Mycoflora of Pecans Treated with Heat, Low Temperatures, or Methyl Bromide for Control of the Pecan Weevil

John M. Wells and Jerry A. Payne

Plant Pathologist and Entomologist, respectively, Southeastern Fruit and Tree Nut Research Station, Agricultural Research Service, U.S. Department of Agriculture, Byron, Georgia 31008.

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ABSTRACT

Fungus infection of weevil-damaged pecan kernels was reduced to percentages of 8.0 to 15.3% by hot water dips at 60 C for 10 and 15 minutes, or at 77 C for 3 minutes, and by steam treatments for 3 minutes, compared with infection of 81.6% in the controls. Average infection on pecans stored at −6 C was reduced to 56.3%, but was not affected by storage at 0 or at 6 C. Methyl bromide fumigation reduced infection on pecans treated at 1.6 and 3.3 kg/100 m^3, but not at 0.8 kg/100 m^3.

Genera of fungi isolated and identified from untreated weevil-damaged kernels were *Penicillium, Alternaria, Pestalotia, Monochaeta, Cladosporium, Fusarium, Phoma,* and *Aspergillus. Penicillium* was the most common genus in heat-treated or fumigated pecans. Chloroform extracts of 28 of 92 representative *Penicillium* isolates, and one of 10 *Aspergillus* isolates were toxic to 1-day-old cockerels.

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The pecan [*Carya illinoensis* (Wang) K. Koch] is a semi-perishable nut susceptible to insect infestation in the orchard. Nuts damaged by the pecan weevil, *Curculio caryae* (Horn), are particularly subject to fungal decay. Weevil larvae, hatched from eggs deposited in the nuts, eat a portion of the kernels and emerge through a perforation in the shell (1). Emergence may occur before the nuts are harvested and thus provide an avenue of infection by decay-causing fungi when moisture conditions are favorable for their development. Weevil-damaged pecans are mechanically shelled and processed along with undamaged nuts, and thus fragments of infected kernels may enter food channels.

In a survey of bakery and market pecans, Lillard et al. (4) isolated 71 cultures of *Aspergillus flavus* Link and *A. parasiticus* Speare, most of which produced aflatoxins in vitro. Schindler et al. (6) reported that the mycotoxins sterigmatocystin, patulin, and ochratoxin, as well as aflatoxin, were produced by fungi isolated from moldy pecans. Fungal infection of pecans might thus be a potential public health hazard as well as an economic loss to the pecan industry.

In 1972, tests were conducted by the U.S. Department of Agriculture at the Southeastern Fruit and Tree Nut Research Station in Byron, Ga., and at the Stored Products Insect Laboratory in Savannah, Ga., to determine the effects of experimental hot water dips, steam treatments, low-temperature storage, and conventional fumigation practices with methyl bromide on the survival of the inshell pecan weevil. Payne and Wells (5) reported 100% control of weevils with hot water dips at 77 C for 3 minutes or at 60 C for 5 minutes, and with steam treatments for 3 minutes at approximately 0.35 kg/cm^2. Methyl bromide treatments, however, were only partly effective within permissible application levels (3).

Treatments with hot water and steam have long been used in some pecan shelling plants to condition pecans for more efficient shelling; fumigation is standard practice for insect control; and low temperature storage is a conventional commercial practice (7). There is no information, however, on the effects of these treatments or practices on the mycoflora of pecans. We conducted a study, therefore, to determine the nature of the mycoflora in weevil-infested pecans, and the changes caused in the fungal population by treatments with heat, low temperature, and fumigation.

MATERIALS AND METHODS.—In Byron, Georgia, pecans (cultivars Schley and Stuart) were harvested within 2 weeks of drop from trees in experimental orchards previously infested with the pecan weevil. These trees received no insecticide treatments. Nuts were gathered from individual trees, percentage infestation was determined for each tree, and then the nuts were mixed so that lots for each treatment had about the same percentage of infested nuts. Lots of 500 to 1,000 nuts each were treated with hot water, steam, and controlled-temperature storage by methods and with equipment previously described (5). Treatments were steam at 0.35 kg/cm^2 and 100 C for 1, 2, and 3 minutes; water at 60 C for 5, 10, and 15 minutes; at 77 C for 1, 2, and 3 minutes, and at 22 C for 20 minutes; and storage for 3 weeks at −6, 0, 6, and 22 C. Relative humidities were approximately 85% at 6 C and 65% at 22 C. In addition, methyl bromide treatments were tested on approximately 120 pecans per treatment at 0.8, 1.6, and 3.3 kg/100 m^3 for 24 hours at 25 C. Fumigated pecans were then held for 3 weeks at 1 C prior to examination, while those from other treatments were held at 1 C and examined within 3 days of treatment. All treatments were replicated three times.

Subsamples of 100 pecans were drawn from each treatment lot and held for 1 week at 22 C, then shelled to determine weevil mortality. An average of 72.5% of the pecans were infested with weevils. Only treatments which caused 100% weevil mortality were considered effective.

Treated pecans were cracked, and weevil-damaged kernel pieces were surface-sterilized for 5 minutes with 10% sodium hypochlorite. Sections of damaged tissue were placed on Difco malt-salt agar plates. One hundred to 360 kernel sections were plated from each treatment lot, and incubated for 3 to 4 weeks at 22 C. Fungal colonies from infected kernel sections were classified by

TABLE 1. Percent infection by fungi of kernel sections from weevil-infested pecans treated with hot water or steam

Treatment			Infection of kernels[x] in replicate				Average weevil
Method	Temp. (C)	Time (min)	1 (%)	2 (%)	3 (%)	Average[y] (%)	mortality (%)
Water	60	5	4.2	86.7	22.5	37.8 cd	100.0 a
Water	60	10	3.3	51.2	30.8	28.4 c	100.0 a
Water	60	15	0.8	15.8	29.2	15.3 ab	100.0 a
Water	77	1	51.7	60.0	78.3	63.3 d	34.4 c
Water	77	2	34.2	11.7	14.2	20.0 ab	97.3 ab
Water	77	3	17.5	0.8	5.8	8.0 a	100.0 a
Steam	100	1	65.0	93.3	46.7	68.3 d	62.9 b
Steam	100	2	55.0	89.2	36.7	60.3 d	86.0 b
Steam	100	3	9.1	13.3	20.0	14.1 ab	100.0 a
Water	22	20	88.3	79.2	77.4	81.6 d	11.7 c

[x]Based on 360 kernel sections examined per treatment.
[y]Averages not followed by the same letter are significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 2. Percent infection by fungi of kernels from weevil-infested pecans fumigated with methyl bromide for 24 hours at 25 C

Treatment rate[y] (kg/100 m^3)	Infected kernel[x] in replicate 1 (%)	2 (%)	3 (%)	Average[z] (%)
0	77.5	75.0	78.3	76.9 b
0.8	66.7	63.3	83.3	71.1 b
1.6	17.5	7.5	8.3	11.1 a
3.3	10.8	3.3	5.8	6.7 a

[x]Based on 120 kernel sections examined per treatment.
[y]Commercial application rates of 1.6 and 3.3 kg are not permitted by the U.S. Department of Agriculture Plant Protection and Quarantine programs.
[z]Averages not followed by the same letter are significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 3. Percent infection by fungi of kernel sections from weevil-infested pecans stored for 3 weeks at different temperatures

Storage temperature (C)	Infection of kernels by fungi[x] in replicate 1 (%)	2 (%)	3 (%)	Average[y] (%)	Average weevil mortality (%)
22	76	97	89	87.3 a	14.8 a
6	87	88	65	80.0 a	20.0 b
0	82	74	65	73.7 a	22.9 b
−6	63	48	58	56.3 b	23.1 b

[x]Based on 100 kernel sections examined per treatment.
[y]Averages not followed by the same letter are significantly different ($P = 0.10$) by Duncan's multiple range test.

genera and enumerated. Representative isolates of *Penicillium* and *Aspergillus* were transferred to potato-dextrose agar slants for subsequent bioassay for avian toxicity.

Fungal isolates were assayed for toxicity to day-old DeKalb cockerels by a method based on that of Kirksey and Cole (2). Test fungi were cultured in 500-ml Erlenmeyer flasks containing one Shredded-wheat biscuit (approximately 25 g wheat) and 50 ml of a 2% yeast extract plus 15% sucrose broth. Cultures, after incubation for 21 days, were blended with 200 ml chloroform for 1 minute. The extract was filtered through 40 g sodium sulfate, mixed with 5 ml corn oil, then placed on a steam bath for approximately 2 hours until all traces of chloroform evaporated. For each extract, five cockerels were dosed by crop intubation, with a catheter, with 1 ml of the corn oil preparation and observed for 72 hours. Extracts were considered toxic if three of the five cockerels died, or showed unusual and debilitative clinical symptoms of intoxication. The activity of toxic extracts were confirmed with two additional tests. Control chicks were intubated with corn oil.

RESULTS.—An average of 81.6% of surface-sterilized, weevil infested kernels were infected by fungi (Table 1). Hot water dips at 60 C for 15 minutes, at 77 C for 3 minutes, and steam treatments for 3 minutes reduced the percentage of infected kernels. Reductions also were significant for dips at 60 C for 10 minutes and for dips at 77 C for 2 minutes. The hot water treatments at 60 C for 5 minutes or at 77 C for 1 minute, and steam treatment for 1 or 2 minutes were not effective.

Weevil mortality was 100% in pecans treated with the water dips at 60 C for 10 and 15 minutes, or at 77 C for 3 minutes, and the steam treatment for 3 minutes. These treatments, therefore, controlled inshell weevils and reduced fungal infection.

Fumigation with methyl bromide significantly reduced the average fungal infection of infested kernels only at concentrations higher than those permitted by state regulatory agencies (Pest Control Act, Exterior Quarantine No. 4, New Mexico Dept. of Agric. 1972) and by the USDA (U.S. Dept. of Agric., Plant Protection and Quarantine Programs, Title 7 — Agriculture Post 319 — Fruits and Vegetables, 1972). Fungus infection of kernels fumigated at the permitted rate was not significantly different from that in the unfumigated controls (Table 2). At the higher rates, the average percentage levels of infection were 11.1% and 6.7%. The fumigation treatments did not provide 100% control of the pecan weevil (3).

Storage of weevil-infested pecans for 3 weeks at or above 0 C did not kill all inshell weevils, nor significantly affect the fungal infections of kernels (Table 3). Storage at −6 C, however, did reduce infection to 56.3%.

Over 60% of the fungi isolated from weevil-damaged pecans belonged to the *Penicillium* and *Alternaria* genera (Table 4). *Pestalotia* and *Monochaeta, Cladosporium, Fusarium, Phoma, Aspergillus,* and miscellaneous genera constituted the remainder.

Heat and methyl bromide treatments qualitatively and quantitatively changed the mycoflora of weevil-damaged pecans. *Penicillium* became relatively more important in treated pecans. *Aspergillus,* similarly, constituted a negligible portion of the mycoflora isolated from

TABLE 4. Percent incidence of major genera of fungi isolated from pecans treated with heat, methyl bromide fumigation, or low temperatures for control of inshell weevils

Genus	Percent incidence of isolates					
	Heat treatments		Fumigation		Cold storage	
	Check (%)	Treated[x] (%)	Check (%)	Treated[y] (%)	Check (%)	Treated[z] (%)
Penicillium	38.0	65.7	21.8	73.8	25.8	10.5
Alternaria	29.0	5.7	43.7	16.1	37.7	61.2
Pestalotia/Monochaeta	14.2	0.0	13.2	0.0	14.5	13.4
Cladosporium	11.2	10.0	1.9	1.5	12.7	6.1
Fusarium	3.5	0.0	11.9	0.0	5.6	7.6
Phoma	1.5	8.6	4.5	0.9	2.7	0.9
Aspergillus	0.5	7.1	0.0	7.7	0.0	0.2
Miscellaneous	2.1	2.8	2.9	0.0	0.9	0.0
Total number of colonies	608	70	311	65	387	818

[x]Averages from pecans treated with 60 C water for 10 and 15 minutes, 77 C for 2 and 3 minutes, and steam at 0.35 kg/cm^2 for 3 minutes.
[y]Averages from pecans fumigated with methyl bromide at 1.5 and 3.3 kg/m^3.
[z]Averages from pecans held for 3 weeks at 6, 0, and −6 C.

untreated pecans (0 to 0.5%), but composed 7-8% of the isolates from heat-treated or fumigated lots.

Genera that decreased in frequency of occurrence on heat-treated or fumigated pecans were *Alternaria, Pestalotia,* and *Monochaeta,* and *Fusarium.*

Storage at low temperatures did not significantly change the incidence of different genera.

Toxic metabolites were produced by 28 of 92 representative *Penicillium* isolates bioassayed, seven of which produced toxins that induced severe muscular tremors and occasional convulsions in the day-old chicks before death. One of the 10 *Aspergillus* isolates bioassayed was toxic. Research is in progress to identify and chemically characterize the toxins.

DISCUSSION.—Heat treatments significantly reduced the mycoflora isolated from weevil-damaged pecan kernels and controlled inshell weevil infestation. Neither hot water nor steam, within the limits used in these tests, adversely affected the appearance, flavor, or shelf-life of treated pecans (R. Forbes, U.S. Dept. of Agric., Athens, Ga., *unpublished*). Methyl bromide fumigation at permitted application rates or low temperature storage for 3 weeks were not effective in controlling fungi or weevils.

The fungal genera isolated from weevil-damaged pecan kernels include species known to produce toxins (4, 6). Extracts of representative *Penicillium* and *Aspergillus* isolates grown on artificial medium frequently were toxic to day-old cockerels. Production of toxin in vitro by fungi isolated from a commodity is not evidence of a public health hazard. In good commercial practice, however, all traces of insect-damaged or discolored kernels should be removed at the shelling plants. Postharvest heat treatments would be useful to kill inshell weevils and to reduce the incidence of fungi in damaged kernels. Pest control in the orchard should be particularly emphasized.

Further study is needed on the fungi associated with insect-damaged pecan kernels. *Penicillium* and *Alternaria* were the most common genera isolated. *Penicillium* constituted over 65% of the total mycoflora of heat-treated or fumigated pecans, and about 30% of these were toxigenic under the conditions tested. Emphasis in current mycotoxin research is on the incidence of aflatoxin-producing isolates of *Aspergillus flavus* and *A. parasiticus* (4, 6). *Aspergillus,* however, constituted less than 1% of the total mycoflora isolated from weevil-damaged nuts, and about 7% of that in heat-treated and fumigated nuts. Mycotoxin research on pecans should also be directed towards toxigenic species of *Penicillium,* and identification of their toxins.

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