# Association of Formamide Hydro-lyase with Fungal Pathogenicity to Cyanogenic Plants

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ABSTRACT

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To estimate the occurrence of formamide hydro-lyase (FHL) among fungi, 31 species of fungi were tested for FHL. Induction by low levels (1.0 mM) of HCN generally caused at least a 50-fold increase in detectable activity. Formamide hydro-lyase was produced by one of six fungi not pathogenic to plants, nine of fourteen pathogens of non-cyanogenic plants, and all eleven pathogens of cyanogenic plants. Thus each of these pathogens of cyanogenic plants had a means of detoxifying HCN through conversion to formamide. Large differences in FHL-specific activities in cell-free homogenates were detected among pathogens of cyanogenic plants (4-66 μmoles/min/mg protein) and among pathogens of non-cyanogenic plants (0-72 μmoles/min/mg protein). The mean specific activities for pathogens of cyanogenic plants, pathogens of non-cyanogenic plants, and non-plant pathogens, were 30.1, 7.4, and 0.1 μmoles/min/mg protein, respectively.

The role of toxic compounds in plant resistance is not well established. A frequently cited example is the onion smudge disease, in which amounts of catechol and protocatechuic acid, found in pigmented onion scales are correlated with resistance of onions to *Colletotrichum circinans* (12, 13, 14, 15, 30). However, there is little conclusive evidence in other pathogen-plant systems that toxic compounds in the plant are involved in resistance to pathogens. For example, although high alkaloid content in potatoes was not associated with resistance to *Phytophthora infestans*, *Verticillium albo-atrum*, or *Streptomyces scabies* (5, 7), there have been conflicting interpretations of the significance of alkaloids in resistance of potatoes to *Alternaria solani* (7, 24).

Confusion surrounding the role in plant resistance of a plant toxicant may be due to differential sensitivities to the toxicant in fungi. For example, *Septoria lycopersici*, a pathogen of tomato, was capable of detoxifying α-tomatine, a glycosidic alkaloid found in tomatoes and potatoes (1). Detoxification occurred via enzymic hydrolysis of one or more sugar residues from α-tomatine (3). Tomato pathogens generally were less sensitive to α-tomatine than were fungi not pathogenic to tomatoes (2).

Our studies concern the role of hydrogen cyanide (HCN) in pathogenesis of cyanogenic plants by fungi. Hydrogen cyanide is released upon infection by hydrolysis of cyanogenic glycosides (18, 19). As is true for many toxic plant compounds, correlations between plant resistance and cyanogenic glycoside content have not been observed consistently (25, 27, 28). This lack of consistency is understandable if different fungi respond differently to HCN. If pathogens of cyanogenic plants are tolerant of HCN, no correlation between cyanogenic glycoside content and resistance would be expected. At least two fungal pathogens of cyanogenic plants, *Stemphylium loti* [a pathogen of the cyanogenic plant birdsfoot trefoil (*Lotus corniculatus* L.)] and *Gloeocercospora sorghi* [a pathogen of the cyanogenic plant *Sorghum vulgare* Pers.] are relatively tolerant of HCN (8, 10, 19). Tolerance was inducible by HCN and was associated with the appearance of the enzyme formamide hydro-lyase (FHL), which converted hydrogen cyanide to formamide ($HCONH_2$) (9, 11).

Occurrence among cyanogenic plant pathogens of a means to detoxify HCN could explain the lack of consistent correlation between cyanogenic glycoside content and resistance. This study was done to determine whether fungal pathogenicity to a cyanogenic plant is correlated to ability to convert HCN to $HCONH_2$ via FHL and to estimate the extent to which FHL production ability occurs among fungi.

## MATERIALS AND METHODS

**Fungi.**—Thirty-one fungal species were represented in this study. Eleven species (15 isolates) are pathogens of cyanogenic plants; 14 species are pathogens of non-cyanogenic plants; and six species are not plant pathogens. The individual species, source from which they were obtained, and an example of their pathogenic capabilities are indicated in Table 1.

Pathogenicity of fungi reported in the literature to be pathogens of cyanogenic plants (Table 1) was tested in a series of inoculation experiments. Fungi reported to cause disease of foliage (Table 1) were cultured on V-8 juice agar (20) at 22-25 C for 7-14 days. Suspensions of conidia in water containing 0.05% Tween-20 (polyoxyethylene sorbitan monolaurate) were atomized onto foliage of the appropriate host plant. Plants then were kept in a moist chamber at 20-27 C at 100% relative humidity for 24-48 hr, and then removed to a greenhouse bench. Only those fungi that were reported by others (Table 1) to be pathogens of cyanogenic plants and that

TABLE 1. Fungi tested for formamide hydro-lyase capability

| Fungus | Isolate | Disease | Lit. (ref) | Source[a] |
|---|---|---|---|---|
| Pathogens of cyanogenic plants: | | | | |
| *Colletotrichum graminicola* (Cesati) G. W. Wilson | (773) | Red stalk rot, red leaf spot of sorghum | (26) | CWB |
| *Fusarium moniliforme* Sheldon | (Per. exp.) | Fusarium stalk rot of sorghum | (26) | LKE |
| *Gloeocercospora sorghi* Bain & Edgerton | (isolate 1) | Zonate leaf spot of sorghum | (26) | RAF |
| *Gloeocercospora sorghi* Bain & Edgerton | (NEB) | Zonate leaf spot of sorghum | (26) | LDD |
| *Gloeocercospora sorghi* Bain & Edgerton | (Kan) | Zonate leaf spot of sorghum | (26) | LDD |
| *Helminthosporium maydis* Nisikado &Miyake (Race T) | (7544) | Leaf spot of sorghum | (20) | OCY |
| *Helminthosporium sorghicola* Lefebvre & Sherwin | (N1598) | Target leaf spot of sorghum | (26) | KJL |
| *Helminthosporium turcicum* Passerini | | Leaf blight of sorghum | (26) | LDD |
| *Macrophomina phaseoli* (Maublanc) Ashby | (Toothwick) | Charcoal rot of sorghum | (26) | LKE |
| *Mycoleptodiscus terrestris* (Gerd) Ostazeski | (MOM) | Root rot of birdsfoot trefoil | (22) | SAO |
| *Periconia circinata* (Mangin) Sacc. | (Hugaton 21) | Milo disease of sorghum | (26) | LKE |
| *Phoma* sp. | (isolate 1) | Leaf spot of cassava | (17) | JCLT |
| *Phoma* sp. | (isolate 2) | Leaf spot of cassava | (17) | JCLT |
| *Stemphylium loti* Graham | (1073) | Leaf spot of birdsfoot trefoil | (6) | WEF |
| Pathogens of non-cyanogenic plants: | | | | |
| *Ascochyta imperfecta* Pk. | (K) | Spring black stem of alfalfa | (6) | RLM |
| *Botrytis cinerea* Pers. ex Fr. | (504) | Gray mold of fruits and vegetables | (4) | OCY |
| *Cladosporium cucumerinum* Ell. & Arth. | (301) | Scab of cucumber[b] | (4) | CWB |
| *Fusarium oxysporium* f. sp. *lycopersici* (Sacc.) Snyder & Hansen 1940 | (302) | Wilt of tomato | (4) | REW |
| *Helminthosporium carbonum* Ullstrup | (HC-C race 1) | Leaf spot of corn | (23) | OCY |
| *Helminthosporium victoriae* Meehan & Murphy | (HV5) | Blight of oats | (6) | OCY |
| *Phyllosticta maydis* Arny & Nelson | (24) | Yellow leaf blight of corn | (23) | OCY |
| *Pythium ultimum* Trow | (cucumber isolate) | Damping-off of various seedlings[b] | (4) | DJP |
| *Rhizoctonia solani* Kühn | (A73) | Damping-off of various seedlings | (4) | DJP |
| *Rhizopus stolonifer* (Fr.) Lind. | (501) | Rot of fruits and vegetables[b] | (29) | RLM |
| *Stemphylium botryosum* Wallr. | (653) | Leafspot of alfalfa | (6) | RLM |
| *Stemphylium sarcinaeforme* (Cav.) Wilt. | (60) | Leafspot of red clover | (6) | RLM |
| *Ustilago maydis* (DC.) Cda. | (1003) | Smut of corn | (23) | RLM |
| *Verticillium albo-atrum* Reinke & Berth | (302) | Wilt of potatoes and tomatoes | (4) | REW |
| Nonpathogens of plants: | | | | |
| *Mucor heimalis* Wehm | (M-241) | | | RPK |
| *Neurospora crassa* Shear and Dodge | (M-440) | | | RPK |
| *Phycomyces blakesleanus* Burg. | (M-439) | | | RPK |
| *Saccharomyces cerevisiae* Meyea | (M-442) | | | RPK |
| *Schizophyllum commune* Fr. | (M-428) | | | RPK |
| *Zygorhynchus moelleri* | (M-251) | | | RPK |

[a]Individuals from whom fungi were obtained: C. W. Boothroyd (CWB), W. E. Fry (WEF), R. P. Korf (RPK), R. L. Millar (RLM), R. E. Wilkinson (REW), O. C. Yoder (OCY), Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853 USA; J. Carlos Lozano T. (JCLT), Centro Internacional de Agricultura Tropical, CIAT, Apartado Aereo 6713, Cali-Valle, Colombia, S.A.; L. D. Dunkle (LDD), Univ. Nebr., Lincoln, NB 68503 USA; L. K. Edmunds (LKE), Kansas State Univ., Manhattan, KS 66502 USA; R. A. Fredericksen (RAF), Texas A & M Univ., College Station, TX 77843 USA; K. J. Leonard (KJL), No. Carolina State Univ., Raleigh, NC 27607 USA; S. A. Ostazeski (SAO), Plant Genetics & Germplasm Inst., Northeast Region, Agric. Research Center-West, ARS, USDA, Beltsville, MD 20705 USA; D. J. Pieczarka (DJP), Dept. Plant Pathology, NYS Agric. Experiment Stn., Geneva, NY 14456 USA.

[b]Confirmation of this pathogenicity was not attempted.

produced expanding lesions on appropriate hosts from which the fungus could sporulate were considered to be pathogens of cyanogenic plants in our survey. Some fungi, such as *Helminthosporium victoriae* and *H. carbonum* produced very small lesions (2-3 mm in diam.) on our sorghum genotypes, but these did not expand and thus these two fungi were grouped with pathogens of non-cyanogenic plants.

Fungi reported to be pathogens of roots of cyanogenic plants were cultured on V-8 juice agar (20) or on V-8 juice broth (200 ml centrifuged V-8 juice, 800 ml water, 50 mmoles phosphate buffer, at a final pH of 7.0) at 22-25 C. Conidia and fragmented mycelium of *Fusarium moniliforme*, *Macrophomina phaseoli*, and *Periconia circinata*, were added to seedling roots of sorghum in a moist chamber. Appearance of root lesions indicated pathogenicity. Pathogenicity of *Mycoleptodiscus terrestris* to birdsfoot trefoil seedlings was assessed by planting seeds in soil infested with fragmented mycelium of *M. terrestris*. Pots containing seed and infested soil were maintained in the greenhouse at 18-30 C. Preemergence damping-off indicated pathogenicity. In all pathogenicity tests, plants or seedlings treated with autoclaved conidia or mycelium served as controls, and all pathogenicity tests were repeated at least once.

The appearance of expanding lesions on roots, foliage or damping off of seedlings caused by a fungus indicated to us that the fungus could colonize cyanogenic plant tissue. There was no attempt to reproduce field symptoms or to assess the relative amounts of disease caused by various fungi.

The pathogenicity of *S. sarcinaeforme* necessitates explanation, for it is our view that it should be grouped with pathogens of non-cyanogenic plants rather than with pathogens of cyanogenic plants. Some investigators (cited in ref. 31) have reported, based on greenhouse inoculations (31), that *S. sarcinaeforme* is a pathogen of the cyanogenic plant, white clover (*Trifolium repens* L.). However, in other experiments inoculation of *S. sarcinaeforme* onto white clover foliage resulted in restricted lesions (1-2 mm in diameter) from which the fungus did not sporulate as long as the leaf remained attached to the plant (31). Therefore, we grouped *S. sarcinaeforme* with pathogens of non-cyanogenic plants.

Pathogenic ability of most of the pathogens of non-cyanogenic plants was confirmed in a manner similar to that described for pathogens of cyanogenic plants. The lack of pathogenicity of the six fungi generally regarded as saprophytes (Table 1) was not investigated.

**Cell-free homogenates.**—Cultures were started by transfer of mycelium to 25 ml of V-8 juice broth in a 125-ml Erlenmeyer flask. Cultures in flasks were maintained on a rotary shaker (100 rotations/min) at 21-23 C under continuous fluorescent light. Mycelium was harvested after 5-10 days growth, and the culture fluid was discarded. Mycelium from each flask in 10 ml of 0.05 M Tris buffer (pH 8) was fragmented for 15-20 sec in a Waring Blendor and then homogenized (at 4 C) by shaking with glass beads (0.45 - 0.50 mm diameter) for 60 sec at 4,000 cycles/min in a Bronwell MSK cell homogenizer. The supernatant fluid was decanted and the beads were washed once with 5 ml of 0.05 M Tris buffer pH 8, and this washing was added to the supernatant. Cell debris was removed by centrifugation for 20 min at 13,000 rpm (20,000 *g*) at 4 C in a Sorvall Superspeed RC2-B centrifuge with a SS-34 rotor.

**Formamide hydro-lyase measurements.**—Activity of FHL in cell-free homogenates was estimated by measuring formamide over time at 25 C. Reaction mixtures contained 0.7 ml of HCN (100 mM) and 0.3 ml of the appropriate dilution of the cell-free homogenate to achieve a final volume of 1.0 ml. All components of the reaction mixture contained Tris (hydroxymethyl) amino methane (50 mM) adjusted to pH 8. Formamide was measured colorimetrically by producing the hydroxamic acid derivative as previously described (10). Most assays

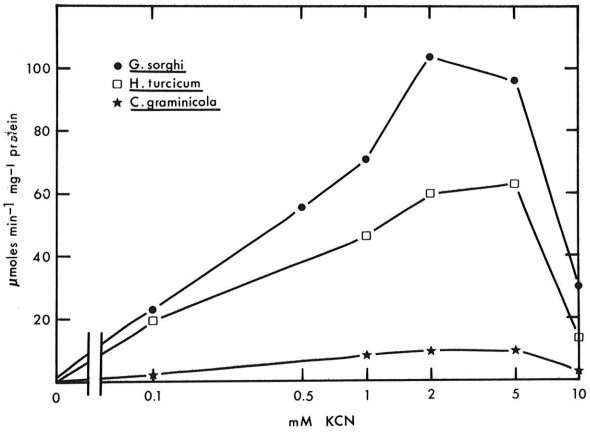


**Fig. 1.** Effect of HCN concentration on induction of formamide hydro-lyase in *Colletotrichum graminicola*, *Gloeocercospora sorghi*, and *Helminthosporium turcicum*. Solutions of HCN in buffer were added to cultures in liquid media to attain the indicated concentration. Mycelia were homogenized 24 hr after addition of HCN. Activity of FHL was assayed by colorimetric measurement of the hydroxamic acid derivative of formamide. Data are averages of several experiments.

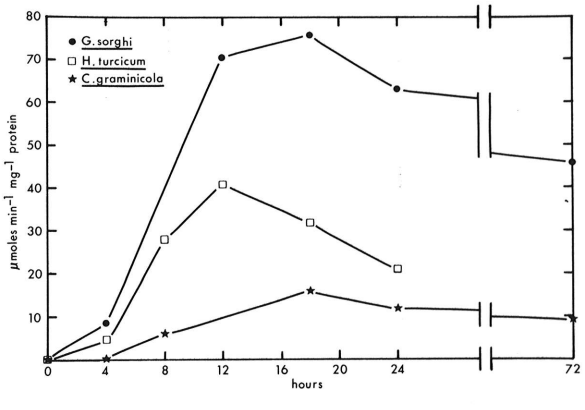


**Fig. 2.** Effect of induction time on formamide hydro-lyase activity in *Colletotrichum graminicola*, *Gloeocercospora sorghi*, and *Helminthosporium turcicum*. Mycelium was homogenized at the indicated intervals after addition of HCN (25 μmoles) to fungi growing in liquid culture (25 ml). Activity of FHL was assayed by colorimetric measurement of the hydroxamic acid derivative of formamide. Data are results of typical experiments.

were conducted within 24 hr of homogenization. Activity is expressed as μmoles formamide produced per minute.

Activity of FHL was estimated both in homogenates of induced and non-induced mycelium and protein concentration was estimated according to the method of Lowry (16). Mycelium was induced by incubating it in 1.0 mM HCN 18 hr prior to homogenization (see Results section). Non-induced mycelium had not been exposed to HCN.

For homogenates which contained FHL activity greater than 1.0 μmoles/min/mg protein, formamide production was confirmed by gas-liquid chromatography of the reaction mixture utilizing a Varian Aerograph Model 1520. The 635 × 2 mm stainless steel column was packed with Tenax GC, 246- to 147-μm (60- to 80-mesh) (Applied Science Laboratories, Inc., State College, PA 18602 USA). Samples of reaction mixture containing approximately 20 mM formamide were injected in 2-μliter aliquots; carrier gas was helium at 15 ml/min, and compounds were detected by means of flame ionization. Acetamide was used as an internal standard. Under these conditions the average retention time of formamide was 126 sec at 130 C.

Unless indicated differently, each experiment was repeated at least once.

## RESULTS

**Estimation of optimum conditions for formamide hydro-lyase induction.**—Formamide hydro-lyase is induced by HCN (11), but the concentration of HCN and the time interval between HCN addition and homogenization of mycelium (induction period) which allowed maximum FHL production had not been established. Preliminary experiments indicated that *Colletotrichum graminicola* produced relatively low FHL activities and that *Gloeocercospora sorghi* and *Helminthosporium turcicum* had high FHL activities. Experiments were conducted with these three fungi to determine the HCN concentration and induction period which enabled maximum FHL activities to be expressed and to determine whether these values varied among fungi with different capacities to produce FHL. Different amounts of HCN in 0.25 ml Tris buffer (50 mM) pH 8 were added to 5 to 10-day-old cultures (25 ml) of *C. graminicola*, *G. sorghi*, and *H. turcicum*. HCN was added to the cultures only once, and the initial concentrations presumably decreased during the incubation period due to FHL activity. The mycelium of each fungus was homogenized 24 hr after addition of HCN and FHL activity was assayed. Maximum FHL activity generally was attained with 1-5 mM HCN (Fig. 1). The optimum induction period was estimated with the same species. Mycelium was homogenized and tested at intervals after addition of HCN (25 μmoles in 0.25 ml 50 mM Tris buffer pH 8) to 5- to 10-day-old cultures (25 ml). Maximum activity was attained at 12-18 hr after addition of HCN (Fig. 2). Because of these findings and because some fungi were likely to be more sensitive to HCN than the three test species, the lower HCN concentration (1.0 mM) and longer induction time (18 hr) were selected as the conditions to induce FHL in the remaining species.

**Formamide hydro-lyase activity in the fungi.**—For most fungi, determinations of FHL specific activities

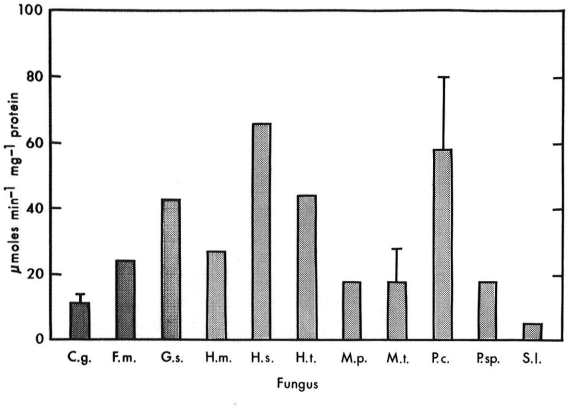


**Fig. 3.** Specific activities of formamide hydro-lyase (FHL) in crude cell-free homogenates of fungi known to be pathogens of cyanogenic plants. Activity of FHL was assayed by colorimetric measurement of the hydroxamic acid derivative of formamide. Mycelium was induced by making the culture media 1.0 mM in HCN 18 hr prior to homogenization. Values represent the average of at least two experiments. Standard deviations of the mean are indicated for *Colletotrichum graminicola*, *Mycoleptodiscus terrestris*, and *Periconia circinata* by the lines shown. Legend: C.g. = *Colletotrichum graminicola*, F.m. = *Fusarium moniliforme*; G.s. = *Gloeocercospora sorghi*; H.m = *Helminthosporium maydis* race T; H.s. = *Helminthosporium sorghicola*; H.t. = *Helminthosporium turcicum*; M.p. = *Macrophomina phaseoli*; M.t. = *Mycoleptodiscus terrestris*; P.c. = *Periconia circinata*; P.sp. = *Phoma* sp.; and S.l. = *Stemphylium loti*.

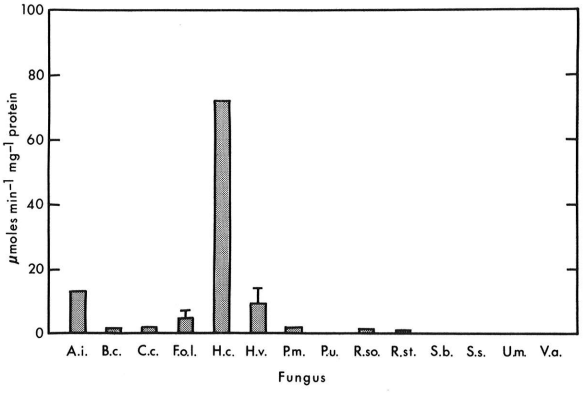


**Fig. 4.** Specific activities of formamide hydro-lyase (FHL) in crude cell-free homogenates of fungi pathogenic to non-cyanogenic plants. Activity of FHL was assayed by colorimetric measurement of the hydroxamic acid derivative of formamide. Mycelium was induced by making the culture medium 1.0 mM HCN 18 hr prior to homogenization. Values represent the averages of at least two experiments. Standard deviations of the mean are indicated for *Fusarium oxysporum* f. sp. *lycopersici* and *Helminthosporium victoriae* by the lines shown. Legend: A.i. = *Ascochyta imperfecta*; B.c. = *Botrytis cinerea*; C.c. = *Cladosporium cucumerinum*; F.o.l. = *Fusarium oxysporum* f. sp. *lycopersici*; H.c. = *Helminthosporium carbonum*; H.v. = *Helminthosporium victoriae*; P.m. = *Phyllosticta maydis*; P.u. = *Pythium ultimum*; R.so. = *Rhizoctonia solani*; R.st. = *Rhizopus stolonifer*; S.b. = *Stemphylium botryosum*; S.s. = *Stemphylium sarcinaeforme*; U.m. = *Ustilago maydis*; and V.a. = *Verticillium albo-atrum*.

were consistent for replicate experiments, and the average of these two values is reported. However, for a few fungi, assessments of FHL specific activities were not consistent in replicate experiments. For these fungi, FHL determinations were repeated, and the standard deviation of the mean is indicated in Fig. 3 and 4. For all except one of the fungi which had specific activities of FHL $> 1.0$ $\mu$moles/min/mg protein the product of catalysis was assayed by gas-liquid chromatography. In all cases, a peak at the retention time of formamide was observed which corresponded to the concentration estimated via the colorimetric assay. The product of catalysis by FHL from *Fusarium oxysporum* f. sp. *lycopersici* was not assayed.

Pathogens of cyanogenic plants produced moderate to high amounts of FHL after induction by HCN (Fig. 3). The specific activities ranged from approximately 4 to 66 $\mu$moles/min/mg protein. The average induced specific activity for all pathogens of cyanogenic plants was 30.1 $\mu$moles/min/mg protein (standard deviation of the mean = 5.9). Four species (*C. graminicola*, *F. moniliforme*, *Macrophomina phaseoli*, and *S. loti*) produced no detectable non-induced (constitutive) FHL. For other pathogens of cyanogenic plants constitutive FHL activity ranged from 0.1 to 1.2 $\mu$moles/min/mg protein and the activity detectable in induced mycelium ranged from 50- to 300-fold greater than that in non-induced mycelium.

Nine of fourteen fungal pathogens of non-cyanogenic plants produced detectable levels of induced FHL. Four fungi (*Ascochyta imperfecta*, *F. oxysporum* f. sp. *lycopersici*, *H. carbonum*, and *H. victoriae*) had moderate to high levels of induced FHL activity (Fig. 4). The other nine species had either low or undetectable levels of FHL activity. Only three species (*Botrytis cinerea*, *H. carbonum*, and *Rhizoctonia solani*) had detectable constitutive FHL activity. The increases of FHL activity in induced mycelium relative to that of non-induced mycelium for these three fungi were 4-, 48-, and 5-fold, respectively. The mean induced FHL activity for this group of fungi was 7.4 $\mu$moles/min/mg protein (standard deviation of the mean = 5.1). The mean of FHL activity in this group of fungi was significantly less ($P = 0.02$) than the mean for pathogens of cyanogenic plants.

Of six nonpathogenic fungi, FHL was detectable only in homogenates of induced *Neurospora crassa* mycelium. The mean induced activity in this group of fungi was 0.1 $\mu$moles/min/mg protein. We could detect no FHL activity in cell-free homogenates of 10 fungi. In a modification of these experiments, eight of these 10 fungi were tested again by inducing them with 0.1 mM as well as 1.0 mM HCN, and mycelial fragments were assayed for FHL. Again no evidence for FHL activity could be observed.

To estimate the statistical significance of the FHL-producing abilities of the three different groups of fungi, each fungus was placed into one of four categories of FHL-producing ability: 0 to $< 0.1$; 0.1 to $< 1.0$; 1.0 to $<$ 10.0; and greater than 10 $\mu$moles/min/mg protein. The distributions of individuals according to FHL-producing capability for pathogens of cyanogenic plants and for saprophytes were significantly different from the distribution for all fungi tested ($P = 0.05$). However, the distribution pattern of FHL capability among pathogens of non-cyanogenic plants was not significantly different from that for all fungi tested.

## DISCUSSION

Our hypothesis is that a pathogen of a cyanogenic plant must have a means of coping with HCN because toxic concentrations of this compound may be released into the environment of that pathogen during infection. Detoxification of HCN by conversion to $HCONH_2$ via FHL is such a process. The induction of FHL activity in all pathogens of cyanogenic plants tested is consistent with our hypothesis.

Formamide hydro-lyase was constitutive or inducible in 9 of 13 fungi pathogenic to noncyanogenic plants and in *Neurospora crassa*. We do not extrapolate to suggest that fungi with FHL ability should be pathogens of cyanogenic plants. We view the ability to cope with HCN as a necessary characteristic of a pathogen exposed to a toxic concentration of HCN upon pathogenesis, and ability to produce FHL seems now to be the most likely mode by which pathogens cope with HCN. We do not view the ability to produce FHL as the only factor necessary to confer pathogenicity to a fungus.

The range of FHL-specific activities induced in pathogens of cyanogenic plants and in pathogens of non cyanogenic plants was very wide. We regard the pattern of FHL distribution in each group to be a more meaningful parameter than the overall average level of FHL activity. The pattern of occurrence of FHL activity clearly associates pathogens of cyanogenic plants with FHL activity.

We cannot now account for the presence of FHL-producing ability in a nonpathogen or pathogen of a non-cyanogenic plant. Several explanations are plausible. First, some of these fungi may indeed be pathogens of cyanogenic plants. For example *H. carbonum* and *H. victoriae*, included as pathogens of non-cyanogenic plants in our study, produced high levels of FHL. In our tests these organisms did not produce expanding lesions on any of several sorghum genotypes. However, in other tests, these fungi were regarded as pathogens of sorghum (21). If these two fungi should be regarded as pathogens of cyanogenic plants, then the association between level of induced FHL activity and pathogenesis of cyanogenic plant tissue is even more striking. Second, FHL may be used to detoxify HCN which these fungi may encounter during saprophytic growth. Third, FHL may have a function other than HCN metabolism. We feel this possibility is not likely because in preliminary tests only HCN served as a substrate or as an inducer of FHL. Stable nitriles such as acetonitrile, and propionitrile, at 1.0 mM did not induce FHL (W. E. Fry and P. H. Evans, *unpublished*).

## LITERATURE CITED

1. ARNESON, P. A., and R. D. DURBIN. 1967. Hydrolysis of tomatine by Septoria lycopersici: A detoxification mechanism. Phytopathology 57:1358-1360.
2. ARNESON, P. A., and R. D. DURBIN. 1968. The sensitivity of fungi to $\alpha$-tomatine. Phytopathology 58:536-537.
3. ARNESON, P. A., and R. D. DURBIN. 1968. Studies on the mode of action of tomatine as a fungitoxic agent. Plant

Physiol. 43:683-686.
4. CHUPP, C., and A. F. SHERF. 1960. Vegetable diseases and their control. Ronald Press, New York. 693 p.
5. DEAHL, K. L., R. J. YOUNG, and S. L. SINDEN. 1973. A study of the relationship of late blight resistance to glycoalkaloid content of fifteen potato clones. Am. Potato J. 50:248-253.
6. DICKSON, J. G. 1956. Diseases of field crops. 2nd edn. McGraw-Hill, New York. 517 p.
7. FRANK, J. A., J. M. WILSON, and R. E. WEBB. 1975. The relationship between glycoalkaloids and disease resistance in potatoes. Phytopathology 65:1045-1049.
8. FRY, W. E., and R. L. MILLAR. 1971. Cyanide tolerance in Stemphylium loti. Phytopathology 61:494-500.
9. FRY, W. E., and R. L. MILLAR. 1971. Development of cyanide tolerance in Stemphylium loti. Phytopathology 61:501-506.
10. FRY, W. E., and R. L. MILLAR. 1972. Cyanide degradation by an enzyme from Stemphylium loti. Arch. Biochem. Biophys. 151:468-474.
11. FRY, W. E., and D. C. MUNCH. 1975. Hydrogen cyanide detoxification by Gloeocercospora sorghi. Physiol. Plant Pathol. 7:23-33.
12. JONES, H. A., J. C. WALKER, T. M. LITTLE, and R. H. LARSON. 1946. Relation of color-inhibiting factor to smudge resistance in onion. J. Agric. Res. 72:259-264.
13. LINK, K. P., H. R. ANGELL, and J. C. WALKER. 1929. The isolation of protocatechuic acid from pigmented onion scales and its significance in relation to disease resistance in onions. J. Biol. Chem. 81:369-375.
14. LINK, K. P., A. D. DICKSON, and J. C. WALKER. 1929. Further observations on the occurrence of protocatechuic acid in pigmented onion scales and its relation to disease resistance in the onion. J. Biol. Chem. 84:719-725.
15. LINK, K. P., and J. C. WALKER. 1933. The isolation of catechol from pigmented onion scales and its relation to disease resistance in onions. J. Biol. Chem. 100:370-383.
16. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265-275.
17. LOZANO, J. C., and R. H. BOOTH. 1976. Diseases of cassava (Manihot esculenta Crantz). Centro Internacional de Agricultura Tropical (CIAT) Series DE-5. June 1976. Cali, Colombia, S. A. 46 pages.
18. LUDTKE, M., and H. HAHN. 1953. Über den Linamaringehalt gesunder und vol. Colletotrichum lini befallener junger Leinpflanzen. Biochem. Z. 324:433-442.
19. MILLAR, R. L., and V. J. HIGGINS. 1970. Association of cyanide with infection of birdsfoot trefoil by Stemphylium loti. Phytopathology 60:104-110.
20. MILLER, P. M. 1955. V-8 juice agar as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.
21. NELSON, R. R., and D. M. KLINE. 1962. Intraspecific variation in pathogenicity in the genus Helminthosporium to gramineous species. Phytopathology 52:1045-1049.
22. OSTAZESKI, S. A. 1967. An undescribed fungus associated with a root and crown rot of birdsfoot trefoil (Lotus corniculatus). Mycologia 59:970-975.
23. SHURTLEFF, M. C. (Chmn.) 1973. A compendium of corn diseases. Cooperative Extension Service of the University of Illinois and the Extension Service of the U.S. Department of Agriculture, cooperating. Am. Phytopathol. Soc., St. Paul, Minn. 64 p.
24. SINDEN, S. L., R. W. GOTH, and M. J. O'BRIEN. 1973. Effect of potato alkaloids on the growth of Alternaria solani and their possible role as resistance factors in potatoes. Phytopathology 63:303-307.
25. SNYDER, E. B. 1950. Inheritance and associations of hydrocyanic acid potential, disease reactions, and other characters in sudan grass, Sorghum vulgare var. sudanensis. Ph.D. Thesis, Univ. of Wisconsin, Madison. 86 p.
26. TARR, S. A. J. 1962. Diseases of sorghum, sudan grass and broom corn. Commonwealth Mycological Institute, Kew, Surrey, England. 380 p.
27. 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.
28. TRIONE, E. J. 1960. The HCN content of flax in relation to flax wilt resistance. Phytopathology 50:482-486.
29. WALKER, J. C. 1957. Plant pathology. McGraw-Hill, New York. 707 p.
30. WALKER, J. C., and M. A. STAHMANN. 1955. Chemical nature of disease resistance in plants. Annu. Rev. Plant Physiol. 6:351-366.
31. WILKINSON, H. T. 1976. The association of host and pathogen linamarase with cyanogenesis in white clover infected by Stemphylium sarcinaeforme. M.S. Thesis, Cornell Univ., Ithaca, New York. 122 p.