The first toxicity studies on ethoxyquin, involving the effects on rats, were reported in 1959 by Wilson and De Eds. More recently, in common with several other feed antioxidants, ethoxyquin has been demonstrated to modify the acute toxicity of certain mutagenic and carcinogenic chemicals to the mouse (Cumming and Walton, 1973) and the rat (Skaare et al., 1977). Also, ethoxyquin potentiated the antimicrobial activity of the antioxidant butylated hydroxytoluene (Turcotte and Saheb, 1978). Most interestingly, significant effects of ethoxyquin on aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus* have very recently been reported (Foudin et al., 1978). This latter report indicated that ethoxyquin had the unique capability of selectively altering aflatoxin synthesis in *A. parasiticus* strains which produce both B and G type aflatoxins. The possible importance of the ethoxyquin conversion product 2,4-dimethyl-6-ethoxyquinoline in phenomena such as these is unknown, but clearly should be considered in future studies.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Norbert Morales who ran the NMR spectra and Walter Hyde who ran the mass spectra. Also we acknowledge the financial assistance of the Iowa Beef Industry Council and D. J. Zaffarano which made R. T. Gallagher's stay possible.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", Washington, DC, 1975.
Batterham, T. J., "NMR Spectra of Simple Heterocycles", Wiley, New York, 1973a, p 314.
Batterham, T. J., "NMR Spectra of Simple Heterocycles", Wiley, New York, 1973b, p 307.
Cumming, R. B., Walton, M. F. *Food Cosmet. Toxicol.* **11**, 547 (1973).
Foudin, A. S., Leaich, L. L., Ayres, J. C., *J. Food Sci.* **43**, 731 (1978).
Issaq, H. J., Barr, E. W., Zielinski, W. L., Jr., *J. Chromatogr.* **132**, 115 (1977).
Moss, M. O., in "Phytochemical Ecology", Harborne, J. B., Ed., Academic Press, New York, 1972, pp 125–144.
Nekipelova, T. D., Gagarina, A. B., *Dokl. Akad. Nauk SSSR* **231**, 392 (1976); *Chem. Abstr.* **86**, 106338g.
Schroeder, H. W., *Appl. Microbiol.* **16**, 946 (1968).
Seitz, L. M., Sauer, D. B., Mohr, H. E., Burroughs, R., Paukstelis, J. V., *J. Agric. Food Chem.* **23**, 1 (1975).
Shotwell, O. L., Shannon, G. M., Goulden, M. L., Milburn, M. S. Hall, H. H., *Cereal Chem.* **45**, 236 (1968).
Skaare, J. U., Dahle, H. K., *J. Agric. Food Chem.* **23**, 1091 (1975).
Skaare, J. U., Nafstad, I., Dahle, H. K., *Toxicol. Appl. Pharmacol.* **42**, 19 (1977).
Stahr, H. M., Ed., "Analytical Toxicology Methods Manual", Iowa State University Press, Ames, IA, 1977.
Stefaniak, B., *J. Chromatogr.* **44**, 403 (1969).
Tung, C. C., *Tetrahedron* **19**, 1685 (1963).
Turcotte, P., Saheb, S. A., *Can. J. Microbiol.* **24**, 1306 (1978).
Vaughan, W. R., *Org. Synth.* **28**, 49 (1948).
Wilson, R. H., De Eds, F., *J. Agric. Food Chem.* **7**, 203 (1959).
Zobian, E. J., Kelley, W. S., Dunathan, H. C., *J. Org. Chem.* **29**, 584 (1964).

Received for review May 7, 1979. Accepted August 17, 1979.

Diplodiol: A New Toxin from *Diplodia macrospora*

Horace G. Cutler,* Farrist G. Crumley, Richard H. Cox, Richard J. Cole, Joe W. Dorner, Frances M. Latterell, and Albert E. Rossi

A toxic metabolite, *trans*-6-ethyl-5-hydroxy-3-hydroxymethyl-5,6,7,8-tetrahydrochromone, trivial name diplodiol, was isolated from *Diplodia macrospora*, a pathogen of corn. It was toxic to day-old cockerels and the calculated LD_{50} was 88.4 mg/kg. The toxin may contribute to chick mortality in Mexico and Central and South America.

Diplodia macrospora Earle, a fungal pathogen of corn, causes ear- and stalk-rot, and under humid conditions, a severe leaf spot. The fungus has been known for many years in the southern corn belt of the U.S. as a minor cause of ear- and stalk-rot, but not often as a leaf pathogen (Eddins, 1930). In the humid tropics and subtropics of Mexico and the countries of Central and South America, we have found *D. macrospora* to be widely distributed, occurring most commonly as a leaf pathogen, but also present as an ear-rotting organism, along with *D. maydis* (Berk.) Sacc., *Fusarium* spp., and other fungi.

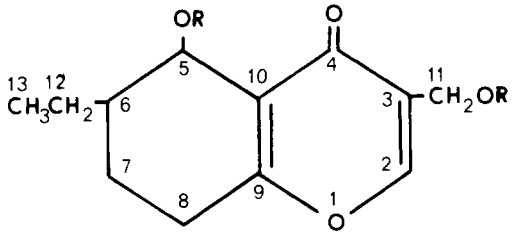
In 1970, De León and Perez reported from Mexico on severe maladies in chicks that were fed corn grains infected with *Diplodia* spp., including heart and liver enlargement, effusion of major organs, rupturing of blood vessels in the skin, and premature death. They attributed the injury to a mycotoxin produced by *D. maydis*. Steyn et al. (1972), working in South Africa, isolated and defined the structure of a toxic metabolite from *D. maydis*, "diplodiatoxin". Further work on the toxicity of *D. maydis* to ducklings and rats was reported by Rabie (1977).

We report the isolation of a new toxin from cultures of *D. macrospora*, *trans*-6-ethyl-5-hydroxymethyl-5,6,7,8-tetrahydrochromone (I) (Figure 1), to which we have assigned the trivial name diplodiol, and describe its effect on chicks.

MATERIALS AND METHODS

Production and Purification of the Toxin. *Diplodia macrospora* (ATCC accession no. 36896) was isolated from

U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Georgia Coastal Plain Experiment Station, Tifton, Georgia 31794 (H.G.C., F.G.C.), The National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 (R.H.C.), U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, National Peanut Research Laboratory, Dawson, Georgia 31742 (R.J.C., J.W.D.), and U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Plant Disease Research Laboratory, Frederick, Maryland 21701 (F.M.L., A.E.R.).



I. R = H

II. R = C(=O)CH3

Figure 1. Structure of diplodiol (I) and diplodiol diacetate (II).

infected corn plants (*Zea mays* L.) in Turrialba, Costa Rica. The fungus was cultured on potato dextrose agar slants at approximately 26 °C for 10 days and was then maintained at 5 °C until transferred to Fernbach flasks (2.8 L), each containing 100 g of shredded wheat, 200 mL of Difco mycological broth (pH 4.8), 2% yeast extract, and 20% sucrose (Kirksey and Cole, 1974) for production of the toxin. Inoculated flasks were incubated in the laboratory at about 24 °C for 19 days, then 300 mL of acetone was added to each flask. The mycelia and substrate were macerated with a Super Dispax homogenizer, and the suspension was strained through cheesecloth to remove the pulp. The liquid was filtered through Whatman No. 1 filter paper on a Buchner funnel and the clarified filtrate was reduced under vacuum at 50 °C to yield an aqueous phase. This was extracted twice with ethyl acetate; each volume of solvent was equal to twice that of the aqueous portion. Combined ethyl acetate extracts were dried over anhydrous sodium sulfate, reduced to a small volume under vacuum, and placed on a silica gel (70–230 mesh) chromatography column (9.0 × 10 cm) that had been slurry packed in benzene. Stepwise elution of the column was completed with 1.0 L each of benzene, ethyl ether, ethyl acetate, acetone, and methanol. Individual solvents drained to the top of the silica gel before addition of the next sequential solvent. The acetone fraction, which exhibited biological activity, was further fractionated by first reducing it to a small volume under vacuum at 50 °C and placing it on a silica gel (70–230 mesh) column (4.0 × 50 cm) that had been slurry packed in benzene. Then 250 mL of benzene was added to the top of the packing material and immediately a linear gradient of benzene (a further 1.0 L) to acetone (1.0 L) was used for elution. Twenty-milliliter fractions were collected, evaporated to approximately 2 mL, and further examined by thin-layer chromatography and bioassay. During the isolation procedure, purification of the metabolite was monitored on silica gel 60, F-254 (E.M. Laboratories, Inc.) with a toluene/ethyl acetate/formic acid (5:4:1, v/v/v) developing solvent. The metabolite was seen as a dark spot under shortwave UV.

Physical and Chemical Analyses. Ultraviolet (UV) spectra of diplodiol were determined in 95% ethanol solution with a Beckman Model DB-G recording spectrophotometer. Infrared (IR) spectra were obtained with a Beckman IR 4210 spectrophotometer using a 4X beam condenser. Samples were prepared as thin films on KBr windows, except diplodiol diacetate which was analyzed in $CHCl_3$ solution.

Proton and ^{13}C nuclear magnetic resonance spectra were obtained on a Varian Associates XL-100-12 spectrometer equipped with the 620 L disk data system. All spectra were obtained in $CDCl_3$ solution using 5-mm sample tubes with tetramethylsilane as the internal reference. The ^{13}C spectra were obtained using the Fourier transform mode with the following parameters: spectral width, 5 kHz; pulse angle approximately 30°; repetition time between pulses, 2 s; data points, 8K; exponential broadening, −1.0; and squarewave modulated proton decoupling, 2 kHz. Single-frequency, off-resonance proton decoupling (sford) was used to aid in the assignment of the ^{13}C spectra.

Low-resolution (LRP) mass spectra were obtained on a Finnigan Model 3300 spectrometer. High-resolution (HRP) data were gathered with an AEI MS-9 spectrometer. Samples were introduced into the instrument by the direct probe method. Ionization was by electron impact at 70 eV. Melting points were observed with a Hoover capillary melting point apparatus and are uncorrected.

Synthetic Preparation. The diacetate of I was prepared by dissolving approximately 30 mg of I in 1 mL each of pyridine and acetic anhydride and allowing the mixture to stir overnight at room temperature. Evaporation of the solution on a rotary evaporator, followed by drying under high vacuum, yielded a pale-yellow oil that was used without further purification.

Bioassays. One-day-old DeKalb cockerels were used for the vertebrate bioassay of the toxin. Chicks were dosed via crop intubation with corn oil as the inert carrier (1 mL of corn oil/chick). Samples were prepared for dosing by dissolving the toxin in acetone. Corn oil was added and the acetone was removed under vacuum on a rotary evaporator at 70 °C. After apparent removal of all the acetone (when no further acetone distilled over), the evaporator reservoir was emptied and dried. Again the sample was placed under vacuum and subjected to further rotary evaporation to ensure complete removal of the acetone (Kirksey and Cole, 1974). Controls were prepared in an identical way.

For median lethal dose determination (LD_{50}), diplodiol was administered to groups of chicks, each consisting of a lot of four chicks, at the following concentrations: 1.25, 2.5, 5.0, and 10.0 mg/chick. Mortality data were taken up to 4 days after dosing, and Weil's tables were used for calculating the LD_{50} data (Weil, 1952).

Wheat seeds (*Triticum aestivum* L. cv. Wakeland) were sown on moist sand in trays and grown in the dark at 22 ± 1 °C for 4 days (Hancock et al., 1964). The etiolated seedlings were removed from the trays and the roots and caryopsis were removed from the shoots. The latter were placed in a Van der Weij guillotine and the apical 2 mm were cut off and discarded. The next 4 mm of the coleoptiles were removed and used for bioassay. All manipulations were done under a green safelight (Nitsch and Nitsch, 1956). Fractions to be assayed for biological activity were added to text tubes (approximately 20 μL/tube) and evaporated under nitrogen to dryness. Diplodiol and diplodiol diacetate were assayed at concentrations of 10^{-3}, 10^{-4}, and 10^{-5} M in duplicate assays. Two milliliters of phosphate–citrate buffer containing 2% sucrose at pH 5.6 (Nitsch and Nitsch, 1956) were added to each test tube. Following the placement of ten coleoptiles in each test tube, the tubes were rotated at 0.25 rpm in a roller tube apparatus for 24 h at 22 °C in the dark. The coleoptiles were measured by projecting their images (×3) from a photographic enlarger (Cutler and Vlitos, 1962). Data were statistically analyzed (Kurtz et al., 1965).

RESULTS AND DISCUSSION

Physical and Chemical Characteristics. *Diplodia macrospora* produced 650 mg of diplodiol from 50 flasks of shredded wheat media in 19 days. Biological activity was first observed in the acetone fraction obtained from stepwise elution silica gel column chromatography. On further separation, with a linear gradient elution series

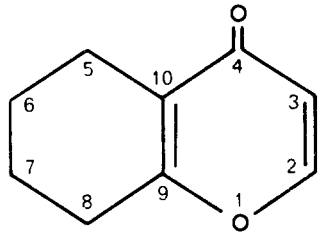


Figure 2. Structure of 5,6,7,8-tetrahydrochromone.

from benzene to acetone on silica gel, activity was associated with tubes 64–76 (1.28–1.52 L of total solvent). They were combined, reduced in volume under vacuum, and stored in a refrigerator at 5 °C. Fine white crystals precipitated within 72 h and were collected on a fine porosity fritted glass funnel under vacuum. The crystals were washed with acetone at −17 °C, then collected and dried at room temperature.

The R_f value of diplodiol (I) was 0.33–0.36 on silica gel 60 thin-layer plates developed with toluene/ethyl acetate/formic acid; diplodiol diacetate (II) had a R_f value of 0.55–0.59. Both were observed as dark spots under shortwave UV. The uncorrected melting point for I was 47–48 °C; II did not crystallize.

UV analysis of I in ethanol showed λ_{max}^{EtOH} 217 (log ϵ 3.90) and λ_{max}^{EtOH} 251 (log ϵ 3.90). The major IR bands and probable assignments for I were 3400 (broad, OH), 2960 (CH_3), 2930 (CH_2), 2870 (CH_2), 1653 (strong:γ-pyrone), 1585 (γ-pyrone), 1450 (CH_2), 1315 (weak), 1235 (weak), 1180 (weak), 1125 (weak), 1020 cm^{-1}.

The HRP mass spectral analysis of I gave a molecular ion peak (M^+) at m/e 224.1051 (calculated mass for $C_{12}H_{16}O_4$, 224.1048) and molecular formula $C_{12}H_{16}O_4$. Fragment ions were observed at m/e 208 ($M^+ - O$), 206 ($M^+ - H_2O$), 193 ($M^+ - CH_2OH$), and 188 ($M^+ - 2H_2O$).

The 100-MHz ^1H NMR spectrum of I showed a singlet at 7.79 ppm (1 H), a doublet at 4.59 ppm (1 H, J = 7.4 Hz), a singlet at 4.46 ppm (2 H), a multiplet at 2.59 ppm (2 H), a five proton multiplet between 1.15 and 1.57 ppm, and a methyl triplet at 0.98 ppm (J = 7.5 Hz). In addition, two broad peaks typical of hydroxyl protons were observed at 3.40 and 4.50 ppm. Thus, 16 protons were indicated in the spectrum of I. The 25-MHz proton-decoupled, natural abundance ^{13}C NMR spectrum exhibited a total of 12 peaks. Single-frequency, off-resonance proton decoupled (sford) spectra showed the 12 peaks to consist of one methyl carbon, four methylene carbons, three methine carbons, and four quaternary carbons. Chemical shift considerations suggested that the carbons may be classified further as a carbonyl carbon, one methine and three quaternary vinyl carbons, primary and secondary aliphatic alcohol carbons, and one methine, three methylene, and one methyl aliphatic carbons.

The ^1H and ^{13}C NMR spectra, along with the mass spectrum, suggested that I has the molecular formula $C_{12}H_{16}O_4$. Since three of the oxygens were accounted for by the carbonyl and two alcohol functional groups, the possibility of an ether-type oxygen was suggested for the remaining oxygen. The carbonyl region of the IR spectrum of I suggested the possibility of a γ-pyrone ring. This possibility was also consistent with the ^{13}C NMR spectrum because the carbonyl carbon and two of the vinyl carbons had chemical shifts, indicating that they were attached to an oxygen. Thus, the data indicated that I is a 5,6,7,8-tetrahydrochromone (Figure 2).

Further considerations of the ^1H and ^{13}C NMR spectra showed that only C(2) of the γ-pyrone ring was attached to a proton. A small coupling observed in the ^1H spectrum between the peaks at 7.79 and 4.46 ppm suggested that a $-CH_2OH$ group was attached to C(3) of the γ-pyrone ring. This left a hydroxyl and ethyl group to be located on the cyclohexene ring. The fact that the proton (4.59 ppm), which was attached to the same carbon as the hydroxyl group, exhibited a coupling of 7.4 Hz indicated that the hydroxyl and ethyl group were vicinal and trans to each other and that the hydroxyl group was located on either C(5) or C(8). Otherwise, the proton attached to the same carbon as the hydroxyl group would have shown additional splitting due to coupling with other protons. That the proton on the carbon attached to the hydroxyl group is coupled to only one proton was confirmed by spin-decoupling experiments at 220 MHz.

Table I. ^1H[a] and ^{13}C NMR Chemical Shifts of I and II

carbon	I	II[b]
2	152.05 (7.79)	153.76 (7.86)
3	123.43	119.46
4	180.29	175.95
5	68.48 (4.59)	66.99 (5.89)
6	41.22 (1.40)	37.96 (1.28)
7	24.17 (2.05, 1.75)	21.88 (1.92)
8	22.96 (2.60)	20.26 (2.54)
9	165.91	166.75
10	126.80	123.14
11	57.73 (4.46)	57.70 (4.97)
12	26.73 (1.67)	23.49 (1.28)
13	11.28 (0.98)	11.58 (1.00)

[a] ^1H chemical shifts in parentheses. [b] Acetate methyls 20.88 and 21.12 and carbonyls 169.73 and 170.61.

The diacetate derivative (II) of I was prepared to differentiate between the two possibilities for the hydroxyl group. Major IR absorptions and their probable assignments were 3020, 3010, 2965 (CH_3), 2930 (CH_2), 2880 (CH_2), 1730 (C=O of acetoxyl), 1662 (γ-pyrone), 1625, 1600 (γ-pyrone), 1440 (CH_2), 1370, 1315, 1225, 1180, 1140, 1125, 1110, 1020, 960, 900, 865 cm^{-1}. The 3400 cm^{-1} (broad band, OH) of I was not present in II and indicated acetylation. The mass spectrum showed a molecular ion peak at M^+/e 308 with other peaks at m/e 265 ($M^+ - CH_3CO$) and 223 (265 − CH_2CO) for the loss of two acetate fragments. Additional, intense peaks were found at m/e 206, 205, 189, 177, and 150.

Both ^1H and ^{13}C NMR spectra were consistent with the addition of two acetate groups. The ^1H NMR spectrum of II showed a singlet at 7.86 ppm (1 H), a doublet at 5.89 ppm (1 H, J = 2.0 Hz), a singlet at 4.97 ppm (2 H), a multiplet at 2.54 ppm (2 H), a multiplet at 1.92 ppm (2 H), a multiplet at 1.28 ppm (3 H), a triplet at 1.00 ppm (3 H, J = 7.5), and two singlets at 2.06 and 2.10 ppm for the acetate methyl groups. The ^{13}C NMR spectrum of II (Table I) showed that the two carbons attached to oxygen in the γ-pyrone ring were shifted downfield with respect to I, whereas the two carbons attached to the carbonyl carbon were shifted upfield. Furthermore, the aliphatic carbons of the cyclohexene ring moiety were also shifted upfield in II compared to I. These shifts are similar to those observed for 2-bromo-1-hydroxy-1,2,3,4-tetrahydronaphthalene and its acetate derivative (Cox, 1979).

The ^{13}C shifts of II compared to I suggested that the hydroxyl group is attached to C(5). Otherwise, opposite shift behavior would have been observed for the two carbons in the γ-pyrone ring attached to oxygen and the two attached to the carbonyl carbon. Therefore, all of the spectral data taken together established the structure of I as *trans*-6-ethyl-5-hydroxy-3-hydroxymethyl-5,6,7,8-tetrahydrochromone.

Assignments of the NMR spectra of I and II are given in Table I. Some additional features of the NMR spectra

of I and II deserve comment. The coupling constant of 7.4 Hz observed between the protons on C(5) and C(6) of I is consistent with diequatorial orientation of the hydroxyl and ethyl substituents. On the basis of Drieding models, this orientation would also lead to the maximum in hydrogen bonding between the hydroxyl proton and carbonyl oxygen. The smaller value of 2 Hz observed for this coupling constant in II indicates a conformational change for the cyclohexene ring moiety of II compared with I. Relief of steric strain in II is the most likely driving force for this conformation difference. In some experiments in which the ^1H spectrum of I was obtained with added lanthanide shift reagent [$Eu(FOD)_3$], both primary and secondary alcohols appear to be complexing with Eu to about equal extents. However, the singlet observed for the protons of C(11) in I splits into an AB pattern (J = 14 Hz) upon addition of the shift reagent. This result indicates that rotation about the C–C bond is slow with respect to the NMR time scale in the complex and probably results from complexation of Eu with both oxygens of the hydroxyl group as well as the carbonyl oxygen.

Bioassays. During the 4-day test period with chicks, the clinical signs observed at all dosage levels were lethargy and anorexia. General deterioration continued at the two higher dosage levels and resulted in death of all chicks. However, at the two lower levels all chicks appeared to be fully recovered from the initial effects when the test was terminated. From these data the LD_{50} was calculated to be 3.5 mg/chick or 88.4 mg/kg body weight.

Diplodiol did not inhibit wheat coleoptile growth at any concentration tested. However, the diacetate (II) significantly ($P < 0.01$) inhibited coleoptiles 19% at 10^{-3} M relative to controls. While this is not in the range of activity of, say, the plant growth inhibitor (±) abscisic acid, it was repeatable from one test to another. Of significance here is the general rule that those natural products exhibiting plant growth inhibitory activity are those that have, among other considerations, free hydroxyl groups in the molecule. Alteration of those groups by acetylation, or methylation, may reduce or eliminate activity, for example, hydroxyterphenyllin vs. terphenyllin tetraacetate (Cutler et al., 1978), orlandin vs. kotanin (Cutler et al., 1979). In the case of diplodiol diacetate the reverse is true. It is possible that the diacetate, being lipophilic, is more readily translocated to an active site and is then subjected to hydrolysis, thus exhibiting biological activity, whereas diplodiol per se cannot be translocated. But for now the observation remains a curious anomaly.

We have reported (Latterell et al., 1976) that because of the increased use of minimum tillage in corn cultivation, the possibility exists that *Diplodia macrospora*, which until now has been a minor pathogen, may change roles and become a major pathogen in the southeastern corn belt of the United States. If this happens, it may be necessary to consider the consequences of potential diplodiol contamination in animal feed.

NOTE

During preparation and after transmittal of this manuscript, a recent publication by A. A. Chalmers, C. P. Gorst-Allman, N. P. J. Kriek, W. F. O. Marasas, P. S. Steyn, R. Vleggar [*S. Afr. J. Chem.* **31**, 111 (1978)] reported the isolation of diplosporin [(5S,6X)-6-ethyl-5-hydroxy-3-hydroxymethyl-5,6,7,8-tetrahydrobenzo[*b*]pyran-4-one] from *D. macrospora*.

ACKNOWLEDGMENT

The authors thank H. Shindo, NIH, for the 220-MHz ^1H NMR spectra and W. J. Wiswesser for suggesting the trivial name diplodiol and pointing out that chromone = benzopyran-4-one. WLN (Wiswesser Line Notation) descriptions are diplodiol = T66 BO EV&TJ D1Q GQ H2 and diplodiol diacetate = T66 BO EV&TJ D1OV1 GOV1 H2.

LITERATURE CITED

Cox, R. H., unpublished results, 1979.
Cutler, H. G., Vlitos, A. J., *Physiol. Plant.* **15**, 27 (1962).
Cutler, H. G., LeFiles, J. H., Crumley, F. G., Cox, R. H., *J. Agric. Food Chem.* **26**, 632 (1978).
Cutler, H. G., Crumley, F. G., Cox, R. H., Hernandez, O., Cole, R. J., Dorner, J. W., *J. Agric. Food Chem.* **27**, 592 (1979).
De León, C., Perez, J., Proceedings of the VI Congreso Nacional Fitopatología, Chapingo, Mexico, 1970.
Eddins, A. H., *Phytopathology* **20**, 439–448 (1930).
Hancock, C. R., Barlow, H. W., Lacey, H. J., *J. Exp. Bot.* **15**, 166 (1964).
Kirksey, J. W., Cole, R. J., *Mycopathol. Mycol. Appl.* **54**, 291 (1974).
Kurtz, T. E., Link, R. F., Tukey, J. W., Wallace, D. L., *Technometrics* **7**, 95 (1965).
Latterell, F. M., Rossi, A. E., Moreno, R., *Proc. Am. Phytopathol. Soc.* **3**, 228 (1976).
Nitsch, J. P., Nitsch, C., *Plant Physiol.* **31**, 94 (1956).
Rabie, C. J., *Proc. Int. Mycol. Congr., 2nd*, 549 (1977).
Steyn, P. S., Wessels, P. L., Holzapfel, C. W., Potgieter, D. J. J., Louw, W. K. A., *Tetrahedron* **28**, 4775 (1972).
Weil, C. S., *Biometrics* **8**, 249 (1952).

Received for review May 21, 1979. Accepted August 28, 1979. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or National Institute of Environmental Health Sciences and does not imply their approval to the exclusion of other products or vendors that may also be suitable.