Inhibition of Soft-Rotting *Erwinia* spp. Strains by 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one in relation to their Pathogenicity on *Zea mays*

G. H. Lacy, S. S. Hirano, J. I. Victoria, A. Kelman, and C. D. Upper

Respectively, research associate, research associate, research assistant and professor, Department of Plant Pathology; and research chemist, Federal Research, Science and Education Administration, U.S. Department of Agriculture, University of Wisconsin, Madison, 53706.

Present addresses: G. H. Lacy, Department of Botany and Plant Pathology, The Connecticut Agricultural Experiment Station, New Haven, CT 06504; and J. I. Victoria, Instituto Colombiano Agropecuario, Palmira, Colombia.

This research was supported by NSF Grant BMS-11786, NIH Postdoctoral Training Grant 1-T32-BM07194 in Pathobiology (senior author), and Graduate Fellowships from the Rockefeller Foundation and from the Instituto Colombiano Agropecuario (third author).

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply approval to the exclusion of other products that also may be suitable.

We gratefully acknowledge the assistance of O. S Vicen, who prepared some of the figures; F. Vojtik, who maintained the growth chambers; J. A. Steele, who adapted his nonlinear regression computer program for our use; and those who supplied cultures or seed.

Accepted for publication 1 February 1979.

ABSTRACT

LACY, G. H., S. S. HIRANO, J. I. VICTORIA, A. KELMAN, and C. D. UPPER. 1979. Inhibition of soft-rotting *Erwinia* spp. strains by 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one in relation to their pathogenicity on *Zea mays*. Phytopathology 69:757-763.

The susceptibility of 68 *Erwinia* strains to 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA), an inhibitory compound often found in corn, was measured with an agar diffusion assay. The strains included 34 *Erwinia chrysanthemi* from corn (EC_z), 25 *E. chrysanthemi* from hosts other than corn (EC_o), and nine *E. carotovora* (EC). Twenty-seven (80%) of the EC_z strains were relatively resistant to DIMBOA, whereas 96% of the EC_o and 89% of the EC strains were relatively susceptible. Genetically related corn plants containing (*BxBx* genotype) or lacking (*bxbx* genotype) DIMBOA were inoculated with EC_z strains resistant and susceptible to DIMBOA. Significant differences generally were not noted in percentage of plants infected, proportion of resistant plants, and mean effective doses (ED_{50}'s) required to cause stalk rot symptoms in the resistant and susceptible plant populations. Furthermore, at least seven DIMBOA-susceptible EC_o strains were moderately pathogenic and produced typical stalk rot symptoms in DIMBOA-containing corn plants of the inbred line $W117^{Ht}$. Therefore, DIMBOA is not the primary means of resistance of corn to *E. chrysanthemi*. However, since 80% of the EC_z strains were resistant to DIMBOA, this compound in corn tissue probably exerts selection pressure for *E. chrysamthemi* strains pathogenic to corn, at some level other than that of primary host resistance.

Additional key words: corn, cyclic hydroxamates, soft-rot.

Bacterial stalk and top rot of corn (*Zea mays* L.) is geographically widespread and occasionally has been very destructive in tropical areas and under overhead irrigation in temperate zones (16,17). Although the causal agent has been assigned several names (16,17,19), it has the greatest taxonomic affinity for the *Erwinia chrysanthemi* Burkholder, McFadden, and Dimock group (1,7,22). For convenience, strains of *E. chrysanthemi* from diseased corn will be designated as EC_z and strains from hosts other than corn as EC_o.

Hartman et al (6) found that lyophilized, aqueous extracts of corn inhibited the growth of EC_z strains less than that of soft-rotting *Erwinia* spp. strains nonpathogenic to corn. The inhibitory effect was manifested as an extension of the lag phase of bacterial growth, with no major effect on log-phase growth. Hartman et al (6) suggested that the differentially inhibitory fraction might contribute to the resistance of corn to soft-rotting bacteria. The major inhibitory component in Hartman's corn extracts was identified as the cyclic hydroxamate, 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3-(4*H*)-one (DIMBOA), by Corcuera et al (3). When purified DIMBOA and ethyl acetate extracts of corn homogenates were added in equivalent hydroxamate concentrations to a bacterial growth medium, inhibitory responses were similar in strains of EC_z, EC_o, and *E. carotovora* (Jones) Bergey, Harrison, Breed, Hammer, and Huntoon. Fifty to 100% of the bacteriostatic activity of corn extracts was attributable to DIMBOA. Furthermore, extracts of DIMBOA-lacking (*bxbx*) corn plants did not inhibit the *Erwinia* strains that were tested.

Either DIMBOA or its degradation product, 6-methoxybenzoxazolinone (MBOA), are toxic to several plant pathogens and insect pests including *Bipolaris* (*Helminthosporium*) *turcicum* (14), *Fusarium nivale* (24), *Erwinia stewartii* (25), the European corn borer (*Ostrinia nubilalis*) (11), and the corn leaf aphid (*Rhopalosiphum maidis*) (13). The possible involvement of DIMBOA or MBOA in resistance of corn to these organisms has been suggested (11,13,14,24,25).

This research was conducted to determine whether the degree of growth inhibition of soft-rotting *Erwinia* strains by DIMBOA was related to their pathogenicity to corn plants. Part of the information presented in this paper has been reported previously (12,23).

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Stock bacterial cultures were stored in capped tubes of sterile distilled water at ambient temperatures (20–26 C). Fresh cultures were prepared by streaking the stock bacterial suspension on a tetrazolium agar medium (TZC) (10). Single colonies were selected for experimentation from TZC plates after incubation for 48 hr at 30–32 C (24 C for *E. carotovora* var. *atroseptica* [Hellmers and Dowson] Dye). The bacterial strains most frequently used were EC_z SR-120, SR-80, SR-78, and *E. carotovora* var. *carotovora* strain SR-53.

Assays for inhibition by DIMBOA. Two assay procedures were used to measure inhibition of *Erwinia* growth.

Liquid turbidimetric assay. The liquid turbidimetric assay was based on the procedure described by Hartman et al (6) and modified by Corcuera et al (3). The assay medium contained 1%

sucrose, mineral salts (9), and 0.1% vitamin-free, acid hydrolyzed casein (Casamino Acids, Difco, Detroit, MI 48232). The medium was buffered with 0.1 M sodium phosphate buffer (pH 6.75) or with 0.1 M succinic acid buffer (pH 5.5).

Purified DIMBOA from corn extracts (2) was dissolved in absolute ethanol and quantitated spectrophotometrically (MW 211, $E_{262\,nm} = 10{,}000$). Required volumes of the DIMBOA solution were dried under vacuum and stored at −20 C. Within 2 hr before the experiment, DIMBOA was dissolved in the assay medium at 28 C, then filtered through 0.45-μm pore size membranes (Millipore, Bedford, MA 01730). Four-milliliter portions of the sterile, DIMBOA-containing medium were dispensed into sterile, cotton-plugged 25-ml Erlenmeyer flasks.

For preparation of inocula for the assay, single bacterial colonies were transferred from 48-hr TZC plates to 25 ml of the assay medium. After incubation at 28 C in a water bath with shaking for about 15 hr, the bacterial cultures were diluted to approximately 10^8 colony-forming units per milliliter (cfu/ml) with sterile assay medium. A 0.1-ml portion of diluted cell suspension was added to each flask of medium with DIMBOA. Two flasks per treatment were inoculated in each experiment. The flasks were incubated at 28 C in a water bath with reciprocal shaking (133–138 cycles per minute). Bacterial density was measured turbidimetrically at 2-hr intervals with a colorimeter (Model 900-3, Klett-Summerson, New York, NY 14603) equipped with a No. 66 filter.

The effect of DIMBOA on bacterial growth was expressed as relative inhibition (RI), which is defined as the ratio of the time required to attain a turbidity of 100 Klett units (KU) ($\sim 10^9$ cfu/ml) in the presence of DIMBOA to the time required to attain the same turbidity in the absence of DIMBOA (3). The determination of time to 100 KU was facilitated by a program for the Wang 2200 computer (Tewksbury, MA 01876) which corrected

TABLE 1. Origin of bacterial strains used to study inhibition of *Erwinia* spp. by 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxain-3(4*H*)-one

Strain[a]	Host	Location of isolation	Source
Erwinia chrysanthemi Burkholder, McFadden & Dimock			
SR-56 (EC-209)	corn (*Zea mays* subsp. *mays*)	Egypt	Sabet
SR-58, SR-59, SR-60C	corn	North Carolina	Kelman
SR-61, SR-75-1, SR-78, SR-79, SR-80	corn	Wisconsin	Kelman
SR-84, SR-90, SR-91, SR-92, SR-93	corn	India	Payak
SR-94B, SR-96-1, SR-97	corn	North Carolina	Kelman
SR-120 (071-1230)	corn	Hawaii	Hayward
SR-140-1 (221-IPV-BO, NCPPB-2347), SR-141-1 (226-IPV-BO, NCPPB-2348), SR-142 (228-IPV-BO)	corn	Italy	Mazzucchi
SR-144-1 (74V, NCPPB-552)	corn	Israel	Quinn
SR-145 (NCPPB-377)	corn	Rhodesia	Quinn
SR-171, SR-172-1, SR-258	corn	Colombia	Victoria
SR-260-1	corn	South Africa	Mildenhall
SR-261	corn	Costa Rica	Madrigal
SP-26, SP-111, SP-221, SP-222, SP-271, SP-272	corn	Wisconsin	Victoria
D-23	stonecrop (*Sedum* sp.)	New York	Dickey
D-52 (ATCC-11662)	*Chrysanthemum* sp.	New York	Dickey
D-79	poinsettia (Euphorbia sp.)	Ohio	Dickey
D-103	carnation (*Dianthus* sp.)	Pennsylvania	Dickey
D-175, D-430 (NCPPB-1609)[b]	*Dahlia* sp.	Netherlands	Dickey
D-242, D-262	*Philodendron* sp.	Florida	Dickey
D-249	*Dracena* sp.	Florida	Dickey
D-290	*Syngonium* sp.	Florida	Dickey
D-362	banana (*Musa paradisiaca* var. *sapientum*)	Honduras	Dickey
D-378	*Dieffenbachia* sp.	Honduras	Dickey
D-431 (NCPPB-1849)	*Parthenium* sp.	U.S.A.	Dickey
D-438 (NCPPB-2309)	Shasta daisy (*Chrysanthemum maximum*)	Italy	Dickey
D-439 (NCPPB-2340)	*Chrysanthemum* sp.	England	Dickey
D-469 (CNBP-1361)	African violet (*Saintpaulia ionantha*)	France	Dickey
D-578 (CNBP-722)	tomato (*Lycopersicum esculentum*)	France	Dickey
D-587 (NCPPB-898)	geranium (*Pelargonium* sp.)	Comoro Island	Dickey
D-600	sweet potato (*Ipomea batatas*)	Georgia	Dickey
SR-30, SR-31 (ICPB-EC-176), SR-32 (ICPB-EC-16)	chrysanthemum	New York	Starr
SR-146-2, SR-147-2	grass	Australia	Quinn
SR-149	sugar cane (*Saccharum officinarum*)	Australia	Quinn
Erwinia carotovora var.*atroseptica* (Hellmers and Dowson) Dye			
SR-8	potato (*Solanum tuberosum*)	Wisconsin	Kelman
SR-54 (ICPB-EA-143)	potato	England	Starr
SR-55 (ICPB-EA-155, NCPPB-549)	potato	Scotland	Starr
SR-246	potato	Arizona	Stanghellini
Erwinia carotovora var. *carotovora* Dye			
SR-44 (ICPB-EC-15)	potato	California	Starr
SR-53 (ATCC-495, ICPB-EC-208)	carrot (*Daucus carota*)	Vermont	Starr
SR-162	potato	North Dakota	Kelman
SR-164	potato	Wisconsin	Kelman
SR-204	potato	New York	Burkholder

[a] Strain designations of the Department of Plant Pathology, University of Wisconsin, Madison, 53706 (other designations in parentheses).
[b] Correction of National Collection of Plant Pathogenic Bacteria (NCPPB) list of cultures by R. S. Dickey (*personal communication*).

KU for deviation from Beer's law and determined the best fit for plotting exponential growth through 100 KU versus time (26,27).

Agar diffusion assay. The assay medium and inocula were prepared as described for the liquid turbidimetric assay. Ten milliliters of assay medium (pH 6.75) containing 1.7% Noble agar (Difco) were mixed with 0.1 ml of bacterial suspension (10^8 cfu/ml) in a 90-mm diameter polystyrene petri dish. After the agar had solidified, two circular wells (6.5 mm in diameter) were cut in the agar in each plate. Either 0, 25, 50, or 100 μg of DIMBOA, dissolved in 0.01 ml of absolute ethanol, was added to the wells. The highest concentration of DIMBOA was used for routine screening. The diameter (in mm) of the zone of inhibition surrounding each well was measured after incubation at 30 C for 48 hr and was used to calculate the area of inhibition (AI) in square millimeters. The AI values were corrected for any inhibition by the solvent and for the area of the well.

Corn lines. The derivation of *BxBx* and *bxbx* corn lines from Hamilton's (5) original selection is shown in Fig. 1. Corn lines 1552 (*BxBx*) and 1455 (*bxbx*), developed by Corcuera et al (3), and the Wisconsin public inbred line $W117^{Ht}$ were used in this study.

Corn seedlings from lines 1552 and 1455 contained DIMBOA at 2.6 mmol/kg and less than 0.007 mmol/kg fresh weight, respectively (3). Line $W117^{Ht}$ contained DIMBOA at 3.2 mmol/kg fresh weight (26).

The presence or absence of hydroxamates in corn seedlings was confirmed by squashing root tips on Whatman No. 1 filter paper (W. R. Balston, Maidstone, Kent, England) treated with an acidic-alcoholic solution of ferric chloride (1 ml of 12 N HCl, 10 g of $FeCl_3 \cdot 6H_2O$ dissolved in 100 ml of 95% ethanol) (3). The treated paper was blue in the presence of hydroxamates and colorless or pink in their absence.

Growth of corn plants. Corn seeds were germinated for 72 hr at room temperature (20–26 C) between layers of paper towels moistened with distilled water. The germinating seeds were planted in a steamed mixture of sand and muck soil (1:2 v/v, pH 7, 16% organic carbon) in 12.7-cm diameter plastic pots (six to seven seeds per pot). The plants were grown for 18 days in a growth chamber (Percival P6W 144, Boone, IA 50036) maintained on a regime of 16 hr light (26 C, 63% relative humidity [RH], ~ 1.9×10^4 lux at the top of the pots) and 8 hr dark (21 C, 71% RH). The corn plants were watered every other day. The first two applications were with a modified Hoagland's solution containing: $Ca(NO_3)_2 \cdot 4H_2O$ (6.3 mM), KNO_3 (6.3 mM), KH_2PO_4 (2.5 mM), Fe (EDTA) (0.16 mM), H_3BO_3 (70 μM), $MnCl_2 \cdot 4H_2O$ (140 μM), $ZnSO_4 \cdot 7H_2O$ (1.2 μM), $CuSo_4 \cdot 5H_2O$ (0.32 μM), and $H_2MoO_4 \cdot H_2O$ (0.88 μM). Subsequent applications were with deionized water.

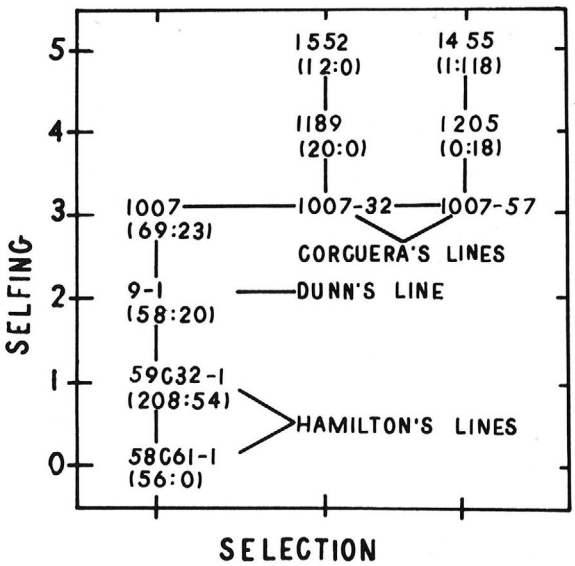

Fig. 1. Derivation of selfed (S_1 to S_5) *BxBx* and *bxbx* corn lines from Hamilton's original selection from open-pollinated cultivar Gehu Yellow Dent. The ratios in parentheses are the number of segregates that contained hydroxamate (*BxBx*) to the number that did not (*bxbx*), as determined by the ferric chloride test (3). Note that "9-1" was Corcuera's designation for material received from G. M. Dunn, Department of Plant Science, University of New Hampshire, Durham, 03824.

Inoculation procedure. Corn plants were inoculated by the procedure developed by Victoria (21,22). Single colonies from 48-hr TZC plates were streaked on nutrient agar (Difco) slants supplemented with glucose (8 g/L) and incubated for 24 hr at 30 C. The resulting bacterial growth was suspended in and diluted with sterile distilled water to a final turbidity of 0.5 OD_{600nm} units determined with a colorimeter (Spectronic 20, Bausch and Lomb, Rochester, NY 14603). For counts of viable cell, the suspensions were diluted serially and then plated on CPG medium (TZC medium without triphenyltetrazolium chloride) and incubated for 24 hr at 32 C.

Corn plants were inoculated by injecting 0.2 ml of diluted cell suspension into the center of the pseudostem just above the apical meristem with a 0.25-mm diameter (30-gauge) needle. The needle wound was sealed immediately with autoclaved petrolatum (Amojell, American Oil, Chicago, IL 60646). The inoculated plants were placed in a growth chamber maintained at constant temperature (32 C). The relative humidity was 64% during the daily 12-hr photoperiod and 78% during the dark period.

Analyses of host response. The corn plants were scored for disease development 6 days after inoculation as follows: 1 = no visible symptoms; 2 = slight discoloration of the pseudostem at the point of inoculation; 3 to 12 = increasing degrees of rotting of the pseudostem at or near the point of inoculation (= 3), spreading internally to the apical meristem (= 6) and to the outside of the pseudostem (= 9) with lodging (= 10) and complete soft rot (= 12) (21,23). The scores were converted to percentages for determination of the percentage of infection on a per-plant basis by the method of Horsfall and Barratt (8).

Quantal host responses (ie, all-or-none) also were determined to graded inoculum concentrations of EC_z. Plants with disease development scores of 3 or higher were considered to be positive and plants with scores of less than 3 were considered to be negative. The untransformed data were analyzed by nonlinear regression with the independent-action or single-hit model (4,20,21) for single-, double-, and triple-component response-probability functions based on the equation:

$$R = 1 - \alpha_1 e^{-0.69\,(d/d\,)} - \alpha_2 e^{-0.69\,(d/d_2)} \ldots - \alpha_n e^{-0.69\,(d/d_n)}$$

in which:

R = diseased fraction of plants at each inoculum concentration;

$1 = \alpha_1 + \alpha_2 \ldots + \alpha_n$;

d = number of cells inoculated per plant;

d_n = mean effective dose (ED_{50}) of the α_n fraction; and

α_n = the fraction of plants represented by each component of the response-probability function.

Values for α_n and d_n were estimated by an iterative, nonlinear least-squares algorithm described by Marquardt (15) and adapted for the Wang 2200 system by J. A. Steele (WARF Institute, University of Wisconsin, Madison 53706) from a program described by Ryshpan and Henkel (18).

RESULTS

Agar diffusion assay for effect of DIMBOA on *Erwinia* strains. All 68 soft-rotting *Erwinia* strains were tested for susceptibility to DIMBOA by agar diffusion assay. When the strains were ranked in order of increasing susceptibility to 100 μg DIMBOA (Fig. 2), it was apparent that the EC_z strains were, in general, more resistant to DIMBOA than the EC_o and *E. carotovora* (EC) strains. Twenty-seven of 34 EC_z strains (80%) either were resistant to DIMBOA or produced areas of inhibition less than 175 mm^2. In contrast, only one of 25 EC_o and one of nine EC strains showed similar levels of resistance to DIMBOA. The other seven EC_z strains were as

susceptible as most of the EC_o and EC strains tested. With the exception of the DIMBOA-resistant strain D-431, the EC_o strains exhibited a broad continuum of susceptibility to DIMBOA, with areas of growth inhibition ranging from 175–885 mm^2.

The data were analyzed by the use of the F test for means with unequal numbers of observations. The mean area of inhibition of the EC_z group, 129.9 ± 80.5 (SD) mm^2, was significantly different from the means of the EC_o and EC groups, 311.7 ± 151.2 (SD) mm^2 and 243.1 ± 59.3 (SD) mm^2, respectively, at $P < 0.001$. The means of the EC_o and EC groups were not significantly different from each other at this probability level.

Effect of DIMBOA on four *Erwinia* strains by the liquid turbidimetric assay. The response of EC_z SF-78 (DIMBOA-susceptible) to various concentrations of DIMBOA was compared to the responses of EC_z SR-120 and SR-80 (DIMBOA-resistant) as well as EC SR-53 (DIMBOA-susceptible and nonpathogenic to corn) in broth assay media buffered at pH 6.75 (Fig. 3) and pH 5.5 (Fig. 4).

Inhibition of the *Erwinia* spp. strains by DIMBOA was made manifest by increase of the lag phase of growth. The responses of strains SR-53, SR-80, and SR-120 to DIMBOA in the assay were similar to previous observations of these strains (3,27). The responses of SR-78 and SR-53 to increasing concentrations of DIMBOA were very similar to each other at both pH 6.75 and 5.5 and were unlike those of EC_z strains SR-120 and SR-80. Both SR-78 and SR-53 were more susceptible to DIMBOA at pH 5.5 than at 6.75, whereas SR-120 was more resistant to DIMBOA at the lower pH. Unlike SR-120, SR-80 was no more susceptible to DIMBOA at pH 6.75 than at pH 5.5. Concentrations of DIMBOA higher than 0.2 mM were bactericidal to SR-78 and SR-53 at pH 5.5.

Relationship between DIMBOA susceptibility in vitro and pathogenicity of EC_z strains on *BxBx* and *bxbx* corn lines. The genetically related DIMBOA-containing (genotype *BxBx*, line 1552) and DIMBOA-lacking (genotype *bxbx*, line 1455) inbred corn lines were inoculated with the SR-78, (DIMBOA-susceptible) SR-120 and SR-80 (DIMBOA-resistant) strains of EC_z. Eleven concentrations of inoculum were tested with each bacterial strain.

In a representative pathogenicity experiment (Table 2), SR-120 was the most pathogenic, SR-78 was intermediate, and SR-80 was the least pathogenic. No significant differences in stalk rot damage were noted between *BxBx* and *bxbx* corn plants inoculated with either SR-120 or SR-80. However, damage was greater on *bxbx* than on *BxBx* plants at four of 11 inoculum concentrations with SR-120 and at six of 11 concentrations with SR-80. In plants inoculated with SR-78, the mean damage was greater on *bxbx* corn plants at nine of 11 inoculum concentrations. However, only at three concentrations were these differences significant. The trend for greater damage on *bxbx* than on *BxBx* corn inoculated with SR-78 was confirmed in three experiments.

Iterative nonlinear regression analyses of the results of the inoculation experiments with the independent-action model (4,20,21), for one-, two-, and three-component exponential response-probability curves (for values of α_1 to α_3 and d_1 to d_3) indicated that the two-component curve most accurately represented the host response data. A more extensive discussion of the heterogenous response (susceptible and resistant populations) of inbred corn lines to disease caused by various levels of EC_z inoculum has been reported (21,23). The fraction of resistant plants (α_2) and the ED_{50}'s of the susceptible (d_1) and resistant (d_2) populations were estimated from the two-component exponential response-probability curves for the *BxBx* and *bxbx* plants inoculated with three bacterial strains at eleven concentrations each (Table 3). The proportion of resistant plants and the ED_{50} of the resistant plant populations were slightly higher in *BxBx* corn plants inoculated with SR-78 and SR-80 than in the comparable *bxbx* plants, but these differences were not significant. The proportions of resistant plants were similar in *BxBx* and *bxbx* plants inoculated with SR-120.

Relationship between DIMBOA susceptibility in vitro and pathogenicity of EC_z and EC_o strains on the $W117^{Ht}$ corn line.

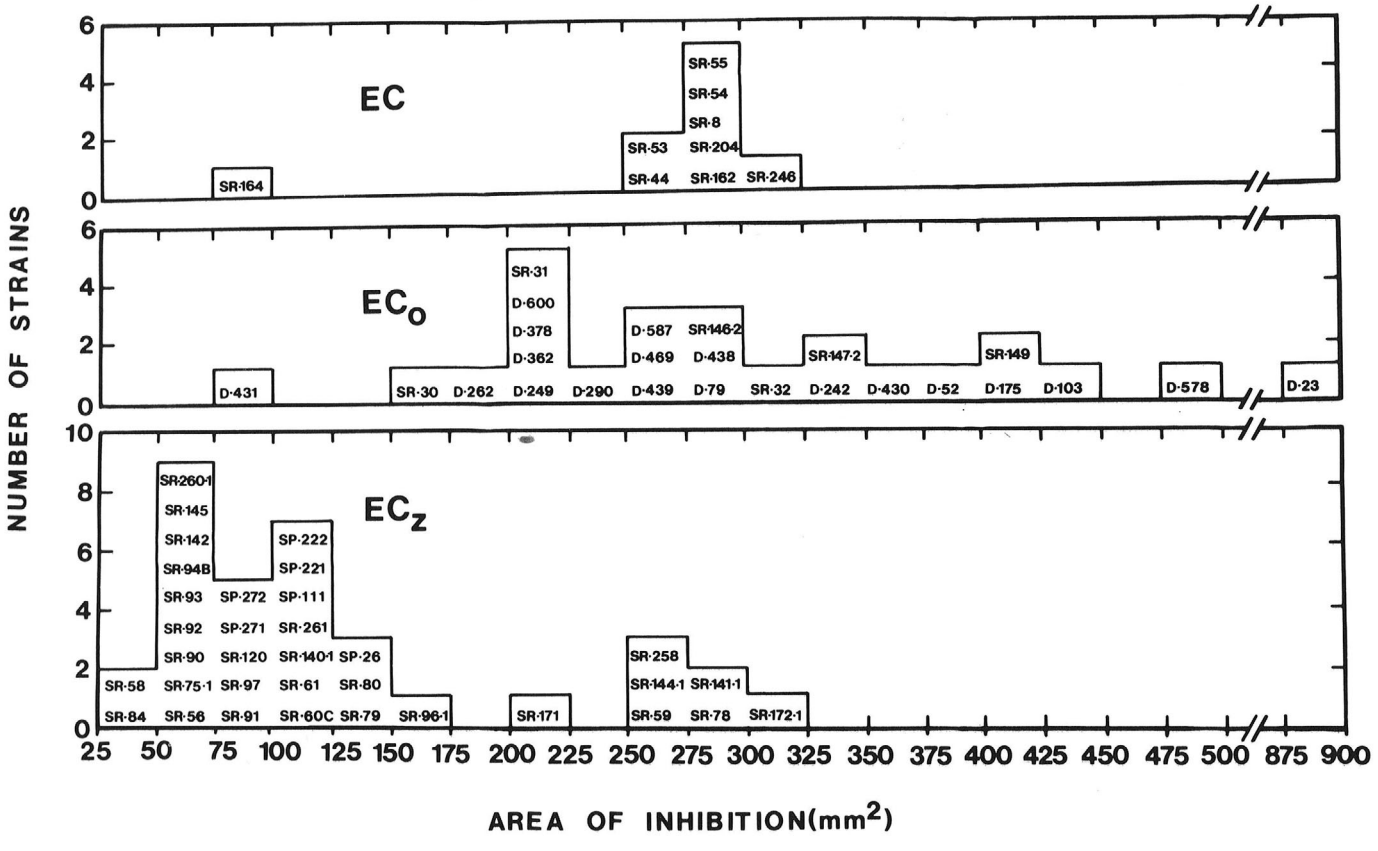

Fig. 2. Responses of 68 strains of *Erwinia* spp. to 100 μg 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) as determined by inhibition of growth in the agar diffusion assay. The *E. chrysanthemi* strains included 34 from corn (EC_z) and 25 from other hosts (EC_o). The *E. carotovora* group (EC) included five strains of *E. carotovora* var. *carotovora* and four of *E. carotovora* var. *atroseptica*.

Twenty-eight EC_z and 24 EC_o strains were inoculated into plants of the inbred corn line $W117^{Ht}$ which contains DIMBOA at 3.2 mmol/kg of fresh weight. Six of the 28 EC_z and 23 of the 24 EC_o strains were susceptible to DIMBOA in the agar diffusion assay. Each strain was inoculated at $\sim 2 \times 10^7$ cfu/plant into at least 40 corn plants. Host response, expressed as percentage of infection per plant, was determined six days after inoculation (Fig. 5).

The ability to induce stalk rot in corn was not limited to the EC_z strains (Fig. 5), which were isolated originally from diseased corn plants. At least seven EC_o strains (D-600, isolated from sweet potato; D-469, from African violet; D-242, from philodendron; D-438, from Shasta daisy; D-290, from syngonium; D-79, from poinsettia; and D-249, from dracena) were moderately pathogenic (caused more than 35% infection) on corn and produced typical stalk rot symptoms, despite being susceptible to DIMBOA in the agar diffusion assay. Although several EC_z strains did not produce stalk rot symptoms on corn line $W117^{Ht}$, most EC_z strains were moderately or highly pathogenic (caused more than 55% infection). Six EC_z strains found to be relatively susceptible to DIMBOA by agar diffusion assay produced the following percentages of infection per plant: 1.5% (SR-172-1), 4.5% (SR-78), 14.6% (SR-141-1), 19.6% (SR-171), 42% (SR-59), and 55% (SR-258).

The coefficient of correlation between the levels of pathogenicity, based on percentage of infection per plant, and

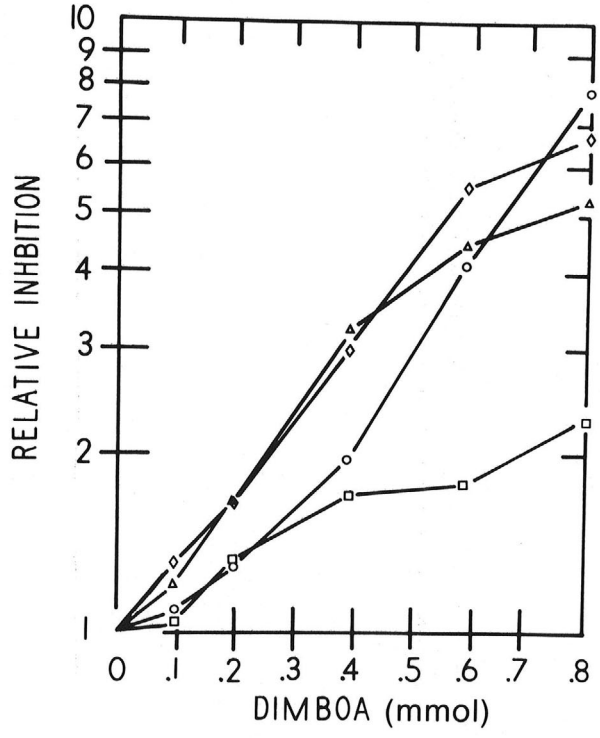

Fig. 3. Relative inhibition (see definition in text) of four *Erwinia* spp. strains caused by increasing concentrations of 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) in bacterial growth medium buffered at pH 6.75. The times required for controls (no DIMBOA added) to attain a turbidity of 100 Klett units were as follows: 7.0 hr for SR-120 (o), 14.0 hr for SR-80 (□), 9.5 hr for SR-78 (Δ) (all *E. chrysanthemi* from corn) and 8.0 hr for SR-53 (◇) (*E. carotovora*). Each point represents the average of two replicates.

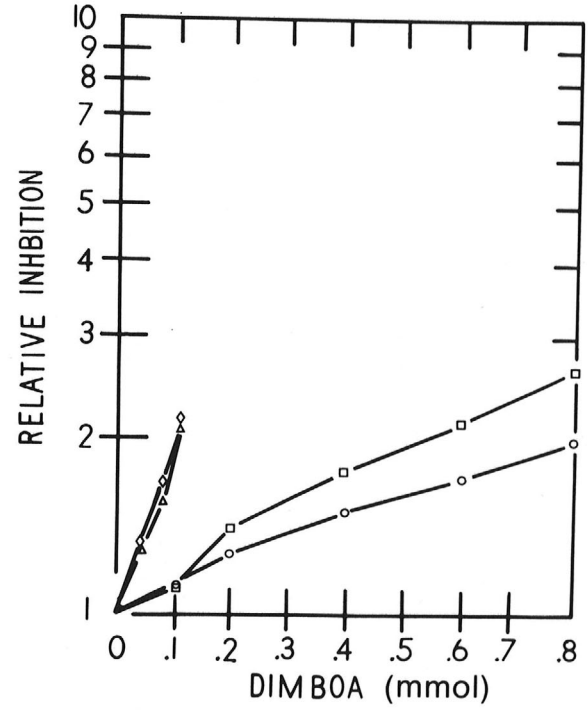

Fig. 4. Relative inhibition (see definition in text) of four *Erwinia* spp. strains caused by increasing concentrations of 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) in bacterial growth medium buffered at pH 5.5. The times required for controls (no DIMBOA added) to attain a turbidity of 100 Klett units were as follows: 10.4 hr for SR-120 (o), 15.0 hr for SR-80 (□), 13.4 hr for SR-78 (Δ) (all *E. chrysanthemi* from corn); and 16.8 hr for SR-53 (◇) (*E. carotovora*). Each point represents the average of two replicates.

TABLE 2. Pathogenicity of three strains of *Erwinia chrysanthemi* on DIMBOA-containing (*BxBx*) and DIMBOA-lacking (*bxbx*) corn plants[a]

	Disease development (% of plant diseased[b]) six days after inoculation					
	Strain SR-120		Strain SR-80		Strain SR-78	
Inoculum dilution factor	*BxBx*[d]	*bxbx*	*BxBx*	*bxbx*	*BxBx*	*bxbx*
Undiluted[c]	100.0	95.2	11.0	18.6	63.8**[d]	99.8**
5^{-1}	97.9	90.0	5.3	13.4	64.3	79.4
5^{-2}	85.1	100.0	1.0	9.6	34.9	64.5
5^{-3}	75.0	90.0	4.0	9.9	13.8	26.3
5^{-4}	62.3	85.0	0.0	9.0	42.9	70.2
5^{-5}	89.5	80.0	0.0	14.0	17.5	35.0
5^{-6}	36.8	55.4	6.2	5.1	22.6*	54.7*
5^{-7}	58.0	48.1	11.9	3.1	26.5	11.7
5^{-8}	67.9	55.0	2.8	0.0	17.0	10.2
5^{-9}	16.7	10.5	0.0	0.0	10.5	19.4
5^{-10}	15.8	10.2	0.0	0.0	4.8*	8.5*

[a] DIMBOA is 2, 4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one.
[b] Each value is the mean of 16 to 21 plants.
[c] Undiluted inoculum levels (cfu/plant): SR-120, 7.7×10^7; SR-80, 8.2×10^7; and SR-78, 7.8×10^7.
[d] Corn lines were 1552 (*BxBx*) and 1455 (*bxbx*).
[e] According to Duncan's multiple range test, the designated pairs of means were significantly different at $P < 0.05$ (*) and $P < 0.01$ (**).

TABLE 3. Proportion of resistant plants (α_2) and ED_{50}'s for resistant (d_1) and susceptible (d_2) populations of *BxBx* and *bxbx* corn plants inoculated with three strains of *Erwinia chrysanthemi* from corn

E. chrysanthemi strain	Proportion of resistant plants (α_2)		ED_{50} (colony-forming units per plant)			
			Resistant population (d_1)		Susceptible population (d_2)	
	BxBx	*bxbx*	*BxBx*	*bxbx*	*BxBx*	*bxbx*
SR-120	0.43[a]	0.49	8.62×10^5	8.62×10^4	4.95×10^1	5.65×10^1
SR-80	0.92	0.86	2.41×10^8	1.90×10^8	6.90×10^1	1.39×10^3
SR-78	0.80	0.72	3.21×10^7	1.25×10^7	5.12×10^2	2.04×10^3

[a]The values of α_2, d_1, and d_2 were estimated from untransformed data by iterative fitting and nonlinear regression of two-component host response-probability functions based on the independent-action model for infection (4,20,21).

DIMBOA susceptibilities, based on area of inhibition measured by the agar diffusion assay, for all *E. chrysanthemi* strains was −0.57 (50 df, significant at the $P < 0.01$). When *E. Chrysanthemi* strains were grouped according to host origin, the correlation coefficients were −0.61 (26 df, significant at the $P < 0.01$) and −0.25 (22 df) determined for the EC_z and EC_o strains, respectively.

DISCUSSION

Hartman et al (6) and Corcuera et al (3) suggested that DIMBOA could be a factor in the resistance of corn to soft-rotting *Erwinia* strains, since DIMBOA inhibited soft-rotting *Erwinia* strains pathogenic to corn less than soft-rotting bacteria nonpathogenic to corn. We have since found by liquid turbidimetric assay that *Erwinia* strain SR-78, which was isolated from diseased corn in Wisconsin in 1966, is as susceptible to DIMBOA as is *E. carotovora* SR-53, which is nonpathogenic to corn. Unlike EC_z SR-120 or SR-80, EC_z SR-78 closely resembled EC SR-53 in its response to increasing concentrations of DIMBOA at both pH 5.5 and 6.75.

When 68 *Erwinia* strains from several different hosts were screened for susceptibility to DIMBOA, a relationship between host origin and resistance to DIMBOA was demonstrated. Most (80%) of the *E. chrysanthemi* strains isolated from diseased corn plants (EC_z) were resistant to DIMBOA, whereas only 4% of the *E. chrysanthemi* strains from hosts other than corn (EC_o) and 11% of the *E. carotovora* strains (EC) were resistant. The relationship between host origin and resistance to DIMBOA may reflect an evolutionary response of the pathogen to the habitat within a corn plant. That is, the DIMBOA in corn may exert selection pressure

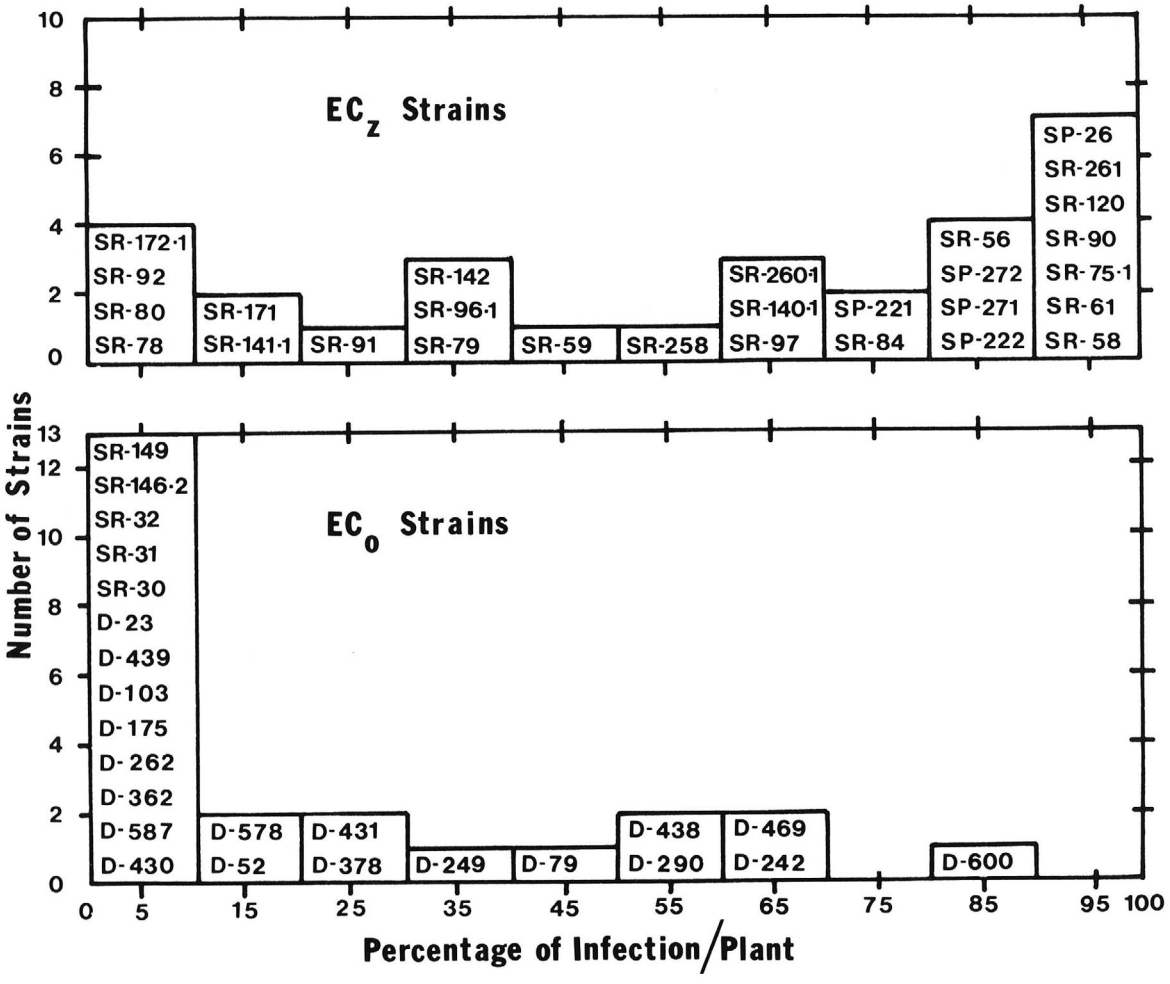

Fig. 5. Pathogenicity of *Erwinia chrysanthemi* strains from corn (EC_z) and from other plants (EC_o) compared on plants of the inbred line $W117^{Ht}$ six days after inoculation. Each strain was inoculated at one inoculum concentration (2×10^7 cfu/plant). The percentage of infection per plant was the average rating of at least 40 corn plants.

for resistance to DIMBOA among *E. chrysanthemi* strains that can infect corn. The ability to cope with toxic host metabolites, in general, may be advantageous to a pathogen, both during infection and, perhaps, for survival after pathogenesis is completed within host tissue.

Genetically related DIMBOA-containing (*BxBx*) and DIMBOA-lacking (*bxbx*) corn plants were not significantly different in susceptibility to EC_z strains SR-120, SR-80, and SR-78 in pathogenicity experiments despite the differences in the in vitro DIMBOA susceptibility demonstrated by the three corn pathogens.

At least seven EC_o strains were moderately pathogenic on DIMBOA-containing corn plants of the inbred line $W117^{Ht}$ in a second series of pathogenicity experiments despite being relatively susceptible to DIMBOA. Thus, with the inoculation procedure used, DIMBOA does not play a major role in host resistance to the EC_z or the EC_o strains pathogenic on corn. If inhibitory levels of DIMBOA were generated during the infection process, then the DIMBOA-susceptible pathogens must have an alternate method of coping with the toxic compound in corn tissue.

The resistance of corn to *E. carotovora* also could not be attributed to DIMBOA. Strain SR-53 did not rot whole *bxbx* (DIMBOA-lacking) corn plants even with inocula of up to 10^8 cfu per plant (21). It is apparent that factors other than DIMBOA are active in the defense mechanism of corn to these soft-rotting *Erwinia* strains.

The higher frequency of DIMBOA resistance in EC_z than in EC_o and EC strains may be interpreted that DIMBOA is active in natural selection for DIMBOA resistance in bacterial strains pathogenic to corn. In support of this hypothesis is the observation that other bacterial corn pathogens, such as *E. stewartii* and *Pseudomonas syringae*, are as resistant as EC_z strains to Hartman's differentially inhibitory fraction (containing DIMBOA) (6). Presumably, toxic plant metabolites need not be active specifically at the level of host resistance to the infecting pathogen to be a factor in the coevolution of host and pathogen. However, evolved resistance to these metabolites may be important in the success of potential pathogens infecting other available hosts and thus continuing the disease cycle.

LITERATURE CITED

1. BRENNER, D. J., G. R. FANNING, and A. G. STEIGERWALT. 1977. Deoxyribonucleic acid relatedness among Erwiniae and other enterobacteria. II. Corn stalk rot bacterium and Pectobacterium chrysanthemi. Int. J. Syst. Bacteriol. 27:211-221.
2. CORCUERA, L. J. 1974. Identification of the major active component present in corn extracts inhibitory to soft rot Erwinia species. Ph.D. Dissertation, University of Wisconsin, Madison. 81 pp.
3. CORCUERA, L. J., M. D. WOODWARD, J. P. HELGESON, A. KELMAN, and C. D. UPPER. 1978. 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one, an inhibitor from Zea mays with differential activity against soft-rotting Erwinia species. Plant Physiol. 61:791-795.
4. ERCOLANI, G. L. 1967. Bacterial canker of tomato. I. Analysis of some factors affecting the response of tomato to Corynebacterium michiganense (E.F. Sm.) Jens. Phytopathol. Mediterr. 6:19-29.
5. HAMILTON, R. H. 1964. A corn mutant deficient in 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one with an altered tolerance of atrazine. Weeds 12:27-30.
6. HARTMAN, J. R., A. KELMAN, and C. D. UPPER. 1975. Differential inhibitory activity of a corn extract to Erwinia spp. causing soft rot. Phytopathology 65:1082-1088.
7. HOPPE, P. E., and A. KELMAN. 1969. Bacterial top and stalk rot disease of corn in Wisconsin. Plant Dis. Rep. 53:66-70.
8. HORSFALL, J. G., and R. W. BARRATT. 1945. An improved grading system for measuring plant disease. (Abstr.) Phytopathology 35:655.
9. HUSAIN, A., and A. KELMAN. 1958. Relation of slime production to mechanism of wilting and pathogencity of Pseudomonas solanacearum. Phytopathology 48:155-165.
10. KELMAN, A. 1954. The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
11. KLUN, J. A., W. D. GUTHRIE, A. R. HALLAUER, and W. A. RUSSELL. 1970. Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one and resistance to the European corn borer in a diallel set of eleven maize inbreds. Crop Sci. 10:87-90.
12. LACY, G. H., S. S. HIRANO, J. I. VICTORIA, and A. KELMAN. 1977. Relative inhibition of soft-rotting Erwinia strains by DIMBOA in relation to host origin and pathogenicity to corn. (Abstr.) Proc. Am. Phytopathol. Soc. 4:140-141.
13. LONG, B. J., G. M. DUNN, J. S. BOWMAN, and D. G. ROUTLEY. 1977. Relationship of hydroxamic acid content in corn and resistance to the corn leaf aphid. Crop Sci. 17:55-58.
14. LONG, B. J., G. M. DUNN, and D. G. ROUTLEY. 1975. Relationship of hydroxamic acid content in maize and resistance to northern corn leaf blight. Crop Sci. 15:333-335.
15. MARQUARDT, D. W. 1963. An algorithm for least squares estimation of nonlinear parameters. J. Soc. Industrial Appl. Math. 2:431-441.
16. MAZZUCCHI, U. 1972. A bacterial stalk rot of maize (Zea mays L.) in Emilia. Phytopathol. Mediterr. 11:1-5.
17. RANGARAJAN, M., and B. P. CHAKRAVARTI. 1971. Erwinia carotovora (Jones) Holland as the inciting agent of corn stalk rot in India. Phytopathol. Mediterr. 10:41-45.
18. RYSHPAN, J., and J. HENKEL. 1972. Nonlinear regression routines: Reference manual for the 1110. Academic Computing Center, University of Wisconsin, Madison. 83 pp.
19. SABET, J. A., A. S. SAMRA, and H. A. EL-SHAFEY. 1964. On the systematic position of the coliform bacteria responsible for stalk rot of maize. Ann. Appl. Biol. 53:267-274.
20. SHORTLEY, G., and J. R. WILKINS. 1965. Independent-action and birth-death models in experimental microbiology. Bacteriol. Rev. 29:102-141.
21. VICTORIA, J. I. 1977. Resistance in corn (Zea mays L.) to bacterial stalk rot in relation to virulence of strains of Erwinia chrysanthemi. Ph.D. dissertation, University of Wisconsin, Madison. 179 pp.
22. VICTORIA, J. I., F. ARBOLEDA, and S. MUÑOZ. 1975. La pudricion suave del tallo de maize (Zea mays L.) en Colombia. Not. Fitopatol. 4:136-147.
23. VICTORIA, J. I., and A. KELMAN. 1977. Resistance in corn to bacterial stalk rot in relation to virulence of strains of Erwinia chrysanthemi. (Abstr.) Proc. Am. Phytopathol. Soc. 4:140.
24. VIRTANEN, A. I., and P. K. HIETALA. 1955. 2(3)-Benzoxazolinone, an anti-Fusarium factor in rye seedlings. Acta Chem. Scand. 9:1543-1544.
25. WHITNEY, N. J., and C. G. MORTIMORE. 1961. Effect of 6-methoxy-benzoxazolinone on the growth of Xanthomonas stewartii (E.F. Smith) Dowson and its presence in sweet corn (Zea mays var. saccharata Bailey). Nature 189:596-597.
26. WOODWARD, M. D. 1975. Biological and chemical properties of 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one and related compounds from maize. Ph.D. dissertation, University of Wisconsin, Madison. 148 pp.
27. WOODWARD, M. D., L. J. CORCUERA, J. P. HELGESON, A. KELMAN, and C. D. UPPER. 1978. Factors that influence the activity of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one on Erwinia species in growth assays. Plant Physiol. 61:803-805.