

Distribution and Persistence of Diazinon in a Cranberry Bog

Sunny Y. Szeto,* Michael T. Wan,† Patricia Price, and Jens Roland

Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, British Columbia, Canada V6T 1X2

Diazinon 5G at 6 kg of AI/ha was applied twice on a cranberry bog to control the larvae of the cranberry girdler moth, *Chrysoteuchia topiaria*. After each application, diazinon concentrations in water were highest in the irrigation ditches inside the treatment plot (338, 456 ppb). Lower concentrations were detected in the adjacent reservoir (78.5, 58.1 ppb), the waterways outside the dyke (29.1, 3.1 ppb), and the tributaries 100 m downstream from the treatment plot (2.8, 1.8 ppb). Some residues accumulated in sediments of the irrigation ditches (21 200 ppb maximum) and the reservoir (2380 ppb maximum). However, only low levels of residues were detected occasionally in sediments of the waterways (80 ppb maximum) and the tributaries (10 ppb, detected once). Diazinon disappeared rapidly and was no longer detected in water (detection limit 0.1 ppb) and in sediments (detection limit 10 ppb) 66 days after the second application, except in the irrigation ditches where 120 ppb still remained. Residues were 12, 6, and 7 ppb, respectively, in fruit collected 14, 21, and 28 days after the second application.

The cranberry girdler moth, *Chrysoteuchia topiaria* (Zeller), is an important pest of cranberry. The larvae feed on the bark and wood, sometimes chewing completely through a runner. Girdling, or removal of the bark and inner conductive layer, interferes with the transfer of water and nutrients in the plant and leads to death of the vine. In recent years this pest has caused severe economic loss for commercial cranberry growers in the Lower Fraser Valley. No insecticide is currently registered in Canada for control of the cranberry girdler, but granular diazinon 14G at 3.36 kg of AI/ha is registered and recommended for this purpose in the United States (Capizzi et al., 1988). Diazinon is known to be toxic to some aquatic organisms. Its 96-h LC₅₀ to rainbow trout is 90 ppb (Kenaga, 1979). Therefore, the irrigation water within a cranberry bog cannot legally be discharged within 3 days of application. To properly assess the impact of diazinon on the aquatic organisms as required for registration, research was conducted in a cranberry bog at Fort Langley, British Columbia, to determine the distribution and persistence of diazinon, including its toxic metabolite diazoxon in irrigation water and sediments at various locations within and outside the cranberry bog, after two applications of granular diazinon. In addition, residues of diazinon including diazoxon in fruit were determined at various intervals after the second application. Our findings are reported here.

MATERIALS AND METHODS

Field Trials. Studies on the distribution and persistence of diazinon were conducted on a cranberry bog of Coast Cranberries Ltd., Fort Langley, British Columbia. The treatment plot was a 19-ha planting consisting of nine beds of cranberries. The irrigation system of the treatment plot consisted of ditches surrounding each bed, reservoirs surrounding the treatment plot, and waterways linking the reservoirs to two small, natural tributaries of the Fraser River (Figure 1). Early in the growing season water was pumped from the tributaries via the linking waterways into the reservoirs for irrigation of the cranberry bog.

Water was supplied by the reservoirs to the sprinklers in each bed. There were gates between the irrigation ditches and the reservoirs, and their linking waterways, to regulate the water level at each irrigation component and to prevent the outflow of water from the treatment plot into the tributaries of the Fraser River. Therefore, irrigation water from the treatment plot could be retained indefinitely within the system without being released until after harvest. Six stations were selected for sampling of the water and sediment within and outside the treatment plot (Figure 1): one at the irrigation ditches inside the treatment plot (station 1); one at the reservoir adjacent to the treatment plot (station 2); two at the waterways outside the dyke (stations 3 and 5); one at each of the two tributaries approximately 100 m downstream from the edge of the treatment plot (stations 4 and 6). The distances from stations 4 and 6 to the Fraser River were about 200 and 1800 m, respectively.

Diazinon 5G at 6 kg of AI/ha was applied to the treatment plot by a fixed-wing aircraft on July 26 and again on Aug 8, 1988. Following each application, the diazinon granules were watered in with approximately 5 cm of water by the sprinklers to the mat where the larvae were located. Samples of water and sediment were collected from each sampling station 10 days before the first application and at various intervals after each application. A sample of 1 L of water including the surface film was collected by holding the bottle against the water flow at about 10 cm below the water surface. Sediment samples were collected from various points within each sampling station with a stainless steel scoop (15-cm diameter) fitted to a 3.5-m wooden handle. Each sediment sample consisted of 10 scoops each of approximately 45 g of sediment from the top 5-cm layer.

Water samples were stored in 1-L amber glass bottles and sediment samples in 500-g wide-mouth amber glass jars that had been solvent-washed and heated at 120 °C overnight prior to use. Water and sediment samples were extracted within 24 h after sampling, and all crude extracts were stored at 4 °C prior to cleanup and GC analysis.

Cranberries were randomly collected at intervals of 14, 21, 28, and 36 days after the second application. Fruit from each bed of the treatment plot comprised the four composite samples of approximately 500 g on each date, for residue determination.

Extraction and Cleanup. Water samples were extracted as sampled, i.e., without filtering. Aliquots of 500 mL of water including all suspended particulate matter were extracted three times with 50 mL of dichloromethane (pesticide grade) by liquid/liquid partitioning in 1-L separatory funnels. The amber glass bottle that contained the water sample was rinsed with dichloromethane to remove any diazinon residues adsorbed on the

† Present address: Environment Canada, Conservation and Protection, Environmental Protection, Pacific and Yukon Region, Capilano 100, Park Royal, West Vancouver, British Columbia, Canada V7T 1A2.

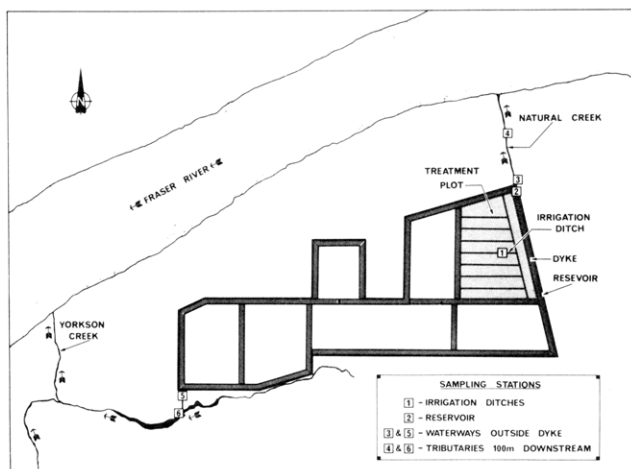


Figure 1. Cranberry bog of 19 ha near Fort Langley, BC, treated twice with diazinon 5G at 6 kg of AI/ha. Shown are the irrigation, drainage, and holding systems and location of stations for sampling water and sediment. Map drawn from aerial photograph.

glass wall. The rinsing was then used to extract the water sample. All extracts were dried with anhydrous Na_2SO_4 that had been treated at 120 °C for 48 h prior to use. The combined extracts were concentrated just to dryness in a flash evaporator at 38 °C. The residues were dissolved in 2 mL of ethyl acetate for GC analysis without further cleanup.

Sediment samples were thoroughly mixed by stirring with a glass rod prior to extraction. Aliquots of 50 g of sediment were extracted three times with 125 mL of a 3:2 (v/v) mixture of dichloromethane and acetone (pesticide grade). The sediments and the extracting solvents were transferred to 250-mL glass-stoppered conical flasks and then shaken for 30 min with a wrist-action shaker. The extracts were filtered through Whatman No. 1 filter paper into a 1-L separatory funnel. The combined extracts were washed with 250 mL of 2% NaCl solution, and the dichloromethane phase was dried with anhydrous Na_2SO_4 and then concentrated to 10 mL with a flash evaporator at 38 °C for further cleanup.

Cranberry samples were macerated and thoroughly mixed with a food processor. Aliquots of 50 g were extracted three times with 125, 75, and 75 mL of a 3:2 (v/v) mixture of dichloromethane and acetone by blending for 1 min with a Polytron homogenizer. The homogenates were filtered through glass fiber filter paper into a 500-mL separatory funnel. The combined filtrates were washed with 150 mL of 2% NaCl solution, and the dichloromethane phase was dried with anhydrous Na_2SO_4 and then concentrated to 10 mL with a flash evaporator at 38 °C for further cleanup.

Crude extracts of sediment and cranberry were purified by gel filtration column chromatography. A Pharmacia column, Model SR 25 (Pharmacia Fine Chemicals, Sweden) (45 cm \times 2.5 cm (i.d.)), was packed with Bio-Beads S-X12 (Bio-Rad Laboratories). The beads were swelled in a 1:1 (v/v) mixture of dichloromethane and cyclohexane overnight before the column was packed. An Eldex Model B-100-S high-pressure pump (Eldex Laboratories, Inc., San Carlos, CA 94070) was used for solvent delivery, and a Valco sample injection valve (Valco Instruments Co., Houston, TX 77024) equipped with a 5-mL injection loop was used to inject samples onto the column. After introduction of a 1-mL aliquot of crude extract representing 5 g of sediment and cranberry, the column was eluted with the 1:1 (v/v) mixture of dichloromethane and cyclohexane. Fraction 1, consisting of the first 58 mL, was discarded, and fraction 2, consisting of the next 150 mL, was collected. The purified extracts were concentrated just to dryness in a flash evaporator at 38 °C, and the residues were dissolved in 2 mL of ethyl acetate for GC analysis.

GC Analysis. A Hewlett-Packard Model 5880 gas chromatograph equipped with a flame photometric detector at phosphorus mode was used for determination of diazinon and diazoxon. A Hewlett-Packard cross-linked methylsilicone ultraperfor-

mance capillary column (25 m \times 0.20 mm (i.d.), 0.33 μm thick) was used. The operating parameters were as follows: detector temperature, 200 °C; injector temperature, 220 °C; splitless injection. Column temperature was programmed as follows: initial, 80 °C for 0.5 min; first program rate 25 °C/min to 185 °C, second program rate 5 °C/min to 225 °C and hold for 2 min. Helium was the carrier gas at 125 kPa. Detector gas consisted of hydrogen at 100 mL/min and air at 100 mL/min. Nitrogen was the makeup gas at 30 mL/min. Detector response was calibrated daily with authentic analytical standards of diazinon and diazoxon. Quantification was based on peak areas of the external standards injected before and after the sample.

Evaluation of Analytical Methods. Samples of water, sediment, and cranberries were collected from the untreated plots for analytical method evaluation. No gas chromatographic response was detected in any of the samples that interfered with the analysis of diazinon and diazoxon. Acetone solutions containing both diazinon and diazoxon at 1, 10, 100, and 500 $\mu\text{g}/\text{mL}$ were prepared. Quadruplicate samples were fortified with both compounds at 1 and 100 ppb for water and at 100 and 5000 ppb for sediments and cranberries by adding 0.5 mL of the appropriate acetone solutions to 500 mL of water and 50 g of sediment or cranberries. The fortified water samples were allowed to equilibrate for 5 min before extraction whereas the fortified sediment and cranberries were equilibrated at room temperature in a fume hood for about 30 min before extraction. All the fortified samples were extracted, purified, and analyzed as described to determine the recoveries of diazinon and diazoxon.

RESULTS AND DISCUSSION

Efficiency of Analytical Methods. The percentage recoveries of diazinon and diazoxon from water, sediment, and cranberries at two fortification levels are given in Table I. Excellent recoveries were obtained from water, but the recoveries from sediment and cranberries were rather low. Diazinon and diazoxon are known to be unstable at acidic pH (Gomaa et al., 1969). Homogenized cranberries have pH 2.8, so that added diazinon and diazoxon would be expected to hydrolyze rapidly. When homogenized cranberries were fortified directly with 100 ppb, the recoveries were only 55.6% for diazinon and 6.2% for diazoxon. For a better assessment of our analytical methods, the homogenized cranberry tissues were neutralized with NaHCO_3 prior to fortification; the recoveries at two levels are given in Table I. Sediments from the irrigation ditches were acidic (pH 4.4–6.0), which may account for the relatively low recoveries of diazinon and diazoxon. The data generated from our studies here are not corrected in accordance with recoveries. The limits of detection were 0.1 ppb for water, 10 ppb for sediment (wet weight), and 5 ppb for cranberry tissues.

Distribution and Persistence. Diazoxon was not detected at the limit of detection in any of the samples of water, sediment, or cranberries.

Prespray samples of water and sediment from all six sampling stations contained no detectable residues of diazinon although diazinon 50 EC at 1.4 kg of AI/ha had already been used in May to control the blackheaded fireworm, *Rhopobota naevana*. Concentrations of diazinon in water and sediment collected from all sampling stations at various intervals after the applications of diazinon 5G are given in Tables II and III. Since the findings were similar for the two sampling stations at the waterways outside the dyke (stations 3 and 5) and in the tributaries 100 m downstream from the edge of the treatment plot (stations 4 and 6), the residues given in Tables II and III are means of the two readings.

As anticipated, the highest concentrations were detected in water of the irrigation ditches located inside the treatment plot (station 1). These were 338 ppb 1 day after

Table I. Percent Recovery of Diazinon and Diazoxon from Fortified Water, Sediment, and Cranberries

compd	% recovery \pm SD ($n = 4$)					
	water		sediment		cranberry ^a	
	1 ppb	100 ppb	100 ppb	5000 ppb	100 ppb	5000 ppb
diazinon	102 \pm 1.7	103 \pm 4.0	72.1 \pm 2.6	75.6 \pm 1.7	102 \pm 2.0	71.4 \pm 3.4
diazoxon	110 \pm 3.3	102 \pm 3.7	68.7 \pm 0.3	70.2 \pm 2.3	86.8 \pm 9.4	69.6 \pm 4.9

^a Homogenized cranberries were neutralized with NaHCO₃ prior to fortification with diazinon and diazoxon.

Table II. Diazinon Residues (ppb) in Water of a Cranberry Bog Treated Twice with Diazinon 5G at 6 kg of AI/ha^a

time of sampling, days	irrigation ditches (station 1) $n = 1$	reservoir (station 2) $n = 1$	waterways outside dyke (stations 3 & 5) mean, $n = 2$	tributaries 100 m downstream (stations 4 & 6) mean, $n = 2$
prespray	nd	nd	nd	nd
postspray				
1 (1st spray)	338	13.1	7.5	
2	278	78.5	29.1	0.8
3	175	31.5	9.3	2.6
4	6.6	16.7	9.1	2.8
5	1.3	11.3	4.9	0.5
6	0.8	8.8	3.3	0.7
7	1.4	8.7	2.4	0.3
8	3.6	7.9	2.7	0.2
13	10.4	2.6	0.4	0.3
14 (2nd spray)	456	39.7	0.4	0.2
15	110	21.7	2.6	1.1
16	78.8	52.3	0.7	0.8
17	47.8	58.1	3.1	1.8
18	21.1	19.0	0.7	0.7
19	34.8	16.7	0.2	0.4
20	10.3	16.3	0.1	0.2
21	45.2	8.0	2.5	0.2
22	13.6	9.9	2.4	0.9
28	8.3	4.6	0.7	0.7
35	0.2	1.3	0.2	0.1
42	nd	0.3	0.1	nd
51	nd	0.3	nd	nd
79	nd	nd	nd	nd
137	nd	nd	nd	nd

^a nd = not detected; detection limit 0.1 ppb.

the first application and 456 ppb 1 day after the second application (Table II). The concentrations decreased to below 100 ppb within 3–4 days after each application and to 8.3 ppb 28 days after the first application (i.e., 10 days after the second application). Diazinon was no longer detected in water from the irrigation ditches 42 days after the first application (i.e., 24 days after the second application). Similar trends were observed in water from all other sampling stations, but the concentrations were much lower. The highest residues were 78.5 ppb in the reservoir adjacent to the treatment plot (station 2), 29.1 ppb in the waterways outside the dyke (stations 3 and 5), and 2.8 ppb in the tributaries about 100 m downstream from the edge of the treatment plot (stations 4 and 6). The minute amounts of diazinon (2.8 ppb maximum) that persisted for 4–5 days in the tributaries about 100 m downstream from the edge of the treatment plot were 32 \times below the 96-h LC₅₀ to rainbow trout (90 ppb of diazinon) (Kenaga, 1979) and should have a minimal impact on fish.

Diazinon is known to be readily adsorbed onto sediments and soils, the amount sorbed being proportional to their organic content (Miles and Harris, 1978; Sharon et al., 1980). Water and sediment in cranberry bogs usually have high organic content. In fact, the sediment consists mostly of organic detritus derived from decayed cranberry vines. Therefore, the chief means of transporting diazinon from the treatment plot is surface runoff. Since the water inside the treatment plot and the reservoirs is

retained for irrigation until harvest, it is anticipated that little diazinon would be moved from the treatment plot to the adjacent aquatic environment. Our data support this argument. Only low levels of diazinon (2.8 ppb maximum) were occasionally detected in water from the tributaries about 100 m downstream from the edge of the treatment plot, which came probably from the leakage of irrigation water through the gate between the reservoir and the waterway outside the dyke. We believe that the residues in the tributaries can be further reduced by the installation of leak-proof gates. The fact that little surface runoff occurred was further evidenced by water samples collected during heavy rainfall 21 days after the first application (i.e., 8 days after the second application). The levels were 45.2 ppb in the irrigation ditches, 8.0 ppb in the reservoir, 2.5 ppb in the waterways, and 0.2 ppb in the tributaries (Table II).

Diazinon was detected in sediments from the irrigation ditches inside the treatment plot (station 1) and the adjacent reservoir (station 2) (Table III). The highest concentrations were 21 200 ppb (wet weight) in sediments of the irrigation ditches 4 days after the first application and 8920 ppb 3 days after the second application or 21 days after the first. Little diazinon was detected in sediments from the waterways outside the dyke (stations 3 and 5). The highest was 80 ppb detected 1 day after the first application. Diazinon was detected only once at 10 ppb in sediments collected 4 days after the first application from the tributaries 100 m downstream

Table III. Diazinon Residues (ppb, Wet Weight) in Sediments of a Cranberry Bog Treated Twice with Diazinon 5G at 6 kg of AI/ha^a

time of sampling, days	irrigation ditches (station 1) <i>n</i> = 1	reservoir (station 2) <i>n</i> = 1	waterways outside dyke (stations 3 & 5) mean, <i>n</i> = 2	tributaries 100 m downstream (stations 4 & 6) mean, <i>n</i> = 2
prespray	nd	nd	nd	nd
postspray				
1 (1st spray)	1960	2380	80	nd
4	21200	400	nd	10
8	1970	710	nd	nd
13	500	10	nd	nd
14 (2nd spray)	1500	30	20	nd
17	6110	110	10	nd
21	8920	40	10	nd
22	2290	40	nd	nd
28	520	10	nd	nd
35	340	nd	10	nd
42	90	nd	nd	nd
51	170	10	nd	nd
79	120	nd	nd	nd
137	20	nd	nd	nd

^a nd = not detected; detection limit 10 ppb.

from the edge of the treatment plot (stations 4 and 6). These results further demonstrated that little transport of diazinon occurred from the treatment plot to the linking waterways and the tributaries.

Diazinon dissipated rapidly in water and sediment (Tables II and III). The major mechanism for dissipation is probably chemical hydrolysis. Diazinon is known to be stable in neutral and slightly basic media but is hydrolyzed readily in acidic media (Gomaa et al., 1969). These workers studied the kinetics of hydrolysis of diazinon and diazoxon. They reported that the hydrolysis of diazinon and diazoxon follows first-order kinetics in buffers of pH 3.1–10.4 and in natural water. At 20 °C the half-lives are 22.8 min at pH 3.1, 30.7 h at pH 5.0, 693.5 h at pH 7.4, 441.2 h at pH 9.0, and 10.1 h at pH 10.4. The pHs of water at various stations of the cranberry bog were as follows: 5.1 at the irrigation ditches (station 1), 5.2 at the reservoir (station 2), 6.2 at the waterways (stations 3 and 5), 6.6 at the tributaries (stations 4 and 6). Therefore, diazinon in water from the irrigation ditches and the reservoir degraded rapidly because of the relatively acidic pH (Table II). As water in the cranberry bog contained significant amounts of particulates and organic matter derived from decayed vines, much of the diazinon residue may also adsorb onto the suspended material. Nevertheless, hydrolysis of the sorbed residues should follow similar kinetics as the unsorbed. Macalady and Wolfe (1985) studied the effects of sediment sorption on hydrolysis of diazinon, chlorpyrifos, and fenclorophos. Their investigations with well-characterized sediments showed that the rates of neutral hydrolysis of the three compounds were unaltered when the compounds were sorbed to sediments.

The pHs of the sediments in the cranberry bog were as follows: 4.4 in the irrigation ditches (station 1), 5.0 in the reservoir (station 2), 5.7 in the waterways (stations 3 and 5), 6.0 in the tributaries (stations 4 and 6). Since diazinon is unstable in acidic media, its residues in sediments from cranberry bogs will degrade rapidly as demonstrated here (Table III). Not much information on the persistence of diazinon in sediments is available in the scientific literature. Studies in turf and submerged soil (Kuhr and Tashiro, 1978; Sethunathan and MacRae, 1969) have shown that diazinon is nonpersistent. In turf treated with diazinon 5G at 6.72 kg of AI/ha, the insecticide was washed into the soil with 1.2 cm of water by a sprinkler. After application, the residues remained at about 10 ppb

for 2 weeks and decreased to levels from 0.17 to 0.65 ppm after 6 weeks (Kuhr and Tashiro, 1978). The dissipation in sediments of the cranberry bog as reported here was much faster probably because of the acidity (pH 4.4–5.0).

In their studies on biodegradation of diazinon in submerged soils, Sethunathan and MacRae (1969) found that only 2–6% of the originally applied diazinon remained in the soils after 50–70 days. Losses of the insecticide from sterilized samples of two of the soils were much slower than from nonsterilized samples whereas the disappearance was just as rapid from sterilized samples of the third soil, an acidic clay of pH 4.7, as from nonsterilized ones. In our studies, sediments from the irrigation ditches inside the treatment plot (pH 4.4) and the adjacent reservoir (pH 5.0) were similar in acidity and disappearance of diazinon from these sediments was just as rapid. Other factors that may contribute to the rapid degradation of diazinon in sediments include microbial degradation as demonstrated by Sethunathan and MacRae (1969) in submerged soils, photolysis (Burkhard and Guth, 1979), and enhanced degradation resulting from repeated applications (Forrest et al., 1981).

Residues in Cranberries. Diazinon is currently registered in Canada for control of the blackheaded fireworm, *R. naevana* in cranberries, and tolerance of residues is 250 ppb. Cranberries were collected for residue determination from the treatment plot on Aug 23, i.e., 14 days after the second application. There was 12 ppb of diazinon, but diazoxon was not detected. The total residues decreased to 6 and 7 ppb, respectively, in cranberries after 21 and 28 days and to nondetectable after 36 days. Our results clearly showed that residues accumulated in cranberries would be well below the 250 ppb tolerance set for diazinon.

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Registry No. Diazinon, 333-41-5.

LITERATURE CITED

Burkhard, N.; Guth, J. A. Photolysis of Organophosphorus Insecticides on Soil Surfaces. *Pestic. Sci.* **1979**, *10*, 313–319.

- Capizzi, J., Fisher, G., Homan, H., Stoltz, R., Antonelli, A., Mayer, D., Eds. *Pacific North West Insect Control Handbook*; Agricultural Communications, Publications Orders, Oregon State University: Corvallis, 1988; p 112.
- Forrest, M.; Lord, K. A.; Walker, N. The Influence of Soil Treatments on The Bacterial Degradation of Diazinon and Other Organophosphorus Insecticides. *Environ. Pollut.* 1981, 24, 93-104.
- Gomaa, H. M.; Suffet, I. H.; Faust, S. D. Kinetics of Hydrolysis of Diazinon and Diazoxon. *Residue Rev.* 1969, 29, 171-190.
- Kenaga, E. Acute and Chronic Toxicity of 75 Pesticides to Various Animal Species. *Down Earth* 1979, 35, 25-31.
- Kuhr, R. J.; Tashiro, H. Distribution and Persistence of Chlorpyrifos and Diazinon Applied to Turf. *Bull. Environ. Contam. Toxicol.* 1978, 20, 652-656.
- Macalady, D. L.; Wolfe, N. L. Effects of Sediment Sorption and Abiotic Hydrolyses. 1. Organophosphorothioate Esters. *J. Agric. Food Chem.* 1985, 33, 167-173.
- Miles, J. R. W.; Harris, C. R. Insecticide Residues in Water, Sediment and Fish of The Drainage System of The Holland Marsh, Ontario, Canada, 1972-1975. *J. Econ. Entomol.* 1978, 71, 125-130.
- Sharon, M. S.; Miles, J. R. W.; Harris, C. R.; McEwen, F. L. Behavior of Twelve Insecticides in Soil and Aqueous Suspensions of Soil and Sediment. *Water Res.* 1980, 14, 1095-1100.
- Sethunathan, N.; MacRae, I. C. Persistence and Biodegradation of Diazinon in Submerged Soils. *J. Agric. Food Chem.* 1969, 17, 221-225.

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Evidence for the Natural Occurrence of Fumonisin B₁, a Mycotoxin Produced by *Fusarium moniliforme*, in Corn

Eric W. Sydenham,* Wentzel C. A. Gelderblom, Pieter G. Thiel, and Walter F. O. Marasas

Research Institute for Nutritional Diseases, South African Medical Research Council, P.O. Box 70, Tygerberg 7505, South Africa

Fusarium moniliforme, a common fungal contaminant of corn, was recently shown to produce a group of mycotoxins, the fumonisins, in culture. Moldy home-grown corn collected from an area of the Transkei, southern Africa, was analyzed for the presence of the fumonisin mycotoxins. Fumonisin B₁ (FB₁) was detected in the sample extract, as independently prepared derivatives, by two high-performance liquid chromatographic procedures. A capillary gas chromatographic-mass spectrometric procedure was used to confirm the identity of the tricarballic acid moiety, present in the esterified hydrolysates of the fumonisins. This is the first conclusive report of the natural occurrence of FB₁ in corn.

Fusarium moniliforme Sheldon, a common fungal contaminant of corn throughout the world (Booth, 1971), has been implicated in animal and human diseases (Marasas et al., 1984b). Various strains of the fungus are known to be highly toxic (Kriek et al., 1981a,b) and carcinogenic (Marasas et al., 1984a; Jaskiewicz et al., 1987) in animals. Since *F. moniliforme* has been associated with human esophageal cancer risk in the Transkei, southern Africa (Marasas, 1982; Marasas et al., 1981, 1988a) and in China (Li et al., 1980; Yang, 1980), recent investigations have focused on the characterization of the carcinogenic compounds produced by this fungus.

The mutagenic activities exhibited by various strains of *F. moniliforme* in the *Salmonella* mutagenicity test resulted in the characterization of the potent mutagen, fusarin C (Wiebe and Bjeldanes, 1981; Gelderblom et al., 1984a; Gaddamidi et al., 1985). However, the lack of carcinogenicity of fusarin C (Gelderblom et al., 1986) makes it unlikely that this mutagen is involved in the carcinogenic effects of the fungus. Recently, several strains of *F. moniliforme* were found to exhibit cancer-promoting activity in a short-term cancer initiation/promotion bioassay in rats using diethylnitrosamine (DEN) as a cancer initiator and the induction of γ -glutamyltranspeptidase positive foci in the liver as end point (Gelderblom et al., 1988b). With use of this bioassay as a monitor for cancer-promoting principles, the fumonisins were iso-

lated (Gelderblom et al., 1988a) and chemically characterized (Bezuidenhout et al., 1988) from culture material of *F. moniliforme* MRC 826, previously shown to be hepatocarcinogenic to rats (Marasas et al., 1984a; Jaskiewicz et al., 1987). In addition to its cancer-promoting ability, fumonisin B₁ (FB₁; Figure 1), the major compound, also exhibits toxic effects in rats similar to that of the culture material of *F. moniliforme* MRC 826 (Gelderblom et al., 1988a). Recently Marasas et al. (1988b) induced the equine neurotoxic disease leukoencephalomalacia (LEM) in a horse by intravenous injection of FB₁ isolated from strain MRC 826.

A sample of home-grown corn from a high esophageal cancer risk area of the Transkei, southern Africa, has previously been shown to be naturally contaminated with at least four *Fusarium* mycotoxins, i.e. moniliformin, zearalenone, deoxynivalenol, and fusarin C (Thiel et al., 1982; Gelderblom et al., 1984b). This paper details the chemical analysis of this corn sample for the presence of FB₁.

EXPERIMENTAL SECTION

Analytical Standards. FB₁ was extracted from *F. moniliforme* MRC 826 as previously described (Gelderblom et al., 1988a). The purity of the analytical standard was assessed by thin-layer chromatography (TLC; see TLC analyses). Visual inspection of the plate showed the presence of a minor contam-