examined by mass spectrometry. However, no tryptophan derivatives were found with either column. Additional studies are intended to further explore this question.

Other Hydrolyzed Protein Preparations. The commercial PIB-7 hydrolyzed protein bait is produced by using an acid hydrolysis. There are indications that enzyme-hydrolyzed protein baits are more effective as attractants. Mass spectrometry–capillary GLC analysis was also carried out on the vacuum steam volatile oil from an enzymatic hydrolyzed brewers yeast protein (Autolysate). This showed many similarities to the acid-hydrolyzed protein. Phenylacetaldehyde and acetic acid were again prominent volatiles. Methional was also present. There were much lower amounts of sugar degradation products and higher concentrations of aliphatic acids, particularly isobutyric, isovaleric, hexanoic, octanoic, and decanoic acids.

ACKNOWLEDGMENT

We thank Dr. K. S. Hagen of the University of California for advice and helpful discussion and L. Lepow of the California/USDA Medfly Eradication Program for Preliminary tests on samples.

Registry No. I, 21834-92-4; II, 85407-25-6; acetaldehyde, 75-07-0; 2-methylpropanol, 78-84-2; 2-methylbutanal, 96-17-3; 3-methylbutanal, 590-86-3; 2-methylbutanol, 137-32-6; 3-methylbutanol, 123-51-3; acetic acid, 64-19-7; butyric acid, 107-92-6; 3-methylbutyric acid, 503-74-2; pentanoic acid, 109-52-4; hexanoic acid, 142-62-1; levulinic acid, 123-76-2; methyl levulinate, 624-45-3; ethyl levulinate, 539-88-8; γ-pentalacetone, 108-29-2; γ-hexaloctone, 695-06-7; dimethyl sulfide, 75-18-3; methional, 3268-49-3; 3-phenylthiophene, 2404-87-7; benzaldehyde, 100-52-7; phenylacetaldehyde, 122-78-1; guaiacol, 90-05-1; 2-phenylethanol, 60-12-8; 2-phenyl-2-butenal, 4411-89-6; 4-methyl-2-phenyl-2-pentenal, 26643-91-4; 4-methyl-2-phenyl-2-hexenal, 26643-92-5; 4-vinylguaiacol, 7786-61-0; 2-pentylfuran, 3777-69-3; 2-methyl-3-oxotetrahydrofuran, 3188-00-9; furfural, 98-01-1; 5-methylfurfural, 620-02-0; 2-propionylfuran, 3194-15-8; 2-acetylfuran, 1192-62-7; furfuryl alcohol, 98-00-0; 2-methyl-5-propionylfuran, 10599-69-6; 3-phenylfuran, 13679-41-9; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; 2-ethyl-6-methylpyrazine, 13925-03-6; 2-ethyl-3-methylpyrazine, 15707-23-0; 2-acetylpyrrole, 1072-83-9; *N*-methyl-2-formylpyrrole, 1192-58-1.

LITERATURE CITED

Beroza, M.; Alexander, B. H.; Steiner, L. F.; Mitchell, W. C.; Miyashita, D. H. *Science (Washington, D.C.)* **1960**, *131*, 1044.
Buttery, R. G. *J. Agric. Food Chem.* **1973**, *21*, 31.
Buttery, R. G.; Kamm, J. A. *J. Agric. Food Chem.* **1980**, *28*, 978.
Buttery, R. G.; Kamm, J. A.; Ling, L. C. *J. Agric. Food Chem.* **1982**, *30*, 739.
Hagen, K. S.; Greany, P.; Sawall, E. F.; Tassan, R. L. *Environ. Entomol.* **1976**, *5*, 458.
Hodge, J. E.; Mills, F. D.; Fisher, B. E. *Cereal Sci. Today* **1972**, *17* (2), 34.
Miller, J. R.; Haarer, B. K. *J. Chem. Ecol.* **1981**, *7*, 555.
Morton, T. C.; Bateman, M. A. *Aust. J. Agric. Res.* **1981**, *32*, 905.
Nunomura, N.; Sasaki, M.; Asao, Y.; Yokotsuka, T. *Agric. Biol. Chem.* **1976a**, *40*, 485.
Nunomura, N.; Sasaki, M.; Asao, Y.; Yokotsuka, T. *Agric. Biol. Chem.* **1976b**, *40*, 491.
Nunomura, N.; Sasaki, M.; Asao, Y.; Yokotsuka, T. *Agric. Biol. Chem.* **1978**, *42*, 2123.
Nunomura, N.; Sasaki, M.; Yokotsuka, T. *Agric. Biol. Chem.* **1980**, *44*, 339.
Stenhagen, E.; Abrahamsson, S.; McLafferty, F., Eds. "Registry of Mass Spectral Data"; Wiley: New York, 1974.
van Emden, H. F.; Hagen, K. S. *Environ. Entomol.* **1976**, *5*, 469.
van Praag, M.; Stein, H. S.; Tibbetts, M. S. *J. Agric. Food Chem.* **1968**, *16*, 1005.

Received for review September 20, 1982. Revised manuscript received February 7, 1983. Accepted March 8, 1983.

Insecticide Inhibition of Aflatoxin Production in Corn

Frances A. Draughon,* Mohammad E. Elahi, and Dennis R. West

Inhibition of fungal growth and aflatoxin production by insecticides in culture medium and in field corn was investigated. Bux, carbofuran, carbaryl, fonofos, fensulphothion, EPN, heptachlor, and toxaphene were added to culture medium at levels of 0, 10, 50, and 100 ppm. Bux, naled, carbaryl, and fonofos at 100 ppm inhibited aflatoxin production by 97, 100, 55, and 64%, respectively. Bux, naled, and carbaryl were applied to *Asperigillus parasiticus* inoculated corn in the field at levels of 100 ppm biweekly. Corn inoculated but not treated with insecticide had a mean aflatoxin concentration of 126 ppb. Bux and carbaryl reduced aflatoxin in inoculated corn to 47 and 49 ppb, respectively. In uninoculated corn, carbaryl and Bux reduced aflatoxin from 27 ppb to less than 5 ppb. Application of naled to corn did not effectively reduce aflatoxin production.

Preharvest contamination of corn produced in the Southeastern United States is a serious agricultural problem, especially under adverse growing conditions. Contamination of preharvest corn by aflatoxins was not thought to be a problem until the 1970s (Shotwell, 1977). Aflatoxin contamiination of corn before harvest became a major agricultural problem in the southeastern states in 1977. Of the 198 million bushels of corn produced in the Southeastern United States in 1977, 56% were contaminated with aflatoxins at concentrations greater than the 20 ppb permitted by FDA in interstate commerce (CAST, 1979). These findings have resulted in much research on methods of decontamination and detoxification.

The use of antifungal agents such as propionic acid to control growth and mycotoxin production on grain during storage has shown promise (Vandegraft et al., 1975); however, little research has been performed on controlling fungal growth and toxin production on grain in the field.

Although certain pesticides are effective inhibitors of aflatoxin production in the laboratory (Hsieh, 1973; Dutton

Department of Food Technology and Science (F.A.D. and M.E.E.) and Department of Plant and Soil Science (D.R.W.), University of Tennessee, Knoxville, Tennessee 37901.

0021-8561/83/1431-0692$01.50/0

and Anderson, 1980; Draughon and Ayres, 1981), only the insecticide Gardona has been tested on corn in the field (Widstrom et al., 1976). Gardona did not inhibit aflatoxin production in corn in the field (Widstrom et al., 1976) or in the laboratory in culture medium (Dutton and Anderson, 1980).

This study was undertaken to screen pesticides commonly used on corn for inhibition of aflatoxin production and growth by *Aspergillus parasiticus* in culture medium. The pesticides with the most antifungal activity were then taken to the field and applied to corn to determine if they inhibited aflatoxin production under field conditions.

MATERIALS AND METHODS

Microorganism. *A. parasiticus* NRRL 3174 was used throughout the study. All cultures were maintained on agar slants containing 1.5% agar, 20% sucrose, and 2% yeast extract (YES medium) and stored at 4 °C.

Semipermanent preservation of cultures was accomplished by the silica gel method of Perkins (1962). Tubes were kept tightly closed and held in a desiccator at 4–5 °C until needed.

Medium. To measure aflatoxin production and growth, *A. parasiticus* was grown in YES broth containing 2% yeast extract and 20% sucrose. Broth (50 mL) was dispensed into 250-mL Erlenmeyer flasks and autoclaved at 121 °C for 15 min. Each flask was inoculated with 1 mL of a spore suspension containing approximately 10^6 spores/mL. Spore suspensions were prepared by flooding a 10-day-old slant with 5.0 mL of sterile Triton X-100 (Sigma Chemical Co., St. Louis, MO) and shaking vigorously. Cultures were incubated for 7 days at 27 ± 1 °C without agitation.

Insecticides: Preparation and Source. The names of the insecticides used in this study for treating fungal cultures are shown in Table I together with source and respective chemical family. Before addition to culture medium, 1 g of insecticide was dissolved in 9 g of dimethyl sulfoxide. Appropriate amounts of the insecticide solution were added to the cultures to achieve the desired concentrations of 1, 10, 50, or 100 μg/mL. Control cultures were treated with equal amounts of dimethyl sulfoxide carrier solution.

Aflatoxin Isolation and Identification. YES broth cultures were extracted with 100 mL of chloroform and filtered through Whatman No. 1 filter paper. After extraction, the mycelial mat was dried for 24 h at 60 °C and weighed to measure dried weight as growth. The broth was transferred to a separatory funnel and the extraction repeated twice with equal volumes of chloroform. The combined extracts were dried by filtering through anhydrous sodium sulfate and evaporated to dryness by flash evaporation at 55 °C. Each sample was dissolved in 10 mL of benzene for assay.

Aflatoxins were identified and quantitated by comparison with standards obtained from Calbiochem, LaJolla, CA. Aflatoxins were separated by the use of a high-pressure (performance) liquid chromatograph (HPLC) (Waters Associates, Inc., Milford, MA) equipped with a Model 440 UV detector [278-nm filter (Engstrom et al., 1977)], M6000 pump, U6K septumless injector, fluorescence detector (360 nm, 440 nm), and μ-Porosil column with a solvent system of benzene–acetic acid (90:10 v/v) at a flow rate of 1 mL/min (Tong and Draughon, 1982).

Growth Studies in the Field. A commercial corn hybrid (Pioneer Brand 3184) was grown at the University of Tennessee, Knoxville, Plant and Soil Science Research Station. The experiment consisted of four randomized subplots as described below (each subplot consisted of 10

Table I. Insecticides Used in Study with Chemical, Common, and Trade Names, Chemical Family, LD_{50}, Source, and Registration (Martin, 1972)

insecticide chemical name	common name	chemical family/trade name	LD_{50},[a] mg/kg	registered[b] by EPA	supplier
m-(1-methylbutyl)- and *m*-(1-ethylpropyl)phenyl *N*-methylcarbamate	none	carbamate/Bux	87	no	Chevron (Richmond, CA)
1-naphthylcarbaryl	carbaryl	carbamate/Sevin	307	yes	Union Carbide (Jacksonville, FL)
2,3-dihydro-2,2-dimethylbenzofuran-7-yl methyl carbamate	carbofuran	carbamate/Furadan	5	yes	FMC (Middleport, NY)
diethyl 4-(methylsulphinyl)phenyl phosphorothionate	fensulphothion	organophosphate/Dasanit	2	yes	Chemagro (Kansas City, MO)
O-ethyl *S*-phenyl ethylphosphonodithioate	fonofos	organophosphate/Dyfonate	8	yes	Stauffer (Richmond, CA)
ethyl 4-nitrophenyl phenylphosphonothioate	EPN	organophosphate/EPN	7	yes	Du Pont (Wilmington, DE)
1,2-dibromo-2,2-dichloroethyl dimethyl phosphate	naled	organophosphate/Dibrom	40	no	Velsical (Chicago, IL)
heptachlorodicyclopentadiene	heptachlor	chlorinated hydrocarbon/none	430	no	Chevron (Richmond, CA)
mixture of chlorinated camphenes (67–69% chlorine)	toxaphene	chlorinated hydrocarbon/Toxaphene	40	yes	Hercules (Wilmington, DE)

[a] Oral LD_{50} in the male rat. [b] Registered for insect pests of corn (USDA, 1982). Toxaphene may not be used on potential silage corn intended for dairy animals or animals being finished for slaughter.

corn plants; two ears were collected from each plant): (1) no insecticide and no fungal inoculation; (2) insecticide with no fungal inoculation; (3) no insecticide but with fungal inoculation; (4) insecticide and fungal inoculation. All the corn ears were cut from the silks to middle through the husk in order to have consistency among the ears throughout the experiment. Those ears scheduled for fungal inoculation were inoculated along the slash 20 days after silk development by injecting approximately 10 mL of a spore suspension of 10^8 spores/mL *Aspergillus parasiticus* (stock). Corn ears scheduled for insecticide were treated with Bux, carbaryl, and naled. Insecticide was applied by spraying a 100-pm insecticide solution once weekly starting with first silks. Ears were covered with plastic bags for 4 days after inoculation and then plastic bags were removed and discarded. Waterproof paper bags were then placed over each ear. Waterproof paper bags were removed only when insecticides were applied weekly. Twenty ears from each treatment were harvested 71 days after silking. Ears were shelled and samples were frozen until analysis. The experiment was replicated once in 1980 and once in 1981. Each replicate represented a separate planting (total of four plantings for each subplot).

Aflatoxin production was measured for each ear. For toxin extraction each corn sample was ground and placed into a 500-mL Erlenmeyer flask, and 250 mL of methanol was added to each flask (Seitz and Mohr, 1974). They were shaken for 30 min. The methanol extract was collected by filtering the liquid phase through Whatman No. 1 filter paper and placed into a 500-mL separatory funnel. Approximately 200 mL of 20% aqueous ammonium sulfate solution and 100 mL of hexane were added to the separatory funnel and shaken for 1 min with venting. The methanol–H_2O layer was collected and the hexane layer was discarded. The methanol–H_2O layer was returned to the separatory funnel and after the addition of 100 mL of chloroform was shaken for 1 min. The chloroform extraction was repeated twice. The combined chloroform extracts were dried by filtering through anhydrous sodium sulfate and evaporated to dryness in a rotary flask evaporator at 60 °C. Each sample was dissolved in 5 mL of benzene for assay.

The extracted samples were quantitatively analyzed by HPLC as described earlier. An analysis of variance was performed and significant differences between means were determined by using Duncan's Multiple Range Test with a confidence level of 95%.

RESULTS AND DISCUSSION

The effects of pesticides on aflatoxin production and growth of *A. parasiticus* are presented in Table II. Addition of 100 ppm of Bux and 100 ppm of carbofuran to the broth significantly ($P < 0.05$) reduced the growth of *A. parasiticus* (approximately 50% inhibition, Table II). Carbaryl did not signficantly inhibit growth at the levels tested. Of the three carbamate insecticides, Bux caused the greatest inhibition in aflatoxin production (97%), followed by carbaryl (55%) and carbofuran (47%), respectively.

Four organophosphorus insecticides were added to broth cultures of *A. parasiticus*. The organophosphorus insecticide naled completely inhibited growth and aflatoxin production by *A. parasiticus* NRRL 3174 when applied in YES broth at a concentration of 100 mg/L (ppm) (Table II). Application of 50 ppm of naled to *A. parasiticus* in YES broth inhibited growth by 98% and toxin production by 99%. These results are comparable to those reported by Draughon and Ayers (1981) for *A. parasiticus* NRRL 2999. Fensulphothion, fonofos, and EPN demonstrated only moderate inhibition of growth of *A. parasiticus* (Table II). Fonofos significantly ($P < 0.05$) inhibited aflatoxin B_1 production; however, fensulphothion and EPN did not reduce aflatoxin production.

Table II. Effect of Insecticides on Growth of *A. parasiticus* in YES Broth and Aflatoxin B_2 Production[a]

insecticide	concn, mg/L	mycelial wt, g	toxin production, μg/50 mL	toxin, % inhibited
Bux	0	3.27 a	513.87 a	
	10	3.01 a	456.34 ab	21
	50	2.63 b	149.15 b	74
	100	1.54 c	18.47 c	97
carbaryl	0	2.28 a	383.40 a	
	10	2.23 a	195.26 b	49
	50	1.99 a	176.80 b	54
	100	1.88 a	173.50 b	55
carbofuran	0	2.78 a	393.94 a	
	10	2.53 a	319.17 a	19
	50	2.39 a	276.44 a	33
	100	1.40 b	209.06 a	47
fensulphothion	0	2.63 a	163.93 a	
	10	2.52 a	144.40 a	12
	50	2.32 b	142.06 a	14
	100	2.00 b	124.85 a	24
fonofos	0	2.49 a	239.59 a	
	10	2.33 a	142.41 b	41
	50	2.18 b	133.10 bc	45
	100	2.04 b	87.93 c	64
EPN	0	3.49 a	160.01 a	
	10	2.97 b	145.82 a	9
	50	2.81 b	138.30 a	14
	100	2.80 b	132.91 a	17
naled	0	2.50 a	556.22 a	
	10	2.06 a	254.93 b	55
	50	0.07 b	3.63 c	99
	100	0.00 c	0.00 c	100
heptachlor	0	2.30 a	286.29 a	
	10	2.29 a	282.98 a	2
	50	2.17 a	272.65 a	5
	100	2.15 a	241.80 a	16
toxaphene	0	2.16 a	701.82 a	
	10	2.11 a	474.49 a	33
	50	1.83 ab	472.19 a	33
	100	1.73 b	468.35 a	34

[a] Values within treatments sharing the same letter are not significantly different ($P < 0.05$).

The chlorinated hydrocarbon insecticides heptachlor and toxaphene did not significantly ($P < 0.05$) reduce aflatoxin production in culture at the highest level tested (100 ppm). Although toxaphene inhibited growth moderately (20% inhibition), inhibition was not sufficient for practical application.

Naled was selected for application in field corn since it was highly inhibitory in the laboratory. Naled is not registered for use on corn (USDA, 1982); however, the information on its antifungal activity in corn was sought to determine the efficacy of taking laboratory information and applying it to a field situation.

Because of field space limitations, only two additional insecticides could be selected for application to corn. Bux was selected because it was highly inhibitory to aflatoxin production in the laboratory. Carbaryl was selected for field application because of its widespread use against many insect pests of corn and becuase of its low toxicity (Martin, 1972). In additional, carbaryl was comparable to naled in its ability to inhibit aflatoxin production at a concentration of 10 ppm (Table II). This ability to inhibit aflatoxin production at lower concentrations was a desirable attribute because of the rapidity of deterioration of most carbamate and organophosphate insecticides in the environment.

Table III. Effect of Insecticides on Preharvest Aflatoxin B_1 Production in Pioneer Brand Corn Harvested 71 Days after Silking (All Data Are the Mean of 80 Samples[a])

treatment	*A. parasiticus* spores added	aflatoxin B_1 in corn,[a] $\bar{X}$, ppb (μg/kg)
no pesticide	10^8	126 a[b]
naled	10^8	109 b
Bux	10^8	47 c
carbaryl	10^8	49 c
no pesticide	no spores	27 d
naled	no spores	18 d
Bux	no spores	4 e
carbaryl	no spores	1 e

[a] The 80 samples per treatment (subplot) were composed of 20 ears per treatment times four plantings. [b] Those values followed by the same letter are not significantly different ($P < 0.05$).

Data from the field study showing application of naled, Bux, and carbaryl to corn are presented in Table III. Inoculating corn with spores of *A. parasiticus* increased aflatoxin B_1 production from 27 ppb in uninoculated corn to 126 ppb in inoculated corn. Application of Bux and carbaryl to inoculated corn reduced aflatoxin B_1 from 126 to 49 and 47 ppb, respectively (Table III). However, these levels were still greater than the 20-ppb level established as the action level by FDA for interstate commerce.

Application of naled to corn reduced aflatoxin B_1 levels from 126 to 109 ppb. Although naled showed some inhibition in aflatoxin production in corn, it was not a very effective inhibitor of aflatoxin production in the field. The highly inhibitory action of naled in the laboratory (100% inhibition) was not a good indicator of inhibitory action in the field. Some possible reasons for the loss of inhibitory activity in the field are the rapid deterioration of naled upon exposure to the elements since naled has a fairly short persistence (<7 days) and loss of naled to the environment due to its volatile nature.

Corn not inoculated with *A. parasiticus* was treated with Bux, carbaryl and naled to represent a typical plot of corn undergoing insecticide application (Table III). Preharvest development of aflatoxin B_1 averaged 27 ppb in corn receiving no fungal inoculum or insecticide treatment. Application of naled to uninoculated corn did not reduce preharvest development of aflatoxin. However, both Bux and carbaryl significantly reduced aflatoxin B_1 production to 4 and 1 ppb, respectively. Therefore, they show promise as protective agents against *A. parasiticus*.

In the past, it has not been economically feasible to apply pesticides to field corn during growth even to control insects, although, a preemergence systemic pesticide is usually applied to the soil at planting. Serious infestations of armyworm as well as earworm in recent years have contributed to the high levels of aflatoxin as well as other mycotoxins such as ochratoxin and zearalenone in corn (CAST, 1979). In 1977 in the Southeastern United States alone, 570.2 million dollars was lost on the corn crop due to drought and aflatoxin contamination (Olson and Stoloff, 1977).

With other commodities such as peanuts, it is estimated that 10–12 million dollars was lost for the years 1972–1978 (CAST, 1979). Approximately 9 million dollars was lost in Arizona on the 1977 cottonseed crop due to aflatoxin contamination.

High aflatoxin levels in crops such as cottonseed and corn are almost always associated with insect damage and adverse weather conditions (Ashworth et al., 1971). If certain insecticides are also antifungal agents, they may be of value in preventing situations such as occurred in 1977 and 1980.

Registry No. Bux, 8065-36-9; carbofuran, 1563-66-2; carbaryl, 63-25-2; fonofos, 944-22-9; fensulphothion, 115-90-2; EPN, 2104-64-5; heptachlor, 76-44-8; toxaphene, 8001-35-2; malid, 300-76-5; aflatoxin B, 1162-65-8.

LITERATURE CITED

Ashworth, L. J., Jr.; Rice, R. E.; McMeans, J. L.; Brown, C. M. *Phytopathology* **1971**, *61*, 488.

CAST "Aflatoxin and other Mycotoxins: An Agricultural Perspective"; Council for Agricultural Science and Technology: Ames, IA, 1979; Report No. 80, p 13.

Draughon, F. A.; Ayres, J. C. *Appl. Environ. Microbiol.* **1981**, *41*, 972.

Dutton, M. F.; Anderson, M. S. *J. Food Prot.* **1980**, *43*, 381.

Engstrom, G. W.; Richard, J. L.; Cysewski, S. J. *J. Agric. Food Chem.* **1977**, *25*, 833.

Hsieh, D. P. H. *J. Agric. Food Chem.* **1973**, *21*, 468.

Martin, H. "Pesticide Manual"; British Crop Protection Council: Worcester, England, 1972; pp 80, 83, 85, 238, 261, 272, 281, 336, 358.

Olson, M. C.; Stoloff, L. "Analyses of aflatoxin problem in corn in Southeast U.S."; Food and Drug Administration: Washington, DC, 1977; p 30.

Perkins, D. D. *Can. J. Microbiol.* **1962**, *8*, 591.

Seitz, L. M.; Mohr, H. E. *Cereal Chem.* **1974**, *51*, 487.

Shotwell, O. L. *J. Am. Oil Chem. Soc.* **1977**, *54*, 216A.

Tong, C. H.; Draughon, F. A., Annual Meeting of the Institute of Food Technologists, Las Vegas, NV, June 1982, Abstract No. 94.

USDA *U.S., Dep. Agric., Agric. Handb.* **1982**, *No. 584*, 331.

Vandegraft, E. E.; Hesseltine, C. W.; Shotwell, O. L. *Cereal Chem.* **1975**, *52*, 79.

Widstrom, N. W.; Lillehoj, E. B.; Sparks, A. N.; Kwolek, W. F. *J. Econ. Entomol.* **1976**, *69*, 677.

Received for review February 11, 1982. Accepted February 28, 1983. This work was supported in part by Pesticide Impact Assessment Program Grant R11-2015-11.