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1998

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Wicklow, Donald T.; Weaver, David K.; and Throne, James E., "Fungal Colonists of Maize Grain Conditioned at Constant Temperatures and Humidities" (1998). *Publications from USDA-ARS / UNL Faculty*. 2064.  
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Pergamon

*J. stored Prod. Res.* Vol. 34, No. 4, pp. 355–361, 1998  
Published by Elsevier Science Ltd  
Printed in Great Britain  
0022-474X/98 \$19.00 + 0.00

PII: S0022-474X(98)00021-6

## Fungal Colonists of Maize Grain Conditioned at Constant Temperatures and Humidities

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(Accepted 4 May 1998)

**Abstract**—Fungal colonization of shelled maize (Pioneer 3320) harvested from a field near Furman, South Carolina, in 1992 was determined after 348 and 751 days of continuous storage at each of seven temperatures (10, 15, 20, 25, 30, 35, or 40°C) and four constant relative humidities, giving equilibrium grain moisture contents ranging from 9.4% to 17.5% m.c. in 28 grain conditioning environments. Twenty fungal species infected surface sterilized seeds and were recorded from these conditioned grain treatments, including species commonly found in preharvest maize [e.g. *Acremonium zeae*, *Aspergillus flavus*, *Fusarium moniliforme* (syn. *F. verticillioides*), *Penicillium pinophilum* (syn. *P. funiculosum*), etc.]. *Eupenicillium cinnamopurpureum* and *Monascus ruber* were recorded only from conditioned grain treatments. *Eurotium chevalieri* colonized 50–96% of the kernels from grain conditioning treatments with the highest moisture content for each incubation temperature. Grain samples with >33% *E. chevalieri* infection had a decreased occurrence of *F. moniliforme* and *A. zeae*, and no kernels from these samples germinated. No fungi colonized more than 50% of the kernels conditioned at 30–40°C and 9.4–14.2% m.c. The results of this study indicate that individual patterns of fungal colonization during grain conditioning were a function of the survival rates for preharvest fungal colonists and their potential replacement by *E. chevalieri*. Published by Elsevier Science Ltd

**Key words**—maize, storage, temperature, humidity, *Eurotium*, fungi

### INTRODUCTION

The best known examples of fungal succession in stored grain bulks are associated with dramatic increases in grain moisture (damp grain pockets) and temperatures resulting from: (1) unsafe storage of high-moisture grain; (2) leaks in a storage structure; (3) moisture migration and condensation; (4) fungus-induced hot spots; and (5) insect-induced hot spots (Christensen and Kaufmann, 1969; Miller, 1995; Sauer *et al.*, 1992; Sinha, 1992; Wicklow, 1995). Less atten-

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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tion has been given to interpretations of fungal colonization patterns in large storage bulks with consistent environmental conditions. At harvest, maize (*Zea mays* L.) kernels may be infected, colonized or contaminated with fungi representing different ecological groups including seed replacement pathogens, mildly parasitic colonists of the phylloplane, stem-rot and ear-rot pathogens, endophyte mutualists, saprotrophs and yeasts in dust from soil and/or crop residues (Wicklow, 1995). In storage, the growth and survival of these fungi is affected by tolerance to 'extreme conditions' (Lacey, 1980), grain moisture content, temperature, gas composition (Bottomley *et al.*, 1950; Quasem and Christensen, 1958; Mislevic and Tuite, 1970; Marin *et al.*, 1995), fungal species interactions (Wicklow *et al.*, 1980, 1988; Wicklow, 1988), fungal interactions with granary insects and mites (Dharmaputra *et al.*, 1994; Sinha, 1992; Barney *et al.*, 1995), and active seed resistance to fungal infection (Moreno-Martinez and Christensen, 1971; Cantone *et al.*, 1983; Yao and Tuite, 1989; Stroshine and Yang, 1990).

The present study examines patterns of fungal occurrence on undamaged maize grain conditioned for 348 and 751 days at each of seven temperatures (10–40°C) and four relative humidities (40–80% r.h.). These grain conditioning treatments were selected because they overlap environments in which 'storage molds' can grow and/or stored product arthropods (granary insects and mites) are reported to be active inhabitants (Arbogast and Mullen, 1988). The goal of this research was to understand how the grain storage environment impacts fungal colonization patterns in the absence of disturbance. Although it is known that fungi interact with arthropods, stored-product arthropods were necessarily excluded as confounding variables. In low-moisture environments, which are unfavorable for fungal growth, and in the absence of stored-product arthropods, the death of preharvest fungal colonists should result in fungal-free (uncolonized) grain.

## MATERIALS AND METHODS

### *Grain source and handling*

A commercial dent maize hybrid (Pioneer 3320) was grown to maturity in a field near Furman, SC in 1992. Grain was combine-harvested at 18–22% moisture content (m.c.) in late August, to limit preharvest field infestation by stored-product insects, and then dried at 40°C to 15% m.c. The grain was then cleaned with a sieve to remove broken kernels and foreign material (BCFM), delivered to the Savannah Laboratory in paper bags (50 lbs), and immediately fumigated with phosphine ('Phostoxin,' Degesch, Laudenbach, Germany) at 1 g/28.3 m<sup>3</sup> for 4 days (at 20°C; 50% r.h.) to kill any incipient insect infestation. The bags were then placed on wooden pallets in cold storage at 4.4°C; with 20–30% r.h. The interval from grain harvest to cold storage did not exceed 14 days. A follow-up method of arthropod disinfestation included exposing the grain to –20°C for 14 days immediately preceding moisture equilibration treatments.

### *Storage environments*

Grain equilibration treatments were conducted using plastic boxes (40 × 27.5 × 16 cm) as humidity chambers that could be placed in temperature cabinets. Grain samples (300 g) were placed in individual acrylic tube containers 76 mm internal diameter × 71 mm high, with openings at the lid and base ends covered by nylon mesh (64 µm pore size). Two tube containers were placed in each humidity chamber. Constant humidities were produced and maintained at each of seven temperatures (10, 15, 20, 25, 30, 35 and 40°C) using saturated salt solutions of KCL, NaCl, NaBr, and K<sub>2</sub>CO<sub>3</sub> (Winston and Bates, 1960; Greenspan, 1977). Approximately 600 ml of each solution was poured into each box and a perforated false floor was placed in the bottom of each box to support the acrylic tubes containing individual grain samples above the salt solutions (Arthur *et al.*, 1991). Grain moisture content was monitored in one of the containers, using a Motomco Model 919 automatic grain moisture tester (Dickey–John, Auburn, IL). The other container was not disturbed until the end of the study. Up to 7 wk incubation was required for grain samples to achieve equilibrium water balance at the lowest moisture contents. Equilibrium grain moisture contents (Table 1) were maintained by adding fresh saturated salt solution as needed. Response surface equations were fitted to the moisture content data using TableCurve 3D (Jandel Scientific, San Rafael, CA) with initial sorting specified by the magni-

Table 1. Equilibrium moisture content (%) wet weight basis of maize grain conditioned at constant humidities and temperatures using saturated salt solutions\*

Temperature (°C)	KCl		NaCl		NaBr		K <sub>2</sub> CO <sub>3</sub>	
10	17.5	(88.0)	15.5	(76.5)	13.8	(63.0)	11.6	(47.0)
15	16.5	(86.5)	15.4	(76.0)	13.5	(61.0)	11.3	(44.0)
20	16.4	(85.0)	15.3	(76.0)	13.3	(59.0)	11.1	(44.0)
25	15.9	(85.0)	14.9	(75.5)	12.6	(57.5)	10.7	(43.0)
30	15.5	(84.5)	14.6	(75.5)	12.1	(56.0)	10.4	(43.5)
35	15.0	(83.0)	14.2	(75.5)	11.5	(54.5)	9.9	(NL)
40	14.3	(82.0)	13.7	(75.0)	10.6	(53.0)	9.4	(40.0)

\*Relative humidity (r.h.) values in parentheses (Winston and Bates, 1960); NL = not listed.

tude of the regression F-statistic. Fitted biologically plausible equations were further evaluated for systematic trends in the residuals and for lack-of-fit (Draper and Smith, 1981). The disinfested grain bulk was subsampled for the following treatments: (1) immediate incubation in one of 28 grain conditioning environments for 751 days; (2) dry cold storage for 403 days followed by 348 days incubation in a parallel set of humidity chambers and temperature cabinets to reproduce each of the 28 grain conditioning environments; (3) dry cold storage for 751 days (control). Following the period of grain conditioning, all samples were placed under a hood and dried to 12–13% m.c. The dried samples were shipped to NCAUR, Peoria, IL, for fungal analyses which were performed within 3 months after the conditioning was terminated. During this interval, the grain samples were stored at 5°C.

#### Fungal evaluations

Fungal colonization of grain incubated at different moisture and temperature combinations was determined on 50 randomly selected kernels (control sample = 100 kernels). Kernels were surface-sterilized in a 1% sodium hypochlorite solution for 2 min, then washed twice in sterile water. Five kernels were plated on each of 10 Petri dishes of malt extract agar (30 g malt extract, 20 g agar, in 1 l water), and incubated for 6 days at 25°C. Fungi growing from the individual plated kernels were identified and their frequency (%) of grain colonization was calculated. Isolations of representative fungal cultures were deposited with the ARS Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, IL 61604. A record was also made of the number of kernels that germinated on the agar.

## RESULTS AND DISCUSSION

The moisture content of the equilibrated grain at different temperatures over various saturated salt solutions was quite stable over the duration of the experiment (Fig. 1). The abrupt change in moisture content at the beginning of the study is associated with the period of equilibration. Twenty fungal species were recorded from grain samples representing 28 conditioning treatments and incubation for 348 or 751 days (Table 2). Representative strains include: NRRL 25143 *Acremonium zeae* W. Gams and Sumn. (syn. *Acremonium strictum*); NRRL 25065 *Aspergillus flavus* Link; NRRL 25066 *Aspergillus niger* v. Tieghem; NRRL 25075 *Eupenicillium cinnamopurpureum* Scott and Stolk; NRRL 25068 *Eurotium chevalieri* Mangin; NRRL 25069 *Monascus ruber* v. Tieghem; NRRL 25071 *Penicillium citrinum* Thom; NRRL 25076 and NRRL 25074 *Penicillium pinophilum* Hedgcock (syn. *P. funiculosum*); NRRL 25144 *Chaetomium globosum* Kunze; NRRL 25147 and NRRL 25457 *Fusarium verticillioides* (Sacc.) Nirenb. (syn. *F. moniliforme*); NRRL 25152 *Mycelia sterilia*. Most of these fungi have been reported from pre-harvest maize, including commonly occurring species such as *A. zeae*, *A. flavus*, *F. verticillioides*, *P. pinophilum*, etc. In the present study, *Eupenicillium cinnamopurpureum* and *Monascus ruber* were recorded from maize grain incubated in several of the 28 grain conditioning environments.

*Eurotium chevalieri* was recorded from 20% of the 'control' grains sampled following 751 days cold storage. This fungus is known to invade corn in storage (Dharmaputra *et al.*, 1994) and was not recorded from maize sampled at harvest throughout North Carolina (Hesseltine *et al.*, 1981). Grain entering cold storage had about 15% m.c., sufficient moisture to support the

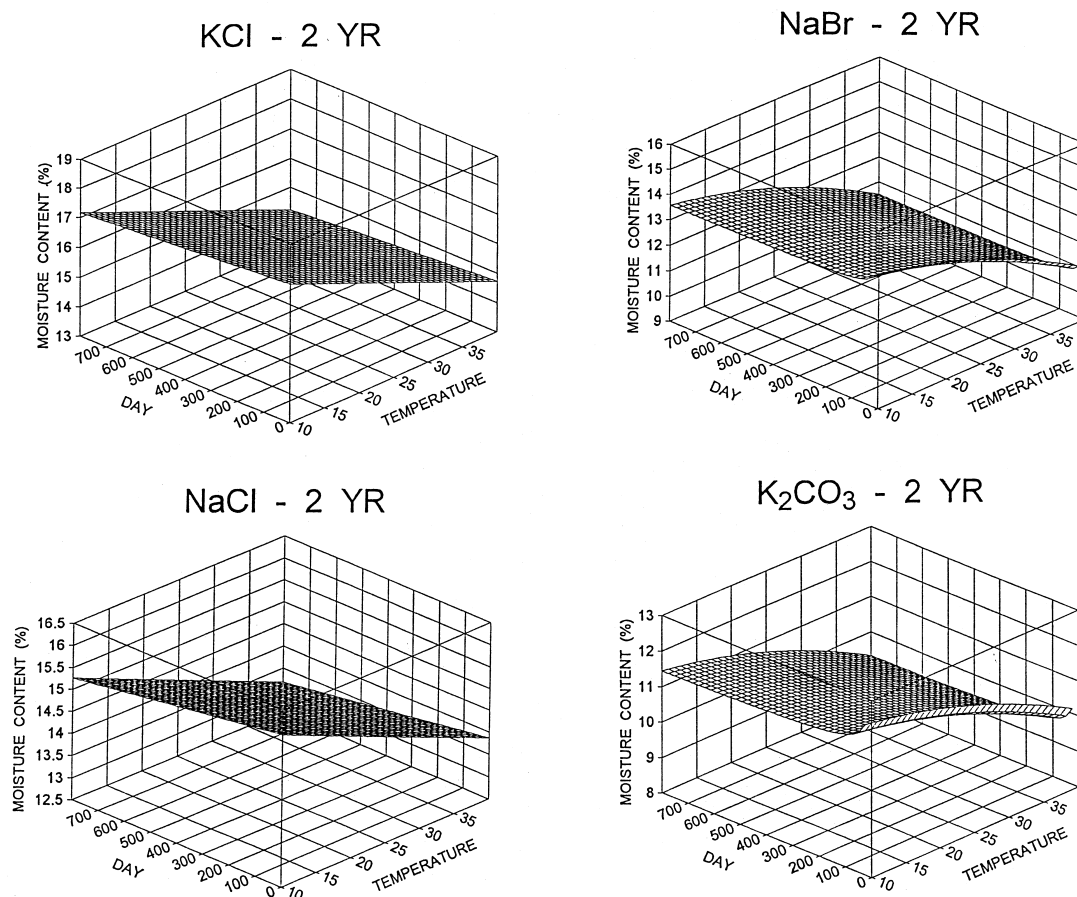


Fig. 1. Fitted response surface showing moisture content at specific temperatures for maize grain equilibrated over saturated solutions of potassium chloride, sodium chloride, sodium bromide, and potassium carbonate for 751 days.

growth of *E. chevalieri* and other members of the '*Aspergillus glaucus* group' which can grow at a minimum temperature of 0–5°C (Christensen and Kaufmann, 1969; Kozakiewicz and Smith, 1994). *Penicillium pinophilum* also infected 20% of the kernels in cold storage while *A. zeae* infected only 6%. However, *A. zeae* showed 20% kernel infection in several conditioning treatments where *P. pinophilum* infected 6% or fewer kernels (Table 2). *Penicillium pinophilum* suppresses growth of *A. zeae* when grown together on malt extract agar in Petri dish cultures incubated at 25°C (Wicklow *et al.*, 1980).

*Eurotium chevalieri* colonized 47–96% of the kernels conditioned at the highest grain moisture content for each incubation temperature (Table 2). In these treatments there was a corresponding decrease in numbers of kernels showing *F. moniliforme* and *A. zeae* and the kernels did not germinate. *Aspergillus flavus* was also replaced by *E. chevalieri* in some of the treatments at 20°C and above. Wicklow (1995) suggests that *Eurotium* spp. may function as mycoparasites in species replacement, killing *F. moniliforme* and other fungal colonists of preharvest grain. Even so, *F. moniliforme* was recorded in significant numbers of kernels from grain samples in which *E. chevalieri* colonized a large majority of the kernels stored at 10 and 15°C. Mycoparasitic behavior may be better expressed at temperatures favorable to the production of fungal cell wall degrading enzymes and/or antifungal metabolites. *Aspergillus flavus* also appears to have grown in storage, infecting larger numbers of kernels at 10°C with 17.5% m.c. Grain conditioned at 30–40°C produced few germinated kernels, with no molds appearing on 50–100% of the kernels incubated at the lower humidities. Here the conditions do not favor survival of fungi infecting grain at harvest nor the growth of *Eurotium* spp. or other 'storage molds.' However, fungal metabolites may persist as chemical contaminants in these mold-free kernels.

Table 2. Kernel infection (%) of maize grain conditioned for 348 and 751 days at constant temperatures and humidities\*

Fungal taxa	Control**	Grain conditioned for 348 days																											
		10°C				15°C				20°C				25°C				30°C				35°C				40°C			
		(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)
<i>Acremonium zeae</i>	6	2	2	30	6	24	16	26	2	4	22	16	8	8	6	20	10	6	6	2	4	6	6	2	4	4	4	2	4
<i>Aspergillus flavus</i>	11	36	26	14	16	22	28	20	12	2	10	10	2	6	20	10	10	6	6	2	2	6	6	2	2	4	4	2	4
<i>Aspergillus niger</i>	3	6	2			2																							
<i>Chaetomium globosum</i>																													
<i>Eupenicillium</i>																													
<i>cinnamomopurpureum</i>																													
<i>Eurotium chevalieri</i>	21	64	6	6	2	80	6	20	2	96	33	8	94	66	14	16	96	48	12	20	92	38	10	78	2	2	2	2	4
<i>Fusarium moniliforme</i>	39	22	76	48	90	22	38	50	52	31	90	38	18	24	18	6	2	2	2	2	2	2	2	2	2	2	2	2	2
<i>Monascus ruber</i>																													
<i>Penicillium citrinum</i>	10	8	2	8	8	6	2	4	6	4	4	6	6	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
<i>Penicillium pinophilum</i>	21																												
<i>Mycelia sterilia</i>																													
Other Molds	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
No Molds	0	0	6	2	0	0	8	6	8	0	31	6	0	32	38	30	0	52	52	56	0	62	98	70	0	96	98	98	96
Germination (%)	68	0	10	76	70	0	10	66	72	0	62	50	0	52	42	0	0	10	24	0	0	0	0	10	0	0	0	0	0

Fungal taxa	Control**	Grain conditioned for 751 days																											
		10°C				15°C				20°C				25°C				30°C				35°C				40°C			
		(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)
<i>Acremonium zeae</i>	6	18	34	26	14	22	28	12	8	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
<i>Aspergillus flavus</i>	11	38	14	8	14	12	16	18	18	2	4	28	8	2	10	18	10	10	10	10	10	10	10	10	10	10	10	10	10
<i>Aspergillus niger</i>																													
<i>Chaetomium globosum</i>	3	2																											
<i>Eupenicillium</i>																													
<i>cinnamomopurpureum</i>																													
<i>Eurotium chevalieri</i>	21	47	12	6	10	86	30	4	2	86	44	34	26	78	56	36	42	86	48	22	18	68	2	32	12	86	2	2	2
<i>Fusarium moniliforme</i>	39	24	48	46	58	6	22	38	53	10	36	28	2	8	2	2	2	8	2	2	2	8	4	4	4	4	4	4	4
<i>Monascus ruber</i>																													
<i>Penicillium citrinum</i>	10	4	4	2	6	6	6	4	2	12	2	2	6	20	6	6	6	14	14	14	14	14	14	14	14	14	14	14	14
<i>Penicillium pinophilum</i>	21																												
<i>Mycelia sterilia</i>																													
Other Molds	8	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
No Molds	0	9	12	6	0	0	18	2	2	0	40	2	16	0	44	42	0	0	50	78	64	18	98	68	84	14	98	98	100
Germination (%)	68	0	12	78	64	0	0	58	69	0	52	22	0	4	2	0	4	2	0	0	4	0	0	0	0	0	0	0	0

\*Saturated salt solutions (a) KCl, (b) NaCl, (c) NaBr, (d) K<sub>2</sub>CO<sub>3</sub> and temperatures giving relative humidities (R.H.) and grain moisture contents listed in Table 1.  
 \*\*Fungal occurrence (%) on 100 kernels sampled from grain placed in cold storage for 751 days.

There were no major differences in fungal colonization pattern after 1 or 2 y of grain conditioning (Table 2). However, the numbers of kernels infected by a fungus increased significantly during the second year of incubation for *E. chevalieri* at 25°C and 12.6 or 10.7% m.c.; *A. zeae* at 10°C with 15.5 or 11.2% m.c., and *Penicillium citrinum* at 20–35°C with 16.4 or 15% m.c. Kernel germination losses also increased at 20°C with 11.1% m.c., and 30°C with 12.1 or 10.4% m.c. In some treatments, the occurrence of infected kernels decreased during the second year: *F. moniliforme* at 15°C with 16.5% m.c., 25°C with 12.6 or 10.7% m.c.; *A. zeae* at 25°C with 12.6 or 10.7% m.c.; *E. chevalieri* at 35°C with 14.2% m.c. Increased numbers of fungal-free kernels were recorded at 35°C with 15% m.c.; 40°C with 14.3% m.c. Christensen (1967) reported that corn stored for 2 y at 14.5% m.c. and 12°C showed 46% kernel infection by *A. glaucus* and 15% kernel germination. For grain stored at 15.2% m.c. and 12°C, nearly all of the kernels were infected with *A. glaucus* group and no kernels germinated.

Storage molds do not usually penetrate and infect corn kernels before harvest (Quasem and Christensen, 1958). *Eurotium* spp. were rarely isolated from corn at harvest in Indiana (Tuite, 1961). At the same time, *E. chevalieri*, *E. amstelodami*, *E. repens* and *E. ruber* were isolated from shelled maize stored in bins throughout Iowa (Semeniuk *et al.*, 1947). Koehler (1938) observed that *A. glaucus* group molds could always be isolated from maize kernels at 14.5–15.5% moisture, sometimes in the absence of other fungi. These molds slowly kill and discolor the germs at moisture contents near the lower limit for growth (14.0–14.5 m.c.) and cause “blue-eye” in corn stored at 14.5–15.0% m.c. (Semeniuk *et al.*, 1947; Christensen and Kaufmann, 1969). Tuite *et al.* (1985) wound-inoculated the germs of intact maize kernels with *E. amstelodami*, *E. repens* and *E. ruber* to show that damage provides necessary entry points for fungi to attack the germ. *Eurotium chevalieri* invaded undamaged, ‘healthy dent kernels’ incubated at 26°C and 98% r.h., producing cleistothecia in the space between the pericarp and the germ (Tsuruta *et al.*, 1981).

In the absence of disturbance or introductions of fungal species by granary insects or mites, the patterns of fungal occurrence are a product of the ability of individual fungi to grow or survive in such environments, the outcome of interactions with other fungi, species replacement and extinction. The results of this study indicate that individual patterns of fungal colonization during grain conditioning were a function of the survival rates for pre-harvest fungal colonists and their potential replacement by *E. chevalieri*. Long-term mycological data are needed to evaluate the practical use of environmental controls (e.g. temperature, moisture content, pH, decreased O<sub>2</sub> and increased CO<sub>2</sub>) to maintain grain quality and in assessing economic cost (Tuite and Foster, 1979; Magan and Lacey, 1984). Grain-infesting insects or mites develop in molded grain, become contaminated with the spores of these storage fungi and carry the spores to clean grain (Griffiths *et al.*, 1959; Sinha, 1992). These data on probable species present might also serve as a guide for interpreting insect behaviors (attractiveness vs. repellency) involving fungal volatiles (Sinha *et al.*, 1988; Kaminski and Wasowicz, 1991) associated with naturally occurring pockets of molded grain in bulk storage, particularly with regard to movement and orientation of primary pests that can attack sound grain, as opposed to secondary pests that prefer damaged grain, and facultatively or truly fungivorous species.

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