APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1983, p. 503–505
0099-2240/83/080503-03$02.00/0

Vol. 46, No. 2

Effect of Pyridazinone Herbicides on Growth and Aflatoxin Release by *Aspergillus flavus* and *Aspergillus parasiticus*†

G. A. BEAN* AND A. SOUTHALL

Department of Botany, University of Maryland, College Park, Maryland 20742

Received 12 May 1983/Accepted 23 May 1983

The influence of pyridazinone herbicides on aflatoxin production by *Aspergillus flavus* and *A. parasiticus* was studied in liquid media. Mycelia production was not affected by 20, 40, or 60 μg of herbicide per ml; however, aflatoxin production by *A. parasiticus* was higher in media with herbicide, whereas *A. flavus* produced lower aflatoxin levels.

Although aflatoxin contamination of corn was once thought to be the result of improper storage conditions, it has been demonstrated that preharvest occurrence of aflatoxins can also occur (4, 6, 11). For example, in the southeastern states, corn growing under water stress or high insect populations appears to be more susceptible to *Aspergillus* spp. and aflatoxin contamination. The increasing use of pesticides, especially with corn grown under no-till conditions, could also be a factor influencing the development of aflatoxins in preharvest corn. Most of the studies involving pesticides and their influence on aflatoxin occurrence have been with insecticides. Rao and Harein (9) reported complete inhibition of aflatoxin production in media containing 10 μg of the insecticide dichlorvos per ml and a 60% reduction in aflatoxins in rice containing the same level of insecticide. Both Lillehoj et al. (7) and Widstrom et al. (12) found that by reducing insect damage in corn with insecticides the levels of aflatoxins were reduced. Draughon (3) has been investigating the influence of insecticides on aflatoxin levels in the field and on growth and aflatoxin production by *A. flavus* in laboratory studies. She found that some insecticides, such as dursban and nellite, increased aflatoxin production by *A. flavus* in liquid media, whereas the insecticide dicrotophos had no influence on aflatoxin production but increased growth of *A. flavus* by 43%. Draughon (3) warned that certain insecticides now being applied to crops for insect control could stimulate growth and aflatoxin production by *Aspergillus* spp.

Few studies have been done on the influence of herbicides or the occurrence of aflatoxin production by *Aspergillus* spp. Cobb (2) did a survey of North Carolina corn in 1977 and was unable to detect a correlation between the use of herbicides and the levels of aflatoxins found in mature corn. In 1979, workers in South Carolina (G. Kingsland, personal communication) found that corn sprayed with Lasso Atrazine and Sutan had a 75% incidence of aflatoxin whereas corn that was not sprayed had a 30% incidence of aflatoxin. The number of *Aspergillus* spp. propagules in the soil in this study was the same in the sprayed and nonsprayed treatments, indicating that the herbicides caused increased susceptibility of the corn plants to *Aspergillus* spp.

The influence of two pyridazinone herbicides {6706–3197 [4-chloro-5-(dimethylamino)-2-α,α,α-trifluro-*m*-tolyl-3(2*H*)-pyridazinone] and 13-338 [4-chloro-5-(dimethylamino)-2-phenyl-3(2*H*)-pyridazinone]} on growth and aflatoxin release by aflatoxigenic strains of *A. flavus* and *A. parasiticus* has been examined. These herbicides were selected because they are relatively inexpensive, are stable at room temperature, possess low mammalian toxicity, and, most important, are systemic (5).

Technical grades of pyridazinone herbicides were used in this study. Each compound was dissolved in 95% ethanol and added to autoclaved growth medium to achieve concentrations of 20, 40, and 60 μg/ml.

The four strains used in this study, *A. parasiticus* NRRL 2999 and ATCC 26871 and *A. flavus* ATCC 15546 and 6432 (U.S. Department of Agriculture, Peoria, Ill.), were maintained on potato glucose agar slants under laboratory conditions. For growth and aflatoxin production studies, a synthetic medium was used which contained sucrose, asparagine ammonium sulfate, potassium phosphate, magnesium sulfate, calcium chloride, and trace amounts of metals (10). A 100-ml volume of medium was added to 250-ml Erlenmeyer flasks which were autoclaved; after cooling, the herbicides and a spore

† Scientific article no. A 3265, contribution no. 6331, of the Maryland Agricultural Experiment Station.

TABLE 1. Effect of pyridazinone herbicides on mycelial dry weight and aflatoxin release by *A. parasiticus* and *A. flavus*

Isolate	Herbicide	Mycelial dry wt[a] and aflatoxin[b] released at given herbicide concn									
		Control		Control (ethanol)		20 μg/ml		40 μg/ml		60 μg/ml	
		Mycelia	Aflatoxin	Mycelia	Aflatoxin	Mycelia	Aflatoxin	Mycelia	Aflatoxin	Mycelia	Aflatoxin
2999	13–338	587	7.29	743	5.88	925	6.25	835	7.26	1,013	7.15
	6706–3179					466	7.59	484	8.29	619	9.16
26871	13–338	512	4.41	846	4.22	605	5.23	799	5.55	608	5.19
	6706–3179					487	5.78	541	5.09	620	5.36
6432	13–338	933	0.56	1,249	0.45	1,035	0.33	1,155	0.34	840	0.34
	6706–3179					1,093	0.34	1,181	0.39	988	0.35
15546	13–338	970	1.29	1,072	1.82	1,212	1.10	1,095	1.17	930	0.99
	6706–3179					1,098	0.86	978	0.90	943	0.97

[a] Expressed as milligrams (dry weight) per flask. Values are means of three replicate samples.
[b] Expressed as milligrams of total aflatoxin per milligram (dry weight).

suspension of the fungus (1.5×10^6 to 3.0×10^6 spores per flask) were added. The cultures were incubated without agitation for 8 days at 25°C in the dark. The mycelial mats were removed from each flask and freeze-dried, and the dry weight was determined. There were three replications for each treatment. A single-day incubation period was used based on the studies of Chipley and Uraih (1) and Moss and Badii (8), who found that the greatest difference between control and inhibitor-treated cultures was after 8 days.

The pH of the culture filtrate was recorded, the filtrate was extracted with chloroform, and the extract was reduced to dryness on a rotary evaporator. Aflatoxins were dissolved in chloroform and separated by thin-layer chromatography on Silica Gel 60-coated plates, using chloroform-acetone-water (88:12:1) as the developing solvent. The spots representing aflatoxin B_1 and G_1 were removed from the plate and quantitated with a Gilford model 240 spectrophotometer.

The influence of pyridazinone herbicides on growth and aflatoxin release by *Aspergillus* spp. is summarized in Table 1. *A. parasiticus* isolates 2999 and 26871 differed from the *A. flavus* isolates both in the amount of mycelia and in the level of aflatoxins released. The *A. flavus* isolates produced more mycelia but less aflatoxin than did the *A. parasiticus* isolates. There did not appear to be any difference in the toxicity of the two herbicides on growth of the fungi except that isolate 2999 was more sensitive to herbicide 6706–3179 than to herbicide 13–338 as indicated by a decrease in mycelium production at comparable levels of the two herbicides.

Of greater importance in this study was the effect of these herbicides on aflatoxin release. In Table 1, the influence of pyridazinone herbicides on aflatoxin production is expressed in terms of the level of aflatoxin released in relation to the amount of mycelial growth. None of the differences observed in aflatoxin release were statistically significant; the isolates of the same species reacted the same to the herbicides, except isolate 2999 had a slightly higher level of aflatoxin release when herbicide 6706–3179 was present in the medium as compared with herbicide 13–338. What was unexpected in this study was the difference in response of the two *Aspergillus* spp. to the herbicides. Whereas the *A. parasiticus* isolates had approximately 13% higher aflatoxin levels in herbicide-amended medium (relative to the control), the *A. flavus* isolates had a 6% lower level of aflatoxins.

We suggest from this study that the application of systemic herbicides, such as pyridazinone, could have an influence on preharvest aflatoxin contamination by directly influencing aflatoxin production by the fungus. Draughon (3) found similar results with some insecticides that were tested. However, and depending upon which isolates or species of the fungus are present in an area, there is likely to be an increase in aflatoxin production or else a decrease in aflatoxin production. Herbicides would be of greater importance if used to reduce or eliminate the stress of weed species growing in competition with corn and thus increase the resistance of corn to *Aspergillus* spp. and aflatoxin contamination. In any field or laboratory studies dealing with growth and aflatoxin production by *Aspergillus* spp., the inherent variability between *A. flavus* and *A. parasiticus* and isolates of the same species should be recognized in the design of the experiment.

We thank J. B. St. John, U.S. Department of Agriculture, Beltsville, Md., for supplying the pyridazinone herbicides used in this study.

LITERATURE CITED

1. **Chipley, J. R., and N. Uraih.** 1980. Inhibition of *Aspergillus* growth and aflatoxin release by derivatives of benzoic acid. Appl. Environ. Microbiol. **40**:352–357.
2. **Cobb, W. Y.** 1979. Aflatoxin in the southeastern United States: was 1977 exceptional? Bull. Assoc. Food Drug Off. **43**:99–107.
3. **Draughon, F. A.** 1983. Control or suppression of aflatoxin production with pesticides. South. Coop. Ser. Bull. **279**:81–86.
4. **Hesseltine, C. W., O. L. Shotwell, W. F. Kwolek, E. B. Lillehoj, W. K. Jackson, and R. J. Bothast.** 1976. Aflatoxin occurrence in 1973 corn at harvest. II. Mycological studies. Mycologia **68**:341–353.
5. **Klingman, G. L., F. M. Ashton, and L. J. Noordhoff.** 1979. Herbicide handbook, 4th ed., p. 274–275, 313–315. John Wiley & Sons, Inc., New York.
6. **Lillehoj, E. B., D. I. Fennell, and W. F. Kwolek.** 1976. *Aspergillus flavus* and aflatoxin in Iowa corn before harvest. Science **193**:495–496.
7. **Lillehoj, E. B., W. F. Kwolek, A. Manwiller, J. A. Durrant, J. C. Laprade, E. S. Horner, J. Reid, and M. S. Zuber.** 1976. Aflatoxin production in several corn hybrids grown in South Carolina and Florida. Crop Sci. **16**:483–485.
8. **Moss, M. O., and F. Badii.** Increased production of aflatoxins by *Aspergillus parasiticus* Speare in the presence of rubratoxin B. Appl. Environ. Microbiol. **43**:895–898
9. **Rao, H. R. G., and P. K. Harein.** 1972. Dichlorvos as an inhibitor of aflatoxin production in wheat, corn, rice and peanuts. J. Econ. Entomol. **65**:988–990.
10. **Reddy, T. V., L. Viswanathan, and T. A. Venkitasubramanian.** 1971. High aflatoxin production on a chemically defined medium. Appl. Microbiol. **22**:393–396.
11. **Shotwell, O. L., W. F. Kwolek, and C. W. Hesseltine.** 1981. Aflatoxin in freshly harvested 1979 Georgia corn and formation after collection. J. Assoc. Off. Anal. Chem. **58**:980–982.
12. **Widstrom, N. W., E. B. Lillehoj, A. N. Sparks, and W. F. Kwolek.** 1976. Corn earworm damage and aflatoxin B_1 on corn ears protected with insecticide. J. Econ. Entomol. **69**:677–679.