical to those produced naturally, developed rarely in culture and then only from single macropycnidiospore or single ascospore cultures. Pycnidia from naturally infected *Opuntia*, as well as from cultures of the *Opuntia* isolate, contained only micropycnidiospores, averaging $9.9 \times 6.6 \mu\text{m}$ in both cases.

Airborne spore dispersal. Pycnidia containing macro- and micropycnidiospores identical to those on infected plants developed on all straw agar plates exposed in the greenhouse. Perithecia, also identical to those produced naturally, were observed on four of the petri plates. Ascospores were forcibly discharged at least 1 cm from the source of inoculum.

Pathogenicity. Water-soaked lesions developed on leaves 3 days after inoculation when plants were kept in the mist chamber. Pycnidia developed in 5 days, perithecia in 10 days. Pycnidia developed on inoculated fruit in 10 days. Leaves and fruits became infected at wound sites only. Infected stems became water-soaked and collapsed in 5 days. Stems were infected at both wound and nonwound sites. Infection progressed on leaves and stems only in the mist chamber.

When plants were removed from the mist chamber at different times up to 96 hr, pycnidia did not develop on any plants before removal. However, 4 days after all plants had been removed from the chambers (8 days after inoculation), they were all returned to the mist chamber, and pycnidia formed within 3 days.

Cross-inoculations of cucumber plants and fruits with isolates from *Opuntia* and of *Opuntia* pads with isolates from cucumber were all successful. When inoculated with the *Opuntia* isolate, cucumber leaves and stems developed water-soaked lesions, with pycnidia, in 5 days. Pycnidia developed on fruit in 10 days. Stems were infected at both wound and nonwound sites. Although perithecia were never observed, pycnidia that formed on cucumber plants and fruits contained both macro- and micropycnidiospores. On wounded *Opuntia* pads, the cucumber isolate produced pycnidia that contained only micropycnidiospores.

DISCUSSION

M. melonis has been reported on commercial greenhouse cucumbers in England (1,2,5), Europe (4), Japan (7), and Canada (8) but has not previously been reported in the United States as a disease of greenhouse cucumber seedlings or of mature plants and fruit. The initial infection on the uppermost leaves of cucumber plants and the experimental evidence of airborne spores in the

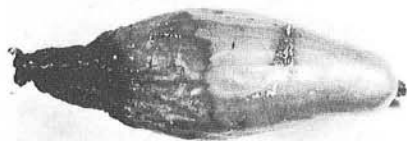


Fig. 1. Cucumber fruit decay caused by *Mycosphaerella melonis*.

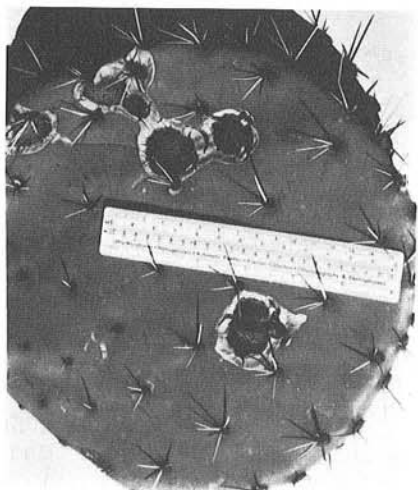


Fig. 2. Lesions on *Opuntia* caused by *Phyllosticta* sp.

greenhouse indicate that airborne ascospores are the primary source of inoculum on mature plants. Development of lesions on leaves directly below those initially infected indicates a drip pattern for the secondary source of inoculum (pycnidiospores). Under experimental conditions in the mist chamber and while disease developed in the greenhouse, infection progressed only in free moisture at about 23 C. Experimental determinations and the daily flux of free moisture in the greenhouse indicate that free moisture is required for pycnidia and perithecia formation.

Because seed assays were negative, inoculum presumably came from contaminated soil debris in the greenhouse and/or from indigenous plants. No field cucumbers are produced commercially within 150 km of the greenhouse, and no field plantings of cucumber are made in the winter months in Arizona.

Pycnidial isolates of *Phyllosticta* sp. from *Opuntia* were both microscopically and culturally similar to those on cucumber. Cross-inoculations between *Phyllosticta* sp. from cucumber and from *Opuntia* indicated possible substrate effects on spore morphology. Isolates from *Opuntia* produced both micro- and

macro-pycnidiospores on cucumber, whereas they produced only micro-pycnidiospores in culture and on naturally infected *Opuntia* tissue. Likewise, the isolate from cucumber produced only micropycnidiospores on *Opuntia*, whereas it produced both micro- and macro-pycnidiospores on naturally infected cucumber leaves. Although perithecia have not been observed on naturally infected *Opuntia*, lesions occurred randomly on pads at varying heights on the plants, indicating that airborne spores initiated the infection.

Widespread occurrences of lesions on *Opuntia* in the Tucson area and results of artificial cross-inoculations implicate *Opuntia* sp. as an indigenous source of primary inoculum. Once introduced, secondary inoculum probably resides inside the greenhouse indefinitely. Isolations from soil debris and spore traps (straw agar plates) between plantings were successful (M. W. Olsen, unpublished).

Because free moisture is necessary for progressive infection, *M. melonis* on greenhouse cucumber can be controlled by ventilation. Preventing the accumulation of free moisture within the

greenhouse might inhibit progressive infection and subsequent development of pycnidia and perithecia.

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