28. McDonald, D., and C. Harkness, Trop. Sci. 6:12 (1964).
29. McDonald, D., and C. Harkness, Samaru Res. Bull. 33, 1964, p. 143.
30. McDonald, D., C. Harkness and W.C. Stonebridge, Trop. Sci. 6:131 (1964).
31. Schroeder, H.W., and L.J. Ashworth, Jr., Phytopathology 55:464 (1965).
32. Widstrom, N.W., J. Environ. Qual. 8:5 (1979).
33. Joffe, A.Z., Plant Soil 31:57 (1979).
34. Kushalappa, A.C., J.A. Bartz and A.J. Norden, Proc. Am. Phytopathol. Soc. 3:255 (1976). (Abstr.).
35. Porter, D.M., and K.H. Garren, Trop. Sci. 10:100 (1968).
36. Kulkarni, L.G., Y. Sharief and V.S. Sarma, Indian Farming 17:11 (1967).
37. Suryanaranana-Rao, K.S., and P.G. Tulpule, Nature (London) 214:738 (1967).
38. Doupnik, B., and D.K. Bell, Proc. Am. Peanut Res. Educ. Assoc. 1:80 (1969).
39. Mixon, A.C., and K.M. Rogers, Agron. J. 64:560 (1973).
40. Nagarajan, V., and R.V. Bhat, Appl. Microbiol. 25:319 (1973).
41. Schroeder, H.W., and L.J. Ashworth, Jr., Phytopathol. 55:464 (1965).
42. Nagarajan, V., and R.V. Bhat, Appl. Microbiol. 25:319 (1973).
43. Priyadarshini, F., and P.G. Tulpule, J. Agric. Food Chem. 26:249 (1978).
44. Mixon, A.C., and K.M. Rogers, Agron. J. 64:560 (1973).
45. Mixon, A.C., and K.M. Rogers, Crop Sci. 15:106 (1975).
46. Bartz, J.A., A.J. Norden, J.C. LaPrade and T.J. Demuynk, Proc. Am. Peanut Res. Educ. Assoc. 8:94 (1966) (Abstr.).
47. Bartz, J.A., A.J. Norden, J.C. LaPrade and T.J. Demuynk, Peanut Sci. 5:53 (1978).
48. Zambettakis, C., Oleagineux 30:161 (1975).
49. Zambettakis, C., A. Bockelee-Morvan, F. Waliyar and J. Rossion, Ibid 32:8 (1977).
50. Kushalappa, A.C., J.A. Bartz and A.J. Norden, Proc. Am. Phytopathol. Soc. 3:255 (1976) (Abstr.).
51. Mixon, A.C., Proc. Am. Peanut Res. Educ. Assoc. 8:54 (1976).
52. LaPrade, J.C., and J.A. Bartz, Phytopathol. 62:771 (1972) (Abstr.).
53. LaPrade, J.C., J.A. Bartz, A.J. Norden and T.J. Demuynk, Proc. Am. Peanut Res. Educ. Assoc. 5:89 (1973).
54. Taber, R.A., R.E. Pettit, C.R. Benedict, J.W. Dieckert and D.L. Ketring, Ibid. 5:206 (1973) (Abstr.).
55. Dickens, J.W., J.B. Satterwhite and R.F. Sneed, Ibid. 5:48 (1973).
56. Dieckert, M.C., and J.W. Dieckert, Oleagineux 33:78, 914 (1977).
57. Pettit, R.E., R.A. Taber, O.D. Smith and B.L. Jones, Ann. Technol. Agric. 26:343 (1977).
58. Taber, R.A., R.E. Pettit, C.R. Benedict, J.W. Dieckert and D.L. Ketring, Proc. Am. Peanut Res. Educ. Assoc. 5:206 (1976) (Abstr.).
59. Zambettakis, C., and A. Bockelee-Morvan, Oleagineux 31:219 (1976).
60. Waliyar, F., and M. Abadie, Ibid. 33:447 (1978).
61. Carter, J.B.H., Ann. Appl. Biol. 74:315 (1973).
62. Lindsey, D.L., and R.B. Turner, Mycopathologia 55:149 (1975).
63. Turner, R.B., D.L. Lindsey, D.D. Davis and R.D. Bishop, Mycopathologia 57:39 (1975).
64. Sanders, T.H., Peanut Sci. 4:51 (1977).
65. Sanders, T.H., and A.C. Mixon, Mycopathologia 66:169 (1978).
66. Pettit, R.E., R.A. Taber, O.D. Smith and B.L. Jones. Ann. Tech. Agric. 26:343 (1977).
67. Amaya-F., J., C.T. Young, A.C. Mixon and A.J. Norden, J. Agric. Food Chem. 25:661 (1977).
68. Mixon, A.C., and K.M. Rogers, Agron. J. 64:560 (1973).

Effect of Drought on Occurrence of *Aspergillus flavus* in Maturing Peanuts

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ABSTRACT

Florunner peanuts were grown in experimental plots with soil moisture and soil temperature modified during the last third of the growing period to produce drought, drought with cooled soil, irrigated and irrigated with heated soil treatments. Twice each week, beginning 97 days after planting, random samples were harvested and maturities of individual pods were determined without destroying pod integrity. The nature and quantity of the microflora associated with the pods and kernels were subsequently assessed. Drought and lower soil temperature resulted in maturity distributions containing higher proportions of immature pods. On peanuts with no visible damage to the pod or kernel, colonization by *Aspergillus flavus* was more frequent in immature than mature kernels. Drought stress increased the incidence of *A. flavus* and irrigation decreased it, except when soil temperatures were modified. *A. flavus* infestation was greatly increased at all maturity levels by pod damage.

INTRODUCTION

Peanuts without obvious damage can be invaded by *Aspergillus flavus* and contaminated with aflatoxin in the field before digging. Although the exact circumstances have not yet been fully delineated, severe, prolonged drought stress during the last 4-6 weeks of the growing season favors invasion of peanuts by *A. flavus* (1-3). The relationship between high *A. flavus* invasion in pods and kernels of peanuts and severe drought was noted in South Africa (4). In Texas, peanuts grown under drought conditions contained more aflatoxin before digging than peanuts grown under irrigation (2). The geographical distribution of rainfall and of farms which produced segregation-3 peanuts in North Carolina suggested that drought after peanuts are formed, but before they are dug, is conducive to their infection with *A. flavus* before digging (1). Data from irrigation experiments indicated that the incidence of kernels with visible *A. flavus*, insect damage and aflatoxin were related to drought conditions before digging (1). Timing of the drought period affects the occurrence and extent of *A. flavus* infection. Dickens et al. (1) found that irrigation during the last 2 months of the growing season was just as effective in reducing aflatoxin contamination as was irrigation throughout the growing season. Reduced metabolic activity due to a decrease in pod moisture content under drought conditions has been suggested to increase the susceptibility of peanuts to fungal invasion (5). Several investigators have reported that *A. flavus* activity was restricted above 30% and below 10% kernel moisture content (5-8). Diener et al. (9) found a higher incidence of

TABLE I

Pod Maturity Profile Class Characteristics

Class	Color[a]	Exocarp characteristics
1	White	Initial development, smooth, soft, watery
2	White	Reaching maximal size, soft, watery, longitudinal venation distinct, net venation on basal segments beginning
3	Very pale yellow	Net venation nearly complete, to complete, slightly rough, somewhat resilient
4	Dark yellow	Somewhat rigid, to rigid structure, distinct reticulation
5	Orange to brownish-orange	Rough, rigid, reticulated
6	Reddish-brown to brown	Rough, very rigid, reticulated
7	Black	Rough, very rigid, reticulated

[a]Median class color of mesocarp at or near the basal seed attachment point.

A. flavus invasion in kernels and pods with less than 30% moisture than in kernels and pods with ca. 48% moisture. These moisture contents were associated with immature, mature and overmature categories and suggest that pods at certain maturity levels may be more susceptibile to invasion by *A. flavus*. Because drought stress and maturity have been suggested as factors contributing to peanut susceptibility to *A. flavus*, the occurrence of *A. flavus* invasion in various maturity stages under various conditions of soil moisture was determined.

MATERIALS AND METHODS

Florunner peanuts were planted on May 10, 1980, in 6 18-ft x 40-ft plots equipped with automatic, mechanized roof systems for moisture control (10). A 36-in. row pattern was used and conventional cultural practices were observed. The plots contained Tifton Sandy Loam soil and were constructed to prevent lateral soil moisture movement. Soil temperature and moisture tension under and between the rows at 2, 12 and 24 in. below the surface were measured every 2 hr with copper-constantan thermocouples and Delmhorst gypsum blocks, respectively, throughout the growing season. In each plot, each depth contained at least 10 sensors of each type. Tensiometers were used to determine when irrigation was required. Different treatment regimes, imposed from 94 days after planting (DAP), were irrigated (I); irrigated/heated (I/H); drought-stressed (D); and drought-stressed with cooled soil (D/C). Soil temperatures were elevated with electric heating cable or lowered with epoxy-coated copper tubing through which cool water was pumped. Twice each week, beginning 97 DAP, 4-6 random plants were hand-dug from each plot. The peanuts were removed and classified into maturity stages according to the Pod Maturity Profile (PMP) method of Drexler and Williams (11). The PMP contains 7 maturity stages based on structure and color of the pod mesocarp after partial removal of pod exocarp (Table I). The classifications range from white, unenlarged gynophores in stage 1 to pods with black, rigid mesocarp and black internal pericarp in stage 7.

The various maturity stages were surface-disinfested, plated on either 10% malt-salt agar or *A. flavus* medium (12) and incubated both at 25 and 37 C for ca. 7 days when incidence of *A. flavus* group fungi was determined.

RESULTS AND DISCUSSION

Peanut plants that have grown to cover row middles during adequate moisture conditions will recede as drought becomes more prolonged and severe. This phenomenon exposes more soil to direct sunlight causing an increase in soil temperature. Soil moisture and temperature data collected during the treatment period are shown in Figures

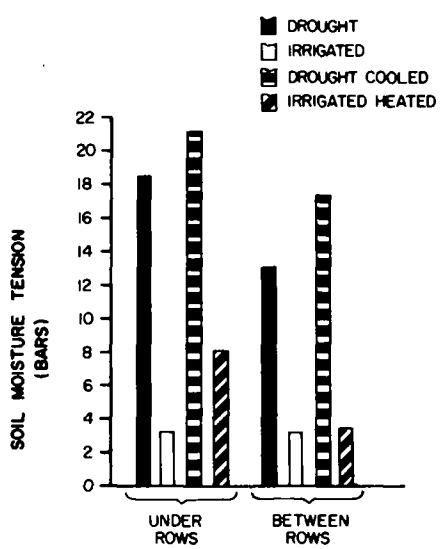

FIG. 1. Mean soil moisture tension 2 in below soil surface for treatment period.

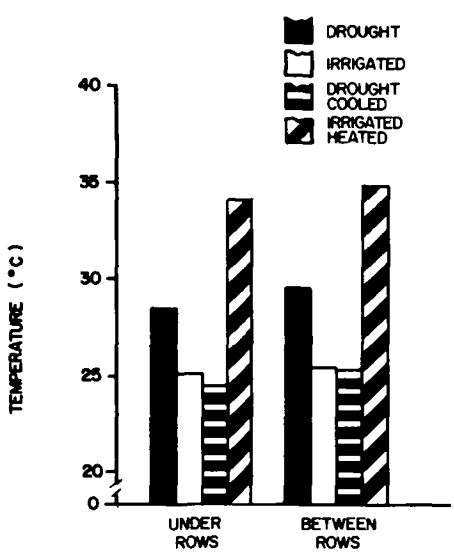

FIG. 2. Mean temperature 2 in below soil surface for treatment period.

1 and 2, respectively. Only data collected at 2 in. are reported herein as this depth corresponds to the fruiting zone of peanuts. Plant uptake of water probably accounts for the consistently higher soil moisture tensions under the rows compared to between the rows. Soil moisture tension in the I treatment plots was similar between and under the rows. A mean soil moisture tension of 3.3 bars at 2 in. below the soil surface in the I treatment plots seems to indicate relatively dry conditions; however, normally low (<0.3 bars) soil moisture tension increased to very high levels just before irrigation and the resulting mean value was disproportionately high. This phenomenon was accelerated in the I/H treatment. The I and I/H treatment plots received water when tensiometers in the plots indicated 0.6 bars tension at ca. 18 in. below the surface. The fact that mean moisture tension of the D/C treatment was consistently higher than that of D alone remains unexplained.

The temperature differences 2 in. below the soil surface between and under rows (no shade vs shade) are evident in all treatments in Figure 2. The differences between and under rows may not affect maturation rates or *A. flavus* invasion of the peanuts distributed on a given plant; however, the difference between I and D temperatures is sufficient to suggest that temperature is a major factor in the relationships between drought stress, peanut maturation and *A. flavus* invasion.

Pod maturation and pod damage were affected by the various treatment regimes (Figs. 3-5). At 111 DAP, the maturity distribution for I and D/C treatments were similar as were the distributions for D and I/H treatments. These similarities possibly reflect early similarities in soil temperature. The treatments with higher soil temperature contained some pods in maturity stage 6 whereas no pods of this maturity were found in the I or D/C treatments. The D and I/H treatment plots also contained damaged pods which were not found in the other treatments. Any visibly damaged pod, regardless of maturity stage, was placed into the damaged category. A large portion of the damaged pods resulted from activity of the lesser cornstalk borer, which has been associated with increased *A. flavus* invasion of peanuts in drought stress situations (1).

At 128 DAP (Fig. 4), some damaged pods were found in 3 or 4 treatments. The percentage damaged remained constant in the D treatment, but no damaged pods were found in the I/H treatment. The maturity distribution in the I/H treatment was more advanced than the I treatment and demonstrates again the effect of increased soil temperature. The D/C maturity distribution is not as advanced as the I treatment and probably reflects the effects of both drought and cooler soil temperature.

At 144 DAP, maturity distributions were similar to those at 128 DAP, except for evidence of an overall increase in maturity. The proportion of stage 3 pods decreased in each treament whereas shifts in other specific stages were related to temperature and moisture availability. Dreyer (13) investigated the growth response of peanuts with different fruiting zone temperature and found

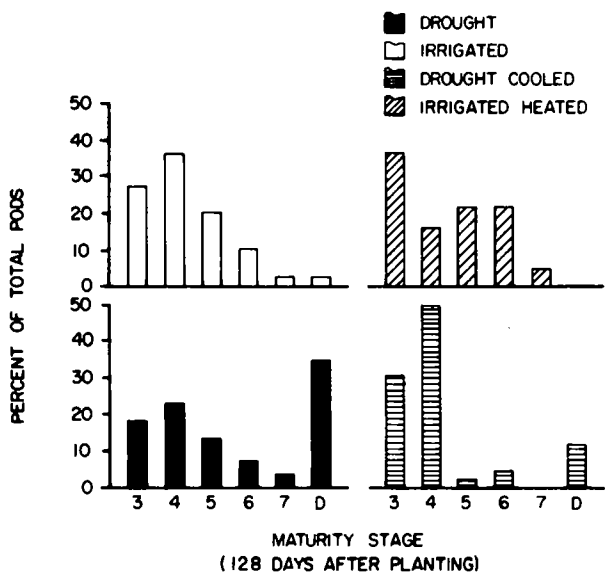

FIG. 4. Peanut maturity distributions at 128 days after planting from various soil moisture and temperature regimes. D = damaged pods of any maturity.

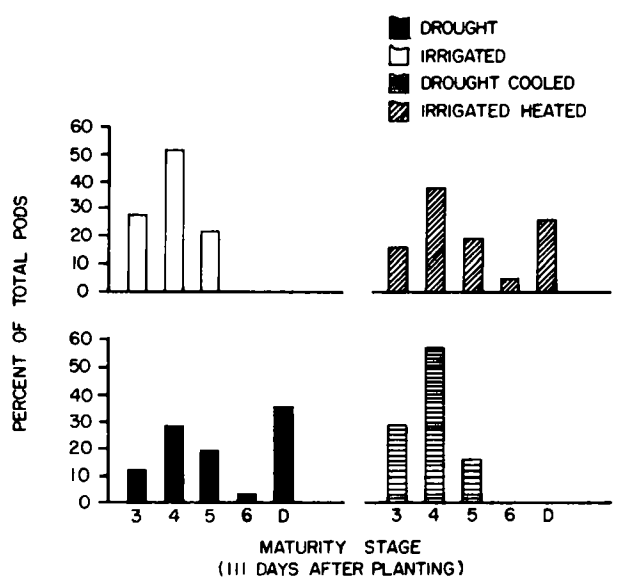

FIG. 3. Peanut maturity distributions at 111 days after planting from various soil moisture and temperature regimes. D = damaged pods of any maturity.

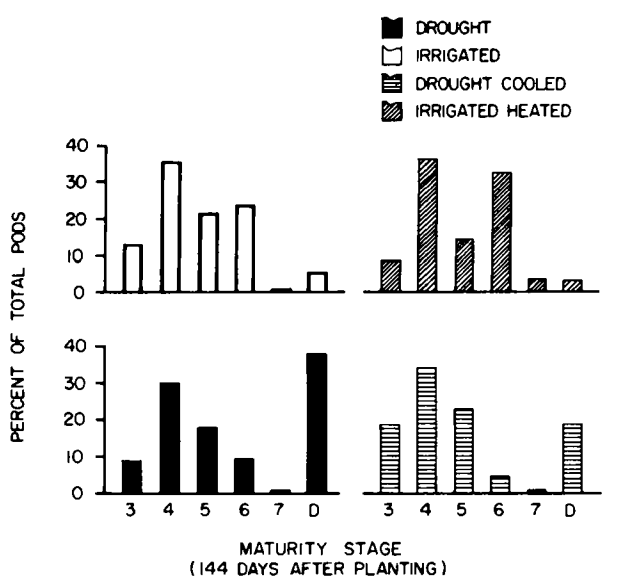

FIG. 5. Peanut maturity distributions at 144 days after planting from various soil moisture and temperature regimes. D = damaged pods of any maturity.

that maturity was advanced by higher temperatures whereas more pegs and pods were formed at lower temperatures.

Incidence of *A. flavus* in maturity stages in all treatments generally increased with time (Figs. 6-8). However, differences between treatments were obvious at 111 DAP or 17 days after the various treatments were imposed (Fig. 6). Pegs (P) and maturity stages 1 and 2 were plated intact because separation of kernels and hulls was generally impossible.

In the I treatment, low infection percentages were found in each maturity stage present whereas in the D treatment, a higher incidence of infection generally occurred. The increased incidence of *A. flavus* in the D treatment is obvious, but increased temperature without drought (I/C) at 111 DAP did not result in a general increase over I in infection percentage in all maturity stages. Drought without increased temperature (D/C) at 111 DAP did not result in increased infection over that found in I.

At 128 DAP (Fig. 7), the incidence of *A. flavus* infection had increased in all treatments, but the smallest increase occurred in the I treatment. In the I and D treatments, the highest infection percentage in undamaged pods was found in pegs. Damaged pods from the 3 treatments in which they occurred had the highest incidence of infection. In the D and D/C treatments, high infection percentages are in some part related to the preponderance of insect-damaged pods. Over 50% of all kernels or pods (P and stages 1 and 2) from the D treatment contained *A. flavus* at 128 DAP (34 days after drought treatment began). This compares to an overall infection percentage of ca. 11% in the I treatment.

At 144 DAP, percentage infection had increased to even higher levels in the D treatment. Kernels from damaged pods continued to be highly infected in the D and D/C treatments with somewhat less infection in damaged in the two other treatments. As at 128 DAP, this difference must be related to the level of damage caused by the lesser cornstalk borer. The low infection percentages found in the I treatment at 111 DAP (Fig. 6) had approximately doubled to an overall average of ca. 14% at 144 DAP.

In the D, D/C and I/H treatments, pegs contained extremely high incidences of infection, indicating an early infection of the developing fruit. A careful examination of the D, D/C and I/H treatment infection percentages may suggest that pegs are infected early, and the infection percentage for other maturity stages is related to survival of the fungus and not necessarily to a new infection at some later maturity stage. Wells and Kruetzer (14) and Griffin and Garren (15) found *A. flavus* associated with flowers and aerial pegs, but the percentage of infection was not nearly as high as found in the study reported here. The data indicate that conditions in D, D/C and I/H treatments were more conducive to infection in all stages of maturity and the highest percentages, except for damaged, occurred in the most immature fruit. This suggests that differences existed in the environment of flowers and pegs in different treatments or that physiology of these organs was sufficiently different in D, D/C and I/H conditions to predispose them to invasion by *A. flavus*.

The data collected in this study provide no indication of why the immature fruit were more highly infected, especially when soil conditions were modified; however, continued studies to elucidate specific environmental and/or physiological factors are in progress.

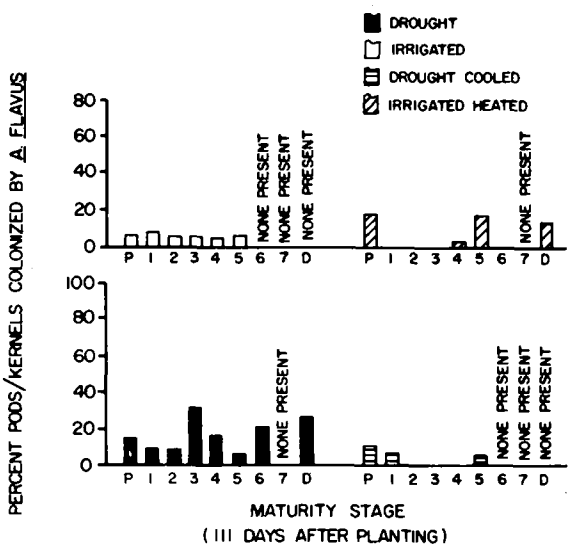

FIG. 6. Percentage of each peanut maturity stage colonized by *Aspergillus flavus* at 111 days after planting. P (pegs) and stage 1 counted as pods due to size. D = damaged of any maturity.

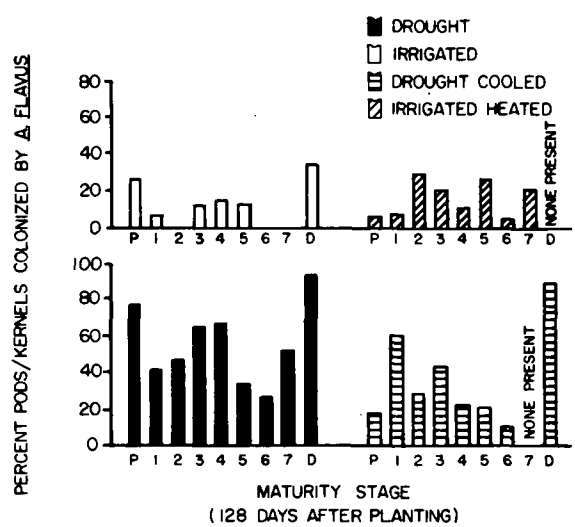

FIG. 7. Percentage of each peanut maturity stage colonized by *Aspergillus flavus* at 128 days after planting. P (pegs) and stage 1 counted as pods due to size. D = damaged of any maturity.

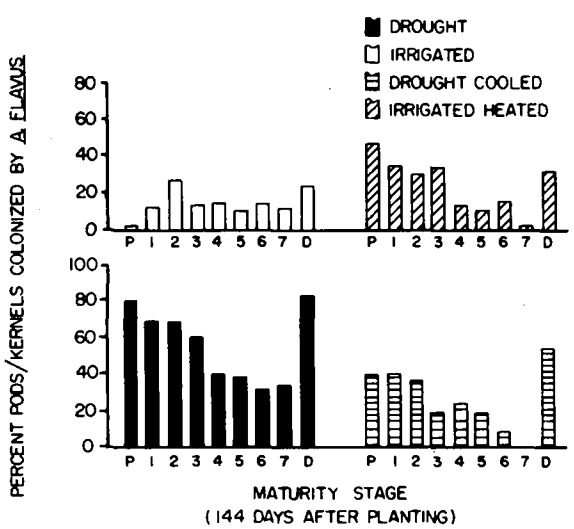

FIG. 8. Percentage of each peanut maturity stage colonized by *Aspergillus flavus* at 144 days after planting. P (pegs) and stage 1 counted as pods due to size. D = damaged of any maturity.

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REFERENCES

1. Dickens, J.W., J.B. Satterwhite and R.E. Sneed, J.Am. Peanut Res. Ed. Assoc. 5:48 (1973).
2. Pettit, R.E., R.A. Taber, H.W. Schroeder and A.L. Harrison, Appl. Microbiol. 22:692 (1971).
3. McDonald, D., and C. Harkness, Trop. Sci. 9:148 (1967).
4. Sellschop, J.P.F., Symp. Mycotoxins in Foodstuff, Agric. Aspects, Pretoria, South Africa, 1954, p. 47.
5. Diener, U.L., and N.D. Davis, AL, Agric. Exp. Sta. Bull. 493 (1977).
6. Austwick, P.K.C., and G. Ayerst, Chem. Ind. (London) 2:55 (1965).
7. McDonald, D., and C. Harkness, Trop. Sci. 6:12 (1964).
8. McDonald, D., and C. Harkness, Ibid. 7:122 (1965).
9. Diener, U.L., C.R. Jackson, W.E. Cooper, R.J. Stipes and N.D. Davis, Plant Dis. Rep. 49:931 (1965).
10. Blankenship, P.D., R.J. Cole and T.H. Sanders, Proc. Am. Peanut Res. Ed. Assoc. 12:46 (1980).
11. Drexler, S.J., and E.J. Williams, Ibid. 11:57 (1979).
12. Griffin, G.J., and K.H. Garren, Phytopathology 64:322 (1974).
13. Dreyer, J., "Growth Response of Peanuts (*Arachis hypogaea* L.) with Different Fruiting Zone Temperatures," Ph.D. dissertation, University of Florida, 1980.
14. Wells, T.R., and W.A. Kreutzer, Phytopathology 62:797 (1972).
15. Griffin, G.J., and K.H. Garren, Ibid. 66:1161 (1976).

Variability in Corn Hybrid Resistance to Preharvest Aflatoxin Contamination

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ABSTRACT

Preliminary field studies suggested evidence for resistance of certain corn hybrids to the preharvest infection of kernels by *A. flavus* and contamination of the kernels with aflatoxin before harvest. A major constraint in evaluating corn hybrids for resistance to the contamination has been the unusual heterogeneity associated with the toxin distribution. A few kernels containing high levels of toxin are routinely responsible for contamination of large sample lots. Extraordinary heterogeneity is also observed in toxin occurrence among fields within a region and among large geographic areas. Edaphic and climatic differences appear to render immature kernels susceptible to aflatoxin accumulation in a discontinuous manner. To reduce intrinsic variability and acquire definitive information on hybrid differences in susceptibility to contamination, several techniques have been developed including: (a) an increase in the number of regional test sites, (b) expansion of the sample sizes, (c) an increase in replication numbers, and (d) elevation of toxin levels in kernels by experimental treatments. Reduction of test variability has allowed for delineation of hybrid differences in aflatoxin resistance. In a diallel set study, genotypes have been identified with heritable qualities of reduced aflatoxin levels in developing kernels. These results provide a basis for further characterization of a genetic facility for resistance to the toxin-producing fungi; these factors have the potential for incorporation into commercial hybrids.

INTRODUCTION

Initial observers of aflatoxin contamination of corn assumed that the toxin accumulated exclusively during storage. Recommendations for controlling the problem stated the well-established techniques for appropriate processing of commodities prior to storage, particularly drying. Subsequent observations of preharvest aflatoxin contamination of corn kernels confronted mycotoxicologists with an entirely new problem. Occurrence of the toxin in the field required consideration of a number of parameters that were relatively alien to traditional storage investigations. To cope with the new dilemma, multidisciplinary groups evolved with technical skills in entomology, corn breeding, plant pathology, agronomy, microbiology and statistics (1). In spite of some of the early problems in acquiring definitive information, the studies provided several important observations: (a) *A. flavus* can infect developing kernels both in southern and midwestern regions of the U.S., but conditions in the South generally favor development of the fungus and toxin production (2); (b) drought and other stress factors appear to render the crop susceptible to attack by fungi (2); (c) kernel damage is routinely associated with *A. flavus* infection (3); and (d) insects feeding on developing kernels often cause the type of damage that is linked to infection by toxin-producing fungi (1).

EARLY OBSERVATIONS OF HYBRID DIFFERENCES

As the awareness of the aflatoxin problem in preharvest corn increased, the question of hybrid variation in susceptibility to *A. flavus* infection became a critical area of inquiry. Initial evidence of hybrid differences in toxin accumulation was detected in an investigation of 6 hybrids grown in South Carolina and Florida (4). The hybrids included 5 South Carolina single crosses developed for the South and a commercial single cross developed for the Corn Belt but widely grown in the South. At maturity, the aflatoxin levels in kernels of the Corn Belt hybrid were significantly higher than in the South Carolina hybrids at both locations. Hybrids that have been developed for the South generally have been selected for enhanced resistance to the corn earworm; this characteristic is routinely expressed through the morphological protection provided developing ears by long, tight husks. Conversely, Corn Belt hybrids are often characterized by shorter, loose husks for rapid ear drying in the field.

In a subsequent investigation of hybrids, 4 varieties were grown in Florida, Georgia, Missouri and South Carolina (5); of the 4 hybrids, 2 were developed for the South and 2 were developed for the Corn Belt. Although the aflatoxin levels in mature kernels were not entirely consistent, most of the samples from hybrids developed for the South had