UNITED STATES DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION

AGRICULTURAL RESEARCH
WESTERN REGION
CALIFORNIA-HAWAII-NEVADA AREA
P.O. BOX 8143
FRESNO, CALIFORNIA 93747

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# 1979 ANNUAL REPORT

Project No. 79-V6

Title: Almond Diseases
Mycotoxin Research - Field and Storage

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

## 1. Objectives:

To study factors that contribute to or influence the occurrence of Aspergillus flavus and aflatoxins in almond hulls, shells and kernels, and to examine the possibility of using these factors to reduce the potential hazard of this fungus and toxin on almonds.

## 2. Interpretive Summary:

Summary (1979): Our results, although not yet complete, indicate that A. flavus and A. parasiticus grow faster than Ulocladium spp. on all substrates at 30°C, but at 20°C, except for growth on ground almond hulls and strained peach, the Ulocladium spp. grow faster than the Aspergillus spp. On ground almond hulls, the Aspergillus spp. grew more slowly than on whole hulls, but faster than Ulocladium spp. Intense browning occurred in the ground hulls. Browning is likely caused by the formation of melanins resulting from oxidation of the injured tissue.

## 3. Experimental Procedure:

Aspergillus flavus, A. parasiticus, Ulocladium atrum, and U. chartarum were grown under controlled conditions. Growth was monitored by measuring the $CO_2$ produced by these fungi at 30°C when cultured on strained peaches, cooked brown rice, almond hulls (whole and ground), or almond kernels.

The almonds used in this study were harvested at hull-split, fumigated with propolyene oxide and held under refrigeration until used. Their moisture content was 80% for hulls and 32% for kernels giving an equilibrium relative humidity of 100% at 20° or 30°C.

Background (1979): A number of fungi are antagonistic to Aspergillus flavus and A. parasiticus. Of the fungi we tested, U. chartarum reduced the isolation of A. flavus and A. parasiticus most significantly. It is possible that the environment on the almond may favor or discourage the development of A. flavus and A. parasiticus over other fungi. We compared the growth of two Ulocladium spp. to that of A. flavus and A. parasiticus under known temperature and moisture conditions and on known substrates.

4. Results:

On sterile strained peaches A. flavus and A. parasiticus grew faster than Ulocladium spp. at both 30° and 20°C (Table 1). The results were somewhat different on cooked sterile brown rice where at 20°C U. chartarum, but not U. atrum, grew faster than Aspergillus spp. (Fig. 1). Both Ulocladia grew faster than Aspergillus spp. when grown at 20°C on whole almond hulls (Fig. 2). The results were different when the hulls were ground. Here, although growth of all fungi was slower than on whole hulls, the A. flavus and A. parasiticus grew faster than Ulocladium spp. at both 20° and 30°C (Fig. 3).

The growth on kernels at 30°C show that the Aspergilli grew faster than Ulocladium spp. (Table 2). We have not completed the study of growth on kernels at 20°C, or on whole hulls at 30°C.

In summary, A. flavus and A. parasiticus grew better than Ulocladium spp. on (1) strained peaches and ground hulls at 20° and 30°C, and (2) on cooked brown rice and kernels at 30°C. Ulocladium spp. grew better than the Aspergilli on whole hulls and on brown rice at 20°C.

5. Discussion:

As previously reported by us and other workers, A. flavus and A. parasiticus grow faster at 30° than at 20°C and at 30° easily grew faster than Ulocladium spp. Thus at 30°C, when competing for substrate, the Aspergillus spp. should be more successful than Ulocladium spp. At 20°C the temperature slowed the Aspergilli more than the Ulocladium, and the Ulocladium spp. were often able to grow faster than the Aspergilli.

Because growth of all fungi was very slow on ground almond hulls, there may be a fungal inhibitor in the ground hulls that is not present in whole hulls. This inhibitor may nearly stop the growth of Ulocladium spp. and if found under natural conditions may suppress the ability of the fungi to compete with the Aspergilli.

The most obvious difference between ground hulls and whole hulls is their color. Intense browning occurred in the ground hulls. This browning is likely the formation of melanin resulting from the oxidation of the injured tissue. In other fruit, the browning of tissue has been related to their ability to resist fungal attacks. There is a possibility that the browning reaction in almonds favors the colonization of almond hulls by Aspergillus flavus and A. parasiticus.

Table 1. The growth of Aspergillus flavus, A. parasiticus, Ulocladium atrum, and U. chartarum on sterile strained peaches at 20° and 30°C (100% relative humidity).

| Temperature | Time (hr) | Accumulative amount of $CO_2$ evolved (mg) | | | |
|---|---|---|---|---|---|
| | | A. flavus | A. parasiticus | U. atrum | U. chartarum |
| 20°C | 24 | 0.2 | 0.2 | 2.5 | 0.5 |
| 20°C | 48 | 0.8 | 0.8 | 5.2 | 1.6 |
| 20°C | 87 | 6.8 | 7.6 | 7.9 | 4.7 |
| 20°C | 98 | 10.6 | 12.5 | 9.3 | 6.9 |
| 20°C | 122 | 24.9 | 31.6 | 13.6 | 14.5 |
| 20°C | 146 | 47.9 | 61.6 | 20.5 | 26.9 |
| 30°C | 24 | 4.5 | 5.5 | 0.1 | 0.1 |
| 30°C | 45 | 19.1 | 13.8 | 0.9 | 1.7 |
| 30°C | 72 | 50.0 | 50.9 | 2.1 | 8.9 |
| 30°C | 94 | 104.4 | 103.2 | 4.9 | 22.1 |
| 30°C | 118 | 203.6 | 178.7 | 11.4 | 40.2 |
| 30°C | 142 | 352.8 | 270.7 | 16.9 | 67.0 |

Table 2. The growth of Aspergillus flavus, A. parasiticus, Ulocladium atrum and U. chartarum on disinfested almond kernels at 30°C (100% relative humidity).

| Time (hr) | Accumulative amount of $CO_2$ evolved (mg) | | | |
|---|---|---|---|---|
| | A. flavus | A. parasiticus | U. atrum | U. chartarum |
| 24 | 2.4 | 2.6 | 0.2 | 0.3 |
| 48 | 14.2 | 21.4 | 2.7 | 1.9 |
| 72 | 46.0 | 69.8 | 9.3 | 6.0 |
| 99 | 151.0 | 219.0 | 26.4 | 18.1 |
| 123 | 300.0 | 397.4 | 44.2 | 34.6 |
| 144 | 469.6 | 595.1 | 65.3 | 54.0 |
| 168 | 682.1 | 826.8 | 95.0 | 83.9 |

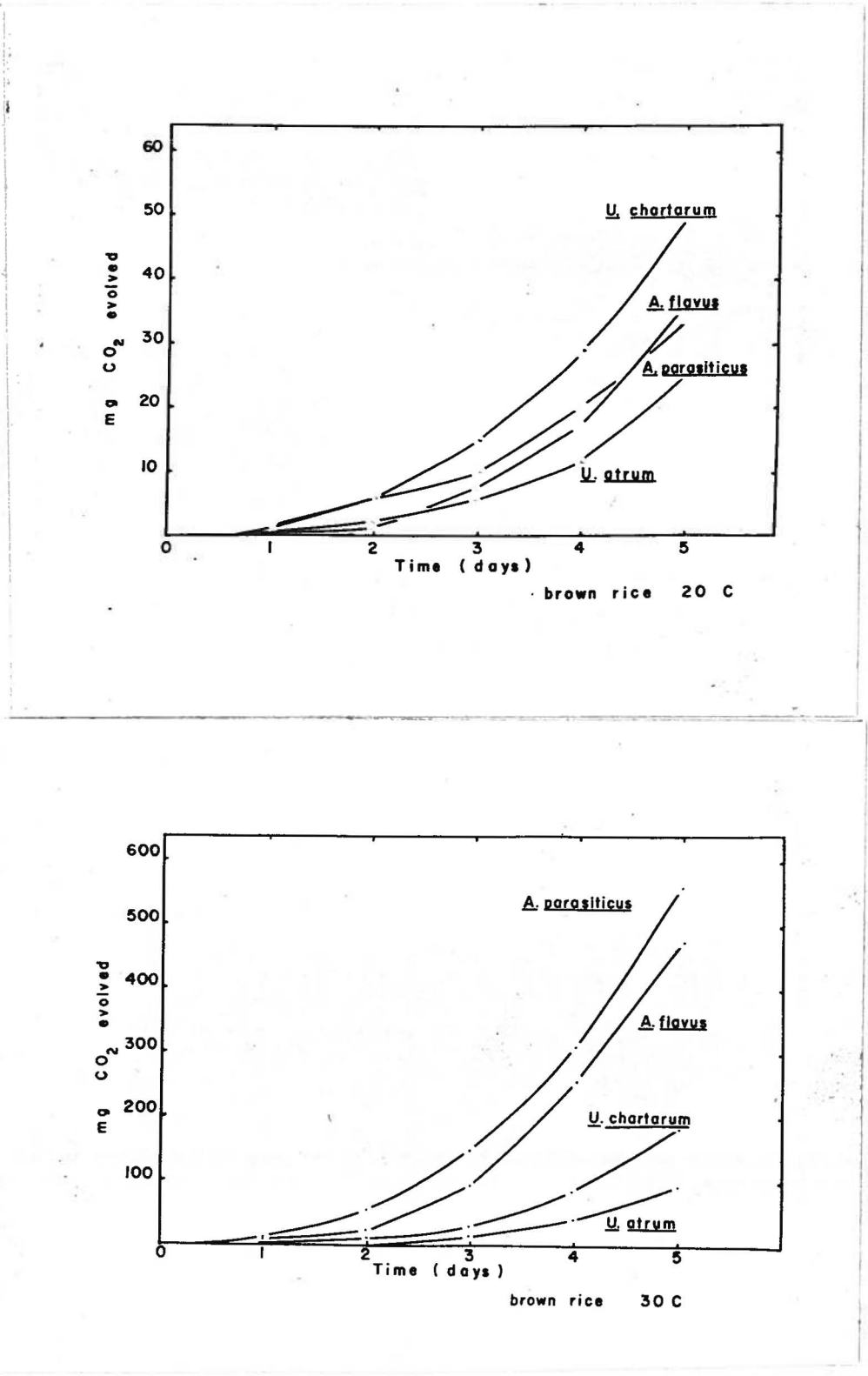



Fig. 1. The growth of Aspergillus flavus, A. parasiticus, Ulocladium atrum, and U. chartarum on cooked brown rice at 20° and 30°C (100% relative humidity).

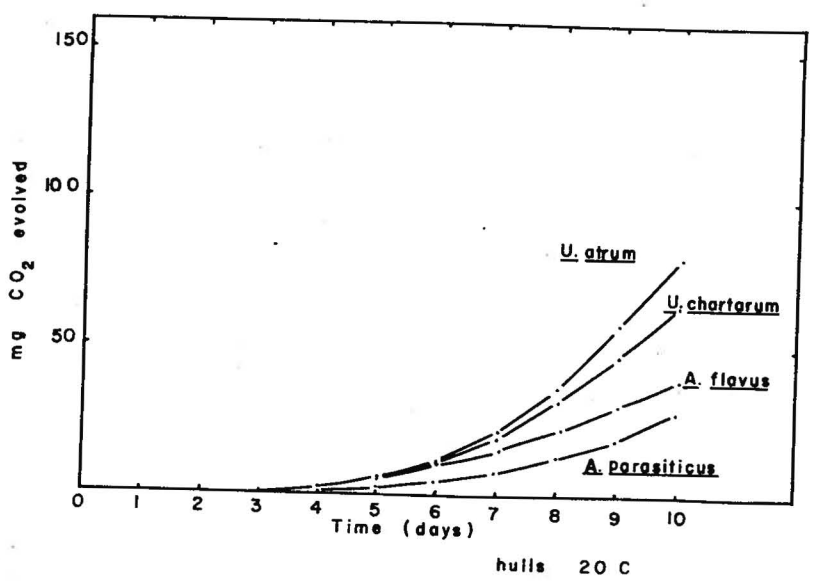
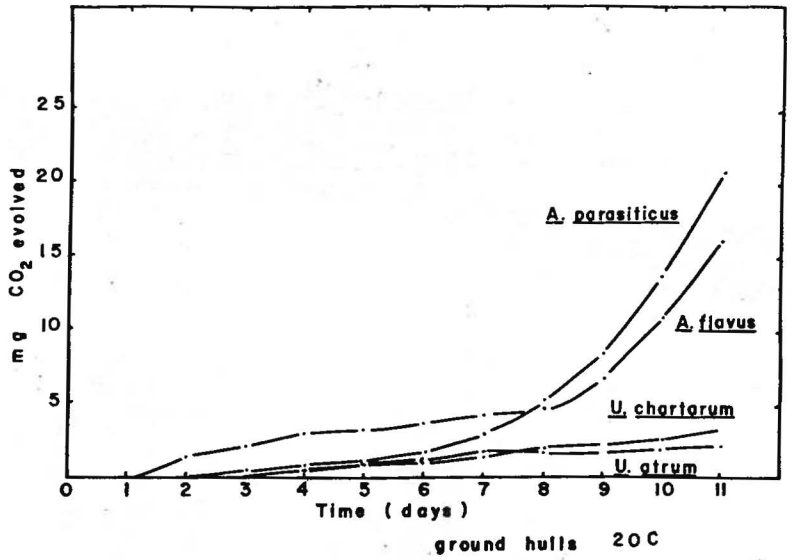



Fig. 2. The growth of Aspergillus flavus, A. parasiticus, Ulocladium atrum, and U. chartarum on sterile almond hulls at 20°C (100% relative humidity).



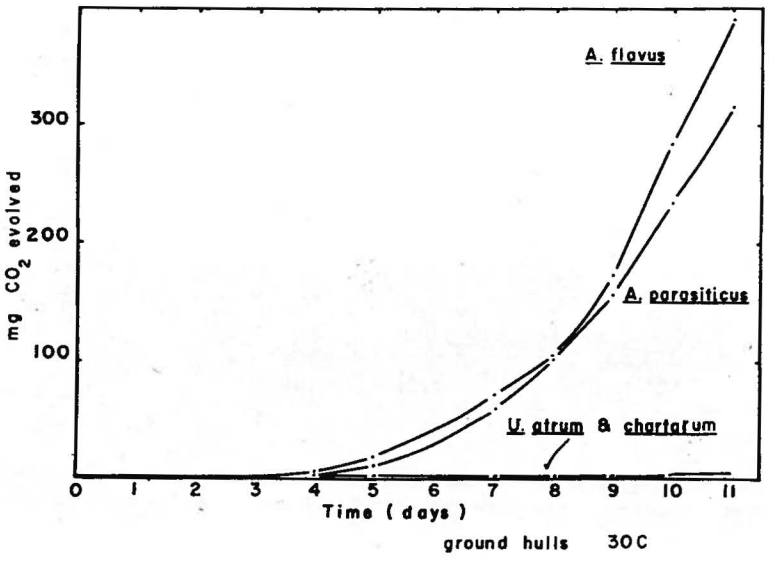




Fig. 3. The growth of Aspergillus flavus, A. parasiticus, Ulocladium atrum, and U. chartarum on sterile ground almond hulls at 20° and 30°C (100% relative humidity).