Pathogenicity of *Penicillia* to Corn Ears

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ABSTRACT

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Eighty-one isolates of 15 *Penicillium* species were screened for pathogenicity to corn ears. At the full-silk and dent stages, spore suspensions either were injected into the ears at the butt and tip or the silks were sprayed. Injection at full silk was the most effective inoculation procedure. *Penicillium oxalicum* was pathogenic to corn. It caused lesions on husks, rotted ears, streaked kernels, and reduced germination and invaded over 50% of the kernels on ears injected at full silk. *Penicillium brevi-compactum, P. chrysogenum, P. citrinum, P. cyclopium, P. expansum, P. frequentans, P. funiculosum, P. martensii, P. palitans, P. purpurogenum, P. tardum, P. variabile,* and *P. viridicatum* were nonpathogenic. *Penicillium funiculosum* caused white streaks in the kernel pericarp and invaded over 50% of the kernels on ears injected at full silk, but failed to rot ears or reduce seed viability. The percentage of kernels invaded by the same isolates of eight *Penicillium* species injected into corn ears at silk 2 yr in succession was not significantly different. Following a 53-day harvest delay, the percentage of kernels invaded by *P. brevi-compactum, P. cyclopium,* and *P. viridicatum* (storage species) significantly increased. *Penicillium oxalicum* colonized and occasionally rotted ears that were artifically injured and spray-inoculated at full silk. *Penicillium funiculosum* colonized, but did not rot ears. Corn from ears injected at full silk with *P. funiculosum* and *P. oxalicum* was not toxic when fed ad libitum to mice.

Additional key words: Penicillium oxalicum, Zea mays

Forty-two species of *Penicillium* have been isolated from dent corn kernels (4). *Penicillium oxalicum* and *P. funiculosum* commonly are isolated from preharvest corn (16,18,21) and *P. cyclopium* and *P. viridicatum* from stored corn (2,15,17,21). In many studies of the mycoflora of corn, *Penicillium* is reported as an unidentified species (3,9,12,23,32,34).

Mycotoxins are produced by several *Penicillium* species commonly isolated from corn. Among these are: oxalic acid by *P. oxalicum* (14,36); penicillic acid by *P. cyclopium, P. martensii, P. palitans,* and *P. puberulum* (11,29); cyclopiazonic acid by *P. cyclopium* (24); and ochratoxin A, citrinin, xanthomegnin, viomellein, and viridicatumtoxin by *P. viridicatum* (7,8,13,28,29,33).

The purpose of this study was to determine the pathogenicity to corn ears of *Penicillium* species isolated from preharvest and stored corn. This knowledge should be valuable in assessing the potential mycotoxin content of corn ears invaded by *Penicillium* before harvest. A preliminary report has been published (6).

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MATERIALS AND METHODS

In a study of *Penicillium* species in freshly harvested and stored dent corn kernels, Mislivec (20) lyophilized a large number of species. Isolates used in our studies were mainly from this collection. Additional cultures either were isolated from dent corn kernels or were obtained from the Northern Regional Research Center, Peoria, IL 61604.

Corn inoculations. Eighty-one isolates of 15 *Penicillium* species were screened for pathogenicity to corn ears in 1969, 1970, and 1971. Field plots of a double cross hybrid, Indiana 253, (Wf9 × W22) × (B14 × Oh43) were grown at the Purdue University Agronomy Farm. Ears were inoculated with spore suspensions at the full-silk stage (90% of the silks evident) and at the dent stage of species development. They were: *P. brevi-compactum, P. chrysogenum, P. citrinum, P. cyclopium, P. expansum, P. frequentans, P. funiculosum, P. martensii, P. oxalicum, P. palitans, P. purpurogenum, P. tardum, P. urticae, P. variabile,* and *P. viridicatum.* Spore suspensions were prepared by adding 5 ml of sterile water containing one drop of Tween-80 per 100 ml to a 7- to 10-day-old malt extract agar slant culture. Spore concentrations

were counted with a hemacytometer and adjusted to $2–4 \times 10^6$ per milliliter. One milliliter of spore suspension was injected into the butt and tip of ears with a 1.24-mm-diameter (18-gauge) hypodermic needle, or the silks were sprayed for 5 sec (approximately 2 ml of spore suspension) with a chromatography sprayer and immediately capped with glassine pollinating bags. The first ear in each treatment row was injected or sprayed with sterile water containing one drop of Tween-80 per 100 ml.

Artificial injury of ears. Ears were artificially injured and inoculated with *P. funiculosum* and *P. oxalicum*, the chief field species. A plug of husk tissue at the ear tip was removed to the kernels with a Number 5 cork borer. In one treatment, the 3–4 exposed kernels were punctured with a scalpel; in another, they were left intact. The exposed kernels were sprayed for 3 sec (approximately 1 ml) with a suspension containing $2–4 \times 10^6$ spores per milliliter and the ears were capped with pollinating bags. In one control, intact ears were sprayed with inoculum; in another, injured ears were sprayed with sterile water. The experiment was conducted 3 wk after full silk when the kernels were in the milk stage.

Corn harvest and sampling. All 18 ears from rows injected with *P. oxalicum* and *P. funiculosum* were hand-harvested and rated for ear rot and streaked kernels. The remaining rows were checked for rot and six randomly selected ears were harvested, dried to approximately 12% moisture at 38 C, mechanically shelled, and the shelled grain was stored at 4 C.

Corn kernel disinfection and plating. Kernels were surface disinfected for 5 min in 5.25% sodium hypochlorite containing one drop of Tween-80 per 100 ml. After two rinses in sterile water, five kernels per petri dish were plated germ side up on corn steep agar (25) containing 100 μg/ml of Tergitol NPX. The Tergitol, which is used to slow the growth of fast-spreading fungi, was autoclaved separately and added prior to pouring. The plates were incubated for 7–10 days at 22–24 C and the *Penicillium* species were identified according to Raper and Thom (25). By plating kernels on this diagnostic medium, it was usually possible to identify *Penicillium* species without transfer.

RESULTS

Corn ear pathogenicity screening trial. *Husk lesions.* Husk lesions occurred only on ears injected with *P. oxalicum* at full silk. Ovoid to oblong lesions up to 4 cm long developed at the basal injection site. At first water-soaked, the lesions became necrotic and supported abundant sporulation, especially just beneath the outer husk (Fig. 1). Sporulation probably also occurred on the outer husk but weathered off.

Ear rot. Penicillium oxalicum was the only one of 15 species that caused ear rot. All nine isolates rotted ears injected at full silk (Table 1, Fig. 2). Rot was more common at the ear tip than at the butt; 42% of the ears were rotted only at the tip, 4% only at the butt, 36% at the butt and tip, 17% had scattered rot, and 0.6% were sound. Ears injected at the dent stage or ears that were sprayed at full silk or dent were sound.

TABLE 1. Corn ear area rotted, seed germination, and kernels invaded on ears injected at full silk with gray and green isolates of *Penicillium oxalicum*[w]

P. oxalicum (isolate no.)	Year inoculated	Isolate type	Ear area rotted (%)[x]	Seed germination (%)[y]	Kernels invaded (%)[y,z]
1	1969	Gray	5 a	96.0 a	64.8 a
2	1969	Gray	8 abc	89.0 a	73.2 abc
4	1969	Gray	12 abcd	94.0 a	84.4 abc
4	1970	Gray	15 bcd	87.4 a	79.2 abc
7	1969	Gray	12 abcd	92.0 a	74.4 abc
11	1970	Gray	15 bcd	89.3 a	81.0 abc
Average			11	91.3	76.2
9	1970	Green	8 ab	91.8 a	75.2 abc
12	1970	Green	6 a	92.8 a	71.0 ab
20	1971	Green	15 d	93.0 a	88.3 bc
21	1071	Green	16 cd	86.0 a	91.0 c
Average			11	90.0	81.4

[w] Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[x] Mean of 18 ears.
[y] Mean of a minimum of three 100-kernel replications plated on agar medium.
[z] Kernels were surface disinfected for 5 min in 5.25% sodium hypochlorite containing one drop of Tween-80/100 ml.

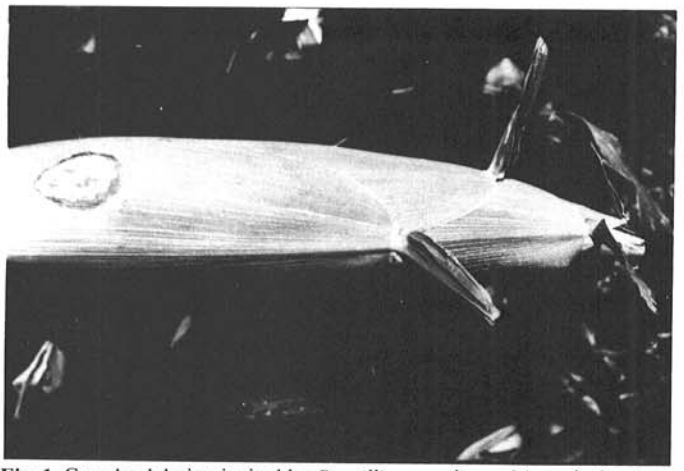

Fig. 1. Corn husk lesion incited by *Pencillium oxalicum* 4 (gray isolate) on an ear injected with a spore suspension at full silk.

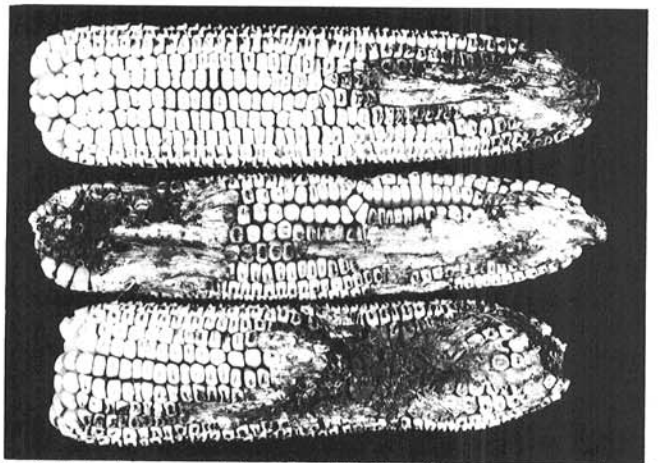

Fig. 2. Rot of corn ears injected at full silk with a spore suspension of *Penicillium oxalicum* 21 (gray isolate).

Isolates of *P. oxalicum* were either gray or green. Gray isolates were storm to castor grey (Plate LII in: Ridgway [26]), odorless, with smooth, broadly elliptical conidia 4.5–5.0 × 3.5–4.0 μm. Green isolates were blue-green or dusky olive green (Plate XLI in: Ridgway [26]) in the young spore areas, becoming dark greenish olive (plate XXX in: Ridgway [26]) or ivy green (Plate, XXXI in: Ridgway [26]) with age. Green isolates had a honeysuckle odor and finely roughened elliptical conidia 5.0–5.5 × 3.0–3.5 μm.

Gray and green isolates caused approximately equal amounts of ear rot (Table 1).

Other *Penicillium* species injected into ears at silk occasionally rotted one to two kernels, but this was probably saprophytic growth that followed inoculation injury.

Germination. The viability of kernels plated on corn steep-Tergitol NPX agar was determined. Kernels were considered to have germinated if the epicotyl or radicle broke through the pericarp. *Penicillium oxalicum* reduced germination nearly 10% with little difference between gray and green isoaltes (Table 1). Nearly 100% of kernels invaded by *P. funiculosum* germinated even though the fungus grew and sporulated abundantly on the plated kernels (Table 2). Species of *Penicillium* other than *P. oxalicum* did not reduce seed viability. All of the kernels from uninoculated ears germinated.

Streaked kernels. Two morphological types of *P. funiculosum* isolated from dent corn kernels were used. A sclerotial type formed sclerotia on malt extract agar after 14 days and produced only a weak aroma. An aromatic type lacked sclerotia, but produced a strong spicy, aromatic odor and fascicles on malt extract agar. Microscopic features of both types agreed with those given by Raper and Thom (25).

In 1969 and 1970, ears were inoculated with sclerotial isolates of *P. funiculosum* and kernels with white streaks in the pericarp often were present near the inoculation site on ears that had been injected at full silk. Usually only a few kernels (no more than 10% of the ear) were affected. In 1971, however, the ear area streaked by four aromatic isolates ranged 12–20% and averaged 18%. Both isolate types occasionally caused vivipary, but only the aromatic isolates sporulated abundantly on the kernels and inner husk.

In 1972, the four sclerotial isolates used in the 1970 trial and the four aromatic isolates used in the 1971 trial were reevaluated. The aromatic isolates streaked a greater ear area than did the sclerotial isolates (Table 2). Since many kernels were streaked on the kernel face and not the crown, the ears were shelled and individual kernels were rated for streaking. Kernel streaking ranged from 13 to 56%, and the four aromatic isolates streaked significantly more kernels than did three of the four sclerotial isolates (Table 2). *Penicillium oxalicum* also streaked up to 50% of the kernels on injected ears.

Invaded kernels. Penicillium funiculosum and *P. oxalicum* invaded 82 and 77%, respectively, of the kernels on ears injected at full silk (Table 3). All 11 *P. funiculosum* and nine *P. oxalicum* isolates of these species invaded over 50% of the kernels. In 1972, three of the four aromatic isolates of *P. funiculosum* invaded significantly more kernels than the four sclerotial isolates (Table 2). Gray and green isolates of *P. oxalicum* invaded approximately an equal number of kernels (Table 1).

Other *Penicillium* species invaded less than 50% of the kernels (Table 3). Of these, *Penicillium variabile* was the most active and invaded 28% of the kernels. One isolate each of *P. citrinum, P. cyclopium,* and *P. expansum* invaded 26–50% of the kernels, but most isolates invaded less than 11%.

On ears sprayed at silk, *P. funiculosum* invaded 6–63% of the kernels (avg 21.3%) and *P. oxalicum* invaded 3–41% (avg 19.6%) (Table 3). The average percentage of kernels invaded by *P. brevi-compactum, P. citrinum, P. cyclopium, P. expansum,* and *P. variabile* was less than 3%. Nevertheless, one or more isolates of these species invaded 3–20% of the kernels.

Inoculation at the dent stage was much less effective than at the silk stage, but the relative activities of the species remained similar. *Penicillium funiculosum, P. oxalicum,* and *P. cyclopium* were the only species that invaded over 3% of the kernels (Table 3).

Penicillium funiculosum and *P. oxalicum* invaded 0.5 to 4.7% of the kernels on uninoculated control ears (Table 4). Approximately 0.5% of the kernels were invaded by the other eight *Penicillium* species. *Cephalosporium acremonium* Cda., *Fusarium moniliforme* Sheld., and *Nigrospora oryzae* (Berk & Br.) Petch were the most common fungi isolated. The number of kernels invaded by *F. moniliforme* on ears with the silks sprayed at full silk was only one third to one half the number of the other treatments. This was probably due to protection from inoculum and insects afforded by the pollinating bags placed over the ears following inoculation.

Comparative isolate pathogenicity. Yearly variations in pathogenicity of a species could be caused by different populations or environmental effects. To assess invironmental effects on isolate performance, ears of Indiana 253 were injected at full silk or the silks were sprayed with the same isolate of nine *Penicillium* species used in 1969 and 1970. Isolates were lyophilized to minimize genetic changes.

In 1969, the ripening period from August through October was cooler and wetter than in 1970. During these 3 mo in 1969, the average temperature was 1.0 C below normal; in 1970, it was 0.4 C above normal. Precipitation averaged 2.0 cm above normal in 1969 and 0.8 cm above normal in 1970.

Penicillium oxalicum 4 rotted 12% of the ear area in 1969 versus 15% in 1970 (Table 1). With the exception of *P. brevi-compactum,* yearly variations in the percent of kernels invaded were not significantly different (Table 5). On ears sprayed at full silk, the percentage of kernels invaded by *P. oxalicum* was fourfold greater in 1969 than 1970. Since spraying the silks with inoculum simulates natural inoculation, wide yearly variations in the

TABLE 2. Corn ear area streaked, kernels streaked, germination, and kernels invaded on ears injected at full silk with sclerotial and aromatic isolates of *Penicillium funiculosum* in 1972

P. funiculosum isolate no.	Isolate type	Ear area streaked (%)[w]	Kernels streaked (%)[x]	Seed germination (%)[y]	Kernels invaded (%)[y,z]
2	Sclerotial	7 a	20 b	100 b	89.3
8	Sclerotial	16 ab	31 c	100 b	93.7
12	Sclerotial	7 a	13 a	100 b	95.0 cd
14	Sclerotial	12 ab	19 ab	99.7 b	82.0 a
Average		10	21	99.9	90.0
15	Aromatic	21 b	56 e	99.7 b	99.0
19	Aromatic	17 ab	38 d	99.3 b	96.3 cd
21	Aromatic	20 b	52 e	97.3 a	100 d
26	Aromatic	25 b	27 c	99.3 b	100 d
Average		21	43	98.8	98.8

[v] Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[w] Mean of 18 ears.
[x] Mean of five 100-kernel replications.
[y] Mean of three 100-kernel replications on agar medium.
[z] Kernels were surface disinfected for 5 min in 5.25% sodium hypochlorite containing one drop of Tween-80/100 ml.

TABLE 3. Corn kernels invaded by *Penicillium* species on inoculated ears

Species	No. of isolates	Kernels invaded (%)[x] Injected at silk[y]	Sprayed at silk[z]	Injected at dent[z]	Sprayed at dent[z]
P. brevi-compactum	8	2.2	1.3	2.2	0.1
P. chrysogenum	1	1.2	0.0	1.0	0.0
P. citrinum	7	11.2	1.9	2.0	0.4
P. cyclopium	8	9.8	2.0	6.9	0.9
P. expansum	7	6.6	0.6	1.6	0.5
P. frequentans	2	4.2	0.0	1.0	0.0
P. funiculosum	11	82.5	21.3	16.2	3.1
P. martensii	2	12.8	0.2	1.2	0.5
P. oxalicum	9	77.0	19.6	6.3	3.1
P. palitans	3	0.5	0.0	0.0	0.0
P. purpurogenum	4	4.8	0.0	1.0	0.1
P. tardum	2	15.4	0.0	1.5	0.0
P. urticae	2	1.4	0.0	0.0	0.0
P. variabile	7	27.6	1.3	3.0	1.1
P. viridicatum	8	4.6	0.4	2.2	0.3

[x] Kernels were surface disinfected for 5 min in 5.25% sodium hypochlorite containing one drop of Tween-80/100 ml.
[y] Mean of a minimum of three 100-kernel replications.
[z] mean of a minimum of one 100-kernel replication.

TABLE 4. Mycoflora of control corn ears inoculated with sterile water

Species	Kernels invaded (%)[x] Injected at silk[y]	Sprayed at silk[y]	Injected at dent[y]	Sprayed at dent[y]
Alternaria alternata	0.9	0.4	0.7	0.5
Aspergillus flavus	0.4	0.5	1.5	0.2
Cephalosporium acremonium	46.5	36.5	37.7	32.9
Chaetomium species	1.7	0.5	3.1	1.6
Cladosporium herbarum	0.8	0.7	1.0	0.1
Fusarium moniliforme	17.1	8.7	24.1	29.5
Gibberella zeae	1.2	1.0	2.4	2.9
Nigrospora oryzae	12.3	21.7	11.5	14.4
Penicillium funiculosum	3.1	0.8	0.5	0.7
Penicillium oxalicum	2.0	1.9	4.7	2.2
Penicillium species[z]	0.5	0.6	0.5	0.6

[x] Kernels were surface disinfected for 5 min in 5.25% sodium hypochlorite containing one drop of Tween-80/100 ml.
[y] Mean of five 100-kernel replications for each of 3 yr.
[z] Included *P. brevi-compactum, P. chrysogenum, P. citrinum, P. cyclopium, P. expansum, P. purpurogenum, P variabile,* and *P. viridicatum.*

TABLE 5. Corn kernels invaded by *Penicillium* on ears inoculated with the same species in 1969 and 1970

Isolates	Kernels invaded (%)[w] Injected at silk[x] 1969	1970	Sprayed at silk[y] 1969	1970
P. brevi-compactum 10	8.8	0.7*[z]	5.5	0.0*
P. citrinum 4	14.7	6.0	0.0	2.0
P. cyclopium 2	24.7	33.5	1.0	0.0
P. expansum 1	21.4	34.8	0.0	1.0
P. funiculosum 2	72.2	78.2	2.4	7.2*
P. oxalicum 4	84.4	79.2	29.7	6.8*
P. palitans 1	1.5	0.0	0.0	0.0
P. variabile 6	27.0	23.7	2.0	0.0
P. viridicatum 1	15.6	12.0	0.0	4.5

[w] Kernels were surface disinfected for 5 min in 5.25% sodium hypochlorite containing one drop of Tween-80/100 ml.
[x] Mean of minimum of three 100-kernel replications.
[y] Mean of four 100-kernel replications for *P. funiculosum* and *P. oxalicum* and one 100 kernel replication for the remaining species.
[z] Means followed by an asterisk are significantly different ($P = 0.05$) from the 1969 values according to Duncan's multiple range test.

occurrence of *P. oxalicum* would be expected and such variation was reported by Mislivec and Tuite (21).

Delayed harvest. In 1970, corn harvest was delayed to determine if the major storage species, *P. brevi-compactum, P. cyclopium,* and *P. virdicatum* and others would develop further. Ears of Indiana 253 were injected at full silk or the silks were sprayed at full silk with isolates that invaded the greatest number of kernels in the ear pathogenicity screening trial. Ears were harvested on 26 October and 53 days later on 18 December.

Penicillium oxalicum rotted 15% of the ear area on ears injected at full silk regardless of the harvest date. No other species incited ear rot. *Penicillium funiculosum* and *P. oxalicum* invaded over 75% of the kernels on ears injected at full silk irrespective of the harvest date (Table 6). *Penicillium brevi-compactum, P. citrinum, P. cyclopium, P. expansum,* and *P. viridicatum* invaded significantly more kernels during a delay in harvest.

Penicillium oxalicum was the only species to reduce germination at the time of normal harvest (Table 6). Following the harvest delay, germination of kernels on ears injected with *P. cyclopium, P. expansum,* and *P. viridicatum* was reduced 6.5, 3.5, and 0.2%, respectively. Even with the harvest delay, *Penicillium* species did not reduce the germination of kernels from ears that had been sprayed at silk.

Artificial injury. A plug of husk tissue at the ear tip was removed and the exposed kernels were punctured and sprayed with inoculum. *Penicillium funiculosum* rotted punctured (but not intact) kernels, and caused streaked kernels in the immediate vicinity of the rotted kernels. On plugged inoculated ears, even though 57–97% of the kernels were invaded, seed germination remained high (Table 7). The aromatic isolate of *P. funiculosum* invaded a higher percentage of kernels than the sclerotial isolate, and both isolates invaded more kernels on plugged inoculated ears with intact kernels than on plugged inoculated ears with punctured kernels. These unexpected results may be explained by the high incidence of *Fusarium moniliforme* on ears with punctured kernels (Table 7). This common, fast-growing field fungus was probably more competitive than *P. funiculosum.*

Penicillium oxalicum generally rotted only injured and immediately adjacent kernels on plugged inoculated ears; however, an average of one ear in 18 was extensively rotted. Seed germination was reduced up to 14% on inoculated plugged ears with punctured kernels and approximately 50% of the kernels on plugged inoculated ears were invaded regardless of kernel condition (Table 7). As with *P. funiculosum,* kernel injury favored colonization by *F. moniliforme.*

Feeding. The toxicity of the nine *P. oxalicum* and 11 *P. funiculosum* isolates used in these studies was evaluated by feeding to mice. Ten weanling male mice (12–15 g) per isolate were fed 4 wk ad libitum a ration of one part purified diet to three parts ground shelled corn from ears injected at full silk. The corn was finely ground in a Wiley mill through a 2-mm screen to prevent sorting by the mice.

Mice fed corn molded by *P. funiculosum* and *P. oxalicum* gained 13.0 and 13.7 g, respectively, versus 15 g gained by the controls. At necropsy, tissues were fixed in 10% buffered formalin and processed for paraffin sections. Tissue sections were stained with hematoxylin and eosin. No gross or histopathologic change was detected in the brain, heart, kidneys, liver, lungs, pancreas, small intestine, spleen, stomach, or testes. Based on the small reductions in weight gains and the lack of pathological symptoms, we concluded that *P. funiculosum* and *P. oxalicum* did not produce significant levels of murine mycotoxins in the field.

DISCUSSION

Christensen (10) classified fungi associated with grains into field fungi (which invade kernels before harvest) and storage fungi (which invade kernels stored at moisture contents [13.5–19.0%] in equilibrium with relative humidities of 70–90%). In the former group he included *Alternaria, Cladosporium, Fusarium,* and *Helminthosporium* and in the latter, *Aspergillus* and *Penicillium.* Based on extensive isolations from freshly harvested and stored

dent corn kernels, Mislivec and Tuite (21) categorized *P. oxalicum, P. funiculosum, P. cyclopium, P. variabile,* and *P. citrinum* as "field penicillia" and list *P. oxalicum* and *P. funiculosum* as the chief field species isolated from unstored corn.

Penicillium oxalicum was a pathogenic field species in our tests. It produced lesions on husks, rotted ears, streaked kernels, reduced germination, and invaded over 50% of the kernels on ears injected at full silk. However, since ear rot was not caused by spraying the silks and only one of 18 ears was rotted in the artificial injury study, it is not considered to be a highly virulent pathogen. *P. oxalicum* also causes a seedling blight of corn (14).

We consider *P. funiculosum* to be a field species, because it invaded a high percentage of kernels on ears injected at full silk. We consider it nonpathogenic because it did not rot ears or significantly reduce seed viability. The white kernel streaks it caused were due to the breakdown of pericarp cells that lost their transparency and became chalky (16). White streaking is also caused by *Cephalosporium acremonium, Fusarium moniliforme,* and *Nigrospora oryzae* (16) and is not considered indicative of pathogenicity.

Several reports (1,5,16,19,31,35) indicate that *Penicillium* infection follows bird and ear worm injury. An increase in the incidence of *P. oxalicum* was associated with bird and ear worm damage by Koehler (15). Mislivec and Tuite (21) reported increased infection by *Penicillium,* especially *P. funiculosum* and *P. oxalicum*, on ears inoculated with *Fusarium*-invaded toothpicks. In our injury study, we simulated bird and insect damage by removing a plug of husk tissue and puncturing the exposed kernels. Since such injury greatly enhanced the occurrence of *P. oxalicum* and *P. funiculosum,* it is likely that bird and insect damage are predisposing factors to invasion.

Penicillium variabile was a minor field species. It invaded 28% of the kernels on ears injected at full silk, but did not increase following a harvest delay. *P. variabile* is a slow-growing fungus that probably cannot compete with fast-growing field fungi.

Penicillium tardum was somewhat less active than *P. variabile* and was differentiated from it primarily by its extremely sparse growth on Czapek's solution agar (25). *Penicillium tardum* was not observed growing out in pure culture from plated kernels, but it overgrew and sporulated abundantly on *P. oxalicum* colonies. It is

TABLE 6. Corn seed germination and kernels invaded by *Penicillium* species on inoculated ears harvested with and without a 53-day delay

Isolate	Seed germination of ears injected at silk (%)[x,y]		Kernels invaded (%)[x,y]			
			Injected at silk		Sprayed at silk	
	No delay	Delay	No delay	Delay	No delay	Delay
P. brevi-compactium 10	100	100	1.0	10.0*[z]	0.0	12.0*
P. citrinum 4	100	100	6.0	19.0*	2.0	1.3
P. cyclopium 2	100	93.5	33.5	44.7*	0.0	0.0
P. expansum 1	100	96.5	20.2	41.0*	1.0	0.0
P. funiculosum 2	100	99.3	78.2	89.2*	7.2	9.0
P. oxalicum 4	87.4	83.8	79.2	98.0*	6.2	6.8
P. palitans 1	100	100	0.0	2.2	0.0	0.0
P. variabile 6	100	100	22.7	13.2*	0.0	0.0
P. viridicatum 1	100	99.8	12.0	41.2*	4.5	1.3

[x] Mean of a minimum of three 100-kernel replications.
[y] Kernels were surface disinfected for 5 min in 5.25% sodium hypochlorite containing one drop of Tween-80/100 ml.
[z] Means followed by one asterisk are significantly different ($P = 0.05$) following the harvest delay according to Duncan's multiple range test.

TABLE 7. Corn seed germination and kernels invaded by *Penicillium funiculosum, P. oxalicum,* and *Fusarium moniliforme* on artificially injured ears inoculated with *P. funiculosum* and *P. oxalicum*

Treatment[v]	Isolate	Seed germ (%)[t,u]	Kernels invaded (%)[t,u]		
			P. funiculosum	*P. oxalicum*	*F. moniliforme*
Ear plugged, kernels punctured, ear inoculated	*P. funiculosum* 2[w]	99.3	57.0	1.0	31.0
	P. funiculosum 26[x]	96.3	83.3	1.7	26.7
	P. oxalicum 4[y]	85.7	0.7	41.7	38.7
	P. oxalicum 12[z]	86.0	3.7	53.3	14.7
Ear plugged, kernels intact, ear inoculated	*P. funiculosum* 2	100	85.0	2.0	4.3
	P. funiculosum 26	99.3	96.7	4.0	7.7
	P. oxalicum 4	96.7	2.3	53.7	16.0
	P. oxalicum 12	95.7	0.7	60.0	4.7
Ear intact, kernels intact, ear inoculated	*P. funiculosum* 2	100	2.7	1.3	1.0
	P. funiculosum 26	100	6.7	3.7	9.0
	P. oxalicum 4	100	0.7	1.3	10.3
	P. oxalicum 12	100	1.0	0.0	13.3
Ear plugged, kernels punctured, ear uninoculated	*P. funiculosum* 2	99.7	19.0	2.3	32.3
	P. funiculosum 26	100	5.0	5.0	47.0
	P. oxalicum 4	100	1.0	6.3	40.3
	P. oxalicum 12	100	2.3	4.0	28.3

[t] Mean of three 100-kernel replications.
[u] Kernels were surface disinfected for 5 min in 5.25% sodium hypochlorite containing one drop of Tween-80/100 ml.
[v] Plug of husk tissue removed near the tip of the ear without kernel injury.
[w] Sclerotial isolate.
[x] Aromatic isolate.
[y] Gray isolate.
[z] Green isolate.

possible that *P. tardum* derived some nutritional factor(s) from *P. oxalicum*.

Penicillium cyclopium and *P. viridicatum* were inactive in the field and are primarily storage species. The delayed-harvest study demonstrated that these species could invade corn kernels in the field if harvest was delayed. However, these species probably are rare in freshly harvested corn, since the advent of combine harvesting and artificial drying have resulted in early harvest.

Penicillium martensii and *P. cyclopium* are morphologically similar and both are in the *P. cyclopium* series of the subsection Fasciculata (25). The identity of representative isolates of *P. cyclopium* in these studies was verified by Dorothy Fennell (formerly a mycologist at the Northern Regional Research Center, Peoria, IL 61604). The two isolates of *P. martensii* (NRRL 3068 and NRRL 3612) that were screened were obtained from the Northern Regional Research Center, since neither we nor Mislivec and Tuite (21) isolated this species. Raper and Thom (25) stated that *P. martensii* differs from *P. cyclopium* "in producing colonies of bluer color, less definitely roughened conidiophores, and elliptical conidia." We found these differentiating characters to be too subjective. Samson et al (27) cultured isolates that showed characterisitics of both *P. cyclopium* and *P. martensii* and concluded "no distinct delimination between these two taxa could be made." They proposed *P. verrucosum* Dierckx var. *cyclopium* (Westling) Samson, Stolk, & Hadlok as a new combination which included *P. cyclopium, P. martensii,* and several other species recognized by Raper and Thom.

Mislivec and Tuite (21) categorized *P. citrinum* as a "field *Penicillium*" based on its field occurrence in 1964 (5.2% of the samples infected). Since *P. citrinum* is xerophytic (22), the extremely dry fall of 1964 may have been responsible for its occurrence. Precipitation in the years of our trials was near normal, and *P. citrinum* invaded a limited number of kernels.

Penicillium expansum, P. purpurogenum, P. frequentans, P. brevi-compactum, P. urticae, P. chrysogenum, and *P. palitans* invaded few kernels and are not field species.

An obvious prerequisite for mycotoxin production on preharvest corn is the ability of a mycotoxigenic fungus to invade and colonize the ears. We found that of the 15 species of *Penicillium* tested, *P. oxalicum* and *P. funiculosum* were the only species with this ability. Fortunately, the main secondary metabolite of *P. oxalicum*, oxalic acid, is a relatively weak nephrotoxin (36). Secalonic acid has been reported as a toxic metabolite of *P. oxalicum* (30), but its occurrence in the field is unknown. *Penicillium funiculosum* is not known to produce a mycotoxin.

Based on our studies, we concluded that in the Midwest, significant levels of known Penicillium mycotoxins are not produced in the field before the normal harvest date because the field penicillia probably are not toxin-producers and mycotoxigenic storage penicillia are rare. Substantial livestock and laboratory animal feeding trials should be done with grain affected by the field penicillia to confirm this assessment.

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