Factors Affecting Seedling Blight of Sweet Corn Caused by Seedborne *Penicillium oxalicum*

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ABSTRACT

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Sweet corn seedlings of the cultivar Jubilee were stunted and blighted in early spring 1985 plantings in Israel. *Penicillium oxalicum* was isolated from seedlings and from fungicide-treated seeds. Pathogenicity and seedborne transmission of the fungus were shown with both naturally infected and artificially inoculated seeds. Among seedlings grown from infected seed, the percentage with rotted mesocotyls was higher and the percentage with shoot height was lower at 15 and 20 C than at 25 and 30 C. Inhibition of seedling growth increased and the occurrence of wilt was more frequent as the inoculum concentration increased. Damage to seedlings from infected seeds was expressed more in sterile loess soil than in sand. The fungus lost its viability in seeds after storage at room temperature (20–25 C) for 12 mo. Methods for assessing seed infection and transmission by seed are described.

During the spring of 1985 in Israel, an unusual amount (5–20%) of seedling blight, loss in stand, and retardation of seedling growth occurred in sweet corn (*Zea mays* L.) cultivar Jubilee. The disease was prevalent mainly in fields planted in early spring. Isolations from diseased seedlings predominantly yielded a species of *Penicillium*. Seed lots used for sowing in the affected fields were examined and a high incidence (51–82%) of a species of *Penicillium* was found. The seed lots, which were imported, had been treated with a mixture of captan, thiram, and carboxin. It appeared that field infection could have been caused by seedborne inoculum.

A large number of *Penicillium* spp. have been isolated from dent corn seeds in the United States (1,4,10,11); *P. oxalicum* Currie & Thom and *P. funiculosum* Thom were commonly isolated from preharvest corn (5,10,13,14). When 15 species of *Penicillium* were tested, only *P. oxalicum* was found to be pathogenic to corn ears (1). The first report of *Penicillium* as a causal agent of seedling blight of corn was by Johann in 1928 (2). Johann and Holbert (3) found that various hybrids of dent corn showed markedly reduced stand and yield when seeds were inoculated at planting with *P. oxalicum*. Large and consistent differences were noted in the reactions of different corn types to artificial inoculation of seeds (6). Schroeder (13) used artificially inoculated seeds to demonstrate the pathogenicity of *P. oxalicum* on several sweet corn cultivars. There are no recent reports on the occurrence of seedling blight caused by seedborne *P. oxalicum* in Jubilee or other field-grown sweet corn cultivars.

Our investigation studied the seedborne nature of *P. oxalicum* and factors affecting its pathogenicity. Preliminary reports of this study have been published elsewhere (8,9).

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MATERIALS AND METHODS

Isolation of causal organism from field-grown seedlings and seed. Seedlings from five affected fields planted with commercial seed lots were examined for disease symptoms and used for isolation of the causal organism. Samples of 20 affected and 10 asymptomatic plants were collected from each field. The samples were then assessed for fungal infection by plating surface-sterilized sections (4–6 mm) on PDA and incubating them at 30 C for six days. Seed lots used to plant the affected fields were assayed for fungal infection using a modified deep-freeze blotter procedure, described later. The sweet corn seeds used throughout this study were cultivar Jubilee. Except for a few untreated samples (as noted), all seed samples had been treated by the producer with a mixture of captan, thiram, and carboxin. We will refer to these samples as commercial seed.

Assay of seed infection. The deep-freeze blotter procedure (7) was modified to detect infection of corn seed by *P. oxaliucum*. Samples of 200 seeds, 50 per tray (18 × 18 × 3 cm), were plated on a three-layer blotter previously soaked in dicloran (Allisan) suspension (0.04%) to prevent contamination by *Rhizopus* spp. (Fig. 1). The trays were covered with plastic bags and maintained for 1 day at 30 C, followed by 1 day at −20 C. The untreated seeds were then incubated at 30 C for 5–7 days; the fungicide-treated seeds were incubated for 10–12 days. This assay will be referred to as the deep-freeze blotter test with Allisan treatment, or DFBTA, method.

Effect of seed size and shape on infection rate. Ten commercial seed lots, each sorted by the producer according to size (large or medium) and shape (flat or round), were assayed for the fungus infection using the DFBTA method.

Location of *P. oxalicum* on seed. The infection rate of seeds in one untreated and two commercial samples were assayed using the DFBTA method both before and after disinfection with 1% NaOCl for 10 or 15 min (100 seeds of each sample per treatment). In addition, 100 surface-disinfected seeds from these samples were soaked individually in sterile water overnight and then aseptically dissected into component parts. Each part was dipped in 1% NaOCl, washed and dried, plated on PDA, and incubated for 6 days at 30 C.

Survival of *P. oxalicum* in stored seed. Seed samples (200 seeds each) from five commercial and two untreated lots were stored in paper bags for 12 mo at either 3 C and 30% RH or at 20–25 C (room temperature). The infection rate of each sample was determined before and after storage. Germinability of the seed was assessed using the International Seed Testing Association standard method.

Seedling growth in sand (sand test) and seed inoculation. We devised the sand test described here to study transmission of *P. oxalicum* and factors influencing disease expression by naturally infected or inoculated seeds. The experimental design of the sand test was the standard procedure used throughout our experiments.

In each treatment, 100 seeds were planted in oven-sterilized quartz sand, either in plastic trays (16 × 16 × 5 cm) with 25 seeds per tray or in plastic pots (15-cm diameter) with 10 seeds per pot. The seeds were planted with the germ side up in 2-cm-deep holes. Except where otherwise indicated, the containers were kept in temperature-controlled chambers at 20 C under white fluorescent light ($162\mu E \cdot m^{-2} \cdot s^{-1}$, 8-hr photoperiod) and watered as necessary. Seedlings were removed from the sand after 21 days or at predetermined intervals and then

sorted according to occurrence of a green spore mass on the seed surface, necrosis of the mesocotyl, and seedling wilt. Shoot height or weight was determined after removing the seed and the root system.

Samples with a relatively low level of infection (6–10%) by *P. oxalicum* were used for seed inoculation, since noninfected seeds were not available. The seeds were disinfected by soaking them in 1% NaOCl for 15 min and then dried overnight. At planting in sand, each seed was inoculated with two drops (0.06 ml) of an aqueous (amended with Tween 20) conidial suspension (5×10^6 spores per ml) of *P. oxalicum* washed from 10- to 15-day-old cultures on PDA. The control seeds received two drops of water.

Effect of seed infection on seedling development and colonization by *P. oxalicum*. Disease symptoms of 30 commercial seed samples with infection rates ranging from 10 to 86%, and six artificially inoculated and respective noninoculated control seed samples were assessed in seedlings developed in sand. The shoot weight of symptomatic and healthy seedlings in each sample was determined. Surface-sterilized sections (4–6 mm) from the rotted mesocotyl and from noninjured stem tissue (1 cm from the lesion) were used to isolate the causal organism (PDA, 6 days, 30 C).

The height of seedlings developed from naturally infected (83%) seed samples were compared with that of seedlings from disinfected (1% NaOCl, 15 min) fungus-free seed; and the height of seedlings from artificially inoculated seed samples was compared with that of seedlings from noninoculated fungus-free seed. Heights were recorded 7, 14, and 21 days after sowing. The experiment was repeated twice.

Temperature, inoculum concentration, and soil type effects on disease expression. Inoculated and noninoculated seeds were grown in sand at 15 C for 28 days, 20 C for 21 days, 25 C for 14 days, and 30 C for 14 days, until the seedlings reached the two-to-three-leaf stage of growth. Emergence, shoot height, and mesocotyl necrosis were recorded.

In a separate study, seeds were inoculated with 5×10^6, 5×10^5 and 5×10^4 spores per milliliter at planting. Wilt rate and shoot weight were recorded after 21 and 28 days.

In a third study, inoculated and noninoculated seeds ware grown in sand and in sterile loess soil (sandy loam), collected from a cornfield, using procedures similar to the sand test. After 28 days, emergence, wilt rate, and shoot weight were recorded.

RESULTS

Disease symptoms, isolation, and identification of causal organisms. On affected seedlings from affected fields, longitudinal streaks of yellow or blue-green became apparent at the three- to five-leaf stage. Leaf margins dried and, after several days, the leaf blades shriveled. Some affected seedlings became wilted; others continued to grow, but were stunted when compared with asymptomatic plants. When the affected plants were dug, masses of blue conidia were observed on the seed surface; the mesocotyl was necrotic on 95% of the seedlings. A species of *Penicillium* was isolated from all symptomatic plants collected in the five fields. Recovery of *Penicillium* from those plants was 80–90% for the root, 90–95% for the mesocotyl, and 50–80% for the upper mesocotyl (uppermost 2 cm). *Aspergillus niger* Tiegh. and *Fusarium* spp. were also isolated from some symptomatic seedlings. In a few cases (5–15%), a species of *Penicillium* was isolated from asymptomatic plants from the five fields in the study. In the commercial seed lots used to plant the fields, seed infection by *Penicillium* spp. ranged from 51 to 82%.

We identified the fungus from the seed and infected seedlings as *Penicillium oxalicum*. This identification was confirmed by the Commonwealth Mycological Institute, Kew, United Kingdom.

Location of *P. oxalicum* on seed. Seed samples assayed using the DFBTA method revealed high rates (71–98%) of seeds with surface contamination by *P. oxalicum* (Table 1). After surface-disinfection with NaOCl for 10 or 15 min, seed infection rates ranged from 7 to 24%. *P. oxalicum* had developed from all seed components, but mainly from the pericarp and endosperm (Table 1).

Survival of *P. oxalicum* in stored seed. *P. oxalicum* remained viable in most seed samples stored at 3 C and 30% RH (Table 2) and its pathogenicity was retained, as determined by the sand test (*data not shown*). However, viability of *P. oxalicum* dropped sharply (to 0–2.5%) in seed samples stored at room temperature (Table 2). Germinability of the seed did not decrease significantly under either storage condition (Table 2).

Effect of seed size and shape on infection rate. Infection rates of large flat, large round, medium flat, and medium round seeds were similar within each seed lot. The mean infection rates of ten seed lots were 63.7% for large flat, 63.7% for large round, 60.4% for medium flat, and 59.8% for medium round.

Seed transmission of *P. oxalicum* and factors affecting disease expression. Seedlings from 30 commercial samples were sorted according to occurrence of disease symptoms. The seed surface of affected plants was covered with a mass of conidia of *P. oxalicum* and the mesocotyl was rotted (Fig. 2). The fungus was isolated from the rotted mesocotyl and from noninjured stem tissue 1 cm from the lesion. *A. niger* and *Fusarium* spp.

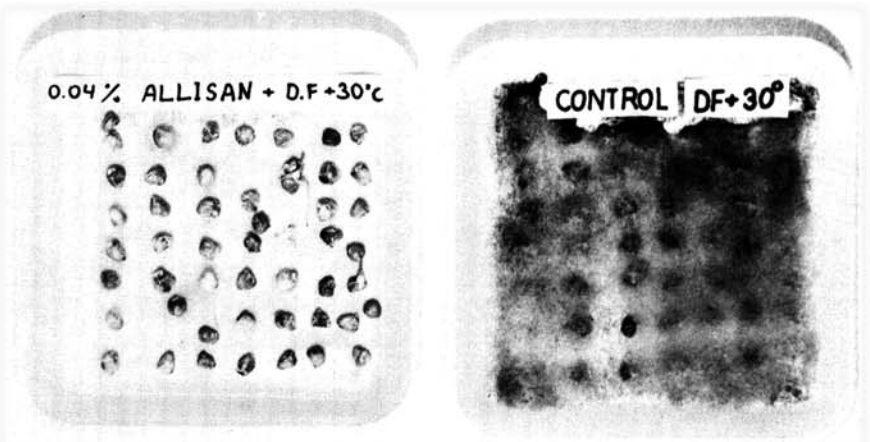

Fig. 1. Blotter test for *Penicillium oxalicum* on sweet corn seeds. Left tray contains blotters soaked in dicloran (Allisan); right tray contains blotters without dicloran (seeds covered with *Rhizopus* spp.).

Table 1. Percent recovery of *Penicillium oxalicum* from whole seeds or from different components of naturally infected seeds

	Whole seeds			Seed components			
		Treated[z]					
Seed sample[y]	Untreated	10 min	15 min	Pericarp	Endosperm	Embryo and scutellum	Tip cap
A	98	24	7	7	6	5	4
B	71	20	9	2	10	4	0
C	87	21	18	8	12	5	1

[y] A = Seeds not treated with fungicides; B and C = seeds treated with a mixture of captan, thiram, and carboxin.
[z] Treated with 1% NaOCl.

were occasionally (5–10%) isolated from diseased mesocotyl; their pathogenicity was not tested.

All seeds inoculated with *P. oxalicum* produced seedlings with disease symptoms similar to those found in seedlings from naturally infected seeds. Seedlings from control seeds did not show any disease symptoms. The shoot weights of seedlings with the described below-ground symptoms were significantly lower than those of asymptomatic seedlings (Table 3). The mean shoot weight of inoculated seeds was 50% that of seedlings from noninoculated seeds (Table 3). The mean shoot weight of affected seedlings in the commercial seed samples was reduced by 20% compared to healthy ones in the same samples, apparently because of a smaller amount of the fungus found on naturally infected seeds.

Figure 3 shows the inhibitory effect of seedborne inoculum on seedling growth rate in sand. During the first week, seedling growth from inoculated and noninoculated seeds was similar. After 14 days, growth inhibition among seedlings from inoculated seed became apparent. At 21 days, there was a significant difference in shoot height between seedlings from inoculated and noninoculated seeds, when the seedlings bore two or three leaves. Seedlings from both naturally infected and artificially inoculated seeds showed similar growth retardation (Fig. 3).

Temperature affected the emergence of artificially inoculated seeds and subsequent seedling growth in the sand test (Table 4). At 15 or 20 C, seedling emergence from inoculated seeds was significantly lower than that from noninoculated seeds; inoculation did not affect emergence at 25 or 30 C. Temperature also affected the expression of seed infection in mesocotyl necrosis and seedling growth (Table 4). At 15 and 20 C, all seedlings had rotted mesocotyls; infection was less frequent and less severe at 25 and 30 C, consisting mainly of partially infected mesocotyl. At lower temperatures, the shoot height of seedlings from infected seeds was approximately 60% of that of seedlings in the control group; at 25 and 30 C, their height was 76% of that of the control seedlings (Table 4).

Inhibition of seedling growth increased as inoculum concentrations increased (Fig. 4). The mean weight of shoots that developed from seed inoculated with 5×10^6 conidia per milliliter was significantly lower than that of shoots from seed inoculated with less-concentrated inoculum. All plants were infected to some degree, but wilt symptoms, which first became apparent at the age of 3 wk, were more frequent at 4 wk (Fig. 4).

When artificially infected seeds were planted in sterile loess soil and in sand, the necrotic area of the mesocotyl of 28-day-old seedlings was larger and deeper in loess soil. The rate of wilting of seedlings from infected seeds was significantly higher in loess soil than in sand (Table 5). Similar results (*not shown*) were obtained from a commercial seed sample.

Table 2. Percent infection of seeds by *Penicillium oxalicum* and percent germination of seed before and after storage at two temperatures for 1 yr

Seed sample[y]	Before storage		After storage			
			Room temperature[z]		3 C	
	Infection	Germination	Infection	Germination	Infection	Germination
A	55.5	97	0.0	98	60.5	98
B	61.0	97	0.5	99	62.0	100
C	23.0	95	0.0	97	17.5	97
D	85.0	97	0.0	94	10.5	97
E	77.0	96	2.5	99	22.5	97
F	80.0	92	...	...	86.0	90
G	81.0	97	...	...	81.5	92

[y] A–E are commercial seed lots treated with a mixture of captan, thiram, and carboxin; F and G were not treated with fungicides.
[z] Generally 20–25 C.

Table 3. Effect of seedborne *Penicillium oxalicum* on shoot weight of 21-day-old seedlings in sand[v]

Type of seed infection	Shoot weight (g)[w]	
	Infected[x]	Healthy[y]
Natural (commercial seed)	0.57 b	0.71 a
Artificial	0.29 b	0.61[z] a

[v] Means of 30 commercial, 6 inoculated, and 6 noninoculated seed samples; 95–100 seedlings per seed sample.
[w] Within rows, values followed by different letters are significantly different ($P = 0.01$), according to LSD.
[x] Seedlings with mesocotyl necrosis and conidia of *P. oxalicum* on seed.
[y] Seedlings without mesocotyl necrosis or conidia of *P. oxalicum* on seed.
[z] Seedlings developed from noninoculated control.

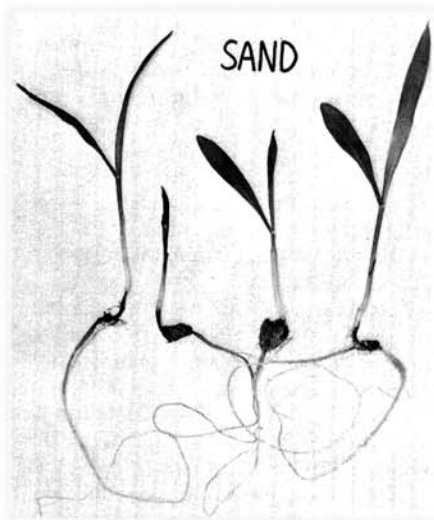

Fig. 2. Necrosis on the mesocotyl of 21-day-old seedlings of sweet corn grown in sand from seeds infected by *Penicillium oxalicum*.

Table 4. Effect of temperature on seedling emergence and damage in sand caused by seedborne *Penicillium oxalicum*

Temperature (C)	Seedling age (days)	Emergence (%)		Seedlings with mesocotyl necrosis (%)		Shoot height (cm)	
		Control seeds	Inoculated seeds	Control seeds	Inoculated seeds	Control seeds	Inoculated seeds
15	28	96 a[y,z]	86 b	5 a[z]	100	11.4 c[z]	6.7 c
20	21	98 a[z]	91 b	7 a[z]	100	16.3 b[z]	9.8 b
25	14	95 a	98 a	7 a[z]	74	25.6 a[z]	19.5 a
30	14	100 a	98 a	5 a[z]	76	25.1 a[z]	21.4 a

[y] Mean of four replicates of 25 seeds each. Within columns, values followed by different letters are significantly different ($P = 0.01$).
[z] Significant difference between control and inoculated seeds in the same treatment ($P = 0.01$). Factorial analyses of variance were done for the effects of temperature and infection, followed by Duncan's multiple range test. For seedling height, a log transformation was applied before the analysis in order to stabilize the variance.

DISCUSSION

Our results show that seedborne *P. oxalicum* can be pathogenic to seedlings of sweet corn cultivar Jubilee. This supports our assumption that the high incidence of seedling blight in Israel in 1985 resulted from a fungus carried by imported commercial seeds.

Contamination by seedborne *Rhizopus* spp. masked the appearance of *P. oxalicum* on seeds in the deep-freeze blotter test. This was especially evident with commercial fungicide-treated seeds, which require a longer incubation period before *Penicillium* becomes detectable. Schroeder (13) also attributed a low rate of seed infection on naturally infected seeds to contamination by *Rhizopus*. We avoided this contamination by soaking the blotter with dicloran (the DFBTA method), which inhibited development of *Rhizopus*. We believe that this is a reliable test for assessing the degree of seed infection by *P. oxalicum*.

Our study shows that low temperatures (15–20 C), which are suboptimal for corn development (15), contribute to disease severity. This may explain the fact that seedling blight and growth retardation were observed in both experimental and commercial field plots in early spring planting, when the soil temperature was 20 C or lower. The fact is that seed disinfection with imazalil, which markedly reduced seedborne *P. oxalicum*, increased seedling stand rate and shoot weight in early spring planting (9). Low temperatures may affect the host more than the pathogen, which also has a high (25–30 C) growth optimum (Halfon-Meiri, *unpublished*). Sweet corn develops faster at higher temperatures, and plants produce adventitious roots in a short time, probably avoiding the damage caused to the infected mesocotyl. Reilly (12) held that corn seedlings that rapidly develop adventitious roots are resistant to *P. oxalicum*.

Our results with inoculated seeds show that the amount of inoculum present on the seed affects the severity of the disease on the seedling. We assume that larger amounts of the fungus on seed favor greater production of oxalic acid, which has been suggested "to be the toxic substance responsible for the injuries observed" (3).

Our study also shows that seed storage at room temperature can inactivate the seedborne pathogen. Storing seeds at room temperature for one year virtually rids the seeds of viable inoculum, without any loss of seed germinability. This procedure may be one method of control for seedborne *P. oxalicum* of corn.

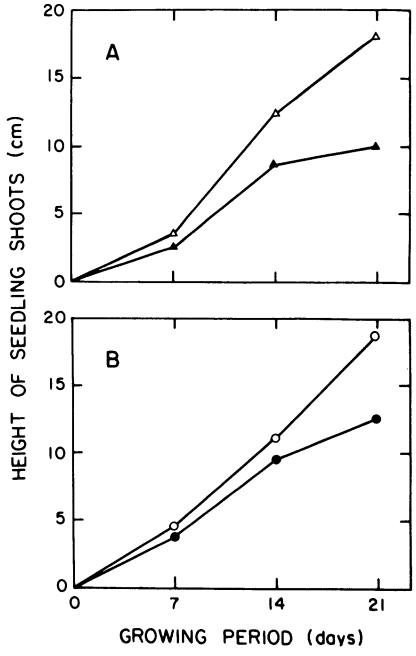

Fig. 3. Effect of seedborne *Penicillium oxalicum* on growth rate of seedlings during 21 days in sand. **(A)** Effect on artificially inoculated seeds; ▲ = inoculated; △ = noninoculated. **(B)** Effect on naturally infected seed; ● = infected; ○ = disinfected (1% NaOCl, 15 min). The LSD ($P = 0.05$) for 7, 14, and 21 days is 1.3, 1.9, and 2.6 for artificially inoculated seeds, and 1.3, 2.8, and 3.6 for naturally infected seeds.

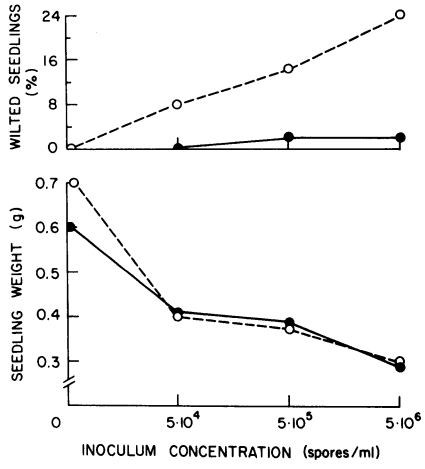

Fig. 4. Effect of concentration of inoculum of *Penicillium oxalicum* carried by seeds on shoot weight and wilting of sweet corn seedlings 21 (●) and 28 (○) days after planting in sand. The LSD ($P = 0.05$) is 0.06 for seedling weight and 5.3 for percent of wilted seedlings.

Table 5. Effect of soil type on severity of damage caused by seedborne *Penicillium oxalicum*

	Emergence (%)		Wilted seedlings (%)		Shoot weight (g)	
Seed treatment	**Sand**	**Loess[y]**	**Sand**	**Loess**	**Sand**	**Loess**
Not inoculated	90 a[z]	94 a	0 a	0 a	0.67 a	0.30 a
Inoculated	88 b	82 b	13 b	72 b	0.30 b	0.16 b

[y] Sandy loam soil.
[z] Within column, values followed by different letters are significantly different ($P = 0.01$) according to LSD.

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