Walnuts' Anti-Aflatoxin Ally

Gallic Acid

Rich, crunchy walnuts are always delicious, whether sprinkled on top of a waffle at breakfast, added to brownies you've whipped up for an after-lunch treat, or tossed with a crisp green salad for dinner. Walnuts are a good source of omega-3 fatty acids, thought to reduce risk of cancer. They also provide protein, several essential vitamins and minerals, and antioxidants, yet are free of trans fats and cholesterol. Today, they're ranked as America's third most popular tree nut.

U.S. walnut growers and processors meticulously inspect walnuts so that only the best make their way from orchards to your kitchen. Part of their quality control involves making sure the nuts don't exceed federal food-safety standards for aflatoxin, a naturally occurring compound that can be converted into chemicals harmful to us.

Two kinds of fungi, *Aspergillus flavus* and *A. parasiticus,* are perhaps the best known of the microbes capable of forming aflatoxin. They have the ability to infect not only walnuts, but also almonds, pistachios, peanuts, corn, and cotton.

But ARS scientists in the Western Regional Research Center's Plant Mycotoxin Research Unit at Albany, California, have found that a commercial walnut variety, Tulare, is remarkably resistant to being contaminated by the fungus. The quest to discover Tulare's secret has led the researchers to gallic acid, a natural compound that's locked up in walnut tannin.

Enzyme Disrupts Aflatoxin-Production Process

Ironically, *Aspergillus* itself helps free up the gallic acid in walnut tannin. An *Aspergillus* protein or enzyme known as tannase breaks down the tannin and unlocks the gallic acid.

In Tulare walnuts, the gallic-acid-containing tannin occurs in levels sufficient to render the fungus incapable of making aflatoxin. The nuts' ability to inhibit aflatoxin production increases as the walnut matures, notes ARS chemist Russell J. Molyneux, who led the research.

The thin outer coating—or pellicle—of Tulare walnuts is removed before analysis for gallic acid.

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Chemist Russell Molyneux prepares walnut pellicle samples (see photo below) for analysis of gallic acid content.

Comparing Gallic Acid Levels

Molyneux and ARS chemist Noreen E. Mahoney at Albany tracked down gallic acid as the key player in Tulare's aflatoxin-defense strategy. Their petri-dish tests revealed that this variety's anti-aflatoxin activity was better than that of the other commercial walnut varieties—and other tree nuts—they screened.

The assays encompassed nearly a dozen leading varieties of English walnuts, the kind most widely marketed in the United States and Europe today, and two species of black walnuts, not as widely grown because their thicker shells are harder to open and their nutmeats don't loosen as easily from the shells.

The Albany scientists collaborated in the screening with Charles A. Leslie, Gale H. McGranahan, and James McKenna of the University of California, Davis. They found that Tulare walnuts contained one and a half to two times more gallic acid than, for instance, Chico—the most aflatoxin-susceptible variety they examined.

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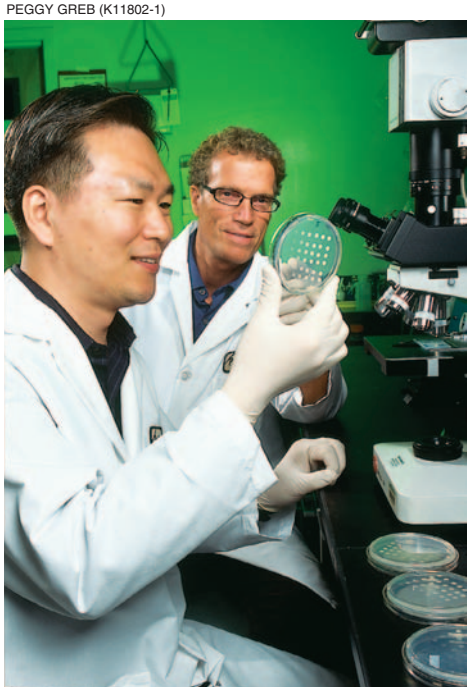

Research associate Jong Kim (left) and research leader Bruce Campbell examine high-throughput bioassays to determine effects of gallic acid on genes that control aflatoxin production.

Researchers elsewhere had already shown, in laboratory experiments, that gallic acid had antimicrobial effects. But the investigations Molyneux directed are likely the first to find that an agricultural crop—susceptible to infection by an aflatoxin-producing *Aspergillus* species—can actually prevent the *Aspergillus* from making aflatoxin.

The work paves the way for moving Tulare's gallic-acid-producing prowess into vulnerable walnut varieties. Conventional walnut breeding is one way to accomplish this.

Discovering More About Gallic Acid

Molyneux and Mahoney pinpointed Tulare walnuts' pellicle—the nutmeat's thin outer coating—as the only source of the gallic acid. Exactly how gallic acid disrupts the fungus's ability to produce aflatoxin isn't clear yet, says Bruce C. Campbell, who heads the Albany research unit.

Preliminary research by Campbell and molecular biologist Jong H. Kim suggests that *Aspergillus* may make aflatoxin in response to environmental stress, such as drought.

More details about the gallic acid research appeared in the *Journal of Food Science.*

The aflatoxin investigations were funded in part by the California-based Walnut Marketing Board. California produces nearly all of the nation's walnut crop. The 2003 harvest of 326,000 tons of in-the-shell walnuts was worth about $355 million to growers.

This research may be good news for growers, as it might lead to new, environmentally friendly strategies to undermine aflatoxin production not only in walnuts and other popular tree nuts but also in other crops.—By **Marcia Wood,** ARS.

This research is part of Food Safety, an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

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Chemist Noreen Mahoney performs analysis of aflatoxin from a petri dish (center) containing Tulare walnuts inoculated with *Aspergillus flavus.* The dish in the foreground (green) contains no walnut tissue and displays extensive growth of *A. flavus.*