Hydrogen Cyanide Sensitivity in Bacterial Pathogens of Cyanogenic and Non-Cyanogenic Plants

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ABSTRACT

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The cyanide sensitivity of bacterial pathogens of cyanogenic and non-cyanogenic plants was determined by observing the effects of HCN on their growth rates in buffered nutrient broth. The HCN concentration that reduced the growth rate of each strain to 50% of its growth rate in the absence of cyanide (EC_{50}) was calculated and used to compare strains. Bacterial pathogens of cyanogenic plants appeared to be only slightly more tolerant of HCN than were bacterial pathogens of non-cyanogenic plants; these differences were not statistically significant ($P = 0.05$). Pseudomonads generally were more sensitive to HCN than were the xanthomonads tested. Among xanthomonads, strains of *Xanthomonas manihotis*, a pathogen of the highly cyanogenic plant, cassava (*Manihot esculenta*), appeared to be most tolerant. None of the bacteria appeared to either metabolize HCN or to adapt markedly to it. In contrast to fungal pathogens of cyanogenic plants, bacterial pathogens of cyanogenic plants are not distinctly more tolerant of HCN than are those of non-cyanogenic plants.

Cyanogenic glycosides are produced by over 1,000 plant species, but their role is a subject of speculation (25). One function may be to protect plants from pests (14,23). However, simple correlations between glycoside content and resistance to a given pathogen have not been consistently identified (16,28,29).

Studies of the copperspot disease of the cyanogenic plant, birdsfoot trefoil (*Lotus corniculatus* L.) reveal that the fungal pathogen *Stemphylium loti* Graham, is tolerant of cyanide (HCN) (17). Tolerance is probably due to an HCN-inducible enzyme, formamide hydro-lyase (FHL), that detoxifies cyanide to formamide (9–11). FHL also was found in *Gloeocercospora sorghi* Bain and Edgerton, a pathogen of the cyanogenic plant sorghum (*Sorghum bicolor* (L.) Moench), indicating that this enzyme is likely to be responsible for HCN tolerance in that fungus. Fry and Munch (12) hypothesized that pathogens of cyanogenic plants cope with HCN if it is released. An association between fungal pathogens of cyanogenic plants and the ability to produce FHL has since been demonstrated. Of 31 species of fungi tested, every aggressive pathogen of a cyanogenic plant has a metabolic means of coping with HCN (8). Furthermore, in vivo detoxification of HCN via FHL is indicated in the pathogenesis of sorghum by *G. sorghi* (19–21) in which the pathogen apparently is exposed to HCN during disease development, but can tolerate the concentrations encountered.

The purpose of our work was to determine if a similar relationship exists for bacterial pathogens, that is, to determine if bacterial pathogens of cyanogenic plants are more tolerant of HCN than bacterial pathogens of non-cyanogenic plants. A preliminary report of this work has been made (24).

MATERIALS AND METHODS

Bacteria. Six species of bacteria in two genera were studied. At least two strains of each species were tested. *Pseudomonas syringae* and *Xanthomonas manihotis* are pathogens of the cyanogenic plants sorghum and cassava (*Manihot esculenta* Crantz), respectively (3,18). *P. lachrymans, P. tabaci, X. malvacearum,* and *X. vesicatoria* were isolated from plants not known to be cyanogenic. Strains of *P. syringae* isolated from wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.), which are not highly cyanogenic, also were tested. The strains used, host of isolation, location, and donor of each strain were tabulated (Table 1). Pseudomonads were maintained on Difco nutrient agar (Difco Laboratories, Detroit, MI 43210) while xanthomonads were maintained on potato-dextrose agar plus 1% glucose (PDA +). This medium was made from 500 ml of water in which 200 g of potato tuber tissue had been autoclaved, 20 g Difco Bacto agar, 30 g glucose, and water added to bring the total volume to 1 L.

HCN sensitivity. The sensitivity of bacteria to HCN was determined by observing its effects on the growth rate of each strain. Growth was determined turbidimetrically. Preliminary inoculum consisted of two loopfuls of cells from a culture which had grown on an agar slant for 24 hr at 27 C. This inoculum was placed into 25 ml of buffered nutrient broth plus 1% (w/v) glucose (BNB+) in a 125-ml Erlenmeyer flask. BNB+ consists of 8 g Difco nutrient broth plus 10 g glucose per L in 0.1 M potassium phosphate buffer, pH 6.8. This culture, which was designated the primary inoculum, was incubated at 27–29 C on a rotary shaker operated at 250 cpm. After 15 hr, 10 ml of the culture was removed and added to 10 ml of fresh BNB+ in a sterile sidearm (nephelo) culture flask made from a 250-ml Erlenmeyer flask with an 18 mm × 150-mm test tube as a sidearm. This culture was designated the secondary inoculum and was maintained under conditions described for the primary inoculum. Secondary inoculum was used to initiate the sensitivity studies when its turbidity, measured at 620 nm with a Bausch & Lomb Model 20 Spectronic colorimeter (Bausch & Lomb Inc., Rochester, NY 14650), attained an optical density (OD) of 0.9 or greater.

The effect of HCN on growth of the different bacteria was determined by using the sidearm flasks as described above. Each flask contained 8 ml fresh BNB+, 1 ml of the secondary inoculum and 1.0 ml of HCN solution. Flasks were plugged with sterile rubber stoppers immediately after addition of the HCN. At the beginning of an assay, each sidearm flask contained a total volume of 10 ml of a BNB+ culture of exponentially growing cells with an $OD_{620nm} = 0.10 \pm .03$ at a desired final HCN concentration ranging from zero to 0.30 mM. Three concentrations of HCN and a zero-HCN control were tested in each experiment. Each treatment was replicated once or twice within an experiment. Cultures were incubated under conditions described for the inoculum and turbidity was determined periodically. Experiments routinely were terminated when the OD of the control (no HCN) flask had increased from the initial OD by 0.60 or more units.

When the average increase in OD_{620nm} (Δ OD_{620nm}) of the two flasks containing no HCN was $0.60 \pm .01$, the Δ OD_{620nm} for each

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experimental flask was determined (Fig. 1). The relative growth, measured as the change in OD_{620nm} (Δ OD_{620nm}) of the culture (the mean of both replicates), was calculated as a proportion of the growth in the flasks containing no HCN and designated "percent growth" for that treatment. Percent growth was plotted against HCN concentration, and the concentration of HCN that inhibited 50% of the growth (EC_{50}) was found by interpolation (Fig. 2).

The HCN sensitivity of each strain was assayed at least twice.

Adaptation. To determine whether bacteria adapted to HCN, the effect of cyanide on the growth rate of bacteria that had been previously exposed to HCN was measured. Bacteria were grown in medium with HCN and then assayed for cyanide sensitivity. Two strains were tested for adaptation. *P. lachrymans* 5 was chosen because it is the pseudomonad with the lowest EC_{50}, and *X. manihotis* 1105 was tested because it is the xanthomonad with the highest EC_{50} value (see Results). Primary inoculum was prepared as described above, but secondary inoculum was initiated with 20 ml of BNB+ culture with an $OD_{620nm} = 0.80 \pm 0.05$, and an HCN concentration either equal to the EC_{50} value for the strain or no HCN for the control. These cultures were grown with shaking as described above for approximately two doubling times. This interval was 2 hr for *P. lachrymans* 5 and 4 hr for *X. manihotis* 1105. A large number of cells was used for the secondary inoculum to insure that the standard condition of $OD_{620nm} \geq 0.9$ would be met for the cyanide sensitivity assay after the appropriate pretreatment period. The HCN sensitivity of HCN-exposed and control secondary inoculum was determined as previously described. Adaptation experiments were repeated at least once for each strain.

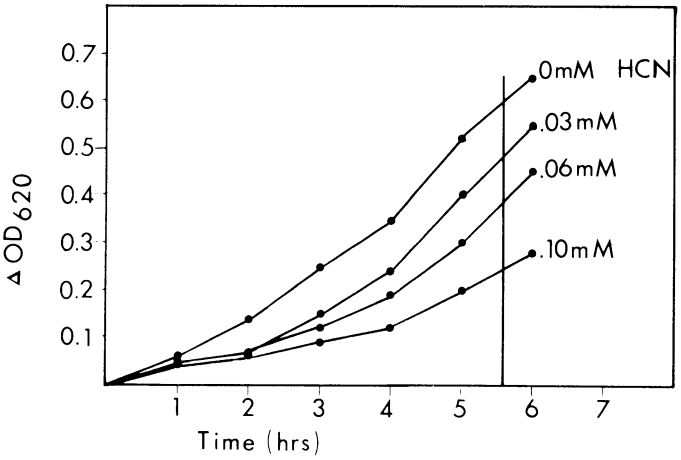

Fig. 1. Effect of HCN on growth of *Pseudomonas lachrymans* 5. The initial turbidity (OD_{620nm}) of all suspensions was approximately 0.1. The vertical line at 5.7 hr indicates the time at which the EC_{50} was calculated.

Determination of HCN concentration. The concentration of HCN in glass-distilled water solutions was determined with alkaline sodium picrate solution (2.5 g picric acid and 2.5 g Na_2CO_3/L) (20). One milliliter of solution to be assayed was added to 4 ml of picrate solution in a 13 mm × 100-mm test tube, sealed with a rubber stopper, mixed, and incubated for 24–48 hr at room temperature. Absorbance was determined at 480 nm in a Bausch & Lomb Model 20 Spectronic colorimeter. A standard curve was used to relate cyanide concentrations to absorbance.

HCN concentration in BNB+ cultures was assayed in a manner similar to that described by Myers and Fry (19) because sodium picrate reacts with compounds other than cyanide in this complex medium (9). A 2-ml BNB+ sample was placed in the center well of polypropylene conway microdiffusion dishes (Bel-Art Products, Inc., Peguannock, NJ 07440). Two drops of 5 N HCl were added to induce volatilization of HCN and inhibit further bacterial growth. The amount of HCN volatilized after 48 hr was determined colorimetrically as described above.

Pathogenicity tests. Pathogenicity of the three *X. manihotis* strains was tested by inoculating 6-wk-old rooted cuttings of cassava cultivar Llanera. Bacteria were cultured on PDA+ at 27 C for 36–48 hr and suspended in sterile distilled water to an OD_{620nm} of 1.0 or approximately 10^8 colony forming units (CFU) per milliliter. Controls consisted of autoclaved bacterial suspensions. Plants were incubated in a growth chamber at 33 C with 100% relative humidity (RH) for 72 hr, then at 70% RH for 3 wk.

Two inoculation procedures were used. In the stem puncture method, plants were inoculated at the fourth leaf axil from the apex by piercing the stem with a needle such that a drop of bacterial suspension remained at each side of the wound. For spray inoculations, leaves were sprayed to the point of run-off with a suspension of bacteria in distilled water containing 0.2% (v/v) Tween 20.

RESULTS

Cyanide reduced the rate of the exponential growth of all phytopathogenic bacteria; the effect on the lag phase was small and difficult to discern. A representative set of growth curves illustrate

TABLE 1. Strains of bacterial pathogens of cyanogenic and non-cyanogenic plants and their cyanide sensitivity in vitro

Species and strain number	Source of Bacterial Strain		Cyanide Sensitivity[a]	
	Host	Location and Donor[b]	EC_{50} (Strain mean)	EC_{50} (Species mean)
Pseudomonas lachrymans 3	cucumber	(R.S.D.)	0.08 ± .01 ef	0.07 x
P. lachrymans 5	cucumber	Canada, (R.S.D.)	0.06 ± .01 f	
P. tabaci 3	tobacco	Indiana, (R.S.D.)	0.08 ± .01 ef	0.08 x
P. tabaci 11	soil	(R.S.D.)	0.08 ± .01 ef	
P. syringae 7	sorghum	South Dakota, (J.D.O.)	0.08 ± .01 ef	0.10 x
P. syringae 19	sorghum	South Dakota, (J.D.O.)	0.08 ± .01 ef	
P. syringae 159	sorghum	South Dakota, (J.D.O.)	0.08 ± .00 ef	
P. syringae 2	corn	(L.D.D.)	0.16 ± .01 ab	
P. syringae 747	wheat	South Dakota, (J.D.O.)	0.10 ± .01 cdef	
Xanthomonas malvacearum 13	cotton	South Carolina, (R.S.D)	0.13 ± .01 bcd	0.11 x
X. malvacearum 14	cotton	Texas, (R.S.D.)	0.09 ± .03 def	
X. vesicatoria 21	tomato	Pennsylvania, (R.S.D.)	0.11 ± .02 cde	0.11 x
X. vesicatoria 27	pepper	New York, (R.S.D.)	0.11 ± .02 cde	
X. manihotis 1060	cassava	Colombia, (J.C.L.T.)	0.09 ± .01 def	0.13 x
X. manihotis 1088	cassava	Africa, (J.C.L.T.)	0.14 ± .01 abc	
X. manihotis 1105	cassava	Nigeria, (J.C.L.T.)	0.17 ± .04 a	

[a] The EC_{50} is the concentration of HCN (mM) at which 50% growth was achieved relative to the zero HCN control. The mean of two or more experiments is reported and standard deviations are indicated. Values followed by the same letter are not significantly different according to an approximate Duncan's multiple range test ($P = 0.05$).

[b] Individuals from whom cultures were obtained: R. S. D. (R. S. Dickey, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853, USA); L. D. D. (L. D. Dunkle, University of Nebraska, Lincoln 68503, USA); J. C. L. T. (J. Carlos Lozano T., Centro Internacional de Agricultura Tropical, CIAT, Apartado Aereo 67-13, Cali-Valle, Colombia, S. A.); J. D. O. (J. D. Otta, Dept. Plant Science, South Dakota State University, Brookings 57006, USA). Strain numbers designated by the donor were retained. *P. lachrymans* 3 is American Type Culture Collection (ATCC) culture 7386.

the reduced growth of *P. lachrymans* 5 in broth amended with HCN (Fig. 1). Where EC_{50} values differed among replicate experiments, the mean is reported in Table 1 and the standard deviation is indicated. The OD_{620nm} for replicates within a treatment never had a variance greater than 0.05.

Xanthomonads generally were less sensitive than were pseudomonads (Table 1). *X. manihotis* was more tolerant of cyanide than were the other species tested (Table 1). The HCN sensitivities of strains of bacteria isolated from cyanogenic plants did not differ significantly ($P = 0.05$) from those of bacteria from non-cyanogenic plants (Table 1).

The pH of BNB+ containing HCN was 6.8, consequently, almost all the cyanide in the sidearm flasks was in the form of HCN. We found that the concentration of HCN in sterile BNB+ had decreased to about half the initial concentration within 2 hr, and then remained stable for at least an additional 6 hr. These findings are consistent with there being an equilibrium of HCN established between the air and broth fractions in the flasks. The ΔOD_{620nm} values used to calculate the EC_{50} were obtained after 3–8 hr of growth. Therefore, the EC_{50} of each strain was calculated after the HCN concentration in the broth had stabilized. The EC_{50} values were calculated on the basis of the concentrations of HCN initially established in the BNB+ cultures; the actual EC_{50} value for each strain may be estimated by multiplying the apparent value from Table 1 by 0.5.

Changes in turbidity reflected changes in CFU. For example, for *P. lachrymans* 5 turbidities of 0.11 and 0.70 (OD_{620nm}) corresponded to 0.8 and 6.6×10^8 CFU per milliliter. When 0.10 mM HCN suppressed turbidity of the culture to 38% that of the control, CFU were reduced to 34% that of the control.

The possibility that *X. manihotis* 1105, *P. lachrymans* 3, and *P. lachrymans* 5 might metabolize HCN was investigated by determining the HCN concentration after termination of HCN sensitivity experiments. In every case the HCN concentration in the bacterial culture was equal to that of a sterile BNB+ control which had been initiated with appropriate cyanide concentration at the beginning of the sensitivity experiment. Thus, no metabolism of HCN occurred. We also found that previous exposure to HCN did not significantly alter ($P = 0.05$) the ability of either *P. lachrymans* 5 or *X. manihotis* 1105 to tolerate cyanide (Table 2), even though the EC_{50} tended to increase.

The HCN sensitivity of bacterial pathogens of cyanogenic plants was not associated with loss of pathogenicity in these isolates. The three *X. manihotis* strains induced symptoms characteristic of cassava bacterial blight (CBB) (15). Plants that had been sprayed with *X. manihotis* suspensions developed angular water-soaked spots that were clearly distinguishable on the abaxial leaf surface 7–10 days after inoculation. Three to 5 days later these spots had coalesced to form large water-soaked areas on the leaf. These angular leaf spots are the most definite diagnostic feature of CBB (27). Plants inoculated by the stem puncture method had vascular discoloration both distally and proximally from the point of wounding 5–7 days after inoculation. The leaf originating at the point of the stem puncture wilted within 5–6 days. Leaves distal and proximal to this point began wilting 1–3 days later.

Bacteria were isolated from a plant inoculated by the stem puncture method with *X. manihotis* 1105. Portions of the stem from an area just beginning to show vascular discoloration were surface disinfested and macerated with a sterile scalpel in sterile water. This suspension was streaked on PDA+ plates and incubated at 27 C for 3 days. A large number of similar colonies developed that appeared mucoid, circular, shiny, cream colored, and convex with entire margins and that resembled those of *X. manihotis*.

DISCUSSION

Bacteria isolated from cyanogenic plants were not strikingly more tolerant of hydrogen cyanide (HCN) than were bacteria isolated from noncyanogenic plants. These observations contrast with those observed for fungal pathogens of cyanogenic plants, which are generally more tolerant of HCN than are fungal pathogens of non-cyanogenic plants (8,9,17). In cyanogenic plants infected by fungal pathogens, cyanogenic glycosides are metabolized to sugars, HCN, and appropriate aldehydes or ketones; thus free HCN occurs in these tissues but it seems to be metabolized by fungal pathogens via FHL (12,19,21). When FHL biosynthesis was inhibited, cyanide sensitivity of fungal pathogens of cyanogenic plants was similar to that of other sensitive fungi (10,22).

Because tolerance to HCN and production of FHL are inducible by HCN in fungi, we investigated the possibility that HCN might induce tolerance mechanisms in bacteria. When bacterial pathogens of plants were assayed for sensitivity to HCN after a preliminary exposure to it, there again appeared to be no major difference between bacterial pathogens of cyanogenic plants and those of non-cyanogenic plants. The EC_{50} value for *P. lachrymans* 5 (a pathogen of a non-cyanogenic plant) increased slightly (but insignificantly) and the EC_{50} for *X. manihotis* 1105 also increased slightly (but insignificantly). Some isolates of *P. syringae* have been assayed for FHL, but none has been detected (Fry and Evans, *unpublished*). Other pathways of HCN metabolism are identified in several species of bacteria (1,2,5,7,26,30). However, if these pathways were operating in the isolates we tested, their influence

TABLE 2. Effect of pre-treatment with HCN on the sensitivity of *P. lachrymans* 5 and *X. manihotis* 1105 to HCN

Species and strain	Pretreatment HCN concentration (mM)	EC_{50} (mM)[a]
P. lachrymans 5	0.00	0.06 ± .01
	0.06	0.08 ± .02
X. manihotis 1105	0.00	0.18 ± .04
	0.14	0.23 ± .05

[a] The EC_{50} is the concentration of HCN at which 50% growth was achieved relative to the zero-HCN control. The mean and standard deviation for each treatment are indicated. The amount of growth was determined turbidimetrically at OD_{620nm} as described in the text.

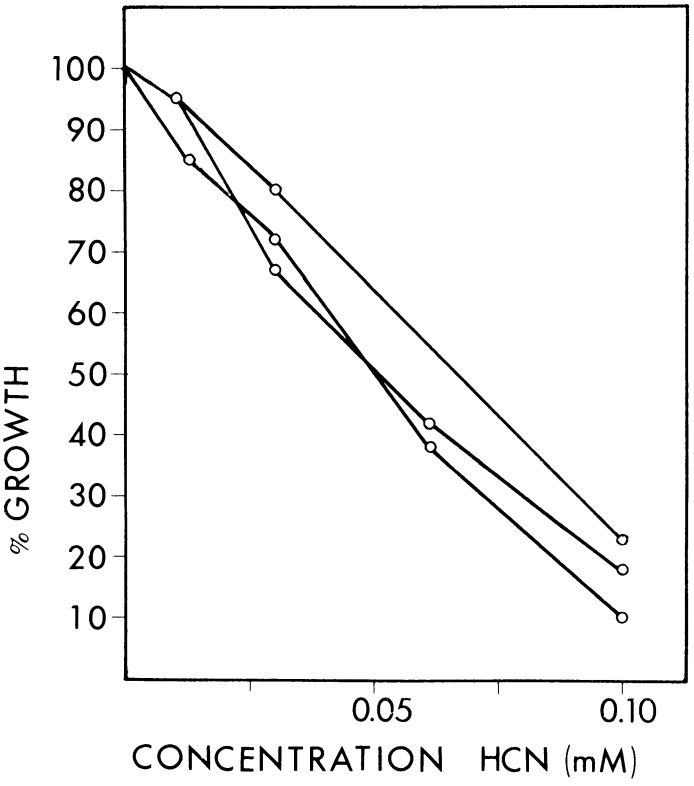

Fig. 2. Effect of HCN on growth of *Pseudomonas lachrymans* 5. Data from three experiments are presented. Growth was determined tubidimetrically (OD_{620nm}) in buffered nutrient broth plus glucose. These three curves represent the results of three separate experiments with this strain.

apparently was not sufficient to cause some isolates to be sensitive and others to be resistant to HCN.

The HCN-sensitivity of strains within some species was diverse, but some species still appeared to be more sensitive than others. For example, strains of *Xanthomonas manihotis*, a pathogen of the highly cyanogenic cassava plant, appeared to be generally more resistant to HCN than to strains of other species. However, even *X. manihotis* strains were not especially resistant compared to HCN-resistant fungi.

Our results with the sensitivity of bacterial isolates to HCN also differed from those obtained with another type of post-inhibition (sensu Ingham [13]). Strains of *Erwinia* spp. not pathogenic to corn were more sensitive to DIMBOA [2,4-dihydroxy-7-methoxy-2H-1, 4-benzoxazin-3(4H)-one] than were strains pathogenic to corn. The effect of DIMBOA was to extend the lag period of bacterial growth. The effect of HCN, in contrast, was to depress the rate of exponential growth phase (6).

In our study, *P. syringae* isolates from corn and wheat were grouped with pathogens from non-cyanogenic plants. However, some genotypes of these plants may produce HCN at concentrations orders of magnitude lower than those found in sorghum and cassava (3,4,18,21).

Our inability to detect markedly greater HCN tolerances among bacterial pathogens of cyanogenic plants relative to tolerances in bacterial pathogens of non-cyanogenic plants was unexpected and is puzzling. The range of sensitivity to HCN of these bacteria was comparable to that of fungi classified as sensitive to HCN (9). If the cyanogenic glycoside of young leaves of sorghum seedlings or of cassava leaves were hydrolyzed to release HCN, the amounts of HCN would be much greater than the EC_{50} values for these bacteria. For example, young leaves of sorghum seedlings contain sufficient glycoside to release up to 100 μmoles of HCN per gram dry wt ~10 mM HCN) (21). Cassava leaves contain even more cyanogenic glycoside. If even a small portion of the cyanogenic glycoside is hydrolyzed, one would expect it to adversely affect the growth of these bacteria. Obviously, the cyanide released is not sufficient to prevent infection. Information concerning the degree to which cyanogenic glycosides in the plant are metabolized and release HCN and the pathway by which they are broken down may contribute to resolving this paradox. If the glycosides are not metabolized or if HCN is not released into the intercellular environment of the leaf, bacterial sensitivity to HCN is irrelevant. If inhibitory concentrations of HCN are released into the environment of the bacterial pathogen, alternative explanations are needed.

LITERATURE CITED

1. ATKINSON, A. 1975. Bacterial cyanide detoxification. Biotechnol. and Bioeng. 17:457-460.
2. BOWEN, T. J., P. J. BUTLER, and F. C. HAPPOLD. 1965. Some properties of the rhodanese system of *Thiobacillus denitrificans*. Biochem J. 97:651-657.
3. BRUIJN, G. H. de. 1973. The cyanogenic character of cassava (*Manihot esculenta*). Pages 43–48 in: Chronic cassava toxicity: Proceedings of an interdisciplinary workshop. 29–30 January 1973. London, England. Int. Develop. Res. Centre Monogr. IDRC-010e.
4. BRÜNNICH, J. C. 1903. Hydrocyanic acid in fodder crops. J. Chem. Soc. 83:788-796.
5. CASTRIC, P. A., and G. A. STROBEL. 1969. Cyanide metabolism by *Bacillus megaterium*. J. Biol. Chem. 244:4089-4094.
6. 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.
7. DUNHILL, P. M., and L. FOWDEN. 1965. Enzymatic formation of β-cyanoalanine from cyanide by *Escherichia coli* extracts. Nature 208:1206-1207.
8. FRY, W. E., and P. E. EVANS. 1977. Association of formamide hydrolyase with fungal pathogenicity to cyanogenic plants. Phytopathology 67:1001-1006.
9. FRY, W. E., and R. L. MILLAR. 1971. Cyanide tolerance in *Stemphylium loti*. Phytopathology 61:494-500.
10. FRY, W. E., and R. L. MILLAR. 1971. Development of cyanide tolerance in *Stemphylium loti*. Phytopathology 61:501-506.
11. FRY, W. E., and R. L. MILLAR. 1972. Cyanide degradation by an enzyme from *Stemphylium loti*. Arch. Biochem. Biophys. 151:468-474.
12. FRY, W. E., and D. C. MUNCH. 1975. Hydrogen cyanide detoxification by *Gloeocercospora sorghi*. Physiol. Plant Pathol. 7:23-33.
13. INGHAM, J. L. 1973. Disease resistance in higher plants. The concept of pre-infectional and post-infectional resistance. Phytopathol. Z. 78:314-335.
14. JONES, D. A. 1973. Co-evolution and cyanogenesis. Pages 213–242 in: V. H. Heywood, ed. Taxonomy and Ecology. Special Vol. 5. Academic Press, New York, NY.
15. LOZANO, J. C., and L. SEQUEIRA. 1974. Bacterial blight of cassava in Colombia: etiology. Phytopathology 64:74-82.
16. LÜDTKE, M., and H. HAHN. 1953. Über die Linamaringehalt gesunder und von *Collectotrichum lini* befallener junger Leinpflanzen. Biochem. Z. 324:433-442.
17. MILLAR, R. L., and V. J. HIGGINS. 1970. Association of cyanide with infection of birdsfoot trefoil by *Stemphylium loti*. Phytopathology 60:104-110.
18. MYERS, D. F. 1978. Relation of hydrogen cyanide potential and hydrogen cyanide metabolism to the development of *Gloeocercospora sorghi* in sorghum leaves. Ph.D. Thesis, Cornell University, Ithaca, NY. 106 pp.
19. MYERS, D. F., and W. E. FRY. 1978. Hydrogen cyanide potential during pathogenesis of sorghum by *Gloeocercospora sorghi* or *Helminthosporium sorghicola*. Phytopathology 68:1037-1041.
20. MYERS, D. F., and W. E. FRY. 1978. The development of *Gloeocercospora sorghi* in sorghum. Phytopathology 68:1147-1155.
21. MYERS, D. F., and W. E. FRY. 1978. Enzymatic release and metabolism of hydrogen cyanide in sorghum infected by *Gloeocercospora sorghi*. Phytopathology 68:1717-1722.
22. RISSLER, J. F., and R. L. MILLAR. 1977. Contribution of a cyanide-insensitive alternate respiratory system to increases in formamide hydro-lyase activity and to growth in *Stemphylium loti* in vitro. Plant Physiol. 60:857-861.
23. ROBINSON, M. E. 1930. Cyanogenesis in Plants. Biol. Rev., Cambridge Philos. Soc. 5:126-141.
24. RUST, L. A., W. E. FRY, and S. V. BEER. 1979. Lack of HCN tolerance in bacterial pathogens of cyanogenic plants. Abstract 548 in: Proc. IX International Congress of Plant Protection, 5–11 August 1979; Washington, DC.
25. SEIGLER, D. S. 1976. Plants of the northeastern United States that produce cyanogenic compounds. Econ. Bot. 30:395-401.
26. SKOWRONSKI, B., and G. A. STROBEL. 1969. Cyanide resistance and cyanide utilization by a strain of *Bacillus pumilus*. Can. J. Microbiol. 15:93-98.
27. TERRY, E. R. 1976. Diagnosis of cassava bacterial blight disease. Pages 5–8 in: G. Persley, E. R. Terry, and R. MacIntyre, eds. Cassava Bacterial Blight. Report of an interdisciplinary workshop held 1–4 November 1976 at ITTA, Ibadan, Nigeria. IDRC-096e. Int'l. Devel. Res. Centre, Ottawa, Canada.
28. TIMONIN, M. I. 1941. The interaction of higher plants and soil microorganisms. III. Effect of by-products of plant growth on activity of fungi and actinomycetes. Soil Sci. 52:395-414.
29. TRIONE, E. J. 1960. The HCN content of flax in relation to flax wilt resistance. Phytopathology 50:482-486.
30. VILLAREJO, M., and J. WESTLEY. 1963. Mechanism of rhodanese catalysis of thiosulfate-lipoate oxidation-reduction. J. Biol. Chem. 238:4016-4020.