

## C O M P E N D I O

*Especies de Alternaria, Aspergillus, Botrydiplodia, Cephalosporium, Curvaluria, Fusarium, Pectalotia y Penicillium fueron aisladas de las envolturas (mesocarpo) y porción exterior de la nuez (endocarpo) de cocos inmaduros y maduros (Cocos nucifera L.). En los mercados locales se compraron aproximadamente 100 cocos pelados; la carne (endosperma) de aquellos que tenían cáscaras rajadas y rotas estaba fuertemente invadida por una variedad de hongos, y la carne de muchos de aquellos con cáscara intacta invadida por Botrydiplodia sp, y unas pocas por especies de Cephalosporium y Fusarium, aunque la carne tenía apariencia sana. En la cubierta exterior (testa) de la carne de algunos de estos cocos estuvieron presentes picnidios de Botrydiplodia. El secado de las muestras a 130 C por tres horas parece ser un procedimiento apropiado para determinar el contenido de humedad de la carne de coco o copra. Los contenidos de humedad por encima de 5,0 - 5,5 por ciento, base peso húmedo, permiten la invasión de la copra por hongos del almacenamiento.*

## Introduction

SEVERAL workers have shown that invasion by fungi is a major cause of deterioration of coconut meats and copra (2, 3, 4). Coconut meat is also an excellent medium for the production of aflatoxin (1). The work here reported was undertaken to learn more about the numbers and kinds of fungi that invade coconuts, when they invade the coconuts, and their location within the nuts. Also, since moisture content is a major factor in determining invasion of stored products by fungi, work was done on moisture content determinations and on moisture content equilibria at relative humidities that might be encountered in harvesting and handling of coconuts.

## Materials and Methods

*Source of coconuts*—Coconuts of different degrees of development, from very young to nearly mature but with the outer layer of the husk (pericarp) still green

were collected from trees in Hawaii and sent to St. Paul via air express, so that only a few days elapsed between the time they were picked and the time we examined them. Newly fallen mature coconuts, with the husks still on, were collected in Florida and carried to St. Paul by auto. Over the course of the work approximately 100 dehusked coconuts were bought in various grocery stores in St. Paul.

*Examination for numbers, kinds, and location of fungi*—Portions of husks (mesocarp), shell (endocarp) and meat (endosperm) were examined microscopically, and pieces were shaken for 1-2 minutes in 2 per cent sodium hypochlorite, placed on agar media, and incubated until the fungi that grew out could be identified. The two culture media most used were acid potato dextrose agar (APDA) and tomato juice agar containing 6.0 per cent NaCl (T-6), both of which have long been used to determine the number and kinds of fungi in grains, seeds, and other plant tissues. APDA is favorable to the growth of a large number of fungi that grow in tissues of high moisture content, and the T-2 is favorable to the growth of many kinds of storage fungi that inhabit material with moisture contents in equilibrium with relative humidities of 70 to 90 per cent. Fragments of various tissues were scraped with a sterile razor blade, and deposited directly onto agar media in petri dishes. Dilution cultures were made by comminuting portions of the meat in a sterile dilution medium in a Waring Blender.

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*Moisture content*—This was determined by three methods: (i) Drying a weighed portion in a circulating air oven at 103 C to constant weight, usually for 72 hours; (ii) drying in a circulating air oven at 130 C for 3 hours; (iii) drying in a vacuum oven for 18 hours at 80 C and 5 mm or less of mercury. Moisture contents of coconuts meat in equilibrium with relative humidities of 75, 80, and 85 per cent were determined by exposing a thin layer of previously air-dried, coarsely grated meat in aluminum weighing dishes over saturated solutions of NaCl,  $(\text{NH}_4)_2\text{SO}_4$ , and KCl, respectively.

### Results

*Numbers and kinds of fungi in husks*—Surface disinfected pieces of husk of the freshly harvested immature coconuts from Hawaii and of the mature nuts from Florida, yielded many colonies of *Botryodiplodia*, *Penicillium*, *Curvularia*, *Pestalotia*, *Cephalosporium*, *Aspergillus flavus*, and *A. niger*. The fungi present in the husks from the coconuts of the two

widely separated places were the same, and were also very similar to those isolated from shells and meats of nuts from local stores. This suggests that the makeup of the fungus flora is determined mainly by the material of the husk, and not by geographic location.

*In the shell*—The fungi listed above, plus *Fusarium* and *Trichoderma* were obtained in abundance from scrapings from the fibrous outer layers (mesocarp) of the shell. From the inner side of the shell *Botryodiplodia*, *Cephalosporium*, *Fusarium* and *Penicillium* were obtained. Dark mycelium, presumably that of *Botryodiplodia*, and pycnidia of *Botryodiplodia* were found in the outer tissues of the germ pore of numerous coconuts, and also, and sometimes in abundance, on the inner side of the shell and outer covering of the meat (the testa).

*In the meat*—No filamentous fungi were found in the meat of the fresh and living coconuts picked from the trees in Hawaii, but yeasts were present in the meat and milk of some of these coconuts when we received them, and so presumably were present when the nuts were picked from the trees. No odor of fermentation was present in these coconuts from which yeasts were isolated. A number of the coconuts bought in stores in St. Paul had obvious cracks in the shell, and all of these were heavily invaded by fungi. A representative sample is shown in Figure 1. Table 1 summarizes the numbers and kinds of fungi detected in dilution cultures of 12 of these coconuts.

Twenty coconuts bought in stores in St. Paul were selected as being free from cracks or breaks. These were broken open, and numerous small pieces of meat from each one were surface disinfected and placed on agar. *Botryodiplodia* was recovered from 18 of the 20 nuts, *Cephalosporium* from 12, and *Fusarium* from 5. The meat of the nuts from which these fungi grew was not obviously discolored or decayed; to the eye it appeared sound. Presumably the fungi had grown into the interior of the nuts through the soft tissues of the germ pore, as illustrated in Figure 2. We do not know when this invasion occurred, but presumably it was some time after the coconuts were harvested and while they still were exposed to an atmosphere of high relative humidity.

*Moisture content*—The moisture contents of samples of meat from 12 freshly opened coconuts, as determined by three methods, are given in Table 2, and moisture contents of samples of grated meat exposed to relative humidities of 75, 80, and 85 per cent are given in Table 3.

### Discussion

The fungi isolated from the husks of coconuts were common saprophytes that one would expect to find in

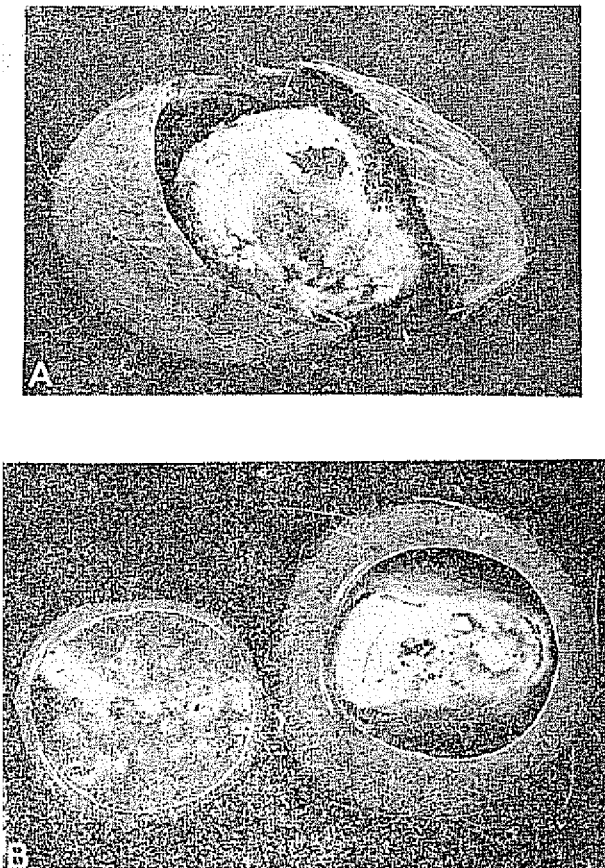


Figure 1. A and B—Coconuts opened to show heavily molded interior.

Table 1.—Numbers and kinds of fungi isolated from the meat of coconuts bought in local markets <sup>a/</sup>

Coconut No	Numbers and kinds of colonies per gram coconut meat						
	Total	<i>A. flavus</i>	<i>A. niger</i>	Cephalo- sporium	Fusarium	Peni- cillium	Yeast
22	257,000	0	0	172,000	0	0	85,000
25	160,500	158,000	72,000	60,000	2,000	3,500	165,000
49	9,500	0	0	7,000	0	0	2,500
68	124,000	0	0	246,000	0	8,000	170,000
69	75,500	0	0	18,500	0	0	27,000
70	93,000	0	0	31,000	0	0	59,000
72	20,000	0	0	3,500	0	0	16,500
73	327,500	0	0	150,000	9,500	0	168,000
74	330,000	0	0	126,000	0	0	204,000
75	15,500	0	18,000	11,000	0	0	16,500
76	12,500	0	0	8,500	0	0	4,000
83	36,500	0	0	19,000	0	0	17,500

<sup>a/</sup> Each figure is an average of four replicate samples from each nut

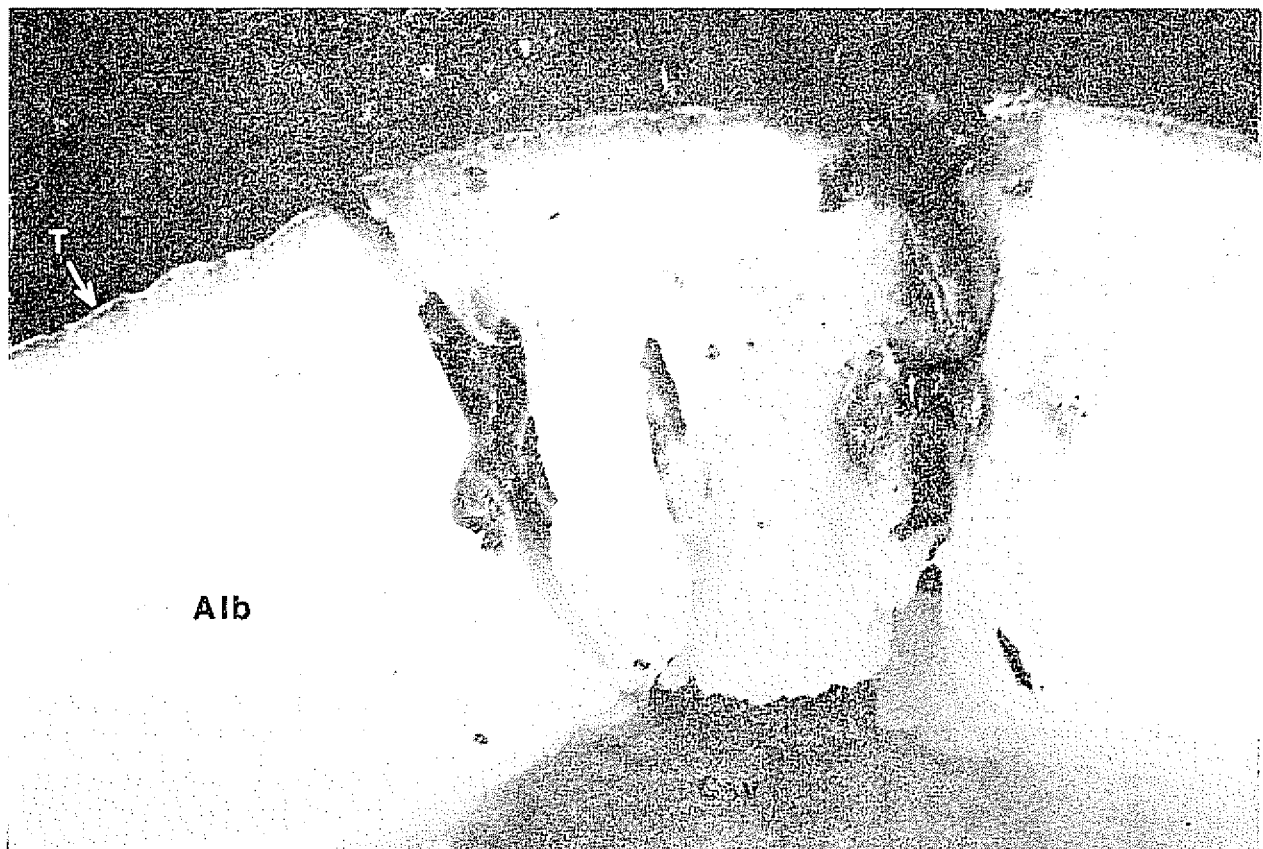


Figure 2.—Section through meat of freshly opened coconut, showing path of invasion by fungus. T = tea. Alb = endosperm, Car = central cavity

Table 2.—Moisture content of fresh coconut meats as determined by three different oven drying methods.

Coconut No	Moisture content of coconut meat as determined by a/ b/		
	Air oven, 103 C, 72 hours	Air oven, 130 C, 3 hours	Vacuum oven, 80 C, 5 mm Hg, 18 hours
	%	%	%
20	35.85	36.02	35.97
21	41.44	41.81	41.62
22	39.26	39.29	39.41
35	37.38	37.29	38.41
36	39.92	40.02	39.98
48	40.03	40.16	39.99
49	36.66	36.73	36.68
50	38.92	39.04	39.01
64	38.14	38.37	38.52
65	36.67	37.17	36.92
78	37.45	37.21	37.49
85	34.37	34.08	33.96
Average	38.01	38.09	38.16
Range	34.37 — 41.44	34.08 — 41.81	33.96 — 41.62

a/ Each figure was an average of 4 replicates

b/ Moisture content is expressed on a wet weight basis

almost any fibrous plant material exposed to conditions favoring fungus invasion. Those found in the meats of nuts with cracked or broken shells included *Aspergillus flavus*, which is of interest because of the possibility of accompanying aflatoxin contamination. Obvious decay of the coconut meat was detected only in the nuts of which the shells were cracked or broken. The presence of *Botryodiplodia* (and, to a lesser extent, *Cephalosporium* and *Fusarium*) in the meat of apparently sound coconuts is interesting but, as of now, of no known practical significance. Further investigation of *Botryodiplodia* is now under way,

The three different methods of determining moisture content gave results in close agreement with one another. From the standpoint of rapidity and convenience, the method involving drying at 130 C for three hours probably is preferable. Judging from the data on equilibrium moisture contents at different relative humidities, a moisture content of 5.0-5.5 per cent, wet weight basis, in copra would be safe for storage, even in a warm climate. A moisture content of 10 per cent or higher would permit invasion of copra by *A. flavus* and possible aflatoxin production.

Table 3.—Equilibrium moisture contents of coconut meats after 15 days exposure to room (30-35%), and 75%, 80%, and 85% relative humidities; each sample consisted of coarsely grated coconut meats dried in laboratory air for 24 hours prior to exposure.

Relative humidity	Moisture content a/	
	Average b/	Range
%	%	%
Room (30-35)	3.43	3.21 — 3.79
75	6.98	6.16 — 8.99
80	9.16	7.45 — 11.08
85	12.62	10.02 — 14.11

a/ Moisture content was determined by drying in oven at 130 C for 3 hours, and is expressed on a weight wt basis.

b/ Each figure was an average of 4 replicates

### Summary

*Alternaria*, *Aspergillus*, *Botryodiplodia*, *Cephalosporium*, *Curvularia*, *Fusarium*, *Pestalotia*, and *Penicillium* species were isolated from the husk (mesocarp) and outer portion of the shell (endocarp) of immature and mature coconuts (*Cocos nucifera* L.). Approximately 100 dehusked coconuts were bought in local markets; the meat (endosperm) of those with cracked or broken shells was heavily invaded and decayed by a variety of fungi, and the meats of many of those with intact shells were invaded by *Botryodiplodia* sp. and a few by *Cephalosporium* and *Fusarium* species, although the meats appeared sound. Pycnidia of *Botryodiplodia* sp. were present in the outer covering (testa) of the meat of some of these. Drying samples at 130 C for three hours appears to be a suitable procedure for determining moisture content of coconut meat or copra. Moisture contents above about 5.0-5.5 per cent, wet weight basis, permit invasion of copra by storage fungi.

### Literature cited

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