The Role of Seedborne Inoculum on the Development of *Macrophomina phaseolina* on Melon

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ABSTRACT

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Studies using seed from artificially and naturally infected melon fruit revealed that the fungus *Macrophomina phaseolina* is found on both the seed coat and cotyledons. Infected seed gives rise to infected seedlings that can transmit the pathogen into the fruit and also increases the inoculum potential in the soil. The role of these findings is discussed in the context of practices used to control the pathogen in commercial plantings.

Macrophomina phaseolina (Tassi) Goid. causes a disease known as charcoal rot on a wide range of plants in many parts of the world (6). The fungus can also cause hollow stem rot, wilt, and preemergence and postemergence damping-off. The pathogen survives in soil as small black sclerotia formed on infected tissues and is liberated into the soil during tissue disintegration. The sclerotia constitute the primary inoculum source (2,4,5). *M. phaseolina* has been found to be destructive to melon plants and fruits (1,3,8). Recently, we reported that this pathogen is a leading cause of a melon collapse syndrome when grown under high-temperature conditions in Israel (7). The appearance of the disease in virgin soils and other field observations led us to investigate the possible role of dissemination of the fungus via melon seed. The purposes of this study were 1) to determine if the fungus can colonize melon stems and grow into the fruit, 2) to study the behavior of the fungus as a seedborne pathogen, and 3) to determine if and to what extent seedborne inoculum can contribute to pathogen buildup.

MATERIALS AND METHODS

Inoculation tests. Five mature fruits of two local melon cultivars, Galia and Tal Devash, were surface-swabbed with 95% ethyl alcohol at the inoculation site and a 0.5-ml sclerotial suspension (5×10^3 ml^{-1}) was injected 1 cm beneath the rind. Control fruits were treated similarly and injected with sterile water. The fruits were transferred to sterilized paper bags and incubated in the dark at 28 C. Isolations were made from plant tissues and from seed obtained from artificially or naturally infected fruit. Seeds from artificially inoculated fruit were taken 10 days after inoculation, and seeds from naturally infected fruit were taken when the fruit showed symptoms of charcoal rot in the field. The seeds were removed from the fruit and thoroughly rinsed with running tap water. Twenty-five seeds were used from each fruit of each cultivar for isolations. The seed coats were removed and they and the cotyledons were plated out separately on acidified potato-dextrose agar (PDA) in petri plates incubated in the dark at 25 C for 10 days, when the frequency of recovery was recorded. In other experiments, melon plants were dug at various intervals after sowing and isolations were made from roots, stems, and the fruit abscission zones. Segments were disinfested with sodium hypochlorite solution, plated out as before, and percent recovery was recorded.

Inoculum potential of infected seeds and their role in soil infestation. After determining degree of seed infection (95% on Tal Devash), the remaining seed was used to study the effect of such seed on pathogen development on the plant, symptom expression, and residual soil inoculum. Fifty seeds were sown in 10 pots (five seeds per pot), each containing 3 kg of autoclaved soil. Three sowings were made, one during the winter and two during the summer. The soil in one of the summer sowings contained root debris from the winter sowing. During the winter, pots were kept in a heated greenhouse (20–25 C, night-day maximum temperature) and during the summer, in a screenhouse (20–30 C). Plants and fruits were hung on plastic wires to prevent contact with the soil.

Pathogenicity studies at emergence. Five seeds from healthy Tal Devash fruit were sown in each of five pots under summer conditions. One hundred milliliters of a sclerotial suspension (3×10^3 ml^{-1}) from 10-day-old cultures of *M. phaseolina* were placed over the seed and covered with soil. An equal number of pots served as controls, and all pots were kept in a screenhouse (20–30 C).

Symptom development on fruit. Artificially and naturally infected fruits were observed during incubation at 28 C. The naturally infected fruits were picked as soon as symptoms were noted in the field.

RESULTS

Occurrence of *M. phaseolina* on seed. Frequency of recovery of the fungus from seeds removed from naturally and artificially inoculated fruit is shown in Table 1. The fungus was isolated from most seed coats and appeared in high percentages of the cotyledons taken from seed of artificially and naturally infected fruit. Although no differences were noted in the recovery of the fungus from the seed coats of the two cultivars, the frequency of isolation from the cotyledons was higher in Tal Devash than in Galia (95 and 25%, respectively). The cause of this differential recovery is under study.

Inoculum potential of infected seeds. The effect of infected seed on fungus plant colonization, symptom expression, and as a soil-infestation source is shown in Table 2. We observed that under winter conditions, infected seed sown in autoclaved soil germinated well and produced healthy seedlings even though a recovery of 80% was obtained from roots of such plants at the end of the experiment. When the soil containing root debris from the winter sowing was

Table 1. Percent recovery of *Macrophomina phaseolina* from seed of two cultivars[a]

Cultivar	Inoculation method	Seed coat	Cotyledons
Tal Devash	Artificial	98	95
	Natural	96	94
Galia	Artificial	98	25
Control			
Tal Devash	Artificial (H_2O)	0	0
Galia	Artificial (H_2O)	0	0

[a]Isolations carried out on 25 seeds from each fruit of each cultivar.

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Table 2. Effect of sowing seed[a] infected with *Macrophomina phaseolina* on plant emergence, recovery from roots, symptoms, and residual soil infestation[b]

Sowing season	Sequence	Emergence (%)	Root recovery (%)	Defoliation (%)
Winter	First sowing	98	80	0
	Second sowing[c]	32	100	98
Summer	First sowing	92	90	46

[a]Tal Devash; 98% infection of seed.
[b]All soil originally autoclaved.
[c]Sowing carried out in soil from winter sowing that contained root debris.

used for summer sowing, however, plant emergence was low and defoliation very high starting 30 days after sowing. In contrast, similarly infected seed sown under the same environmental conditions in autoclaved soil germinated well, onset of defoliation was delayed 45 days, and its degree was much less.

Pathogenicity studies at emergence. The effect of sclerotial inoculum on emergence was drastic and severe preemergence damping-off was obvious. In contrast with 100% emergence of controls, only one plant emerged and survived for 70 days in infested soil. The fungus was recovered from the roots and all upper parts of the plant. Microsclerotia formed on the stem surface. One fruit was formed on this plant and the fungus was recovered from its seed; the fungus penetrated the fruit via the peduncle.

Symptom development on fruit. Fungal penetration progressed rapidly and 8 days after inoculation, the fungus colonized almost the entire fruit, with numerous sclerotia developing on the various fruit parts. Symptom development on naturally infected plants was also monitored in the field, and fungal penetration and colonization was observed via the peduncle through the abscission zone and into the fruit.

DISCUSSION

The results of this study demonstrate that *M. phaseolina* is readily seedborne, both on the seed coat and cotyledons. Results of one inoculation experiment and numerous field evaluations of infected plants showed that the fungus can penetrate fruit via the peduncle and infect the seed as well. This mechanism of fruit infection has not previously been described. We also showed that infected seeds give rise to infected plants, symptom expression depending on environmental conditions, and more severe symptoms under warmer conditions (7).

Of special importance were the results of residual inoculum in soil formed by planting infected seed in autoclaved soil (Table 2). These results have special significance under our conditions because many of our fields are treated with methyl bromide to combat soilborne pathogens (7). The planting of infected seed leading to infected roots could greatly decrease the value of such fumigations because reintroduction of even small amounts of inoculum could lead to rapid buildup on other known hosts in the rotation, such as sorghum or corn. Based on our findings, greater care is now being taken by our seed producers in their choice or treatment of fields where seed crops are being raised. Fumigation is one of the methods being adopted. Our results highlight the importance of obtaining pathogen-free seed, especially for use under conditions of high investment, eg, fumigation, plastic mulching, and tunnels.

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