3892

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# AFLATOXIN OCCURRENCE IN 1973 CORN AT HARVEST. II. MYCOLOGICAL STUDIES [1]

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SUMMARY

Since aflatoxin is formed in corn in the field before harvest, our objectives were to determine at harvest (a) the amount of *Aspergillus flavus*-infected corn kernels, (b) the amount of *A. flavus* spores on the surface of corn, (c) the total amount of fungus-infected kernels, (d) the occurrence of *A flavus* spores in and on insects from corn reported in the first paper of this series, and (e) the correlation between *A. flavus* infection and occurrence of aflatoxin. The corn was collected at harvest from seven counties in northeastern South Carolina and dried to less than 13% moisture as quickly as possible.

Of the 152 aflatoxin-positive samples, 120 showed one or more kernels internally infected with *A. flavus* and of the 145 aflatoxin-negative samples, 59 showed infection. Of the 297 samples examined, 276 had one or more kernels with surface contamination of *A. flavus* spores, and in 75 of the samples every kernel was contaminated. When kernels were surface disinfected, 224 samples (50 kernels each) showed 100% internal mold contamination. One or more kernels of 185 samples were infected with *A. flavus*; this number represents 60% of the total samples. Of the 375 insects collected and examined for *A. flavus* from the corn samples, 247 showed *A. flavus* present. Of the 85 rice weevils, 78 were carrying *A. flavus* spores and of the other 290 insects, 165 were contaminated. Besides *A. flavus*, the predominant infecting fungi internally were two species of *Penicillium* and *Fusarium*. Members of the Mucorales were rarely seen.

The occurrence of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare growing on corn plants in the field before harvest has rarely been recorded (Taubenhaus, 1920). From 0.02 to 0.09% infection of corn kernels before harvest was reported by Tuite (1961) from Indiana. The highest single instance had a 22% infection by *A. flavus*. In 1970, Tuite and Caldwell (1971) found an average incidence of 0.4% kernel infection before harvest in corn also in Indiana. The *A. flavus* incidence in southern Indiana counties was 1.2% as compared to 0.2% in northern counties. Rambo et al. (1974) reported a 0.03 to 0.08% incidence of

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field infection by *A. flavus* of corn kernels in southern Indiana in 1971 and 1972 and a 2.7% infection of physically damaged corn kernels in 1972.

At harvest, Lillehoj et al. (1975) found aflatoxin in corn from northeastern South Carolina in 91 fields out of 184 examined and in 61 of 113 samples from trucks delivering to elevators; four samples contained respectively 3,480, 653, 550, and 564 ppb of aflatoxin $B_1$ and $B_2$. If aflatoxin is produced by members of the *A. flavus* series, then these molds should be found in corn in the field as a result of natural contamination. The objective of this second paper was to examine corn from the same samples, plus eight additional samples for the presence of internal infection, and for external occurrence of some fungi, especially those belonging to the *A. flavus* series. At the same time, the relationship between insect damage and infection by *A. flavus* was considered. Here *A. flavus* refers to the *A. flavus* series, and we do not distinguish between varieties of *A. flavus* and species closely related to *A. flavus*.

## MATERIALS AND METHODS

During the 1973 harvest, samples of corn were collected in a 3-wk period in September from a 2,000-square-mile region of northeastern South Carolina. Collections were made either from combines or from trucks unloading at country elevators where farmers were delivering corn from fields in the area. Immediately upon collection, all samples were dried to stop any further mold growth. Part I describes in more detail the area covered and the manner in which samples were collected.

For mycological examination, we took a 100-g subsample from each 10-lb sample collected from combines in the field or from trucks at the elevators. The 100-g sample was placed in a No. 2 paper bag and the top of the bag folded over and stapled. The sample bags were placed in an air-drying oven at 60 C for 6 h. After drying, the samples were transported to Peoria, Illinois. Test experiments before collecting showed that drying at 60 C for 6 h in a stapled paper bag had no adverse effect on any mold flora. The dry (11% moisture) corn samples upon arriving at Peoria were stored at room temperature until examined.

From each bag, 50 kernels of corn were examined for internal infection. They were surface disinfected with a 1% solution of sodium hypochlorite for 1 min with stirring. After removal from this solution, they were washed twice with sterile water. Then five kernels were placed aseptically on each of 10 plates having a culture medium composed of 30 g of malt extract and 15 g of agar. In general this medium did not permit the growth of bacteria. Glucose was omitted from the medium

in order to reduce mold growth. The plates were incubated at 28 C for 7 da, and then colonies were examined under a dissecting microscope to determine the common fungi present.

To determine surface contamination with *A. flavus*, 10 whole kernels were placed five per plate after being removed directly from the collection bag with sterile tweezers. To detect *A. flavus*, the differential *A. flavus* medium (ADM) described by Bothast and Fennell (1974) was used because other fast-growing fungi were present. It consists of 1.5% tryptone, 1.0% yeast extract, 0.05% ferric citrate, and 1.5% agar. A persistent bright yellow-orange pigment on the bottom of the plate about a corn kernel was considered positive proof of *A. flavus* contamination. No attempt was made to determine the number of conidia of *A. flavus* on any one kernel. The plates were incubated at 28 C and read at 3 da to avoid, as much as possible, the development of extensive growth of other fungi and, hopefully, not to record colonies of *A. flavus* originating from hyphae inside the corn kernel.

Insect damage was determined at the time of plating the 50 kernels. This damage appeared as holes in the kernel from which weevils had exited. Sometimes live insects were still in the kernel. The possibility exists that eggs were deposited in some developing kernels but failed to develop even though the kernel was infected with *A. flavus* conidia at the time of egg deposit.

After plating the corn kernels, each residual sample was examined for the presence of both living and dead insects. Each insect was removed with tweezers and, if alive, killed and placed five to a plate on the same malt agar described above. These plates were incubated at 28 C and after 5 to 7 da, the plates were examined under a dissecting microscope and read for fungi.

Aflatoxin assays were made on 10-lb samples of corn ground in a 12-inch Raymond hammermill with screens containing $\frac{1}{8}$-inch perforations. Ground samples were thoroughly blended and assayed for aflatoxin $B_1$, $B_2$, $G_1$, and $G_2$ according to assay methods approved by the Association of Official Analytical Chemists in Official First Action (1975).

## RESULTS

*Fungi isolated from surface disinfected kernels.*—The mold profile was conducted on 217 of a total of 305 samples of corn each represented by 50 kernels plated per sample (TABLE I). Had the nonsporulating colonies been isolated and examined under appropriate culture and temperature conditions, undoubtedly most of these could have been identified.

TABLE I

FUNGI ISOLATED FROM SURFACE DISINFECTED KERNELS OF 217 CORN SAMPLES AT HARVEST

| Fungus | Samples positive |
|---|---|
| *Penicillium* sp. | 94 |
| *Penicillium funiculosum* Thom | 122 |
| *Penicillium* No. 1 | 200 |
| *Fusarium moniliforme* Sheldon | 217 |
| *Trichoderma viride* Per. ex Fries | 69 |
| *Nigrospora* sp. | 103 |
| *Helminthosporium* sp. | 70 |
| *Acremonium strictum* Gams | 188 |
| *Alternaria* sp. | 12 |
| *Cladosporium* sp. | 6 |
| *Aspergillus niger* v. Tiegh. | 14 |
| *Curvularia* sp. | 63 |
| *Chaetomium* sp. | 4 |
| *Absidia* sp. | 2 |
| Dematiaceous sterile colonies | 183 |
| Moniliaceous sp. | 175 |
| *Aspergillus flavus* Link ex Fries | 134 |

Apparently *Fusarium moniliforme* Sheldon occurred in all samples. A further study of the remaining 88 samples not in the profile likewise showed the presence of *Fusarium* in all of them, even though one had only one kernel infected and another had all 50 kernels infected. In 230 samples, infection was quite high with more than 50% of the surface-disinfected kernels yielding *F. moniliforme*. The second most common genus present was *Penicillium*, occurring in all but four samples (TABLE II). Penicillia, however, were represented by two forms; one *P. funiculosum* Thom, a species not known to produce a mycotoxin, and the second as yet not identified, and recorded as *Penicillium* No. 1, both in the Biverticillata-Symmetrica. This species occurred in one or more kernels in 200 of 217 samples and is under further investigation in the ARS Culture Collection maintained at the Northern Laboratory.

Unlike the usual situation in stored corn, the field Penicillia are mostly restricted to these two forms, with only 95 samples showing other species. Generally, the number of kernels infected by *Penicillium* per samples was much less than *Fusarium*.

TABLE II

DISTRIBUTION OF *Penicillium* IN 305 SAMPLES

| Kernels infected: | 0 | 1–10 | 11–20 | 21–30 | 31–40 | 41–50 |
|---|---|---|---|---|---|---|
| No. samples: | 4 | 151 | 98 | 35 | 13 | 4 |
| Per cent: | 1.3 | 49.5 | 32.1 | 11.5 | 4.2 | 1.3 |

Samples with one or more kernels infected with *A. flavus* totaled 184 out of 305. However, one sample had 46 kernels out of 50 infected; this sample had a total aflatoxin level of 3,480 ppb. The second highest aflatoxin-containing sample (653 ppb) showed 19 kernels out of 50 infected with this fungus. Another common fungus growing in the corn kernels was *Acremonium strictum* Gams. Dr. S. J. Hughes of Ottawa, Canada, identified three isolates of this fungus for us. It occurred in 188 of 217 samples examined and, undoubtedly, would have been found in even more samples had it been possible to recognize it when overgrown by *Fusarium* and other more rapidly growing fungi.

Even though a number of fungus colonies were sterile on agar plates, some of the dematiaceous forms undoubtedly belonged in such genera as *Nigrospora, Alternaria,* and *Curvularia.* Another *Aspergillus* species, *A. niger* v. Tiegh. was seldom encountered. Also conspicuously absent were members of the Mucorales (only two samples contained *Absidia* and in each they grew from only one kernel per sample).

There seems to be no correlation between the numbers of Fusaria- and Penicillia-infected corn kernels and the amounts of aflatoxin produced. Even though much of the corn was heavily infected with fungi, almost all the kernels readily germinated.

*Surface contamination.*—When 10 kernels of corn removed aseptically were examined for *A. flavus* on ADM medium, all but 21 of 297 samples contained one or more kernels that had *A. flavus* conidia present. In almost all the corn then, inoculum existed which under proper moisture and temperature conditions could germinate, infect, and possibly produce aflatoxin (TABLE III). There were no significant differences in surface contamination of corn taken from either a combine or a truck. Surface contamination and internal infection by *A. flavus* were also examined from the eight counties from which the corn samples were collected. There was no significant difference between counties as to internal *A. flavus* infection, but surface contamination was significant.

*Number of infected kernels.*—A record was kept of the number of sterile kernels found in each sample based on an examination of 50 kernels (TABLE IV). Apparently almost all the corn collected at the time of harvest was infected with various molds, especially with *Fusarium* and *Penicillium* species.

*Moisture.*—Since moisture levels were established at the time of harvest, they were compared to the amount of *A. flavus* infection and *A. flavus*

TABLE III

COMPARISON OF SURFACE CONTAMINATION WITH MEAN VALUES[a] OF AFLATOXIN CONTAMINATION BASED ON 297 CORN SAMPLES

| Number of kernels with *Aspergillus flavus* on the surface[b] | Number of samples | Aflatoxin $B_1$ (ppb) mean values |
|---|---|---|
| 0 | 21 | <1 |
| 1 | 21 | <1 |
| 2 | 26 | <1 |
| 3 | 15 | 1.0 |
| 4 | 10 | 1.2 |
| 5 | 20 | <1 |
| 6 | 20 | 1.0 |
| 7 | 16 | 4.0 |
| 8 | 27 | 2.5 |
| 9 | 46 | 13.4 |
| 10 | 75 | 44.1 |
| Overall mean | | 4.9 |
| Significance | | **[c] |

[a] All means are the antilog of mean log of aflatoxin.
[b] In each sample, 10 kernels were examined.
[c] ** = Variation between means significant at 1% level.

spores on the kernel surface (TABLE V). It is quite apparent that total mold ($r = 0.31$) and *A. flavus* ($r = -0.25$) infections were negatively correlated with the moisture content of the corn at the time of harvest. Also, surface contamination with *A. flavus* ($r = -0.23$) and insect damage ($r = -0.16$) were negatively correlated with moisture.

*Relationship of aflatoxin to internal infection by A. flavus.*—In TABLE VI, the various ranges of aflatoxin are given with the number of samples showing one or more kernels infected by *A. flavus* and *P. funiculosum*. Excellent agreement is seen between the presence of aflatoxin in a sample

TABLE IV

KERNELS FROM WHICH NO FUNGI WERE ISOLATED IN 305 SAMPLES OF CORN COLLECTED IN SOUTH CAROLINA

| Number of sterile kernels in 50 | Number of samples |
|---|---|
| 0 | 224 |
| 1 | 43 |
| 2 | 23 |
| 3 | 2 |
| 4 | 2 |
| 5–10 | 6 |
| 11–20 | 3 |
| 21–50 | 2 |

TABLE V

MEAN VALUES OF *Aspergillus flavus* INFECTION AND INSECT DAMAGE WITH SAMPLES GROUPED BY MOISTURE

| Moisture (%) | Number of samples | *Aspergillus flavus* surface contamination/10 kernel sample | *Aspergillus flavus* infection/50 kernel sample | Insect damage | Total mold infection/50 kernel sample |
|---|---|---|---|---|---|
| 14.0 | 9 | 9.0 | 9.0 | 3.1 | 49.9 |
| 14.5 | 11 | 8.0 | 3.5 | 1.4 | 49.7 |
| 15.0 | 17 | 8.2 | 5.3 | 0.8 | 49.9 |
| 15.5 | 12 | 7.7 | 4.5 | 0.3 | 49.8 |
| 16.0 | 24 | 7.4 | 2.9 | 0.7 | 48.8 |
| 16.5 | 20 | 5.5 | 2.6 | 0.3 | 49.7 |
| 17.0 | 29 | 6.0 | 3.8 | 0.8 | 49.9 |
| 17.5 | 20 | 6.3 | 2.6 | 0.1 | 49.8 |
| 18.0 | 25 | 5.4 | 1.2 | 0.7 | 49.6 |
| 18.5 | 13 | 6.2 | 1.2 | 0.9 | 49.5 |
| 19.0 | 22 | 3.3 | 1.2 | 0.3 | 49.6 |
| 20.0 | 16 | 3.2 | 1.6 | 0.3 | 48.9 |
| 20.5 | 8 | 4.8 | 0.5 | 0.0 | 48.4 |
| 21.0 | 13 | 4.4 | 1.0 | 0.1 | 48.5 |
| 22.0 | 12 | 6.3 | 1.1 | 0.2 | 48.5 |
| 23.0 | 5 | 7.2 | 1.6 | 0.0 | 48.2 |
| Overall mean | | 6.3 | 2.8 | 0.61 | 49.3 |
| Significance | | *[a] | ** | ** | ** |

[a] * = Variation between means significant at 5% level. ** = Variation between means significant at 1% level.

and *A. flavus* infection ($r = +0.59$). This agreement is not true when *P. funiculosum* is compared to aflation content.

In TABLE VII, the number of infected kernels in each 50-kernel sample is compared to aflatoxin $B_1$ content.

To look at the resuls in another way, there were 119 samples out of 297 which had aflatoxin that also had *A. flavus* infection; in 33 afla-

TABLE VI

RELATIONSHIP OF *Aspergillus flavus* AND *Penicillium funiculosum* INFECTION TO AFLATOXIN CONTENT

| Fungus | Aflatoxin $B_1$ and $B_2$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ND[a] | <10 | 10–19 | 20–39 | 40–71 | 80–159 | 160–319 | 320–639 | >639 |
| *Aspergillus flavus:* | | | | | | | | | |
| Present | 60 | 12 | 26 | 28 | 25 | 18 | 9 | 2 | 2 |
| Absent | 85 | 9 | 8 | 13 | 1 | 0 | 0 | 0 | 0 |
| (Based on 298 samples) | | | | | | | | | |
| *Penicillium funiculosum:* | | | | | | | | | |
| Present | 37 | 9 | 14 | 19 | 20 | 10 | 5 | 0 | 1 |
| Absent | 57 | 7 | 7 | 9 | 7 | 3 | 3 | 2 | 0 |
| (Based on 210 samples) | | | | | | | | | |

[a] ND = not detected.

TABLE VII

MEAN VALUES OF AFLATOXIN $B_1$ WITH SAMPLES GROUPED BY *Aspergillus flavus* INFECTION

| Number of kernels out of 50 infected with *Aspergillus flavus* | Number of samples | Aflatoxin $B_1$ (ppb) |
|---|---|---|
| 0 | 119 | 1.1 |
| 1 | 59 | 2.4 |
| 2 | 28 | 4.7 |
| 3 | 17 | 8.4 |
| 4 | 14 | 25.0 |
| 5 | 9 | 41.0 |
| 6 | 11 | 26.4 |
| 7 | 12 | 23.6 |
| 8 | 7 | 49.2 |
| 9 | 6 | 83.5 |
| 10 | 2 | 37.2 |
| 11 | 2 | 85.0 |
| 12 | 1 | 22.0 |
| 13 | 1 | 47.0 |
| 15 | 1 | 179.0 |
| 16 | 1 | 607.0 |
| 19 | 1 | 247.0 |
| 20 | 1 | 109.0 |
| 25 | 2 | 86.5 |
| 27 | 1 | 72.0 |
| 28 | 1 | 495.0 |
| 46 | 1 | 3,190.0 |
| Overall mean | | 4.9 |
| Significance | | *[a] |

[a] * = Variation between means significant at 1% level.

toxin-positive samples, no *A. flavus* was detected. On the other hand, of the aflatoxin-negative samples, 145 had no *A. flavus* present and in 59 *A. flavus* occurred.

*Insects.*—A total of 305 corn samples were examined (eight of these were not assayed for aflatoxin) for the presence of living or dead insects, including even parts of insects. There were 132 samples that contained one or more insects. In 114 of these, the insects bore *A. flavus*. Besides the *A. flavus* series, fungi growing from inside or on the surface of the insects were represented by many of the same genera of fungi encountered in the corn, including several dematiaceous nonsporulating species. Undoubtedly, a number of other slower growing fungi or ones not growing well under the conditions we used were present. The various fungi and the actual number of samples showing them were: *Penicillium*, 95; *Fusarium*, 36; *A. niger*, 14; *Trichoderma*, 8; *Rhizopus*, 3; *Mucor*, 3; and *Curvularia*, 8. Other fungi seen were *A. ochraceus* Wilhelm and *Absidia*. Interestingly, *P. funiculosum* was found on insects in 43 of the samples.

With respect to the 375 individual insects from the 132 corn samples containing them, 247 had *A. flavus* present. Of these, 85 were identified as rice weevils with 78 showing the presence of *A. flavus*. The other insects, represented mostly by two or three species, totaled 290 individuals of which 169 were positive for *A. flavus*.

At the time of plating the whole corn kernels for presence of molds, examination was made of exit holes of insects from the corn. Rice weevils lay their eggs in the corn kernel, these hatch and feed internally, eventually emerging through exit tunnels. Sometimes eggs do not develop or the immature insect may not have emerged at the time of our examination. The majority of the samples (217) showed no exit holes from the 50 whole kernels examined, but 82 samples did have from 1 to 11 kernels with exit holes. Out of 1,750 kernels examined, 188 possessed exit tunnels. One of the samples with the highest aflatoxin content (653 ppb) contained eight kernels with insect holes. However, some other highly aflatoxin-contaminated samples had no damage due to rice weevils. In fact, of the six samples with the highest aflatoxin content, three possessed six or more kernels with holes and three had none. Of course, corn damaged by earworms was not examined since only whole kernels were purposely selected.

When a representative number of (121) of insect-attacked kernels, that is the kernels with exit holes, were examined for growth of *A. flavus*, 42.6% of the kernels were positive. If one considers samples in which

TABLE VIII

COMPARISON OF INSECT DAMAGE WITH AFLATOXIN CONTAMINATION AND *Aspergillus flavus* INFECTION

| Insect damage (%) | Number | Aflatoxin $B_1$ (ppb) | Kernels positive[a] (%) | |
|---|---|---|---|---|
| | | | *Aspergillus flavus* surface | internal |
| 0 | 221 | 3.6 | 58 | 4.4 |
| 2 | 41 | 6.1 | 71 | 5.2 |
| 4 | 14 | 5.6 | 73 | 6.2 |
| 6 | 4 | 65.1 | 97 | 15.0 |
| 8 | 6 | 24.6 | 85 | 12.0 |
| 10 | 4 | 23.6 | 85 | 21.6 |
| 12 | 3 | 74.9 | 87 | 18.0 |
| 14 | 1 | 109.0 | 100 | 18.0 |
| 16 | 1 | 607.0 | 100 | 38.0 |
| 22 | 2 | 208.5 | 100 | 28.0 |
| Overall mean | | 4.92 | 63 | 5.6 |
| Significance | | **[b] | * | ** |

[a] 50 kernels/sample for *A. flavus* internal; 10 kernels/sample for *A. flavus* surface.

[b] ** = Variation between means significant at 1% level. * = Variation between means significant at 5% level.

one or more kernels had exit holes, 58 contained one or more infected kernels, whereas only 23 had one or more kernels wtih insect damage that had no *A. flavus* growth.

TABLE VIII gives the mean values of aflatoxin levels in corn samples grouped by insect damage; i.e., emergent holes excluding earworm damage. Apparently, as the percentage of insect damage increases, the amount of aflatoxin in corn increases. The increase in insect damage also agrees well with higher levels of surface contamination and internal infection of corn by *A. flavus*.

## DISCUSSION

Although the occurrence of *A. flavus* in corn in the field is not new, the magnitude of the infection reported here certainly is. *Aspergillus flavus* in corn before harvest has been reported by Taubenhaus in Texas (1920), Eddins in Florida (1930), Butler in New South Wales (1947), and Savel'yev in Russia (1962, 1965).

In three papers by Tuite and associates—Tuite (1961), Tuite and Caldwell (1971), and Rambo et al. (1974)—corn was collected from Indiana fields and examined for the kinds of fungi present. In the years 1956, 1957, and 1958, 306 fields were surveyed and of the storage fungi, *A. flavus* was the second most important group after *Penicillium* sp. In the second paper, based on the 1970 crop, more *A. flavus* was found in the southern Indiana counties (1.2% of the kernels infected) than in the northern (0.2%).

In the third study, published in 1974, no *A. flavus* was found in 156 samples of preharvested 1971 corn and only 0.08% from 369 samples in 1972. Again, the only *A. flavus* samples detected came from southern Indiana counties. In physically damaged corn, contamination by *A. flavus* was 0.6 and 1.6% in 1971 and 1972, respectively. In southern counties, the infection rate was 2.7%, but none was found in central or northern counties or in the 1,388 kernels examined from Kentucky. These data suggest that more *A. flavus* can be expected to occur in corn in southern Indiana, and further projecting the findings, one would expect even more infection to be found throughout the South. Our findings support this belief. It also would be enlightening to know whether rice weevils occurred in the fields in the southern counties of Indiana.

The literature indicates that the rice weevil, *Sitophilus oryza* L., not only is an important storage insect of cereals, including corn, but also is the most important of the storage insects attacking corn before harvest in the southern part of the United States.

Reddy (1950) found that optimum moisture to develop *Sitophilus*

*oryza* in stored wheat was only 17.6% but that this species can develop with a moisture level as low as 9%. Powell and Floyd (1960) studied the effect of corn moisture in the field as it relates to developing rice weevils in Louisiana. Their results, based on caging weevils on ears of corn in the field, indicated oviposition began at 65% moisture and occurred at all levels of moisture below this to 15% with the maximum at 35%. None occurred at 78% moisture. According to them, in the lower South, corn is nearly always infected in the field by the rice weevil, and at least one generation develops before harvest. The insect enters the fields from stored corn where undoubtedly it has been in contact with, if not in fact feeding on, moldy corn in which *A. flavus* is growing. The female lays her egg by eating a small hole into the kernel, depositing an egg, and sealing the hole with a plug. Blickenstaff (1960) did an analysis of corn earworm and rice weevil infestation and damage in 10 Georgia cornfields. Earworm infestation showed no significant difference as to location in a field, but location was highly significant for the rice weevil infestation, which was always greater at the margin of fields than in the center.

Kirk (1965) demonstrated that field infections are spotty, and further investigation showed that insect-infected spots were related to the location of stored corn. This observation was true even if the nearest bin was half a mile away from the cornfield and shielded by woods. Kirk further demonstrated that weevils fly at levels below the tops of corn, and when a field is reached they attack the first ears on the first plants encountered. No further migration occurs. His trapping studies indicated that only a few rice weevils left corn bins during June and early July, but by mid-July the exodus from stored corn was in full swing and continued during August. Kirk and Manwiller (1964) also report that some hybrid corn has resistance to attack by rice weevils, but neglected to describe the nature of the resistance. About 20 years ago, the overall infestation of rice weevils in eastern South Carolina was 65% of the ears with 20–30% of the kernels damaged. In 1964, with better hybrids, the rate in this area was 20% of the ears infected and only 5% of the kernels damaged. An interesting study would be to see if there is a correlation between the resistance of corn hybrids to rice weevils and the levels of *A. flavus* infection and amount of aflatoxin. The scheme used by Kirk and Manwiller in their experimental plots should give some answers even though the nature of the resistance in corn is not known.

According to Floyd and Newsom (1956), at harvest time in Louisiana corn damaged by stored grain insects varied from 1% to an average of 27% depending on the ecological area. The overall damage at harvest for

that state was 8.7%. The insect species responsible for damage in the field in order of importance were the rice weevil (*Sitophilus oryza*), pink corn worm (*Pyroderces rileyi*), square-necked grain beetle (*Cathartus quadricollis*), confused flour beetle (*Tribolium confusum*), saw-toothed grain bettle (*Oryzaephilus surinamensis*), and the Angoumois grain moth (*Sitotroga cerealella*).

A further study covering a 3-yr period showed that corn damage by stored insects in Louisiana was high and that the rice weevil caused the principal damage (30%) (Floyd et al., 1959). Corn was often attacked in the dough stage. Shuck damage caused by birds and corn earworm facilitates initial entry of the adult rice weevils into ears.

*Aspergillus flavus* and its close relative, *A. parasiticus*, are known as insect parasites and inhabitants of insects. Batra et al. (1973) found *A. flavus* in the honey stomach of three species of bees. Furthermore, *A. flavus* and some other Aspergilli are parasites on bees and attack their larvae and prepupae. However, *A. flavus* is the most destructive filamentous fungus, causing the death of 14.1% of all alkali bee larvae in 1,733 cells examined. According to Batra et al. (1973), the only fungi of importance in honey bees are *A. flavus* and *Ascosphaera apis* (Maassen ex Claussen) Olive & Spiltoir. *A. flavus* causes the honey bee larval disease referred to as "stone brood," but it is found also on dead adults and in the comb.

Ragunathan, Srinath, and Majumder (1974), studying the rice weevil in India, found storage fungi to be present internally in grubs, pupae, and adults, but not in eggs. *A. flavus* was the most frequently observed fungus species on rice weevils from rice and wheat, whereas *A. restrictus* G. Smith was dominant in weevils from sorghum.

Apparently *A. flavus* is present in, and on, the rice weevil, which can carry a large population of *A. flavus* spores. It becomes a prime candidate, therefore, for introducing *A. flavus* infection into corn in the field. Rice weevils inhabit corn storage bins during the winter where undoubtedly *A. flavus* is present. In summer, the weevils migrate to the nearest corn fields. Since the females lay their eggs in corn kernels at less than 65% moisture, this penetration offers the opening necessary for *A. flavus* infection to enter. Our work shows that kernels attacked by the rice weevil also are typically infected with *A. flavus*.

## LITERATURE CITED

**Anon.** 1972. Changes in official methods of analysis. Natural poisons. 26.B01–26.B03. *J. Assoc. Off. Anal. Chem.* **55**: 426–427.

**Anon.** 1975. *Association of Official analytical chemists. Natural poisons, Chapter 26.* Publ. Assoc. Off. Anal. Chem., Washington, D.C. 24 p.

**Batra, L. R., S. W. T. Batra, and G. E. Bohart.** 1973. The mycoflora of domesticated and wild bees (Apoidea). *Mycopathol. Mycol. Appl.* **49**: 13–44.

**Blickenstaff, C. C.** 1960. Effect of sample location within fields on corn earworm and rice weevil infestation and damage. *J. Econ. Entomol.* **53**: 745–747.

**Bothast, R. J., and D. I. Fennell.** 1974. A medium for rapid identification and enumeration of *Aspergillus flavus* and related organisms. *Mycologia* **66**: 365–369.

**Butler, F. C.** 1947. Ear, cob, and grain rots of maize. *Agric. Gaz. New South Wales* **58**: 144–151.

**Eddins, A. H.** 1930. Corn diseases in Florida. *Fla. Agric. Exp. Sta. Bull.* **210**: 1–35.

**Floyd, E. H., and L. D. Newsom.** 1956. Protection of stored corn with lindane-impregnated sawdust. *J. Econ. Entomol.* **49**: 753–757.

——, **A. D. Oliver, and J. D. Powell.** 1959. Damage to corn in Louisiana caused by stored-grain insects. *J. Econ. Entomol.* **52**: 612–615.

**Kirk, V. M.** 1965. Some flight habits of the rice weevil. *J. Econ. Entomol.* **58**: 155–156.

——, **and A. Manwiller.** 1964. Rating dent corn for resistance to rice weevils. *J. Econ. Entomol.* **57**: 850–852.

**Lillehoj, E. B., W. F. Kwolek, G. M. Shannon, O. L. Shotwell, and C. W. Hesseltine.** 1975. Aflatoxin occurrence in 1973 corn at harvest. I. A limited survey in the Southeastern U.S. *Central Chem.* **52**: 603–611.

**Powell, J. D., and E. H. Floyd.** 1960. The effect of grain moisture upon development of the rice weevil in green corn. *J. Econ. Entomol.* **53**: 456–458.

**Ragunathan, A. N., D. Srinath, and S. K. Majumder.** 1974. Storage fungi associated with rice weevil (*Sitophilus oryzae* L.). *J. Food Sci. Technol.* **11**: 19–22.

**Rambo, G. W., J. Tuite, and R. W. Caldwell.** 1974. *Aspergillus flavus* and aflatoxin in preharvest corn from Indiana in 1971 and 1972. *Cereal Chem.* **51**: 848–853.

**Reddy, D. B.** 1950. Ecological studies of the rice weevil. *J. Econ. Entomol.* **43**: 203–206.

**Savel'yev, V. F.** 1962. On the characteristics of the mycoflora of maize cobs in S. Ukraine. *J. Microbiol. Kiev.* **24**: 39–44. [*Abstr. Rev. Appl. Mycol.* **41**: 652 (1962)].

——. 1965. Composition and main characteristics of the causal agents of diseases of maize under irrigation in the S. Ukraine. Vikorist. Zroshuv Zem. Kiev 'Urozhai' 247. [*Abstr. Rev. Appl. Mycol.* **46**: 514 (1967)].

**Taubenhaus, J. J.** 1920. A study of the black and the yellow molds of ear corn. *Texas Agric. Sta. Bull.* **270**: 3–10.

**Tuite, J.** 1961. Fungi isolated from unstored corn seed in Indiana in 1956–1958. *Plant Dis. Rep.* **45**: 212–215.

——, **and R. W. Caldwell.** 1971. Infection of corn seed with *Helminthosporium maydis* and other fungi in 1970. *Plant Dis. Rep.* **55**: 387–389.

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