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

D. MORRIS PORTER AND KENNETH H. GARREN

Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Holland, Virginia 23391, and Department of Plant Pathology and Plant Physiology, Virginia Polytechnic Institute, Blacksburg, Virginia 24061

Received for publication 20 March 1970

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 an endocarpic (11) [or endogecarpic (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).

cally 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 microorganisms 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 40%. 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 5% 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*. *Chaetomium*, *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*.

| Year | Freshly dug | Inverted | Random |
|------|-------------|----------|--------|
| 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   |

**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.**
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              |             |        |          |          |
| Chaetomum 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    | 0.7      |          |
| 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      |          |
| Chaetomum spp.     | 3.9         | 2.7    | 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 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.
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

| 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 |
|---------|--------------------------|
| Dug Oct. 5, 1966, plated Oct. 10 | 0.0 |
| Dug Oct. 24, 1966, plated Oct. 31 | 0.11 |
| Dug Oct. 18, 1966, plated Oct. 24 | 0.38 |
| Dug Nov. 2, 1966, plated Nov. 8 | 0.78 |
| Dug Oct. 25, 1968, plated Oct. 31 | 0.0 |
| Dug Nov. 1, 1968, plated Nov. 7 | 0.21 |
| Dug Oct. 4, 1968, plated Oct. 10 | 0.80 |
| Dug Oct. 18, 1968, plated Oct. 24 | 3.48 |

| Change in infestation (- or +)* |
| Random windrow | Inverted windrow |
|----------------|------------------|
| Shell | Seed | Shell | Seed |
| %    | %    | %    | %    |
| -28.2 | -28.4 | -37.3 | -40.6 |
| -3.1 | -26.4 | -19.5 | -28.9 |
| -2.6 | -29.0 | -32.5 | -62.6 |
| +0.1 | +2.4 | -12.8 | -18.7 |
| -12.7 | -53.5 | -28.9 | -66.2 |
| -1.6 | -48.0 | -61.5 | -74.5 |
| -2.7 | -38.0 | -12.2 | -51.0 |
| +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.

* Comparison is with freshly dug.
TABLE 5. Moisture content (wt. weight basis) of freshly dug and windrow-dried Virginia Bunch 46-2 peanut seed during 1967 and 1968 at Holland, Va.

| Year | Freshly dug | Windrowed |
|------|-------------|-----------|
|      | Random | Inverted |        | Random | Inverted |        |
| 1967 | %      | %        | %     | %      | %        | %     |
| 1968 | %      | %        | %     | %      | %        | %     |

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 windrowing 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 Alternaria 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).

LITERATURE CITED

1. Ashworth, L. J., Jr., and B. C. Langley. 1964. The relationship of pod damage to kernel damage by molds in Spanish peanuts. Plant Dis. Rep. 48:875-878.
2. Austwick, P. K. C., and G. Ayerst. 1963. Groundnut microflora and toxicity. Chem. Ind. (London) 41:55-61.
3. Bampton, S. S. 1963. Growth of Aspergillus flavus and production of aflatoxin in groundnuts. Trop. Sci. 5:74-81.
4. Dickens, J. W., and H. E. Pattee. 1966. The effects of time, temperature and moisture on aflatoxin production in peanuts inoculated with a toxic strain of Aspergillus flavus. Trop. Sci. 8:11-22.
5. Diener, U. L., C. R. Jackson, W. E. Cooper, R. J. Stipes, and N. D. Davis. 1965. Invasion of peanut pods in the soil by Aspergillus flavus. Plant Dis. Rep. 49:931-935.
6. Garren, K. H. 1964. Isolation procedures influence the apparent makeup of the terrestrial microflora of peanut pods. Plant Dis. Rep. 48:344-348.
7. Garren, K. H. 1966. Peanut (Groundnut) microfloras and pathogenesis in peanut pod rot. Phytopathol. Z. 55:359-367.
8. Garren, K. H., C. M. Christensen, and D. M. Porter. 1969. The mycotoxin potential of peanuts (groundnuts): The U.S.A. viewpoint. J. Stored Prod. Res. 5:265-273.
9. Jackson, C. R. 1965. Growth of Aspergillus flavus and other fungi in windrowed peanuts in Georgia. Trop. Sci. 7:27-34.
10. Jackson, C. R. 1965. Peanut-pod mycflora and kernel infection. Plant Soil 23:203-212.
11. Jackson, C. R. 1968. A field study of fungal associations on peanut fruit. Ga. Agr. Exp. Sta. Res. Bull., 26.
12. Joffe, A. Z. 1968. Mycoflora of surface-sterilized groundnut kernels. Plant Dis. Rep. 52:608–611.
13. Joffe, A. Z., and S. Y. Borut. 1966. Soil and kernel mycoflora of groundnut fields in Israel. Mycologia 58:629–640.
14. Lancaster, M. C., F. P. Jenkins, and J. M. Philip. 1961. Toxicity associated with certain samples of groundnuts. Nature (London) 192:1095–1096.
15. Martin, J. P. 1950. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69:215–232.
16. McDonald, D., and C. Harkness. 1964. Growth of Aspergillus flavus and production of aflatoxin in groundnuts. Part IV. Trop. Sci. 6:12–27.
17. Panasenko, V. T. 1944. The ecology of molds. Mikrobiologia 13:158–170.
18. Porter, D. M., and K. H. Garren. 1968. An analysis of the endogeocarpic microflora of peanuts in Virginia. Trop. Sci. 10:100–106.
19. Richards, L. A., and G. Ogara. 1968. Thermocouples for vapor pressure measurements in biological and soil systems at high humidities. Science 128:1089–1090.