Mycoflora and Market Quality of Chestnuts Treated with Hot Water to Control the Chestnut Weevil

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ABSTRACT

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Postharvest treatment of Chinese chestnuts in 52 C water for 5, 15, or 30 min reduced the percentage of fungal infections of weevil-damaged and discolored kernels and the number of fungal colonies isolated compared with untreated checks. Immersion for 60 min was significantly more effective against fungi than the shorter treatments, but soluble sugars decreased and starch increased over 5-mo storage at 3 C, thereby slightly lowering market quality. Addition of the fungicide 2,6-dichloro-4-nitroaniline (Botran) reduced the total number of colonies isolated and the percentage of infected kernels more than did the 30-min hot water treatment alone but not more than the 5, 15, or 60 min treatments. *Alternaria, Penicillium,* and *Aspergillus* were the major genera of fungi isolated from the discolored and damaged chestnuts.

Additional key word: mycotoxins

The Chinese chestnut (*Castanea mollissima* Blume) was introduced into the United States because of its resistance to *Endothia* blight (1). The total acreage in the country is estimated at 200 ha, but the size of the domestic market offers potential for expansion. Current annual imports of chestnuts from Europe total about 2,000,000 kg.

One factor limiting chestnut production is susceptibility to the small chestnut weevil (*Curculio sayi* Gyllenhal) (7). Adult weevils infest trees from April to late June and deposit eggs in nearly mature nuts during August and September. The larvae feed on kernel tissues, then emerge by cutting through the shell. Infested nuts may contain several weevil larvae or weevil burrows filled with excreta. Weevil-damaged nuts are likely to harbor various fungi and are subject to spoilage. Many of these fungi are toxigenic and produce mycotoxins in vivo (9,10).

Another problem limiting domestic chestnut production is the perishability of the chestnuts after harvest. Hammar (5) reported 5–10% losses of chestnuts held at 2 C for 1 mo. Wright (13) reported that 62% of chestnut kernels examined soon after harvest contained visible fungal growth. Blossom-end rot, caused by *Glomerella cingulata* (Ston.) Spauld. & Schrenk (3), also can contribute to postharvest losses (4), and Kays et al (6) attributed losses primarily to *Diplodia* sp., *Phoma* sp., *Gloeosporium* sp., and *Fusarium moniliforme* Shelden.

Hot water treatments reduce fungal infections of weevil-damaged pecan kernels (11), and hot water dips are recommended for chestnuts to extend storage life (12) and to control in-shell weevil infestations (8). This report describes the effects of treatments with hot water and fungicide suspensions on the mycoflora, fungal infection, and market quality of chestnuts.

MATERIALS AND METHODS

Chestnuts were gathered in September 1976 from orchards in central Georgia. Nuts were gathered from individual trees, the percentage weevil infestation was determined for each tree, and then the nuts were mixed so that lots for each postharvest treatment had about the same percentage of infested nuts. A treatment lot consisted of 500–1,000 nuts.

Kernels were immersed in water or a fungicide suspension in a 378.5 L capacity tank that was equipped with an agitator, a thermistor, and a butane heater. Treatments included a dry check, a cold water check (21 C for 60 min), and immersion for 5, 15, 30, and 60 min in water at 52 C. Suspensions of the fungicide 2,6 dichloro-4-nitroaniline (Botran) were also tested at 450 and 900 μg/ml for 60 min at 21 C and for 5, 15, 30, and 60 min at 52 C. All treatments were replicated three times. Treated chestnuts were spread out to dry for 6 hr, then stored at 3 C and 85% relative humidity in covered baskets.

Within 48 hr after treatment, samples

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of chestnuts were surface-sterilized for 5 min in 0.52% sodium hypochlorite, and kernels were removed aseptically and categorized as sound, weevil-damaged, undamaged but discolored, or as showing signs of a fungal decay. Quarters of 150 weevil-damaged and discolored kernels (50 per replication) per treatment were plated on Difco malt-salt agar and incubated for 3 wk at 21 C. One set of isolations (150 quarters) was made from sound chestnuts. Fungal colonies from infected kernels were classified by genera and enumerated. Weevil mortality was determined from an additional 250-nut subsample of each treatment.

Within 48 hr of treatment and at monthly intervals for 5 mo, samples of chestnuts were removed from cold storage and examined for blossom-end rot or any other symptoms of fungal infections and for desiccation of kernel tissues (chalkiness). At 1, 3, and 5 mo after treatment (October, December, and February), replicated samples of selected nuts treated with hot water were dried and powdered for sugar and starch analysis by Dowler and King's method (2). Data were subjected to analysis of variance and Duncan's multiple range test.

Table 1. Percent infection and major genera of fungi isolated from discolored and weevil-damaged kernel quarters of chestnuts treated with hot water or heated suspensions of Botran

Postharvest treatments[w]	Number of colonies[x]					Kernels infected (%)[z]
	Alternaria	*Penicillium*	*Aspergillus*	Miscellaneous[y]	Total	
Check						
Dry	100	96	3	28	227	99 a
Wet	102	61	3	29	196	97 a
Botran	97	70	0	11	188	92 a
Hot water (min)						
5	1	78	0	1	80	45 b
15	3	73	6	18	100	52 b
30	6	58	3	11	78	35 bc
60	4	44	0	8	56	30 c
Hot Botran (min)						
5	18	69	6	14	107	43 b
15	0	48	0	14	62	40 b
30	1	23	2	7	31	25 c
60	1	19	10	11	47	29 c

[w] All heated dips at 52 C.
[x] A total of 150 quarters of weevil-damaged and discolored chestnuts were plated per treatment on malt-salt agar and incubated 3 wk at 21 C.
[y] Includes *Pestalotia, Cladosporium, Phoma, Fusarium, Trichothecium*, and *Colletotrichum*.
[z] Means not followed by the same letter are significantly different ($P = 0.05$).

Table 2. Fungal colonies, kernel infection, and weevil mortality for chestnuts treated with hot water and Botran (combine data) and for chestnuts treated with cold water[x]

Treatment (Min C)		Total fungal colonies[y]	Kernel infection (%)	Weevil mortality (%)[z]
Hot water/ Botran				
5	52	187 c	44.3 b	31.3 b
15	52	162 b	46.5 b	96.0 a
30	52	109 a	30.0 a	99.7 a
60	52	88 a	29.7 a	100.0 a
Cold water				
60	21	384 d	94.5 c	1.5 c

[x] Values in columns not followed by the same letter are significantly different ($P = 0.05$).
[y] Each value based on 300 kernel quarters planted on malt-salt agar and incubated 3 wk at 21 C.
[z] From a 250-nut subsample of each treatment.

Table 3. Sugar and starch contents of chestnuts treated with hot water and stored at 3 C

Postharvest treatment[x]	Carbohydrate analyses[y]							
	Sugar (%)				Starch (%)			
	1 mo	3 mo	5 mo	Avg.	1 mo	3 mo	5 mo	Avg.
Dry check	21.9	22.4	22.9	22.4 a	43.6	45.9	46.6	45.4 a
Hot water (min)								
15	19.2	19.0	20.1	19.4 ab	46.2	47.2	49.2	47.2 ab
30	16.1	17.4	17.7	17.1 b	51.0	51.1	49.3	50.4 b
60	16.2	14.7	14.9	15.3 b	54.7	57.6	58.2	56.8 c

[x] Dips in 52 C water.
[y] Percent of dry weight as determined by Dowler and King's method (2); averages not followed by the same letter are significantly different ($P = 0.05$). Each value is an average from three samples analyzed independently.

RESULTS

Nearly 100% of the untreated, weevil-damaged or discolored chestnut quarters were infected with fungi (Table 1), but only 2–3% of the quarters from sound chestnuts were infected.

Immersion for 5-60 min in water or Botran at 52 C reduced kernel infections to a range of 25–52%. Botran improved the effectiveness of the 30-min heat treatment but not the 5, 15, or 60 min treatments. An analysis of the aggregate data from all hot water and Botran treatments showed no significant differences in percent kernel infection among treatments.

The percent infection of kernel quarters from lots of heat-treated chestnuts (combined hot water and hot Botran data) was influenced by time of exposure to the treatment. Infection levels were 30% for chestnuts treated for 30 or 60 min and 44.3–46.5% for treatment times of 5 or 15 min (Table 2).

Heat and time of treatment also affected the total number of fungal colonies isolated and the percent mortality of weevils (Table 2). Five minutes at 52 C (combined hot water and hot Botran data) reduced colony counts from 384 (unheated check) to 187; all other treatments at 52 C also resulted in significant reductions in colony numbers. The 5-min treatments did not control in-shell weevils, however; at least 15 min was required to kill 96% or more of the insects. Thus, 15 min was the shortest exposure time that controlled both weevils and fungi.

Species of *Alternaria* and *Penicillium* were the fungi most frequently isolated from chestnuts on malt-salt agar; other genera isolated were *Aspergillus, Fusarium, Pestalotia, Cladosporium, Phoma, Trichothecium,* and *Colletotrichum* (Table 1). *Alternaria* spp. were dominant in chestnuts that were not treated with heat, and *Penicillium* spp. were dominant in heat-treated samples and were the second most frequently isolated fungi from unheated checks. *Aspergillus* spp., as well as the other miscellaneous fungi, occurred as frequently in heat treated as in untreated lots. Significantly fewer fungi were isolated from kernels from the combined hot Botran treatments (247 colonies) than from those from the combined hot water dips (314 colonies).

Colony counts of *Alternaria* and *Penicillium* decreased as the duration of hot water or hot Botran treatments increased. The 5-min treatments markedly decreased *Alternaria*, from a total of 197

to 19 colonies in kernels from both heat treatments. The number of colonies of *Penicillium* was not markedly affected, however, until treatment times were increased to 30 min.

The overall quality of treated chestnuts in storage was good. During the 5-mo storage, there were no increases in percent kernels discolored or moldy, or in the percentage of kernels with chalky desiccation. However, some heat treatments affected carbohydrate levels. Hot-water treatments for 30 and 60 min significantly reduced sugar contents and increased starch levels (Table 3). Sugar and starch levels in chestnuts treated 15 min did not differ from those in the unheated checks.

DISCUSSION

Fungal contaminations were almost always associated with weevil-damaged and discolored chestnut kernels. Surface-sterilized sound kernels were generally free of fungi. Heat treatments reduced fungal isolations by 50–75%, and treatments of less than 30 min had no detrimental effects on the quality or appearance of chestnuts. An additional benefit of heat treatments, more extensively documented in another report (8), is the effective eradication of insect infestations.

Hot Botran dips resulted in lower fungal colony counts than did hot water treatments, but differences between the effects of hot water and Botran on the percentage of kernels infected were significant only with the 30-min treatments. Hot Botran dips were no more effective than hot water dips in control of in-shell infestations of weevils (8). There would be little basis, therefore, for incorporating this fungicide in hot water treatments, particularly in view of the increasing awareness of the dangers of excessive use of agricultural chemicals.

Treatments of 30 min provided maximum control of fungi in chestnuts and 99% control of weevils. Longer treatments did not further reduce the percentage of infected kernels, total number of viable colonies, or weevil mortality. Treatments of 5 or 15 min provided less protection than treatments of 30 min but nevertheless strongly affected control of fungi.

An effective postharvest treatment, such as a 30-min hot water dip for the control of in-shell insect and fungal contaminations, combined with optimal storage conditions, should preserve the fresh market quality of chestnuts during prolonged storage.

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