Quiescent Endocarpic Floral Communities in Cured Mature Peanuts from Virginia and Puerto Rico

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ABSTRACT

Without hydration, mycelium developed at some incubation temperatures from unseen interior fungi in about two-thirds of the peanut shells (*Arachis hypogaea*) from 1968 Virginia (VA) and Puerto Rico (PR) crops. Few fungi were found in VA and PR seed from unblemished shells. After hydration, fungi were found in 95% of seed in discolored shells and in 40% and 85%, respectively, of VA and PR seed in unblemished shells.

Ten species or genera of fungi were characteristic of one or more of the eight endocarpic communities. Shell communities were more complex. Among the characteristic forms were five toxicogenic types and three peanut pathogens. The PR and VA communities each had three exclusively characteristic forms. Only two forms seemed to invade seeds from shells during hydration.

Aspergillus flavus was rare in PR samples, and in VA samples was not found before hydration. After hydration, *A. flavus* was a characteristic form of VA-unblemished shells and seed. Though no other fungus was found in more than 20% of VA seed, *A. flavus* was found in up to 30% and 50%, respectively, of seed from unblemished and discolored shells. Possibly it was suppressed in discolored VA shells by competition from *Fusarium* spp. and *Rhizopus stolonifera*. Phytopathology 60:1635-1638.

Additional key words: Soil microbiology, ecology of fungi, *Alternaria tenuis, Diplodia gossypina,* microflora.

In the USA, though decay of peanuts in storage is of no major concern, there are still the threats of mycotoxins (2), concealed damage of seed (3), and seed-borne pathogens. Hence, sound bases are needed for classifying endocarpic fungi of peanuts (1) as "toxicogenic," "pathogenic," or "neutral". An interest in this quiescent internal microflora arose with the need for foodstock oilseeds during World War II, but it was not a practical necessity to study the myco-ecology of peanut geocarps until 1961 when aflatoxins from *Aspergillus flavus* Lk. ex Fr. in moldy peanut meal killed thousands of turkey poults in England (8).

Garren & Higgins (3) in 1947 found discrete ecosystems dominated by soil-borne saprophytes in sound, lifted peanut fruit. In Texas in 1956, Norton et al. (10) first showed that ecosystems develop in sound peanuts in the soil. They found *A. flavus* the dominant invader 6 weeks before harvest. Jackson (5) showed that the internal mycoflora of mature peanut seed is usually in or underneath the dead cells of the testae. Seed with concealed damage (3) are exceptions. Joffe & Borut (7) noted that *A. flavus* and all fungi which could be found in peanut fruit at lifting could be found in abundance in soils in which the fruit grew. Thus, in the period 1961-66, many researchers reevaluated the observation (10) that toxicogenic *A. flavus* can readily invade and become quiescent in peanut geocarps, and suspected that other quiescent forms are toxicogenic. As a result, many researchers added to the data on microflora of the peanut fruit.

There is argument as to when *A. flavus,* or any other fungus, establishes itself in the peanut geocarp. McDonald & Harkness (9) incubated fruit 4 days in sterile sand and concluded that few fresh, undamaged peanut seed in Nigeria harbored fungi. Garren (1) and Porter & Garren (11) found *A. flavus* in up to 6% of young, sound peanut fruit and thought, as did Norton et al. (10), that it invaded and became established in the growing geocarp. Gilman (4), using procedures like those used in the USA, put 732 fruit on agar, and 38 yielded fungi; 36, or 5% of the total, yielded *A. flavus*. But to Gilman, "position of the growth suggested aerial contamination".

Garren et al. (2) stressed the quiescent or static nature of peanut endocarpic mycofloral communities in an International Mycotoxin Seminar in July 1968, and the need for careful handling and storing, pointing out the mycotoxin potential inherent in the presence of *A. flavus* and other toxicogenic fungi in endocarpic floras of many mature, lifted peanuts where these floras had been studied in the USA.

We therefore sought to compare the quiescent mycofloral communities in mature, carefully cured peanut fruit grown as far north as they are grown commercially in the USA, with those of fruit grown in the tropics. The objective was to study the ecology and the economic implications of these communities by determining the extent, if any, to which these communities are dominated by toxicogenic or pathogenic fungi. This report emphasizes the ecology of the important toxicogenic species *A. flavus*.

MATERIALS AND METHODS.—Peanuts of the cultivar Va. Bunch 46-2 grown at Holland, Va., were lifted in mid-October of 1968. After lifting, plants were left in windrows for 36 hr, then placed around a pole in stacks 1 m wide, 1.5 m high, and 20 cm above the soil. After 5 weeks in the stacks, fruit were picked with a mechanical picker and stored in burlap bags.

Peanuts of several cultivars grown at Mayaguez, Puerto Rico, were lifted in mid-April of 1969. Fruit were removed by hand and washed. After the fruit had dried for 1 hr in the sun in open-mesh bags, the bags of fruit were packed loosely in burlap bags. These were shipped from San Juan to Washington, D.C., by air express. They were in transit 72 hr. In both cases, the burlap bags with peanuts were stored in laboratories at about 50% relative humidity until the fruit were used in this study.

The procedure for determining the constituents of the endocarpic flora of samples was that used in other studies (2, 11). Two-seeded fruit were shelled, and 1 cm^2 pieces of shell and seed with intact testae were surface-disinfested for 3 min in 0.5% NaOCl and plated (4/plate) on rose bengal-streptomycin agar, a medium which inhibits both fungi and bacteria.

Plates were examined after 7 days' incubation at 25 C. The number of fruit pieces from which (i) no microorganism; (ii) a fungus; or (iii) a bacterium grew were recorded. Almost all fungi were identified to genus or species, but no bacteria were further identified. The readings were converted to isolation frequencies within samples [i.e., an isolation frequency of 15% for *Fusarium* spp. in seed shows that a *Fusarium* sp. grew from 15% of the seed of the particular sample; "isolation density" was used in the same sense in 1968 (11)].

For each sampling, 80 fruit were selected and randomly divided into four replicates. For hydration, the 20 fruit of a replicate were placed on 1-cm^2 metal mesh ("hardware cloth") which held them 3 cm above 1.5 cm of distilled water in the bottom of a 1-liter plastic beaker. The beakers were sealed at the top with a plastic film held on by rubber bands. These hydrators were kept for 5 or 6 days in a BOD incubator, then platings were made of a piece of shell and a seed from each fruit. In a few cases, both seed of a fruit had broken testae; thus, a few samples had one, or at most three, fewer seed than most of the samples.

The following 32 samplings were made with VA or PR fruit with unblemished or discolored shells: 1 to 4, no hydration; 5 to 8, 5 days' hydration at 10 C; 9 to 12, 5 days' hydration at 16 C; 13 to 16, 5 days' hydration at 21 C; 17 to 20, 2 days' hydration at 27 C; 21 to 24, 4 days' hydration at 27 C; 25 to 28, 5 days' hydration at 27 C; and 29 to 32, 6 days' hydration at 27 C.

RESULTS AND DISCUSSION.—Bacteria grew from so few of the fruit pieces that they merited no further consideration. Fungi of 17 different species or genera were recognized. In every sampling, a few growths were not

TABLE 1. Relation of 5 days' hydration at four temp to isolation frequencies of dominant endocarpic fungi in discolored (discol) and unblemished (unblem) shells of Virginia (VA) and Puerto Rico (PR) grown fruit[a]

Fungus	Origin of fruit	Type of shell[b]	Before hydration	Isolation frequencies at various temp			
				10 C	16 C	21 C	27 C
			%	%	%	%	%
No fungus isolated	VA	discol	25	25	15	5	15
	PR	discol	14	10	5	5	0
	VA	unblem	35	30	40	50	35
	PR	unblem	33	0	10	10	0
Alternaria tenuis	VA	discol	0	15	0	5	0
	VA	unblem	40	25	30	10	25
Aspergillus flavus	VA	discol	0	0	0	5	0
	VA	unblem	0	0	0	0	15
	PR	unblem	0	0	0	5	0
Chaetomium globosum	VA	discol	35	5	0	20	35
	PR	discol	0	5	0	0	0
	VA	unblem	0	5	0	25	5
	PR	unblem	3	20	0	10	0
Cylindrocladium sp.	PR	discol	0	5	0	25	10
	PR	unblem	0	0	5	0	0
Diplodia gossypina	PR	discol	24	5	5	15	15
	PR	unblem	8	5	10	15	20
Fusarium spp.	VA	discol	15	10	40	20	15
	PR	discol	29	40	55	30	45
	VA	unblem	8	0	0	0	0
	PR	unblem	25	35	20	40	35
Penicillium spp.	VA	discol	5	0	5	10	0
	PR	discol	11	20	5	10	20
	PR	unblem	9	10	20	0	0
Rhizoctonia bataticola	PR	discol	4	0	5	0	0
	PR	unblem	5	5	15	10	20
Rhizoctonia solani	VA	discol	0	30	0	0	0
	VA	unblem	0	10	0	0	0
Rhizopus stolonifera	VA	discol	10	0	0	25	35
	VA	unblem	0	0	0	0	5

[a] Fungi were considered dominant when they were found at least once in 5% or more of a sample.
[b] When a type of shell is not listed, the fungus was not found at any temp in that type of shell.

easily identified, but in no samples did these unknowns amount to as much as 5%.

Fungi classified as dominants (Tables 1, 2) because they were found at isolation frequencies of 5% or greater were *Aspergillus flavus, Alternaria tenuis* Auct., *Chaetomium globosum* Kunze ex Fr., *Cylindrocladium* sp., *Diplodia gossypina* Cke., *Fusarium* spp., *Penicillium* spp., *Rhizoctonia bataticola* (Taub.) Butler (*Macrophomina phaseoli* [Maubl.] Ashby), *R. solani* Kuehn, and *Rhizopus stolonifera* (Ehr. ex Fr.) Vuill.

The seven species or genera which were present, but never found at isolation frequencies of as much as 5% were *Aspergillus* spp., *A. niger* v. Tieg., *Colletotrichum* sp., *Nigrospora* sp., *Phoma* sp., *Thielavia* sp., and *Trichoderma viride* Pers. ex S. F. Gray.

After hydration at either 21 or 27 C, tufts of mycelium were observed inside some shells. These tufts washed off during surface disinfestation and there was no apparent discoloration of testae or cotyledons, except for the VA seed from which *R. solani* grew. One seed from each fruit was opened and, except where *R. solani* grew from the other seed of the fruit, no discoloration of cotyledons or concealed damage was found.

When the endocarpic communities were named for those fungi found at isolation frequencies greater than 10%, eight distinct communities were apparent:

1) *The Fusarium-Chaetomium-Rhizopus-R. solani-Alternaria community of discolored VA shells.*—Here *Fusarium* seemed to be a medium-temp form, *Chaetomium* and *Rhizopus* high-temp forms, and *R. solani* and *Alternaria* low-temp forms.

2) *The Alternaria-Chaetomium-A. flavus community of unblemished shells.*—Here *Alternaria* seemed to be a poor competitor, as the highest isolation frequency (40%) was in those samplings made before fruits were hydrated. *Aspergillus flavus* seemed to be a high-temp form. The notable differences between the shell communities, and seed communities as well, of these cured VA peanuts and those of freshly dug fruit of the same cultivar (11) are that (i) *Penicillium* and *Trichoderma* are characteristic forms in freshly dug fruits only; and (ii) *Alternaria* and *A. flavus* are characteristic forms in cured fruits only.

3) *The Fusarium-Cylindrocladium-Diplodia-Penicillium community of discolored PR shells.*—Here *Cylindrocladium* seemed to be a medium-temp form; *Penicillium* seemed to be active over the range of hydration temp; and *Diplodia* seemed to be a poor competitor with its highest isolation frequency (24%) before hydration.

4) *The Fusarium-Chaetomium-Diplodia-Penicillium-R. bataticola community of unblemished PR shells.*—Here *Diplodia, Penicillium,* and *R. bataticola* seemed to be high-temp forms and, in contrast to the VA-discolored shell community, *Chaetomium* seemed to be a low-temp form. Also, in contrast to PR-discolored shell community, *Diplodia* seemed to be a good competitor. Possibly the very low frequency of *Cylindrocladium* in the PR-unblemished shell community may be related to this greater activity of *Diplodia* therein.

5) *The A. flavus-Fusarium-Rhizopus community of VA seed from discolored shells.*—Here, in contrast to the VA-discolored shell community, *Fusarium* seemed

TABLE 2. Relation of 5 days' hydration at four temp to isolation frequencies of dominant endocarpic fungi in seed with intact testae from fruit with discolored (discol) or unblemished (unblem) shells of Virginia (VA) and Puerto Rico (PR) grown peanuts[a]

Fungus	Origin of fruit	Type of shell[b]	Before hydration	Isolation frequencies at various temp			
				10 C	16 C	21 C	27 C
			%	%	%	%	%
No fungus isolated	VA	discol	100	65	50	45	5
	PR	discol	71	75	90	50	5
	VA	unblem	98	90	95	90	60
	PR	unblem	97	85	95	55	15
Aspergillus flavus	VA	discol	0	0	25	25	50
	VA	unblem	0	0	0	0	25
Chaetomium globosum	VA	discol	0	5	0	0	0
Cylindrocladium sp.	PR	discol	0	5	0	0	0
	PR	unblem	0	5	2	0	0
Diplodia gossypina	PR	discol	8	15	0	20	65
	PR	unblem	0	0	0	10	70
Fusarium spp.	VA	discol	0	10	0	10	20
	PR	discol	13	0	5	0	10
	VA	unblem	2	5	5	0	5
	PR	unblem	2	5	0	0	0
Penicillium spp.	VA	discol	0	5	0	0	0
	PR	discol	5	0	10	10	15
	VA	unblem	0	5	0	10	0
	PR	unblem	0	5	0	35	10
Rhizoctonia solani	VA	discol	0	10	0	0	0
Rhizopus stolonifera	VA	discol	0	20	20	20	20
	VA	unblem	0	0	0	0	10

[a] Fungi were considered dominant when they were found at least once in 5% or more of a sample.
[b] When a type of shell is not listed in the table the fungus was not found at any temperature in seed in that type of shell.

to be a high-temp form and *Rhizopus* seemed to be active over the range of hydration temp.

6) *The Diplodia-Penicillium-Fusarium community of PR seed from discolored shells.*—Here *Fusarium* seemed to be a poor competitor in that its highest isolation frequency occurred before fruit were hydrated.

7) *The A. flavus community of VA seed from unblemished shells.*—This was the simplest community of all.

8) *The Diplodia-Penicillium community of PR seed from unblemished shells.*—There was much evidence that these were discrete communities with the behavior of one characteristic form usually being influenced by the activity of other forms in the community. Chief among this evidence was the variation from one community to another in the reaction of *Fusarium* spp., *R. stolonifera,* and *D. gossypina* to temp, and the evidence that *D. gossypina* seemed a good competitor in one community and a poor competitor in another.

In addition to *A. flavus,* long known to be a toxicogenic fungus (8), there are four other forms among these dominants which have been shown to sometimes produce metabolites toxic to animals (2). These are *Fusarium* spp., *Penicillium* spp., *C. globosum,* and *A. tenuis.* Also, *R. solani, D. gossypina,* and *R. bataticola* are well known as pathogens of peanut and other plants. This leaves only two characteristic forms of these endocarpic floras, *Cylindrocladium* sp. and *R. stolonifera,* to be classified as "neutral".

Further evidence that these were discrete communities is that *A. tenuis, R. solani,* and *R. stolonifera* were found only in VA samples, and *Cylindrocladium* sp., *D. gossypina,* and *R. bataticola* were found in PR samples only. *Alternaria tenuis,* which was prominent in the VA-unblemished shell community, was not found in seed, though it was found in a few seed of freshly dug fruit (11). Three forms, *A. flavus, Cylindrocladium* sp., and *R. solani,* were the most quiescent in that they were not found in shells or seed until the fruit were hydrated. Neither *C. globosum,* prominent in VA and PR shell communities, nor *R. stolonifera,* prominent in VA-discolored shell community, were found in seed until fruit were hydrated. Thus, only these two forms were clearly shown to have invaded seed from shells.

In a study with freshly dug fruit (11), *A. tenuis* was found in 1% of shells and seed and *A. flavus* was found in about 1% of shells and 5% of seed. This study with carefully cured fruit of the same cultivar grown in the same area showed that *A. tenuis* persisted and proliferated in the shells but not in the seed, and that *A. flavus* persisted in both shells and seed. Also, this study showed *A. flavus* to be more quiescent than *A. tenuis,* but *A. flavus* persisted so that with hydration it became a characteristic species of three of the four VA endocarpic communities and the sole characteristic species of the community in VA seed from unblemished shells. This persistence of *A. flavus* is worthy of much consideration by those involved in the handling of lifted peanut fruit in this area.

More *A. flavus* persisted in VA fruit than in PR fruit. When samples were hydrated for 2, 4, 5, and 6 days at 27 C 18% and 30%, respectively, *A. flavus* was found in VA seed in discolored and unblemished shells. After 5 days' hydration, these isolation frequencies were 50% and 25%, respectively. The 6th day's hydration at 27 C did not increase the amount of *A. flavus* which could be detected in VA samples, nor did the 4 or 6 day's hydration at 27 C reveal any more *A. flavus* in PR samples than that revealed by 5 days' hydration at 21 or 27 C. *Aspergillus flavus* did persist to a limited extent in PR fruit, however. That so little showed up in PR samples hydrated at 21 and 27 C, in comparison with that which showed up in VA samples, could be due to the difference in the procedure for curing the two types of samples, or possibly to the difference in genotypes involved. Possibly competition from two forms exclusively characteristic of PR samples, *D. gossypina* in all PR shells and *R. bataticola* in unblemished PR shells, suppressed growth of *A. flavus* in PR samples. Since Jackson (6) reported suppression of *R. bataticola* by *A. flavus* in endocarpic floras, this leaves *D. gossypina,* found in 65-70% of PR seed at 27 C, to be considered as a competitor of *A. flavus* in the PR samples.

Other than *A. flavus,* only *R. stolonifera* and *Fusarium* spp. reached isolation frequencies as great as 20% in VA seed samples (Table 2). In VA samples, *A. flavus* was predominantly a form of seed rather than shell communities. Apparently, *A. flavus* was effectively suppressed by competition only in discolored VA shells. *Fusarium* spp. and *R. stolonifera* were forms characteristic of discolored VA shells and not of unblemished shells.

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