INFLUENCE OF TEMPERATURE ON RECOVERY OF ASPERGILLUS FLAVUS FROM SOYBEAN SEED[1]

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Abstract

Aspergillus flavus was seedborne in 17 of 20 seed lots of 'Lee 68' soybean harvested in five States in 1970. Of the 20% recovered at 35°C, 2.7% was internally seedborne and 17.3% was externally seedborne. Recovery of *A. flavus* varied from 0 to 45% among individual lots. The fungus was recovered from 17 lots at 30° and 35°C, but recovery was negligible at 20° and 25°. Increased recovery of *A. flavus* was significantly correlated with decreased germination. Germination of 'Amsoy' soybean seed decreased when inoculated with an isolate of *A. flavus* from 'Lee 68' seed with temperature increase (20 to 35°C). *A. flavus* may be important in evaluating soybean seed quality.

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Deterioration of soybean (*Glycine max*) seed caused by various species of *Aspergillus* and *Penicillium* has been reported, particularly during storage (1,2,3,4). *Aspergillus glaucus*, *A. ruber*, and *A. repens* have been particularly noted for causing soybean seed spoilage (1,2, 3,4). We report that *Aspergillus flavus* Lk. ex Fr. reduces soybean viability, and that incubation temperatures influence the recovery of *A. flavus* from both naturally infected seed and from seed inoculated with the test fungus.

MATERIALS AND METHODS

Twenty seed lots of 'Lee 68' soybeans were harvested in 1970 from Kentucky, Louisiana, Mississippi, South Carolina and Texas. They were assayed for externally- and internally-borne *A. flavus*. Results from other assays using these seed lots were reported (5, 6). The presence of *A. flavus* was determined by placing 400 surface-sterilized or nonsurface-sterilized seed from each lot onto autoclaved (121°C for 15 min) filter paper discs in 15-cm culture plates. Free moisture in the plates was removed before seed placement. Twenty seeds were placed in each plate, and five plates of each lot were incubated at 20, 25, 30 or 35°C. Percent germination and presence of *A. flavus* was recorded after 5 days

An isolate of *A. flavus* was obtained from the Lee 68 seeds and used to test the effect of temperature on its pathogenicity on soybean seedlings. Registered 'Amsoy' seed (*in vitro* germination 80-82%) were surface-sterilized (5), air-dried, and infested with dry conidia of *A. flavus* harvested from a 4-day-old, potato-dextrose agar (Difco brand) culture.

Surface-sterilized, noninfested seeds were controls. Twenty-five seeds of each treatment were planted in nonautoclaved field soil and sand mixture (1:1) in plastic trays. They were incubated in an Isco environmental chamber programmed at either 20, 25, 30 or 35°C; 60% relative humidity with 14 hr of day light at 200 ft-c. Percent germination and any disease development on emerging seedlings was recorded at 10 days. Each experiment was done twice, each time with four replications. Data from both experiments were combined for statistical analysis.

RESULTS AND DISCUSSION

A. flavus was seedborne in 17 of the 20 lots when seeds were incubated at 30 and 35°C (Table 1). The fungus was seldom recovered at 20 or 25°C, varying from 0.0 to 45% among individual seed lots. Of the 20% recovered at 35°C, 2.7% was internally seedborne and 17.3% was externally seedborne. Of the 10.9% recovered at 30°, 1.4% was internally seedborne and 9.5%

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Table 1. Correlation between percent recovery of Aspergillus flavus and percent germination in vitro at four temperatures of 20 lots of 'Lee 68' soybean seed harvested in 1970.

Lot No.[a]	Percent recovery of A. flavus and seed germination[b]							
	20°C		25°C		30°C		35°C	
	Recovery	Germination	Recovery	Germination	Recovery	Germination	Recovery	Germination
1	0	75	0	68	10	75	23	75
2	0	11	0	2	0	1	0	0
3	0	93	0	94	8	66	5	92
4	0	94	0	91	9	87	12	87
5	0	96	5	78	15	79	25	77
6	2	69	0	69	8	53	9	65
7	5	76	5	87	21	67	18	67
8	0	76	2	59	12	68	45	30
9	0	96	0	81	6	91	9	85
10	0	100	0	99	10	82	10	88
11	0	96	0	96	3	62	0	69
12	0	93	0	93	3	95	13	79
13	0	96	0	97	5	70	15	65
14	0	99	0	78	0	78	3	76
15	0	95	0	96	11	88	5	84
16	0	96	0	96	7	73	5	93
17	0	96	1	97	7	89	7	74
18	0	96	1	93	18	84	22	66
19	0	97	0	96	19	81	24	71
20	0	95	0	96	35	53	32	79
Avg	0.3	87.0	0.7	83	10	72	14	70

[a]Seed lots 1-2 from Louisiana, 3-4 from Mississippi, 5-9 from South Carolina, 10-17 from Kentucky, and 18-20 from Texas.
[b]Based on five plates of 20 seed each.

externally seedborne. Recovery of A. flavus was highly correlated (correlation coefficient = -0.75) with decreased germination of the seed in vitro at 35°, but not at 20, 25 or 30°.

The effect of A. flavus on emergence of inoculated soybean seed was influenced by temperature. There was no significant difference at 20°C between emergence of noninoculated (40%) and inoculated (39%) seed. At 25, 30 and 35°, there were significant differences at the 1% level between noninoculated (40.5, 39.25, 38.75%) and inoculated (33.5, 10.25, and 8.5%) seeds, respectively. We observed that cotyledons of plants from inoculated seed at 30° and 35° were smaller than those of the controls and/or seedlings grown at 20° and 25°. Also, some of these smaller cotyledons were decayed. A. flavus was isolated from all these diseased cotyledons.

There are several reports on the effects of various fungi on seed quality in storage (1,2, 3,4), but none has indicated the importance of A. flavus on seed deterioration and its effects on soybean seed germination. Based on our work, we feel A. flavus is important in evaluating soybean seed quality. Most previous studies used low incubation temperatures (22-27°C) for the bioassay of fungi. Our results suggest that the presence of A. flavus on soybean seed can be detected more readily at incubation temperatures of 30° and 35° than 20° and 25°.

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