Population patterns of *Aspergillus flavus* group and *A. niger* group in field soils

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**Abstract**

The horizontal distribution of soil-borne fungi in field soils is an important component of fungal ecology. *Aspergillus flavus* group and its antagonist, the *A. niger* group, had moderate and moderate to high degrees of aggregation (patchiness), respectively, in two peanut fields with a history of aflatoxin contamination: Lloyd’s index of patchiness values ranged from 2.32 to 6.55, where 1.0 is a random pattern. For the *A. niger* group, there was evidence that quadrats in close proximity had similar population densities. Clump size, rarely reported for soil-borne fungi, was found to be 13–52 m\(^2\) for *A. niger* group in one field. Mean population densities of *A. flavus* group in both fields were higher than found previously in most Virginia peanut fields and, along with moderate aggregation, may be related, in part, to the occurrence of aflatoxin in these fields. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** Population patterns; *Aspergillus flavus*; *A. niger*

Information on population densities and horizontal patterns of soil-borne fungi is important to management of pathogens. While information on the degree of aggregation of soil-borne fungi has been presented in recent years (Campbell and Benson, 1994; Griffin and Baker, 1991), there is little or no information of the size and location of patches of aggregated (clumped) or high-density inoculum in field soils (Gilligan, 1995; Mihail and Alcorn, 1987). *Aspergillus flavus* group fungi (*A. flavus* Link and *A. parasiticus* Speare) have been responsible for production of carcinogenic aflatoxins in peanuts (*Arachis hypogaea* L.) in soils worldwide. High levels of *A. flavus* group colonization of peanut fruits in soil and aflatoxin production are favored by hot, dry conditions (Diener et al., 1987; Cotty et al., 1994). Both *A. flavus* group fungi (primarily *A. flavus*) and *A. niger* group fungi may be isolated from peanut kernels, and both may colonize peanut fruits in field soils. These fungi have been considered antagonists of each other by some workers (Ashworth et al., 1965; Joffe, 1969; Diener et al., 1987), and little or no information exists on the relative spatial patterns of pathogens and their antagonists in field soils (Gilligan, 1995). The present investigation was undertaken to determine the horizontal pattern of *A. flavus* and *A. niger* groups in two neighboring peanut fields with a recent history of *A. flavus* group colonization and aflatoxin contamination of peanuts in soil.

Field plots were established in two commercial fields, about 4 ha in size, in Virginia, USA, which contained peanuts that tested positive for aflatoxin contamination in the previous crop. Peanuts and corn have been grown in these fields for several decades. The fields were on the same farm and were located about 300 m apart. The fields were sampled in June after peanuts had been planted in the first week of May. Weed and disease control were good. A 25.4 × 25.4 m\(^2\) lattice plot was established in each field and was divided into 49 blocks, each 3.6 × 3.6 m\(^2\). A soil-core sample (2.5 cm diameter by 7.6 cm deep) was collected at the center of each block. These centric-systematic samples (Milne, 1959), or quadrant samples, were placed in plastic bags with pinholes for gas exchange and transported to the laboratory. Soils were placed at 4–5°C and assayed. Soil samples were mixed by hand for 5 min previous to dilution-plate assay on M3S2B medium, selective for low population densities of *A. flavus* group and *A. niger* group, as used previously (Griffin et al., 1975, 1981). Undesired bacteria and fungi are completely or severely inhibited in this isolation procedure, which uses Botran (2 μg of 2,6-dichloro-4-nitroaniline per ml), NaCl (3%), high temperature (35°C), and antibacterial antibiotics to achieve selective inhibition. This medium was able to recover at 10\(^{-1}\) soil dilution very low population densities from soil [1–10

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Table 1
Advantages, disadvantages, and results obtained for five statistical estimates, tests, or plots on *Aspergillus flavus* group and *A. niger* group population densities in two peanut fields with a history of aflatoxin contamination

<table>
<thead>
<tr>
<th>Statistical estimate, test, or plot</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean population density</strong></td>
<td>Provides information on whether high or low overall population densities are present in a field soil</td>
<td>Does not provide information on aggregation, randomness, or location of fungal population densities in a field soil</td>
<td><strong>Field A</strong></td>
</tr>
<tr>
<td><strong>Lloyd’s index of patchiness</strong></td>
<td>Indicates if propagules exist as aggregates or have a random pattern; 1.0 = random pattern and &gt;1.0 indicates increased aggregation</td>
<td>Does not indicate the location or size (area) of aggregates (= clumps, patches), if present in a field soil</td>
<td><strong>33.9 cfu a per g soil</strong></td>
</tr>
<tr>
<td>(or similar Morrisita’s index of dispersion) (Pielou, 1977; Morrisita, 1959)</td>
<td></td>
<td></td>
<td><strong>318.8 cfu a per g soil</strong></td>
</tr>
<tr>
<td><strong>Moran’s I statistic of autocorrelation</strong> (Upton and Fingleton, 1985)</td>
<td>Indicates if similar population densities are in close proximity in a plot of mapped data</td>
<td>Choice of scale used in sampling may markedly affect results</td>
<td><strong>0.173</strong></td>
</tr>
<tr>
<td><strong>Plot of Morrisita’s index of dispersion versus number of samples in a cluster</strong> (Vandermeer, 1981)</td>
<td>Provides information on clump size (area) in a plot of mapped population density data</td>
<td>Does not provide information for specific population densities</td>
<td><strong>13–26 M²</strong></td>
</tr>
<tr>
<td><strong>Krishner Iyer test</strong> (Pielou, 1977)</td>
<td>Indicates if high population densities are randomly distributed in a plot of mapped data</td>
<td>Does not provide information for specific population densities</td>
<td>(NRJ)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td><strong>(P = 0.0336) a</strong></td>
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<td></td>
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<td><strong>z = 1.823</strong></td>
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<td></td>
<td><strong>13–26 M²</strong></td>
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<td>(NRJ)</td>
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<td><strong>(P &gt; 0.05)</strong></td>
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<td><strong>(P &lt; 0.05)</strong></td>
</tr>
</tbody>
</table>

\( a \) cfu = colony-forming units.
\( b \) Values for ranked data. \( P < 0.0001 \) and \( P < 0.0011 \) indicate strong evidence, \( P = 0.0336 \) indicates marginal evidence and \( P = 0.0986 \) indicates very marginal evidence for samples in close proximity having similar values.
\( c \) Indicates if null hypothesis of random distribution was not rejected (NRJ) or rejected (RJ).
colony-forming units (cfu) per g soil] of *A. flavus* group in extensive evaluation tests (Griffin and Garren, 1974; Griffin et al., 1975). Eight dilution plates at $10^{-1}$ or $10^{-2}$ dilution were incubated at $35^\circ$C for 3 days after which colony counts of *A. flavus* group and *A. niger* group (primarily *A. niger* van Tieghem) were made.

Several approaches have been used to study the pattern of soil-borne fungi in field soils (Campbell and Bensen, 1994). Following soil assays, Lloyd’s index of patchiness (Pielou, 1977) was calculated in the present study to give an estimate of the degree of aggregation of propagules in soil (Table 1). Moran’s *I* statistic (Cliff and Ord, 1973; Upton and Fingleton, 1985) was used to evaluate whether similar population densities occurred in quadrats (samples) located in close proximity in a lattice plot. Moran’s *I* for ranked data, found useful here, is given by:

$$I = \frac{12}{S_0(n^2 - 1)} \sum_i \sum_j W_{ij}(X_i - \bar{X})(X_j - \bar{X})$$

where $X_i$ is the rank of population density quadrat *i*, *n* denotes the number of locations;

$$S_0 = 4c(c - 1),$$

where *c* is the number of columns, and $W_{ij}$ the weight assigned to the join between quadrats *i* and *j* using the rook’s definition (quadrats which have touching sides) of contiguity (Upton and Fingleton, 1985). The analyses for ranked and unranked (= population density) data are similar; as indicated by Upton and Fingleton (1985), the equation for Moran’s *I* using ranked data is a simplification of the equation for Moran’s *I* using unranked data. In this study, the conversion of the population density data to ranked data was straightforward (the lowest population density has the lowest rank): thus, the quadrat with the lowest population density value in the lattice plot for *A. flavus* group in both fields and *A. niger* group in field B, for ranked data (Table 1), and for unranked population density data [Moran’s *I* = 0.135, with a *z*-statistic of 1.87 ($P = 0.0307$)], there was strong and marginal evidence, respectively, for quadrats in close proximity having similar values. For *A. flavus* group ranked data in field A, there was marginal evidence for quadrats in close proximity having similar values (Table 1). For *A. niger* group ranked data in field B, there was very marginal evidence for quadrats in close proximity having similar values (Table 1). For *A. flavus* group ranked data in field B, there was no evidence for clustering of quadrats with similar values. As indicated (Table 1), choice of scale may markedly affect the values obtained for neighboring population densities and the results obtained for Moran’s *I*. Ranked data may be less sensitive to scale effects. Ranked data were examined in these tests because of the large differences in population density among the neighboring higher population density quadrats. For *A. niger* group in fields A and B, plots of Morisita’s index versus numbers of quadrats in a cluster, down the crop rows, indicated a cluster size of one to two quadrats (13–26 m²) and one to four quadrats (13–52 m²), respectively. Across the crop rows, a cluster size of one to two quadrats (13–26 m²) was indicated in both fields. The same was found for *A. flavus* group in both fields, either down or across the crop rows.

Mean population densities for *A. flavus* group in fields A and B were 33.9 and 29.2 colony-forming units (cfu) per g soil, respectively. Population densities ranged from 0 (one quadrat only) to 283.8 cfu per g soil for field A and from 2.5 to 216.8 cfu per g soil for field B. Mean population densities
for *A. niger* group in fields A and B were 318.8 and 301.6 cfu per g soil, respectively. Population densities ranged from 24.9 to 4788 cfu per g soil for field A and from 35.5 to 2656 cfu per g soil for field B. Repeat analysis of random soil samples gave similar results.

Box plots of the lattices in fields A and B for *A. flavus* group and *A. niger* group population densities are shown in Fig. 1. Population densities (cfu per g soil) are indicated on the gray scale bar below each field plot. Rows were oriented from top to bottom.

Box plots of the lattices in fields A and B for *A. flavus* group and *A. niger* group population densities are shown in Fig. 1. Population densities (cfu per g soil) are indicated on the gray scale bar below each field plot. Rows were oriented from top to bottom.

random mingling of high-population density quadrats was not rejected \( P > 0.05 \) in either field A or B for the *A. flavus* group by the Krishna Iyer (1949) test (Table 1). However, the null hypothesis of random mingling of high-population density quadrats of *A. niger* group was rejected \( P < 0.05 \) for both fields. All high-density quadrats of *A. niger* group in field A were clustered into one large patch, measuring 316 m², where the patch is the sum of the areas of all joined high population density quadrats. Similarly, all high-population-density quadrats, except one, in field B were aggregated into one large patch measuring 303 m².
Based on Lloyd’s index of patchiness values, the results indicate that A. flavus group had a moderate level of aggregation. Many soil-borne fungi examined previously had Lloyd’s index of patchiness values of less than 2, indicative of low aggregation (Griffin and Baker, 1991). Values greater than 5, as found here for A. niger group in field A, have not been commonly reported. The spatial scale we used falls about mid-way in the table (for grain or quadrat size) given by Campbell and Bensen (1994) for 44 previous studies. Orum et al. (1997) recently pointed out the need to determine the spatial pattern of A. flavus in adjacent fields in order to understand the factors that determine the spatial structure within and between fields. In the present study, the two fields were separated by about 300 m of woodland. Both fields had similar, moderately aggregated spatial patterns and mean population densities of A. flavus group.

The results obtained for Moran’s I statistic, based on ranked data, provided evidence that similar population densities of A. niger group tended to occur in close proximity in both fields. Similar findings were found for unranked population density data from field B. The clump size range for A. niger group, 13–52 m² was found along the rows. Since this clump size was not found across the rows, cultivation of the field may have contributed to the movement of crop debris or fungal propagules in the field and the orientation of clumps. Rarely has clump size in a field been estimated for a soil-borne fungus (Mihail and Alcorn, 1987). The results of the Krishna Iyer test indicated that the quadrats of A. niger group, having greater than the median population density, were not randomly distributed in each field. Patch size estimates of 316 and 303 m² were relatively large. A. niger group is recognized as a common competitor of A. flavus group (Ashworth et al., 1965; Joffe, 1969; Diener et al., 1987), and, like A. flavus group, is favored by higher temperatures. However, A. niger group may not be as competitive as A. flavus group at very low water potentials (Diener et al., 1987). Conversely, somewhat moister areas or sites may favor the development of high-population densities of A. niger in both fields. Low, moist areas were not apparent in either field, but slight differences in topography may be difficult to detect. The texture of all soil samples appeared similar. Generally, areas in the fields with high A. niger group population densities did not have high population densities of A. flavus group. The high A. niger group population densities found here may be a result of moist periods that occurred during crop debris decomposition.

Overall, the population densities found for A. flavus group were higher than found previously in most Virginia peanut field soils (Griffin and Garren 1974; Griffin et al., 1975; 1981), in which aflatoxin occurrence is uncommon. These higher population densities of A. flavus group, along with the moderate aggregation found for this group, may be related, in part, to the occurrence of aflatoxin in these two fields. Moderate aggregation could lead to patches in the field with high population densities (greater than 200 cfu per g soil). For management considerations, the mixing effects of plowing and disking the field may help break-up aggregates and distribute A. flavus group propagules over a greater volume of soil, possibly diluting these higher p-population densities.

Acknowledgements

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