Project No. 80-U7

ALMOND DISEASES
MYCOTOXIN RESEARCH-FIELD

Douglas J. Phillips
and
Dennis Margosan

Objectives:

To study factors that contribute to or influence the occurrence of Aspergillus flavus and aflatoxins in almond kernels, shells and hulls, and to examine the possible use of these factors to reduce the potential hazard of mycotoxins on almonds.

Following are the results of three separate experiments:

Summary:

I. A. flavus and Storage. Dry almonds stored for six months were infested with the navel orange worm and A. flavus. Even when some moisture vapor was added to the storage, not enough moisture was available for aflatoxin to be produced in either insect-damaged or sound kernels.

II. Insect Damage and A. flavus. The occurrence of A. flavus was compared in navel orange worm (NOW) and twig bore (TB) damaged kernels, and in kernels that were mechanically damaged by artificially drilling into them at hull split. A. flavus was present in 0.85% of NOW, 0.0% of TB, and 0.24% of the drilled nuts.

III. Growth of A. flavus and Its Competitors. Growth of A. flavus, A. parasiticus, U. atrum, and U. chartarum was compared on five substrates at 20° and 30°C. Temperature was shown to be a more important factor affecting growth than subtrate in these fungi.

I. A. flavus AND STORAGE

Aflatoxins in almonds are primarily found in insect-damaged kernels. The navel orange worm (NOW) attacks almond fruits on the tree when the hulls split and the kernels dry. After harvest the hull and shells may be removed and the kernels placed into storage. Although the NOW doesn't normally reproduce in storage, some larvae may develop from eggs laid on the kernels prior to storage. If temperatures are warm these "storage" larvae may cause considerable damage to the kernels. Larvae may stimulate fungal activity or may predispose the kernels to fungal attack. We evaluated the effect of NOW larvae on development of A. flavus and aflatoxin and also studied the susceptibility of the insect-damaged kernels to A. flavus when the nuts were stored under warm (25-30°C), dry conditions (less than 0.85 RH).

METHODS AND MATERIALS

Shelled almonds (supreme grade) were placed in sealed canning jars (2 lb/jar) and held at 25°C (the temperature was raised to 30°C periodically about once a month for 24 hr to simulate commercial practice). Ten NOW eggs were placed into 16 of the jars near the beginning of the storage period on October 9 and 20 NOW eggs were placed in the jars on November 28, 1979. By January 1980 large larvae were easily observed on the kernels infested with NOW. On January 17, all the jars were opened and the kernels were fumigated with 0.02 percent hydrogen phosphide for 24 hr to stop NOW activity.

Following the fumigation and subsequent aeration of the almonds, spores of AF were placed into some of the jars and thoroughly mixed with the kernels. Also at this time vials, each containing 25 ml of water, were placed in the top layer of kernels in some of the jars. The vials were upright and open, which allowed the release of moisture vapor to the surrounding dry almond kernels. This procedure provided eight treatment groups in the re-sealed jars: The wormy kernels or sound kernels 1) alone, 2) with water only, 3) with AF only, or 4) with a combination of water and AF.

The kernels were held in the jars until April 1980 (about 6 months of storage). At this time the almonds were analyzed for fungi, moisture content and aflatoxins.

Isolation of fungi

Almond kernels were surface-disinfested by dipping them in 70% (v/v) ethanol/water for 10 sec., then placing them in 0.5% sodium hypochlorite solution for 5 min. The surface-disinfested kernels were tested for the presence of A. flavus, and A. parasiticus by placing the kernels on plates of malt-salt medium containing 2% malt extract, 7.5% NaCl, 2% agar and 13 µg/ml 2,6-dichloro-4-nitroaniline for 1 week at 30°C.

Moisture content on a weight-before-drying basis for each sample was determined from the weights of 50-kernel subsamples before and after oven drying for 48 hr at 86°C.

After analysis for the presence of fungi, all almonds remaining in a sample were ground and analyzed for aflatoxin, using minicolumn and liquid chromatographic methods, (Dr. Ed Steffen, Dried Fruit Association of California, 1855 S. Van Ness Avenue, Fresno, California).

RESULTS

The addition of navel orange worm eggs to the almonds increased worm damage from 0.6% to 3.9%. Adding water in glass vials to the jars containing the kernels increased the moisture content from 3 to 5%, and adding the spores of Aspergillus flavus to the kernels increase the isolation of this fungus from the kernels from 0 to 3%.

None of these factors affected aflatoxin production: no toxin was found in any of the samples studied.

DISCUSSION

At temperatures that favor fungal growth, moisture was not sufficient for fungal growth 1) in almond kernels damaged by NOW in storage, or 2) in almond kernels in the jars in which water was evaporating from the glass-vials. Almonds with 3-4% moisture,. held under reasonably dry conditions, do not readily support fungal growth, even though some navel orange worm activity may occur in storage.

Excessive worm growth and damage to the kernels, or the addition of free-water to the kernels could stimulate fungal growth and aflatoxin production, but these conditions were not covered by this report.

II. INSECT DAMAGE AND A. flavus

Two commonly occurring insects, the navel orange worm (NOW) and the peach twig bore (TB), attack almond fruits on the tree as the hulls split and the kernels dry. Aflatoxins in almond are found in insect-damaged kernels, and we believe that most of this toxin is produced while the almonds cling to the tree during the early stages of hull-split. The fungus Aspergillus flavus may be associated with the NOW or the TB because these insects attack the kernels at a stage when they also are especially susceptible to fungal attack. In the laboratory we isolated fungi from kernels injured by NOW, TB, or by mechanically drilling holes into the fruit at hull-split.

METHODS AND MATERIALS

Non-pareil almond trees in an orchard near Fresno, California were selected as a study plot. One thousand fruits, still on the tree, were

mechanically injured by drilling a 1/16-inch hole into the kernels at hull-split. The injured fruits remained on the trees until normal harvest, at which time the injured fruits were collected, as were insect-damaged kernels found in nearby trees. The insect-damaged kernels were separated into NOW or TB damaged lots, based on the presence of the insect larvae and/or on the typical superficial TB damage as opposed to the more extensive and deeply penetrating damage caused by the NOW. After harvest all samples were fumigated with 0.02 percent hydrogen phosphide for 24 hr to stop insect activity.

The kernels were tested for the presence of A. flavus or A. parasiticus (AF) by placing surface-disinfested kernels on plates of malt-salt medium for 1 week at 30°C.

RESULTS

In general the occurrence of AF was low. In all samples the damaged kernels were heavily colonized by Aspergillus niger. AF occurred in seven out of 822 kernels damaged by NOW, none of 358 kernels damaged by TB, two of the 832 mechanically-damaged kernels and one of 888 undamaged kernels.

DISCUSSION

Because of the low frequency of AF in the samples, no conclusions are possible. However, there is some indication that NOW damage incites more AF invasion than TB damage or the experimentally induced mechanical damage.

III. GROWTH OF A. flavus AND ITS COMPETITORS

Almonds grown in the central valley of California are subject to colonization by members of the genus Aspergillus. Two members of the Aspergillus flavus group fungi, A. flavus Link and A. parasiticus Speare, have been found to produce aflatoxins in almonds. The incidence of A. flavus and A. parasiticus isolated from surface-disinfested nuts was 10-fold greater in nuts damaged by navel orange worm, Paramyelois transitella (Walker), than it was in sound kernels. The incidence of A. flavus and A. parasiticus in almonds was found to increase with the ambient temperature of the almond orchard. Another fungus isolated from almond, Ulocladium chartarum (Pruess) Simmons, was found to be antagonistic to A. flavus and A. parasiticus. Although the nature of the antagonism is not known, the incidence of U. chartarum was greater on almonds in orchards with the lowest ambient temperatures. Temperature is an important factor influencing the relationship between these fungi; substrate may also effect interactions between these fungi.

In order to explore the nature of the antagonism that may exist between U. chartarum and the Aspergillus fungi, a laboratory experiment was designed to measure their fungal growth on five different substrates at 20° and 30°C. The fungi used were A. flavus, A. parastitcus, U. chartarum and U. atrum. The latter was included because it also has been found on almonds, but when previously tested was not antagonistic to the Aspergilli.

The five substrates tested were almond hulls (whole or ground), almond kernels, cooked brown rice, and cooked strained peaches.

MATERIALS AND METHODS

Aspergillus flavus Link, A. parasiticus Speare, Ulocladium atrum Preuss, and U. chartarum (Pr.) Simmons were isolated from almonds grown in the central valley of California. The Aspergillus fungi were aflatoxin producing strains. Growth of the fungi was determined by measuring the amount of CO_2 evolved from colonies grown on the various substrates at 20° and 30°C.

In order to measure the evolving CO_2, a chamber was devised that consisted of a 1000 ml Erlenmeyer flask fitted with a three-hole rubber stopper. A glass tube for the air intake, inserted into one of the holes, came within 2 cm of the flask bottom; an exhaust tube extended 0.2 to 0.3 cm below the inner stopper surface; a short length of glass tubing sealed with a rubber septum was placed in the third hole, which served as the sampling port.

The air intake of the growth chamber was connected to a humidifier, which consisted of a 300 ml Erlenmeyer flask with a two-hole rubber stopper. The air intake of the humidifier consisted of a glass tube that extended to 0.5 cm of the flask bottom. The flask was filled with 200 ml of de-ionized water. The humidified air passed out of the humidifier through a glass tube flush with the inner stopper surface.

The exhaust tube of the growth chamber was attached to a 33 cm length of tygon tubing to prevent atmospheric CO_2 from entering the chamber. The air flow through each chamber was adjusted to 25 ml/minute. Carbon dioxide evolution was measured by taking a 0.5 ml air sample every 24 hours and analyzing it by gas chromatography. The total CO_2 produced in the preceeding 24 hours was estimated from the CO_2 content of this sample and readings for 5 days totaled.

Tests were replicated four times with each of five substrates for each fungus at 20° and 30°C (total 200 tests). A replication consisted of five growth chambers placed in a constant temperature incubator. Each chamber contained an equal amount of the same growth medium. Each of four chambers contained a specific fungus started from 10 germinated spores taken from a plate of water agar, the fifth chamber served as an uninoculated control. The same procedure was repeated for each substrate.

The different substrates were prepared as follows: 1) 135 g of canned, strained peaches (Gerber Products, Fairmont, MI) were aseptically transferred to each chamber; 2) 65 g of brown rice and 80 ml of water were placed in each chamber, which were then sterilized by heating in a pressure cooker at 15 psi for 15 minutes; 3) 100 g of whole or ground almond hulls or whole kernels previously sterilized with propylene oxide were aseptically transferred to each chamber. The almond fruit, collected from an orchard near Fresno, were near the hull split stage when harvested and were not dried before their sterilization.

RESULTS

Considering the average growth of the Aspergilli on all substrates, they produced 16 times more CO_2 than the Ulocladia at 30°C (Table 1). Growth was slower at 20°C, and the Aspergilli overall produced only 1.6 times more CO_2 than the Ulocladia. However, on brown rice, ground almond hulls and almond kernels there was a tendency for the Ulocladia to produce more CO_2 than the Aspergilli.

When the two Aspergilli are compared, A. flavus produced more CO_2 at 20°C than A. parasiticus, but in contrast A. flavus produced less CO_2 than A. parasiticus at 30°C.

There was no difference in CO_2 evolution between U. atrum and U. chartarum at 20°C but U. chartarum produced three times more CO_2 than U. atrum at 30°. At the higher temperature CO_2 production from U. atrum was especially slowed on strained peaches and almond hulls.

DISCUSSION

The growth of these fungi, as measured by CO_2 evolution, suggests that temperature has a greater effect on their growth than does substrate. The minimal effect of substrate relates to the saprophytic characteristics of these fungi and the broad spectra of substrates in nature.

The optimum temperature for growth of the Ulocladium spp. is 25°C, and for the Aspergillus spp. is 35°C, as determined for these fungi grown on potato dextrose agar. These optimal temperatures indicated the relative ability of the respective fungi to colonize a variety of substrates in different orchard conditions.

Ulocladium chartarum colonized the various substrates and grew quite vigorously at both 20° and 30°C. Its antagonistic behavior to A. flavus is related to temperature. When both fungi are present, cool temperatures may be needed for U. chartarum to become established on a substrate and to compete effectively with the Aspergilli.

TABLE 1. Carbon dioxide evolution of four fungi growing on indicated substrates at 20° or 30°C.

Substrate	Temperature	Ulocladium atrum	Ulocladium chartarum	Aspergillus flavus	Aspergillus parasiticus
	°C	mg CO_2 [1]	mg CO_2 [1]	mg CO_2 [1]	mg CO_2 [1]
Strained peach	20	40,960	37,580	142,155	108,329
Brown rice	20	23,667	44,567	39,309	30,617
Almond kernels	20	2,233	5,080	2,802	4,146
Almond hulls					
ground	20	71,469	69,244	48,703	56,048
whole	20	144,657	121,882	170,813	112,244
Column Totals		282,986	278,353	403,782	311,384
Grand total		432,253		715,166	
Strained peach	30	5,603	81,954	181,685	155,783
Brown rice	30	62,223	146,854	477,618	496,721
Almond kernels	30	53,782	115,856	575,165	693,998
Almond hulls					
ground	30	51,303	142,847	1,980,945	5,632,854
whole	30	73,693	233,533	3,748,540	2,300,247
Column Totals		246,604	721,044	6,963,953	9,279,603
Grand total		967,648		16,243,556	

[1] Each datum is the average of four replications and represents the total CO_2 evolved in 5 days.

UNITED STATES DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION

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Identifying Molds on Food Products

Isolating, identifying and counting molds from foods, such as almonds, is often complicated by overgrowth of some colonies in the petri plate by rapidly growing spreading molds. These molds such as Rhizopus and Botrytis species produce woolly or cottony colonies that fill the plate and obscure the majority of the colonies that are slower growing and which form low colonies with only a small amount of aerial growth. These smaller colonies such as Alternaria, Aspergillus, Cladosporium and Penicillium species are frequently the molds of interest since they cause spoilage and can produce mycotoxins such as aflatoxin, citrinin, etc.

We have developed a medium that was designed to inhibit the rapidly growing molds yet still let them form small colonies and at the same time allow development of the remaining mold flora. This medium consists of a nutrient basal medium at a pH that allows optimum growth plus added inhibitors. Chlortetracycline is added to inhibit bacterial growth. Rose bengal and dichloran inhibit the proliferation of the spreading molds while influencing the remaining molds only slightly when added at the appropriate concentrations.

Sterilized agar is poured into petri plates and allowed to solidify. Then the inoculum is added to the surface (0.1 ml) and spread evenly. After 4-5 days at 25°C (77°F) the plates can be examined and individual colonies selected for isolation.

The medium formulation is:

Glucose		10.0 g
Peptone		5.0 g
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	0.5 g
Potassium phosphate, monobasic	KH_2PO_4	1.0 g
Agar		15.0 g
Distilled water		1 l

Final pH 5.6

Rose bengal (certified) is made up in water 0.5 g/20 ml water and 1 ml of this stock solution is added per liter of medium (25 ppm rose bengal final concentration).

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Market Quality and Transportation
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1980 ANNUAL REPORT

Project No. 80-U7

Title: Almond Diseases
Mycotoxin Research - Field

Personnel: Dr. Douglas J. Phillips
Mr. Rodney K. Austin
Mr. Dennis Margosan
Mr. Thor N. Hansen

1. Objectives:

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Following are the results of three separate experiments:

2. Interpretive Summary:

I. *A. flavus and Storage*. Dry almonds stored for six months were infested with the navel orange worm and *A. flavus*. Even when some moisture vapor was added to the storage, not enough moisture was available for aflatoxin to be produced in either insect-damaged or sound kernels.

II. *Insect Damage and A. flavus*. The occurrence of *A. flavus* was compared in navel orange worm (NOW) and twig bore (TB) damaged kernels, and in kernels that were mechanically damaged by artificially drilling into them at hull split. *A. flavus* was present in 0.85% of NOW, 0.0% of TB, and 0.24% of the drilled nuts.

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Publications:

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Purcell, Steven L., Dougals J. Phillips, and Bruce E. Mackey. 1980. Distribution of Aspergillus flavus and other fungi in several almond-growing areas of California. Phytopathology 70(9):926-929.

TABLE 1. Carbon dioxide evolution of four fungi growing on indicated substrates at 20° or 30°C.

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Grand total		967,648		16,243,556	

[1] Each datum is the average of four replications and represents the total CO_2 evolved in 5 days.

Project No. 80-U7

ALMOND DISEASES
MYCOTOXIN RESEARCH-FIELD

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and
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Objectives:

To study factors that contribute to or influence the occurrence of Aspergillus flavus and aflatoxins in almond kernels, shells and hulls, and to examine the possible use of these factors to reduce the potential hazard of mycotoxins on almonds.

Following are the results of three separate experiments:

Summary:

I. A. flavus and Storage. Dry almonds stored for six months were infested with the navel orange worm and A. flavus. Even when some moisture vapor was added to the storage, not enough moisture was available for aflatoxin to be produced in either insect-damaged or sound kernels.

II. Insect Damage and A. flavus. The occurrence of A. flavus was compared in navel orange worm (NOW) and twig bore (TB) damaged kernels, and in kernels that were mechanically damaged by artificially drilling into them at hull split. A. flavus was present in 0.85% of NOW, 0.0% of TB, and 0.24% of the drilled nuts.

III. Growth of A. flavus and Its Competitors. Growth of A. flavus, A. parasiticus, U. atrum, and U. chartarum was compared on five substrates at 20° and 30°C. Temperature was shown to be a more important factor affecting growth than subtrate in these fungi.

Moisture content on a weight-before-drying basis for each sample was determined from the weights of 50-kernel subsamples before and after oven drying for 48 hr at 86°C.

After analysis for the presence of fungi, all almonds remaining in a sample were ground and analyzed for aflatoxin, using minicolumn and liquid chromatographic methods, (Dr. Ed Steffen, Dried Fruit Association of California, 1855 S. Van Ness Avenue, Fresno, California).

RESULTS

The addition of navel orange worm eggs to the almonds increased worm damage from 0.6% to 3.9%. Adding water in glass vials to the jars containing the kernels increased the moisture content from 3 to 5%, and adding the spores of Aspergillus flavus to the kernels increase the isolation of this fungus from the kernels from 0 to 3%.

None of these factors affected aflatoxin production: no toxin was found in any of the samples studied.

DISCUSSION

At temperatures that favor fungal growth, moisture was not sufficient for fungal growth 1) in almond kernels damaged by NOW in storage, or 2) in almond kernels in the jars in which water was evaporating from the glass-vials. Almonds with 3-4% moisture,. held under reasonably dry conditions, do not readily support fungal growth, even though some navel orange worm activity may occur in storage.

Excessive worm growth and damage to the kernels, or the addition of free-water to the kernels could stimulate fungal growth and aflatoxin production, but these conditions were not covered by this report.

II. INSECT DAMAGE AND A. flavus

Two commonly occurring insects, the navel orange worm (NOW) and the peach twig bore (TB), attack almond fruits on the tree as the hulls split and the kernels dry. Aflatoxins in almond are found in insect-damaged kernels, and we believe that most of this toxin is produced while the almonds cling to the tree during the early stages of hull-split. The fungus Aspergillus flavus may be associated with the NOW or the TB because these insects attack the kernels at a stage when they also are especially susceptible to fungal attack. In the laboratory we isolated fungi from kernels injured by NOW, TB, or by mechanically drilling holes into the fruit at hull-split.

METHODS AND MATERIALS

Non-pareil almond trees in an orchard near Fresno, California were selected as a study plot. One thousand fruits, still on the tree, were

mechanically injured by drilling a 1/16-inch hole into the kernels at hull-split. The injured fruits remained on the trees until normal harvest, at which time the injured fruits were collected, as were insect-damaged kernels found in nearby trees. The insect-damaged kernels were separated into NOW or TB damaged lots, based on the presence of the insect larvae and/or on the typical superficial TB damage as opposed to the more extensive and deeply penetrating damage caused by the NOW. After harvest all samples were fumigated with 0.02 percent hydrogen phosphide for 24 hr to stop insect activity.

The kernels were tested for the presence of A. flavus or A. parasiticus (AF) by placing surface-disinfested kernels on plates of malt-salt medium for 1 week at 30°C.

RESULTS

In general the occurrence of AF was low. In all samples the damaged kernels were heavily colonized by Aspergillus niger. AF occurred in seven out of 822 kernels damaged by NOW, none of 358 kernels damaged by TB, two of the 832 mechanically-damaged kernels and one of 888 undamaged kernels.

DISCUSSION

Because of the low frequency of AF in the samples, no conclusions are possible. However, there is some indication that NOW damage incites more AF invasion than TB damage or the experimentally induced mechanical damage.

III. GROWTH OF A. flavus AND ITS COMPETITORS

Almonds grown in the central valley of California are subject to colonization by members of the genus Aspergillus. Two members of the Aspergillus flavus group fungi, A. flavus Link and A. parasiticus Speare, have been found to produce aflatoxins in almonds. The incidence of A. flavus and A. parasiticus isolated from surface-disinfested nuts was 10-fold greater in nuts damaged by navel orange worm, Paramyelois transitella (Walker), than it was in sound kernels. The incidence of A. flavus and A. parasiticus in almonds was found to increase with the ambient temperature of the almond orchard. Another fungus isolated from almond, Ulocladium chartarum (Pruess) Simmons, was found to be antagonistic to A. flavus and A. parasiticus. Although the nature of the antagonism is not known, the incidence of U. chartarum was greater on almonds in orchards with the lowest ambient temperatures. Temperature is an important factor influencing the relationship between these fungi; substrate may also effect interactions between these fungi.

In order to explore the nature of the antagonism that may exist between U. chartarum and the Aspergillus fungi, a laboratory experiment was designed to measure their fungal growth on five different substrates at 20° and 30°C. The fungi used were A. flavus, A. parastitcus, U. chartarum and U. atrum. The latter was included because it also has been found on almonds, but when previously tested was not antagonistic to the Aspergilli.

The five substrates tested were almond hulls (whole or ground), almond kernels, cooked brown rice, and cooked strained peaches.

MATERIALS AND METHODS

Aspergillus flavus Link, A. parasiticus Speare, Ulocladium atrum Preuss, and U. chartarum (Pr.) Simmons were isolated from almonds grown in the central valley of California. The Aspergillus fungi were aflatoxin producing strains. Growth of the fungi was determined by measuring the amount of CO_2 evolved from colonies grown on the various substrates at 20° and 30°C.

In order to measure the evolving CO_2, a chamber was devised that consisted of a 1000 ml Erlenmeyer flask fitted with a three-hole rubber stopper. A glass tube for the air intake, inserted into one of the holes, came within 2 cm of the flask bottom; an exhaust tube extended 0.2 to 0.3 cm below the inner stopper surface; a short length of glass tubing sealed with a rubber septum was placed in the third hole, which served as the sampling port.

The air intake of the growth chamber was connected to a humidifier, which consisted of a 300 ml Erlenmeyer flask with a two-hole rubber stopper. The air intake of the humidifier consisted of a glass tube that extended to 0.5 cm of the flask bottom. The flask was filled with 200 ml of de-ionized water. The humidified air passed out of the humidifier through a glass tube flush with the inner stopper surface.

The exhaust tube of the growth chamber was attached to a 33 cm length of tygon tubing to prevent atmospheric CO_2 from entering the chamber. The air flow through each chamber was adjusted to 25 ml/minute. Carbon dioxide evolution was measured by taking a 0.5 ml air sample every 24 hours and analyzing it by gas chromatography. The total CO_2 produced in the preceeding 24 hours was estimated from the CO_2 content of this sample and readings for 5 days totaled.

Tests were replicated four times with each of five substrates for each fungus at 20° and 30°C (total 200 tests). A replication consisted of five growth chambers placed in a constant temperature incubator. Each chamber contained an equal amount of the same growth medium. Each of four chambers contained a specific fungus started from 10 germinated spores taken from a plate of water agar, the fifth chamber served as an uninoculated control. The same procedure was repeated for each substrate.

The different substrates were prepared as follows: 1) 135 g of canned, strained peaches (Gerber Products, Fairmont, MI) were aseptically transferred to each chamber; 2) 65 g of brown rice and 80 ml of water were placed in each chamber, which were then sterilized by heating in a pressure cooker at 15 psi for 15 minutes; 3) 100 g of whole or ground almond hulls or whole kernels previously sterilized with propylene oxide were aseptically transferred to each chamber. The almond fruit, collected from an orchard near Fresno, were near the hull split stage when harvested and were not dried before their sterilization.

RESULTS

Considering the average growth of the Aspergilli on all substrates, they produced 16 times more CO_2 than the Ulocladia at 30°C (Table 1). Growth was slower at 20°C, and the Aspergilli overall produced only 1.6 times more CO_2 than the Ulocladia. However, on brown rice, ground almond hulls and almond kernels there was a tendency for the Ulocladia to produce more CO_2 than the Aspergilli.

When the two Aspergilli are compared, A. flavus produced more CO_2 at 20°C than A. parasiticus, but in contrast A. flavus produced less CO_2 than A. parasiticus at 30°C.

There was no difference in CO_2 evolution between U. atrum and U. chartarum at 20°C but U. chartarum produced three times more CO_2 than U. atrum at 30°. At the higher temperature CO_2 production from U. atrum was especially slowed on strained peaches and almond hulls.

DISCUSSION

The growth of these fungi, as measured by CO_2 evolution, suggests that temperature has a greater effect on their growth than does substrate. The minimal effect of substrate relates to the saprophytic characteristics of these fungi and the broad spectra of substrates in nature.

The optimum temperature for growth of the Ulocladium spp. is 25°C, and for the Aspergillus spp. is 35°C, as determined for these fungi grown on potato dextrose agar. These optimal temperatures indicated the relative ability of the respective fungi to colonize a variety of substrates in different orchard conditions.

Ulocladium chartarum colonized the various substrates and grew quite vigorously at both 20° and 30°C. Its antagonistic behavior to A. flavus is related to temperature. When both fungi are present, cool temperatures may be needed for U. chartarum to become established on a substrate and to compete effectively with the Aspergilli.

TABLE 1. Carbon dioxide evolution of four fungi growing on indicated substrates at 20° or 30°C.

Substrate	Temperature	Ulocladium atrum	Ulocladium chartarum	Aspergillus flavus	Aspergillus parasiticus
	°C	mg CO_2 [1]	mg CO_2 [1]	mg CO_2 [1]	mg CO_2 [1]
Strained peach	20	40,960	37,580	142,155	108,329
Brown rice	20	23,667	44,567	39,309	30,617
Almond kernels	20	2,233	5,080	2,802	4,146
Almond hulls					
ground	20	71,469	69,244	48,703	56,048
whole	20	144,657	121,882	170,813	112,244
Column Totals		282,986	278,353	403,782	311,384
Grand total		432,253		715,166	
Strained peach	30	5,603	81,954	181,685	155,783
Brown rice	30	62,223	146,854	477,618	496,721
Almond kernels	30	53,782	115,856	575,165	693,998
Almond hulls					
ground	30	51,303	142,847	1,980,945	5,632,854
whole	30	73,693	233,533	3,748,540	2,300,247
Column Totals		246,604	721,044	6,963,953	9,279,603
Grand total		967,648		16,243,556	

[1] Each datum is the average of four replications and represents the total CO_2 evolved in 5 days.

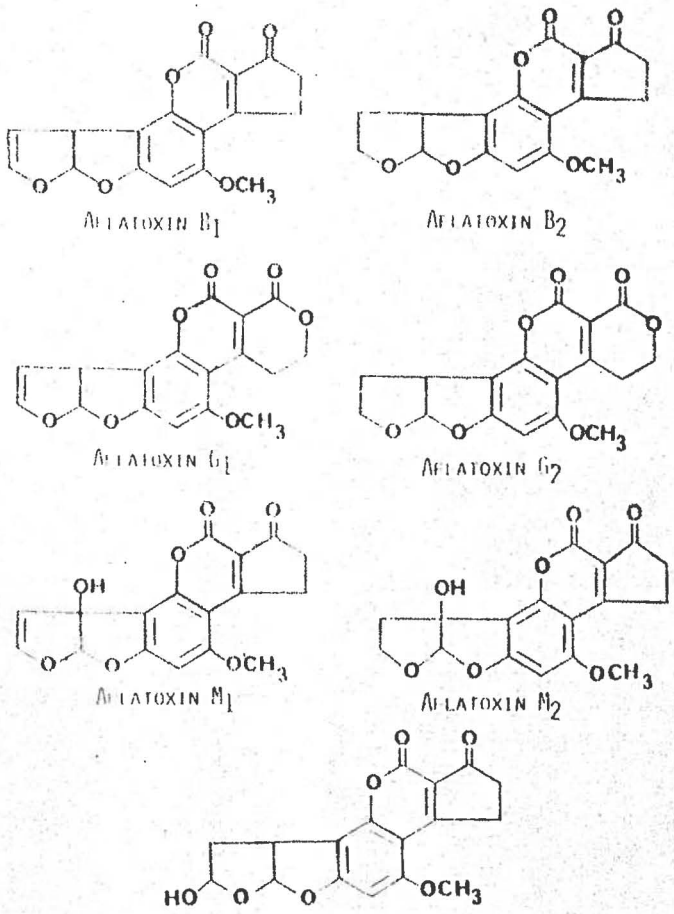
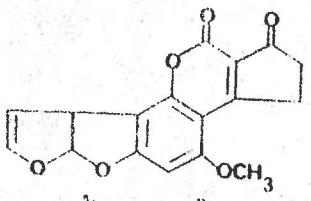
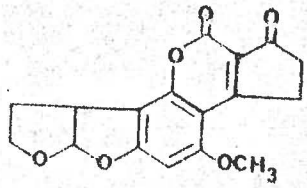


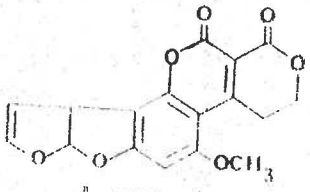
FIG. 1 Structures of aflatoxins of analytical interest in food and feed contamination.



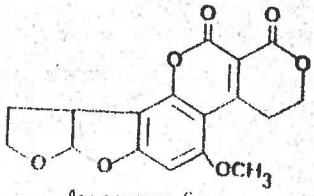
AFLATOXIN B_1



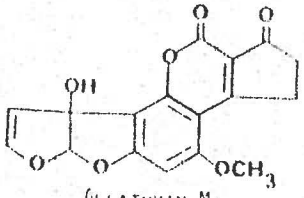
AFLATOXIN B_2



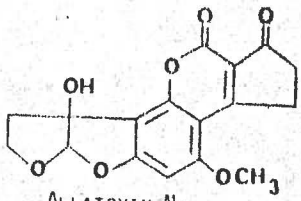
AFLATOXIN G_1



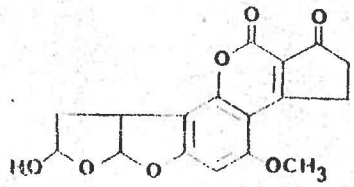
AFLATOXIN G_2



AFLATOXIN M_1



AFLATOXIN M_2



AFLATOXIN B_{2A} (WATER ADDUCT, HEMIACETAL)

FIG. 1 Structures of aflatoxins of analytical interest in food and feed contamination.