Patterns of Fungal Association Within Maize Kernels Harvested in North Carolina

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ABSTRACT

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Eleven common maize-infecting fungi grew out from surface-disinfected maize kernels that were collected at harvest in North Carolina in 1977 and plated on malt extract agar. The fungal species were compared for significant association between pairs in all possible combinations. A negative association was observed for 21 of the 55 pairings of fungal species, whereas only three species pairs were positively associated. *Fusarium moniliforme* infected 52% of the kernels and was negatively associated in pairings with all 10 of the other kernel-invading fungi. This finding lends support to the hypothesis that initial kernel infection by *F. moniliforme* serves as a deterrent to the subsequent establishment of other fungi. In contrast, *Aspergillus flavus* and *A. niger* frequently grew out from the same kernel and showed a highly significant positive association (chi-square = 147.37).

Traditionally, mycologists and seed pathologists have assessed the mold profile of a seed lot by incubating a specified number of seeds on agar media or moist paper, then examining them for the presence of fungi (2,3,15,18,21). Occasionally, it is noted that more than one fungus may colonize the same seed (2,14). The individual fungal taxa colonizing a seed are then identified and quantified (e.g., *Aspergillus flavus* Link: Fr. recorded from 25/50 seeds plated, frequency of occurrence = 50%). Missing in the presentation of such data is information on the association patterns of fungal species for individual seeds. For example, if two species each colonize and infect 50% of the seeds examined, are these the same seeds?

Hesseltine et al (8) conducted a mycological survey of 238 maize samples collected in North Carolina within 24 hr of harvest. They examined a total of 11,900 seeds (238 samples × 50 kernels). Recognizing the potential significance of fungal species interactions in kernel infection (13,23), I examined patterns of fungal species associations among 4,450 individual kernels representing 89 of these maize samples. My interest was in identifying potentially interacting fungi rather than the distribution of the *A. flavus* group among maize samples from counties in North Carolina (8).

MATERIALS AND METHODS

Details of the collection and handling of the maize kernel samples were given by Hesseltine et al (8). The seeds were plated on malt extract agar by R. Rogers. After

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6 days of incubation, I examined individual kernels microscopically and identified the fungal species associated with each. Patterns of fungal species association were determined with a chi-square test for independence in a 2 × 2 table (16). There were 55 pairings of 11 fungal species representing all possible combinations in the analysis.

RESULTS

Eleven fungal species were commonly observed on the plates containing maize kernels from North Carolina (Table 1). Each of these species is known to infest preharvest maize ears (19,23). *Fusarium moniliforme* Sheldon, the most common fungus, grew from 52% of the kernels. *A. flavus* and *A. niger* van Tieghem, two species commonly associated with preharvest maize, grew out from 36 and 19% of the kernels, respectively. Only two species of *Penicillium* were observed; *P. funiculosum* Thom was found in 19% of the kernels and *P. oxalicum* Currie was found in 2%. Other fungi observed included *Acremonium strictum* W. Gams (7%), *Alternaria alternata* (Fr.) Keissler (5%), *Nigrospora oryzae* (Berk. & Br.) Petch (4%), *Curvularia lunata* (Wakker) Boedijn (3%), *Trichoderma viride* Pers.: S. F. Gray (3%), and *Rhizopus* spp. (2%).

A total of 55 species pairings were analyzed (Table 1). Twenty-one of the species pairs were negatively associated, whereas only three species pairs were positively associated. No significant associations were found in 31 of the species pairings. *F. moniliforme* was negatively associated with all 10 of the other fungal species considered. *T. viride* was negatively associated in six of 10 pairings with other fungi and showed no positive associations. The most significant negative association involved *F. moniliforme* and *A. flavus* (chi-square = 377.88). In this example, *F. moniliforme* and *A. flavus* both grew out from 524 (12%) of the kernels we plated, considerably fewer than expected (835 = 19%). This is evidence suggesting a negative association. Each species also occurred in kernels apart from one another (e.g., *F. moniliforme* = 1,776; *A. flavus* = 1,093), and neither species grew out from 1,055 (24%) of the kernels. There were significant positive associations between *A. flavus* and *A. niger* (chi-square = 147.37), between *A. niger* and *P. funiculosum* (chi-square = 20.87), and between *P. funiculosum* and *C. lunata* (chi-square = 10.16). For example, *A. flavus* and *A. niger* both grew out from 458 (10%) of the kernels, significantly more than the number expected (305 = 7%). This is evidence suggesting a positive association. Although *A. niger* grew out from 19% of the individual plated kernels (4,450), it was recorded from 28% of the 1,618 kernels invaded by *A. flavus*.

DISCUSSION

F. moniliforme appears to be an early colonist of preharvest maize ears, infecting the kernels before *Penicillium* and other molds (7,11). Caldwell et al (1) reported that *F. moniliforme* is a "better competitor" in preharvest maize than *P. funiculosum*. Initial kernel infection by *F. moniliforme* may serve as an important deterrent to subsequent kernel invasion by other seed-infecting molds as suggested by Wicklow et al (25). It is not surprising that *T. viride* was negatively associated in species pairings. *T. viride* has been extensively studied because of its antagonism and parasitism against other fungi (5).

The significant positive association between *A. flavus* and *A. niger* is of considerable interest; however, no immediate biological explanation is available. *A. flavus*, associated with 36% of the kernels, was relatively abundant within the corn crop, and 60% of the 238 samples were above the 20-ppb guidelines for aflatoxin (7). *A. niger*, a fungus commonly found associated with *A. flavus* in maize (4,8,20), grew out from 19% of the kernels. Although these species have very similar cardinal values for temperature, *A. flavus* will grow at substrate water potential below the minimum for growth of *A. niger* (9). Both fungi are widespread saprobes and produce quantities of amylolytic, proteolytic, pectolytic, and lipolytic enzymes in solid substrate fermentations (17). *A. flavus* and *A. niger* have also been examined in connection with the ability of *A. niger* to interfere with aflatoxin formation (10,23). Colonization of uninjured maize kernels commonly follows establishment of fungi in kernel tissues damaged by insects or birds (12,19,22). When inoculum of *A. flavus* and *A. niger* was simultaneously applied to toothpick-wounded maturing maize kernels, both molds colonized the same damaged kernels, which also became contaminated with substantial amounts of aflatoxin (24). The positive association of *A. flavus* and *A. niger* among maize kernels at harvest might be expected as a result of their common occurrence in wounded tissues and ability to simultaneously spread from such loci with sufficient inoculum potential (6) to infect the surrounding uninjured kernels.

P. funiculosum and *P. oxalicum* represent two of numerous species of *Penicillium* that have been isolated from preharvest or postharvest maize kernels (1). However, of 15 species of *Penicillium* tested in the field, only *P. funiculosum* and *P. oxalicum* were able to colonize preharvest maize ears and infect kernels (1). Positive association between *P. funiculosum* and *A. niger* could involve mycoparasitism. I commonly observed conidial apparati of *P. funiculosum* relating from the conidial heads and conidiophores of *A. niger*. A superior photograph of this phenomenon was recorded by E. Yuill (17).

Pielou (16) warns that because two species are positively associated in a chi-square test does not necessarily mean that one is beneficial to the other. One also needs to understand the ecological requirements of each fungus and evaluate the potential for mutualistic interactions. To learn more about how each fungus affects the other, it would also be desirable to measure the biomass of each species in individual seeds. One could also determine how fungal distributions within the seed are correlated with positive or negative association values. Although a significant chi-square test for independence does not prove that one fungus is beneficial or detrimental to another, the information is important in guiding a search for potentially interactive fungi. Interpretation of these association

Table 1. Significant association between pairs of fungal species infecting preharvest maize kernels from North Carolina, 1977

	Fm[a]	*Tv*	*Pf*	*Cl*	*An*	*Af*	*No*	*As*	*Po*	*Aa*	*Rh*	Total no. significant associations: Negative	Positive
Fm		−[b]	−	−	−	−	−	−	−	−	−	10	0
Tv	−		−	···	−	−	−	−	···	···	···	6	0
Pf	−	−		+	+	−	···	−	···	−	−	6	2
Cl	−	···	+		···	···	···	···	···	···	···	1	1
An	−	−	+	···		+	···	···	···	−	···	3	2
Af	−	−	−	···	+		···	···	···	···	···	3	1
No	−	−	···	···	···	···		···	···	···	···	2	0
As	−	−	−	···	···	···	···		···	−	···	4	0
Po	−	···	···	···	···	···	···	···		···	···	1	0
Aa	−	···	−	···	−	···	···	−	···		···	4	0
Rh	−	···	−	···	···	···	···	···	···	···		2	0

[a] *Fm* = *Fusarium moniliforme*, *Tv* = *Trichoderma viride*, *Pf* = *Penicillium funiculosum*, *Cl* = *Curvularia lunata*, *An* = *Aspergillus niger*, *Af* = *A. flavus*, *No* = *Nigrospora oryzae*, *As* = *Acremonium strictum*, *Po* = *Penicillium oxalicum*, *Aa* = *Alternaria alternata*, and *Rh* = *Rhizopus* sp.

[b] + = Significant positive association, $P<0.05$; − = significant negative association, $P<0.05$; and ··· = no significant association.

patterns requires information on the ecological requirements and biological attributes of each member of the interacting pair.

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