Endocarpic Microorganisms of Two Types of Windrow-Dried Peanut Fruit (*Arachis hypogaea* L.)

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The endocarpic microorganisms of peanut fruit dried in either a random windrow (plants left as they fell from the digger) or an inverted windrow (plants inverted to expose fruit to sunlight) were different from that of freshly dug fruit. *Chaetomium*, *Penicillium*, *Trichoderma*, *Rhizoctonia*, and *Fusarium* were the dominant fungi found associated with shells (pericarp) of freshly dug fruit. The dominant fungi of shells of windrowed fruit included *Chaetomium*, *Rhizoctonia*, *Fusarium*, *Sclerotium*, and *Alternaria*. Seeds of freshly dug fruit were dominated by *Penicillium* and *Aspergillus*. The only dominant species in seed of windrowed fruit was *Penicillium*. Microorganisms were isolated from shells and seed of freshly dug fruit at a frequency of 79% and 52%, respectively. The percentage of infestation was reduced by drying in the field. This was particularly true of the inverted windrow. The proportion of shells and seed infested with a microorganism was reduced 13% and 36%, respectively, after field drying for 5 to 7 days in random and inverted windrows. Microorganisms were isolated much more frequently from shell pieces (73%) than from seed (36%).

Since the first discovery in 1960 (14) that a fungus commonly associated with peanut fruit (*Arachis hypogaea* L.) could produce a metabolite toxic to some animals, numerous reports on the microorganisms associated with mature fruit (5–7, 10, 12), overmature fruit (3), and damaged fruit (1, 16) have appeared in the literature. As fruits mature in the soil, they become more susceptible to invasion by members of the microbial community of the surrounding soil (13). Thus, there is established an endocarpic (11) [or endoecocarpic (7)] microbial community in the fruit (5, 18). Porter and Garren (18) reported that, from freshly dug fruit, microorganisms were isolated from 90% of the shells and over 63% of the seed. Other reports (2, 3) indicate that fungal invasion may continue after fruits are removed from the soil. Garren (6) showed that isolation procedures, including the use of different media, temperature, and soil additives, influenced the microbial population associated with peanut fruit.

In the now widespread mechanical harvesting of peanuts, plants are frequently lifted from the soil with fruits intact and windrowed in the field until the moisture content is reduced to 20 to 30%. The fruits are then combined, and drying is completed with forced air. During the period of windrow drying, changes in the microbial community associated with peanut fruit have been noted. Jackson (9, 11) found that the "fungal communities from windrowed peanuts were distinctly different from communities which developed in the soil." Dickens (4) found that field-drying was accelerated considerably in the inverted windrow and was less favorable for fungal growth than random windrows.

The objectives of this study were to characterize the dominant endocarpic microorganisms of mature peanut fruit (i) at the time of digging, and (ii) after partial drying in two types of field windrows.

**MATERIALS AND METHODS**

Peanut plants of the cultivar Virginia Bunch 46-2 were grown in a Norfolk fine, sandy loam soil at Holland, Va., in 1966, 1967, 1968, and 1969. Agronomic practices approved for Virginia-type peanuts were used. Planting dates were between 10 and 16 May. Soil fungicides and nematocides were not used. Plants were harvested during the time commercial peanuts were being harvested. Plants were mechani-
Fig. 1. Types of windrow used for field drying of Virginia Bunch 46-2 peanuts at Holland, Va. Plants on right were in an inverted windrow (fruits were directly exposed to sunlight), and plants on left were in a random windrow (most of the fruits were covered by foliage).

Slightly lifted from the soil and windrowed on the following dates: 5, 18, 24 October and 2 November 1966; 3, 10, 20, 25 October and 3 November 1967; 4, 18, 25 October and 1 November 1968; and 2, 9, 21 October 1969.

Plants were exposed for 5 to 7 days in a random windrow or in an inverted windrow (Fig. 1). In the random windrow, plants were left as they fell from the digger with most fruit covered with foliage and in contact with the soil. In the inverted windrow, plants were turned to expose most of the fruit to direct sunlight.

After 5 to 7 days in each windrow, mature hand-picked fruit was shelled, and pieces of shell (ca. 1 cm³) and seed with intact testa were surface-disinfested for 3 min in 0.5% NaOCl and plated (four per plate) on rose bengal-streptomycin-agar (15). Therefore, fungi growing onto this medium from surface-disinfested shells and seed should not have come from surface propagules, but from propagules produced by a thallus well-established therein. After incubation for 7 days at 25°C, most of the thalli that grew on to the medium from shells and seed could be identified. Approximately 1,200 shell pieces and 1,200 seed were plated in each of the 4 years of the study. At each reading, the percentage of shell pieces and seed from which at least one microorganism grew was determined. This was recorded as the proportion of shells and seed infested with some microorganism. We could identify most of the thalli of the fungi that grew from shells and seed. Thus, at each reading, we determined the isolation frequency of the dominant fungi in these shells and seed.

RESULTS

The percentages of shells and seed of freshly dug and windrowed peanut fruit that were infested with some microorganism during the 4 years of the study are given in Table 1. From this data, the following may be deduced. An average of 78.9% (range 72.5 to 88.0) of shells of freshly dug fruit examined during the 4-year period yielded microorganisms. Drying in an inverted windrow reduced the number of shells infested with microorganisms by an average of 20.5%. Drying in a random windrow did not significantly reduce the number of microorganisms associated with shells. An average of 52.1% (range 45.8 to 66.6) of freshly dug seed examined during the 4-year period yielded microorganisms. The proportion of shells and seed infested with a microorganism was reduced by an average of 31.5% by drying in a random windrow and by 60.4% in an inverted windrow. An average of 42.2% fewer seed yielded microorganisms when dried in an inverted windrow than in a random windrow. In the whole of this study, a larger proportion of shells (73%) than seed (36%) was infested with some microorganism.

The dominant shell and seed fungi (classified as a dominant if the isolation frequency from shell or seed was 6% or over) isolated during 1966, 1967, 1968, and 1969 are given in Table 2. The dominant fungi associated with shells of freshly dug peanuts included Chaetomium, Penicillium, Trichoderma, Rhizoctonia, and Fusarium. Chaeto-
mium, Rhizoctonia, Fusarium, Sclerotium, and Alternaria dominated the shells of windrowed fruit. The dominant fungi of seed of freshly dug fruit included Penicillium and Aspergillus while that of seed from windrowed fruit was dominated only by Penicillium.

More than one-fifth of the shells of freshly dug fruit yielded Chaetomium spp., mainly C. glo-
boseum Kunze ex Fr. (Table 2). The isolation fre-
quency of this fungus dropped slightly in the inverted windrow (15.2%) but was not changed.

| Source | Freely dug | Type of windrow |
|--------|-----------|-----------------|
|        | %         | Random | Inverted |
| Shell  | 1966      | 77.3   | 76.1   | 61.2   |
|        | 1967      | 72.5   | 74.0   | 56.5   |
|        | 1968      | 88.0   | 90.8   | 71.7   |
|        | 1969      | 77.8   | 73.1   | 61.6   |
| Seed   | 1966      | 46.1   | 31.5   | 20.7   |
|        | 1967      | 50.0   | 35.5   | 15.5   |
|        | 1968      | 66.6   | 41.6   | 24.7   |
|        | 1969      | 45.8   | 34.4   | 21.8   |

Table 1. Proportion of shells and seed of freshly dug and windrow-dried peanut fruits which were infested with fungi as determined in October 1966 through 1969 at Holland, Va.
in the random windrow (20.5%). The isolation frequency of Chaetomium from seed was much lower than that obtained from shells. In fact, only 3.9% of the seed from freshly dug fruit, 3.7% of the seed from random windrows, and 2.1% from the inverted windrows gave rise to this fungus.

*Penicillium* was isolated at a frequency of 17.5% from shells of freshly dug fruit (Table 2). Each windrow-type greatly reduced the isolation frequency of this fungus. *Penicillium* was the predominant genus in the seed of freshly dug and windrowed fruit, and approximately 28% of all seed plated from freshly dug fruit gave rise to this fungus. Its isolation frequency dropped considerably after drying in the windrow. More shells and seed yielded at least one thallus of *Penicillium* spp. from random windrows than from inverted windrows. Many different species of this fungus were observed; however, on one-half were *P. fumiculosum* Thom. and *P. janthinellum* Biourge.

A higher proportion of freshly dug fruit was infested with *Trichoderma*, mainly *T. viride* Pers. ex Fr. and other species including *Trichoderma*-like isolates that were probably *Gliocladium* spp., than fruit from either windrow type (Table 2). The type of windrow had little effect on the isolation frequency of *Trichoderma*. In all instances, the isolation frequencies of this fungus were higher in shells than in seed.

The isolation frequency of *Rhizoctonia* was greater in windrowed fruit than in freshly dug fruit (Table 2). The average isolation frequency from windrowed fruit was 6.7% compared to 3.5% from freshly dug fruit. More shells than seed were infested with *Rhizoctonia*.

More fruit yielding at least one thallus of *Fusarium* spp. were taken from windrows than were taken from freshly dug lots (Table 2). These thalli included *F. solani* (Mart.) Appel. & Wr. emend. Sny. & Hans., *F. oxysporum* Schlecht. emend. Sny. & Hans., *F. roseum* Lk. ex Fr. emend. Sny. & Hans., and various other species. This fungus was obtained more readily from fruits taken from random windrows than from fruit taken from inverted windrows.

A higher proportion of freshly dug and random-windrowed fruit were infested with *Rhizopus*, mainly *R. stolonifer* (Ehr. ex Fr.) Vuill. and *R. arrhizus* A. Fischer than fruit in the inverted windrow (Table 2). This fungus was found more frequently in shells of random-windrowed fruit (5.5%) than in shells of freshly dug fruit (4.2%). However, twice as many seed with at least one thallus of *Rhizopus* spp. were taken from freshly dug lots than from random windrows.

The isolation frequency of *Sclerotium* from shells was much higher in fruits that were windrowed (8.5%) than from freshly dug fruit (2.7%; Table 2).

More seed than shells yielded at least one thallus of *Aspergillus* spp. (Table 2). These were mainly *A. flavus* and *A. niger* v. Tiegh. *Aspergillus* spp. were isolated more readily from freshly dug fruit than from windrowed fruit. More fruit in the random than in the inverted windrow yielded at least one thallus of *Aspergillus* spp. Some *A. flavus* was isolated each year from shells and seed of freshly dug and windrowed fruit. The isolation frequencies of this species from seed of freshly dug, random, and inverted fruit were 5.2%, 3.9%, and 2.6%, respectively.

Shells with at least one thallus of *Alternaria* spp. were more numerous in windrowed fruit (8.3%) than in freshly dug fruit (0.6%) (Table 2). Seed were rarely infested with this fungus.

In the 4 years of this study, the data (Table 1) show that without exception more shells and seed were infested in 1968 than in any other year. This may be attributed in part to the environmental conditions that prevailed during these years (Table 3). The rainfall in October 1968, was about five times that of 1966, about four times that of 1967, and about two times that of 1969.

### Table 2. Dominant organisms of the endocarpic communities of freshly dug and windrow-dried fruit as determined in October 1966 through 1969 at Holland, Va.

| Organisms          | Avg isolation frequency for the 4 years |
|--------------------|----------------------------------------|
|                    | Freshly dug | Type of windrow |            |
|                    | %           | Random | Inverted |
| Shell              |             |        |         |
| *Chaetomium* spp. | 21.4        | 20.5   | 15.2    |
| *Penicillium* spp. | 17.5        | 2.7    | 3.1     |
| *Trichoderma* spp. | 14.1        | 4.9    | 3.9     |
| *Rhizoctonia* spp.| 6.2         | 10.7   | 9.1     |
| *Fusarium* spp.   | 6.0         | 14.4   | 11.8    |
| *Rhizopus* spp.   | 4.2         | 5.5    | 6.7     |
| *Sclerotium* spp. | 2.7         | 8.0    | 9.0     |
| *Aspergillus* spp.| 1.9         | 1.1    | 1.3     |
| *Alternaria* spp. | 0.6         | 9.5    | 7.0     |
| Seed               |             |        |         |
| *Penicillium* spp. | 27.8        | 8.9    | 6.2     |
| *Aspergillus* spp.| 8.9         | 5.5    | 3.7     |
| *Rhizopus* spp.   | 4.0         | 2.0    | 0.7     |
| *Chaetomium* spp. | 3.9         | 3.7    | 2.1     |
| *Fusarium* spp.   | 2.1         | 2.6    | 2.2     |
| *Trichoderma* spp.| 1.4         | 0.8    | 0.3     |
| *Rhizoctonia* spp.| 0.8         | 4.8    | 2.2     |
| *Alternaria* spp. | 0.1         | 0.2    | 0.0     |
| *Sclerotium* spp. | 0.1         | 1.1    | 0.3     |
The mean temperature for October was slightly higher during 1968 than during the other years. Also, the growing season of 1968 was prolonged because of the lateness of the first killing frost.

The relation of rainfall on the windrow to changes in microbial infestation of peanut shells and seed is shown in Table 4. The differences in microbial infestation of freshly dug fruit and that of samples of the same fruit after 5 to 7 days in the windrow seemed almost inversely proportional to the amount of rain falling on the windrow. If no rain fell on the fruit while they were in the windrow, the proportion of fruit components infested with at least one microorganism decreased greatly and rapidly. For example, no rain fell on those plants dug and windrowed on 5 October 1966 and 25 October 1968, and fewer fruit parts yielding at least one microorganism were found in samples taken from these windrows than in samples taken from the windrows immediately after digging. On the other hand, much rain fell on plants dug on 2 November 1966 and 18 October 1968, and these were the only two instances in which the proportion of fruit parts yielding at least one microorganism in samples taken from windrows was greater than that of the samples taken from the windrows immediately after digging. Frequency of shell infestation was affected more by rain than was the frequency of seed infestation.

The moisture content of freshly dug fruit (determined on wet weight basis after drying for 4 hr at 130 C) during 1967 and 1968 averaged approximately 52% (Table 5). After field-drying for 5 to 7 days, fruit moisture content was reduced to 32% in the random windrow and to 22.7% in the inverted windrow.

**DISCUSSION**

The degree of microbial infestation of shells and seed from windrowed peanut fruit was less than that of freshly dug fruit. Also, fewer shells and seed from fruit dried in inverted windrows were infested with microorganisms than were shells and seed from fruit dried in random windrows. These changes in the microbial community are to be expected, because the environment surrounding the windrowed fruit is different from that of fruit in the soil. At the time of removal from the soil, the fruit moisture averaged over 50%. However, once plants were placed in the windrow, the moisture content dropped rapidly. The drying rate of fruit was much more pronounced in the inverted windrow than in the random windrow. The average moisture content of seed dried for 6 days in the random windrow and inverted windrow in 1967 and 1968 was 31.9% and 22.6%, respectively. Others have reported

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**Table 3. Weather data including rainfall, average temperature, and date of first killing frost at Holland, Va., during October 1966 through 1969**

| Year | Rainfall (inches) | Temp (°F) | Frost * |
|------|-----------------|----------|--------|
| 1966 | 0.97            | 58.1     | Oct. 21 |
| 1967 | 1.31            | 57.6     | Oct. 20 |
| 1968 | 4.72            | 61.1     | Oct. 30 |
| 1969 | 2.69            | 60.5     | Oct. 24 |

* Temperature of 32°F or less.

**Table 4. Relation of amount of rain falling on the windrow to the changes in microorganism infestation of shells and seed of peanuts in those windrows**

| Samples | Rainfall while windrowed (inches) | Change in infestation (- or +)* |
|---------|----------------------------------|--------------------------------|
|         |                                  | Random windrow | Inverted windrow |
|         |                                  | Shell | Seed | Shell | Seed |
| Dug Oct. 5, 1966, plated Oct. 10 | 0.0 | -28.2 | -28.4 | -37.3 | -40.6 |
| Dug Oct. 24, 1966, plated Oct. 31 | 0.11 | -3.1 | -26.4 | -19.5 | -28.9 |
| Dug Oct. 18, 1966, plated Oct. 24 | 0.38 | -2.6 | -29.0 | -32.5 | -62.6 |
| Dug Nov. 2, 1966, plated Nov. 8 | 0.78 | +0.1 | +2.4 | -12.8 | -18.7 |
| Dug Oct. 25, 1968, plated Oct. 31 | 0.0 | -12.7 | -53.5 | -28.9 | -66.2 |
| Dug Nov. 1, 1968, plated Nov. 7 | 0.21 | -1.6 | -48.0 | -61.5 | -74.5 |
| Dug Oct. 4, 1968, plated Oct. 10 | 0.80 | -2.7 | -38.0 | -12.2 | -51.0 |
| Dug Oct. 18, 1968, plated Oct. 24 | 3.48 | +4.2 | -31.3 | -2.5 | -58.0 |

* Comparison is with percentages of infestation by some microorganism of samples taken at the time peanuts were dug, October–November 1966 and 1968, at Holland, Va.

b Comparison is with freshly dug.
similar findings (4, 9). Fruits on plants in a windrow, especially an inverted windrow, undergo rapid dehydration which no doubt retards growth and development of the endocarpic fungi and which may account, in part, for the decrease in prevalence of the microbial populations.

Another, and perhaps even greater, difference between fruit in the soil and fruit on plants in a windrow is relative humidity. The humidity level in the soil is high and remains fairly constant unless the soil moisture is depleted (19). Thus, the subterranean peanut fruits are usually surrounded by an atmosphere of very high humidity, except when surface soil is very dry. On the other hand, fruits on plants in a windrow are exposed to a wide range of relative humidities. Usually the above ground humidity is low during the day except during periods of inclement weather, and the relative humidity at night is high and often exceeds 90%. Most of the fungi found associated with peanut fruit have been called molds. Studies on molds (17) show them to be hydrotolerant, with optimum relative humidities of at least 90%. These high-optimum relative humidities for peanut fruit fungi may account in part for the reduction in the number of microorganisms present in the windrowed fruit. For example, the proportion of seed taken from windrows which yielded at least one thallus of *Penicillium* spp. was only 22 to 32% of that of freshly dug fruit.

These factors, plus others undescribed, may act either separately or in combination to reduce the population of some of microorganisms associated with drying peanut fruit. A reduction in the isolation frequency of a fungus during windrowding may result from dehydration of the fruit and exposure to variable relative humidities. A similar reduction in the inverted windrow may be the result of more rapid dehydration and variable humidities, coupled with the effects of solar radiation.

On the other hand, the isolation frequencies of some of the principal microorganisms of fruit increased while they were drying in the windrow. The isolation frequency of *Fusarium* and *A. naria* increased 4-fold and 16-fold, respectively, when fruits were subjected to windrow drying. Others (11) have also shown that the isolation frequency of *Fusarium* from peanut fruit increased when plants were windrowed. The increase in the isolation frequency of these two genera in windrowed fruit is of significance, especially since Garren et al. (8) recently reported that these fungi were capable of producing mycotoxins.

McDonald and Harkness (16) showed that the isolation frequencies of most of the microorganisms associated with undug peanut fruit increased during periods of rainy weather. In our study, the isolation frequencies of most microorganisms tended to decrease more slowly in shells and seed when fruit drying in the windrows was exposed to appreciable rain than when not so exposed (Table 4). This was probably due to such factors as rehydration of the fruit, increased relative humidities, and lowered light intensities. The possibility of mycotoxin contamination of the fruit also increases under these conditions. Thus, growers must exercise extreme care in the handling of such fruit.

The isolation frequency of the well-known toxicogenic fungus *A. flavus* (14) was relatively low in freshly dug fruit each year of this study. This substantiates the reports of others (2, 3, 5, 7, 10, 12, 16, 18). Windrow drying, particularly the inverted windrow, further reduced the isolation frequency of *A. flavus* but did not eliminate it. Similar findings have been reported by others (9, 16).

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