# Enzymatic Release and Metabolism of Hydrogen Cyanide in Sorghum Infected by Gloeocercospora sorghi

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## ABSTRACT

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The activities of enzymes that may be important in the release or metabolism of hydrogen cyanide (HCN) during disease development were determined in extracts of sorghum infected by *Gloeocercospora sorghi*. The activity of β-glucosidase increased 20-fold during the first 24 hr after inoculation and then remained at that level. This enzyme hydrolyzes dhurrin, the cyanogenic glucoside in sorghum, to glucose and *p*-hydroxymandelonitrile. The increase in β-glucosidase activity preceded the first detectable decline in HCN potential of infected leaves. The activity of oxynitrilase, an enzyme that releases HCN from *p*-hydroxymandelonitrile, remained constant. Of the HCN-metabolizing enzymes examined, activity of formamide hydro-lyase was the highest. No formamide hydro-lyase activity was detected earlier than 12 hr after inoculation, but activity increased at least 200-fold 18–48 hr after inoculation. This increase preceded the decline in HCN potential in diseased leaves. The product of formamide hydro-lyase activity, formamide, did not accumulate in diseased sorghum. No formamide hydrolyase was detected in extracts of diseased older leaves in which no HCN was detected. Activities of β-cyanoalanine synthase and rhodanese (two other HCN-metabolizing enzymes) were detected at low levels in healthy sorghum but were not detected in the pathogen. These activities did not increase during disease development. These data are consistent with the hypotheses that *G. sorghi* is exposed to HCN upon pathogenesis of sorghum leaves and that formamide hydro-lyase may be involved in HCN detoxification.

Hydrogen cyanide (HCN) is released when a cyanogenic plant is subjected to physical (28), chemical (7), or biological stress such as plant disease (19). Because of the toxicity of HCN to most organisms, a role for HCN in plant disease has been predicted (13).

In cyanogenic plants, HCN is bound in glycosides for which no essential metabolic function has been demonstrated conclusively (5, 6). To estimate the HCN potentially available in plant tissue, cyanogenic glycosides are hydrolyzed and the amount of HCN released is measured (21). This amount has been called the HCN potential (28). When young sorghum leaves are infected by *Gloeocercospora sorghi*, the HCN potential of the leaf declines and HCN is produced (21). This decline in HCN potential is probably due to degradation of dhurrin, the cyanogenic glucoside of sorghum (21).

In healthy sorghum, two enzymes probably function in dhurrin degradation. The enzyme β-glucosidase hydrolyzes dhurrin to glucose and *p*-hydroxymandelonitrile (17); an oxynitrilase cleaves *p*-hydroxymandelonitrile to yield *p*-hydroxybenzaldehyde and HCN (2). However, the enzymes responsible for dhurrin degradation in diseased sorghum have not been identified.

Hydrogen cyanide produced during plant disease may be toxic to the pathogen or the host or both. The relative ability of organisms to cope with HCN may be significant to the outcome of the host-parasite interaction. Organisms have evolved mechanisms to cope with HCN. These include HCN detoxification (10), incorporation of HCN in metabolically active compounds (3, 23), and the induction of HCN-insensitive respiration (12, 25). Both sorghum and *G. sorghi* produce enzymes that might enable them to cope with HCN. In sorghum, β-cyanoalanine synthase catalyzes the reaction of cysteine or serine with HCN to produce β-cyanoalanine (8). Rhodanese, an enzyme commonly found in mammals (29) and also in cassava (4), converts HCN to thiocyanate in the presence of a suitable sulfur donor. Rhodanese may also be present in sorghum. *Gloeocercospora sorghi* produces the HCN-inducible enzyme formamide hydrolyase, which condenses HCN with water to produce formamaide (11).

The purpose of our investigation was to identify the enzymes involved either in the decline in HCN potential or in HCN metabolism in sorghum infected by *G. sorghi*.

## MATERIALS AND METHODS

**Inoculation.**—*Gloeocercospora sorghi* D. Bain & Edg. was isolated from diseased sorghum provided by R. A. Fredericksen, Texas A & M University, College Station, TX 77840. The pathogen was maintained on V-8 juice agar (20) under continuous fluorescent light at 22–25 C and remained pathogenic during these studies.

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Grazer, a sorghum [*Sorghum bicolor* (L.) Moench] × sudangrass (*S. sudanense* 'Piper' Stapf.) hybrid was seeded in a sandy loam soil contained in metal flats or clay pots with a 10-cm diameter and maintained under supplemental fluorescent light at about 30 C in a greenhouse.

Inoculum was prepared by washing conidia from 7- to 13-day-old cultures into sterile distilled water and filtering the suspended spores through three layers of cheesecloth. Tween-20 (polyoxyethylene sorbitan monolaurate) was added to the susepension to a concentration of 0.05%, and the spore concentration was adjusted to $4–6 \times 10^5$ spores/ml. Plants were sprayed to runoff with the spore suspension and incubated in a moist chamber at 27 C and 100% RH until harvested.

Primary leaves were used for all studies unless otherwise indicated.

**Host enzyme extraction and determination of enzyme activity.**—For the time course analyses, each sample was stored not longer than 8 hr at 4 C prior to the extraction. Only extraction procedures that maximized enzyme activities are reported here. Enzyme activities were determined within 24 hr after extraction unless otherwise stated. The reported pH optimum for each enzyme was confirmed and its activity measured at optimum pH. For determining enzyme activities, both autoclaved extract and zero time substrate controls were used, and activities were expressed as μmoles of product per hour per milligram of protein. All experiments were performed at least twice.

**Enzyme extraction from plant tissues.**—*Formamide hydro-lyase.*—Leaves were quick-frozen in liquid $N_2$ in a mortar, ground to a fine powder with a pestle, and suspended in 0.1 N citrate-phosphate buffer, pH 7.0, in the proportion of 1:3 (w/v). Then the suspension was homogenized at about 30,000 rpm in a VirTis Model "45" homogenizer kept at 4–10 C. The resulting homogenate was squeezed through one layer of Miracloth (Chicopee Mills, Inc., New York, NY 10018) and centrifuged for 45 min at 20,000 *g*. The pellet was discarded and the supernatant was stored at 4 C.

*Oxynitrilase.*—The extraction procedure for oxynitrilase activity was the same as for formamide hydro-lyase except that after centrifugation, the supernatant fluid was dialyzed at 4 C against 0.1 M citrate-phosphate buffer, pH 7.0, for about 18 hr (about 100 volumes of buffer per extract replaced once after about 9 hr).

*β-Glucosidase.*—Primary leaves were cut into 5–10 $mm^2$ pieces and suspended in 0.25 M NaCl in 0.1 M citrate-phosphate buffer, pH 6.0 (3:1, v/w). The leaf fragments then were homogenized and subsequently treated as described for formamide hydro-lyase.

*β-Cyanoalanine synthase.*—Leaves were frozen in liquid nitrogen and then pulverized. The resulting powder was suspended (3:1, v/w) in 0.1 M sodium ascorbate in 0.1 M Tris buffer (hydroxymethyl amino methane), pH 7.5, and homogenized. Polyvinyl polypyrollidone (1.5 g/g fresh wt of leaves) was added (16); the homogenate was incubated 10–20 min at 4 C and then centrifuged at 20,000 *g*. The supernatant fluid was dialyzed twice in 18 hr at 4 C against about 200 volumes of 0.05 M Tris buffer, pH 7.5.

*Rhodanese.*—Rhodanese was extracted by a modification of procedures developed for cassava (1, 4). Primary leaves were cut into 5–10 $mm^2$ pieces, suspended in glass distilled water (3:1, v/w), and homogenized as for formamide hydro-lyase but at room temperature. The mixture was filtered through one layer of Miracloth and assayed immediately for enzyme activity.

**Enzyme extraction from Gloeocercospora sorghi.**—The fungus was cultured for 7 to 14 days in V-8 juice broth (200 ml of V-8 juice supernatant centrifuged at 20,000 *g* for 20 min, 800 ml of water, 50 mmoles of phosphate buffer, pH 7.0) on a rotary shaker at 21–26 C under fluorescent light. Broth cultures were filtered through glass fiber paper (Grade 934 AH, Reeve Angel, Whatman Inc., Clifton, NJ 07015) on a Büchner funnel. The culture filtrate was retained and analyzed for enzyme activities. The mycelia were fragmented in the VirTis "45" homogenizer at about 20,000 rpm and then homogenized for 60 sec in a Bronwill MSK cell homogenizer (Bronwill Scientific, Inc., Rochester, NY 14606). The homogenate was centrifuged for 45 min at 20,000 *g*.

**Formamide extraction.**—Healthy primary leaves or diseased leaves that had been inoculated 48–72 hr previously were excised, frozen in liquid $N_2$, and ground to a powder with a mortar and pestle. The powder was suspended in glass distilled water and homogenized in the VirTis homogenizer. The mixture then was treated for 15 min at 60 C to inactivate β-glucosidases (2), filtered, and then centrifuged at 20,000 *g* for 45 min. The supernatant fluid was treated sequentially by batch additions of cation (Dowex 50 W-X8) and anion (Dowex 1-X8) exchange resins. Resins were removed by filtration and the solution was extracted with three volumes of chloroform. The aqueous solution was filtered through a Diaflo ultrafiltration UM-05 membrane (Amicon Inc., Lexington, MA 02173) and then was concentrated about 10- to 15-fold by flash evaporation at 40 C. When known concentrations of formamide were combined with healthy or diseased sorghum leaves, nearly 85% of the formamide added was recovered.

**Enzyme activities.**—Formamide hydro-lyase activity in extracts was determined by measuring the amount of formamide produced per hour at 25 C. Reaction mixtures contained formamide hydro-lyase extract, 70 μmoles of HCN, 0.05 mmoles of Tris (hydroxymethyl amino methane) (Fisher) at pH 8.0 in 1 ml. Formamide concentrations were measured colorimetrically at 540 nm by producing the hydroxamic acid derivative (10).

The oxynitrilase activity was determined by measuring the rate of *p*-hydroxybenzaldehyde produced per minute at 25 C. Reaction mixtures contained oxynitrilase extract, 0.35 μmoles *p*-hydroxymandelonitrile [the cyanohydrin of *p*-hydroxybenzaldehyde, prepared as described previously (14)], 0.3 of mmoles citrate-phosphate buffer, pH 5.4, in 3.05 ml. Absorbance at 285 nm (2) was converted to μmoles *p*-hydroxybenzaldehyde based on $\Sigma \, {}^{1\,cm}_{mM} = 14.8$.

The β-glucosidase activity was estimated by measuring HCN produced from *p*-hydroxymandelonitrile at 25 C. The HCN volatilized from the reaction mixture was trapped in alkaline sodium picrate and analyzed colorimetrically (21). The substrate, dhurrin, was obtained from young, healthy, sorghum primary leaves, which were treated in the same manner as for extraction

of formamide hydro-lyase except that after final centrifugation, the extract was heated to 60 C for 15 min. This assay relies on the coupled activities of β-glucosidase and oxynitrilase. Oxynitrilase must not be rate-limiting. Since the oxynitralise activity relative to β-glucosidase was high in our extracts, this enzyme was not rate-limiting in the coupled assay. For each sample, the rate of HCN production was determined a minimum of three times after starting the assay (three replications per sample). Reaction mixtures consisted of β-glucosidase extract, 7.7 μmoles of sodium azide (to prevent microbial contamination), 0.75 mmoles of citrate-phosphate buffer, pH 5.0, and crude dhurrin in 2.9 ml.

The β-cyanoalanine synthase activity was determined by measuring the amount of sulfide produced from cysteine per minute at 25 C (15). Reaction mixtures contained β-cyanoalanine synthase extract, 10 μmoles of cysteine hydrochloride, 10 μmoles of KCN, and 0.11 mmole of Tris, pH 8.4, in a total volume of 3.0 ml. A fresh solution of cysteine hydrochloride was prepared prior to each assay. The reaction mixture was incubated in the main chamber of a stoppered flask provided with a sidearm. The sidearm contained a mixture of 0.5 ml of 0.03 M $FeCl_3$ in 1.2 M HCl and 0.5 ml of 0.02 M *p*-amino-N,N-dimethylaniline in 7.2 M HCl. The flasks were agitated gently several times during each incubation. Then, the contents of the sidearm were tipped into the reaction mixture and the mixture was shaken briefly. After 20 min the precipitate was removed by low-speed centrifugation and the absorbance of the supernatant was read at 650 nm. Absorbance was converted to μmoles of sulfide by reference to a standard curve prepared with sodium sulfide.

The rhodanese activity was estimated by determining the amount of thiocyanate produced per minute at 25 C (4). Reaction mixtures consisted of rhodanese extract, 0.35 mmoles of glycine, pH 9.2, 60 μmoles of sodium thiosulfite, and 20 μmoles of KCN (always added after thiosulfite) in 2.5 ml. The reaction was terminated by adding 0.5 ml of 37% formaldehyde (w/w). Then, 2.5 ml of ferric nitrate reagent [50 g $Fe(NO_3)_3 \cdot 9H_2O$, 525 ml nitric acid sp. gr. 1.40, and glass distilled water to a volume of 2 liters] was added to each assay tube. The reddish brown solution then was centrifuged at 1,146 *g* and the absorbance of the supernatant was measured at 460 nm. Absorbance was converted to μmoles of thiocyanate by reference to a standard curve prepared with sodium thiocyanate.

**Formamide.**—Formamide concentrations in concentrated leaf extracts were assayed colorimetrically (10) and by gas chromatography in a Perkin-Elmer Model 900 chromatograph equipped with a flame ionization detector. The 1,830 × 2 mm glass column was packed with Tenax GC, 60–80 mesh (Applied Science Laboratories Inc., State College, PA 16802). The instrument was programmed from 140 to 170 C at 3 C per minute. The carrier gas was helium at 15 ml/min. The retention time of formamide was about 4.0 min. Formamide, acetamide, and N, N-dimethyl-formamide were used as internal standards.

**Hydrogen cyanide potential.**—The HCN potential in excised sorghum leaves was measured colorimetrically by the chloroform-base method (21).

**Protein.**—Protein concentrations were measured by a modified Lowry procedure (24).

## RESULTS

**Disease development.**—Lesions first were observed between 12 and 18 hr after inoculation. By 48 hr after inoculation, nearly 95% of the inoculated leaf area was affected by the pathogen. Water soaking preceded necrosis and colonization by the pathogen (22).

**Hydrogen cyanide potential.**—The HCN potential of diseased leaves (Fig. 1) declined in a way similar to that described previously (21). The decline was first detected between 24 and 36 hr after inoculation and continued through 84 hr after inoculation. After 84 hr, the HCN potential remaining in diseased leaves was about 10% of the level at the time of inoculation.

**Pathogen enzyme activities.**—Formamide hydrolyase activity has been detected in *G. sorghi* previously and is apparently intracellular (11). Formamide hydrolyase was detected in cell-free homogenates of *G. sorghi*, but no significant activity was detected in culture filtrates. Rhodanese, β-cyanoalanine synthase, or oxynitrilase were not detected in mycelia or culture filtrates of *G. sorghi*. The activity of β-glucosidase against dhurrin was detected in mycelia of *G. sorghi* but not in culture filtrates. The pH optimum for the enzyme activity was about 5.5.

**Host enzyme activities.**—No significant changes occurred in β-glucosidase activity in healthy leaves. During the experiment, the β-glucosidase activities in extracts of healthy leaves remained equivalent to the activities at time of inoculation (Fig. 2). In diseased leaves, β-glucosidase activity increased about 20-fold during the first 24 hr after inoculation and then remained

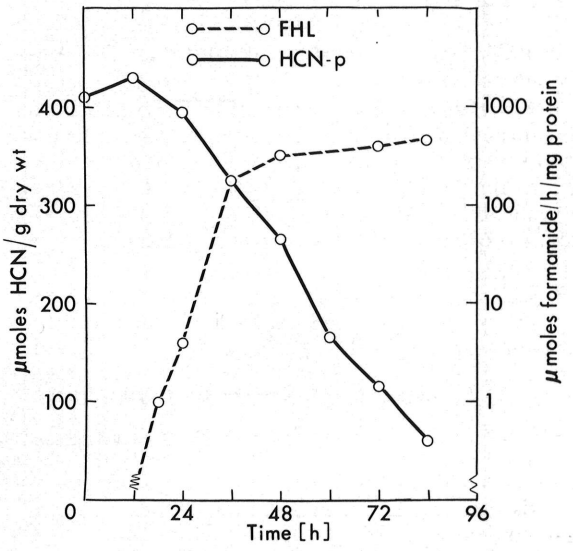


**Fig. 1.** Hydrogen cyanide potential (HCN-p) of diseased sorghum and formamide hydro-lyase (FHL) activity in extracts of diseased sorghum during the development of *Gloeocercospora sorghi* in sorghum primary leaves inoculated 6 days after leaf emergence.

at about that level for the duration of the experiment (Fig. 2). The oxynitrilase activities in extracts of healthy and diseased leaves were equivalent and remained fairly constant through 84 hr after inoculation (Fig. 2). The activity of oxynitrilase was between 7 and 140 times greater than the activity of β-glucosidase (Fig. 2).

Of the three enzymes that may function in HCN metabolism in diseased sorghum, formamide hydro-lyase was the most active. No formamide hydro-lyase activity was detected in extracts of healthy sorghum. In diseased sorghum, activity was first detected 18 hr after inoculation and increased about 200-fold by 36 hr (Fig. 3). After 36 hr, activity continued to increase but more slowly. This increase in formamide hydro-lyase activity in disased sorghum was correlated with the decline in HCN potential (Fig. 1).

To test whether the maximum activities of formamide hydro-lyase were associated with the initial HCN potential of a leaf (prior to inoculation), leaves of various ages differing in HCN potential were inoculated with *G. sorghi*. The maximum formamide hydro-lyase activity (measured between 48 and 72 hr after inoculation) was positively correlated with the initial HCN potential of a leaf (Table 1).

The activity of β-cyanoalanine synthase did not change significantly during the first 36 hr after inoculation; then it decreased slightly (Fig. 3). Activities of β-cyanoalanine synthase in extracts of healthy primary leaves did not change appreciably and were equivalent to the activities at inoculation (0 hr).

A rhodaneselike activity was detected in extracts of healthy and diseased sorghum but did not change significantly during the course of the experiment (Fig. 3). The pH optimum of sorghum rhodanese activity was between 9.0 and 10.0, and the temperature optimum was between 45 and 55 C.

**Formamide.**—We did not detect formamide in concentrated extracts of healthy or diseased sorghum by

TABLE 1. Maximum activity of formamide hydro-lyase (48–96 hr after inoculation with *Gloeocercospora sorghi*) in extracts of sorghum leaves from plants of various ages and differing in initial hydrogen cyanide potential (HCN-p) prior to inoculation

| Leaf position[a] | Plant age (wk) | HCN-p[b] (μmoles HCN/ g dry wt) | Activity[c] (μmoles formamide/ hr/mg protein) |
|---|---|---|---|
| 1 | 1.5 | 464.8 | 340.0 |
| 2 | 1.5 | 144.0 | 156.7 |
| 3 | 1.5 | 49.3 | 46.2 |
| 7 | 5 | 9.9 | 2.2 |
| 8 | 7 | 3.2 | 0 |
| 9 | 9 | 0 | 0 |

[a]Leaf 1 was the first true leaf produced; leaf 2 was the second leaf, etc.

[b]The HCN-p was determined by the chloroform-base method in microdiffusion dishes (D. F. Myers and W. E. Fry 1978. Phytopathology 68:1037-1041).

[c]HCN-p and formamide hydro-lyase activities were ranked from highest to lowest (r = 0.9867, significant at $P = 0.05$).

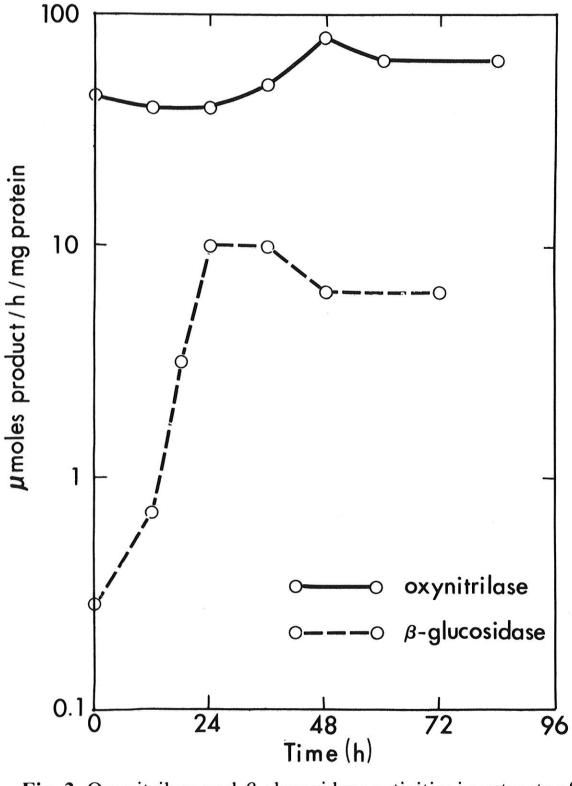


**Fig. 2.** Oxynitrilase and β-glucosidase activities in extracts of diseased sorghum during the development of *Gloeocercospora sorghi* in sorghum primary leaves inoculated 6 days after leaf emergence.

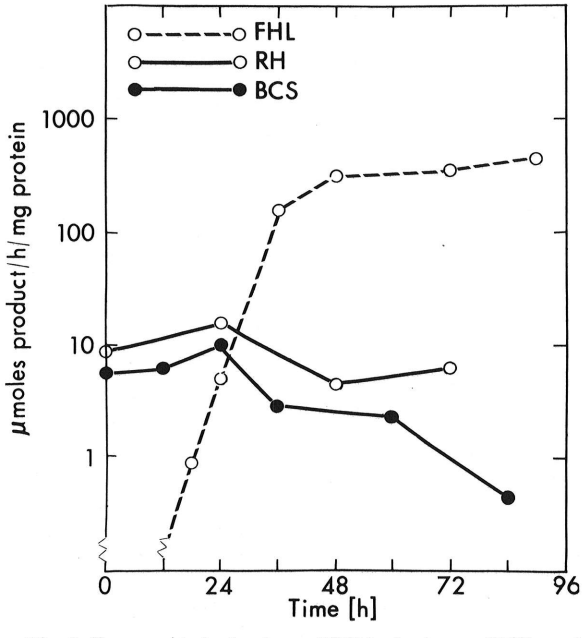


**Fig. 3.** Formamide hydro-lyase (FHL), rhodanese (RH), and β-cyanoalanine synthase (BCS) activities in extracts of diseased sorghum during the development of *Gloeocercospora sorghi* in sorghum primary leaves inoculated 6 days after leaf emergence.

either colorimetric or gas chromatographic procedures. To test the hypothesis that formamide metabolism may preclude the accumulation of formamide in diseased leaves, 10 healthy or diseased primary leaves (48 hr after inoculation) were incubated in separate 10-ml amounts of 25 mM formamide-salts solution (26) in 50-ml Erlenmeyer flasks. Some leaves were autoclaved or surface sterilized in 10% bleach (v/v) in 0.15% Tween-20 (v/v) for 2 or 5 min. The flasks were incubated under fluorescent light at 25 C on a rotary shaker. In diseased leaves, 80 μmoles of formamide were metabolized, compared with 17 μmoles in healthy leaves (Table 2). Surface sterilization significantly reduced the amount of formamide metabolized with diseased leaves but had no significant effect on the amount metabolized by healthy leaves (Table 2).

## DISCUSSION

The two enzymes involved in dhurrin degradation in healthy sorghum, β-glucosidase and oxynitrilase, were present diseased sorghum. The 20-fold increase in sorghum β-glucosidase activity during the first 24 hr after inoculation with *G. sorghi* may be due to the production of this enzyme by *G. sorghi* in vivo or to an increase in host enzymatic activity. Previous investigators have demonstrated that pathogens of cyanogenic plants produce, in vivo, β-glucosidases that are active on cyanogenic glycosides (18, 30). We did not determine the source of the increased β-glucosidase activity in diseased sorghum. Activity of oxynitrilase does not appear to limit HCN production in diseased sorghum, since its activity was at least seven times greater than β-glucosidase activity in all extracts examined [the $K_m$ of host β-glucosidase (17) and of oxynitrilase (2) is identical]. However, oxynitrilase may not be necessary for HCN production. The substrate for oxynitrilase, *p*-phydroxymandelonitrile, decomposes nonenzymatically to *p*-hydroxybenzaldehyde and HCN at pH 6.0 or greater.

The HCN potential of diseased sorghum primary leaves at 84 hr after inoculation with *G. sorghi* was only 10% that of healthy leaves (21). If loss of HCN potential in diseased sorghum results in the production of HCN, there are several possible fates for this HCN, there are several possible fates for this HCN. First, it may be volatilized (21), and second, it may be bound to cell constituents or be metabolized by host or pathogen enzymes.

Pathogen-produced formamide hydro-lyase activity, which was first detected prior to the decline in HCN potential, was 50 times that of the host enzymes rhodanese and β-cyanoalanine synthase during the decline in HCN potential (24–84 hr after inoculation). If these measurements reflect the relative activity of these enzymes in vivo, rhodanese and β-cyanoalanine synthase would seem less important than formamide hydro-lyase. The need for additional substrates at concentrations comparable to HCN may limit the activity of rhodanese and β-cyanoalanine synthase in diseased sorghum.

On the basis of enzyme activity and substrate availability, we hypothesized that formamide hydro-lyase was most likely responsible for HCN metabolism in diseased sorghum. However, the product of its activity, formamide, could not be detected in extracts of diseased sorghum. The formamide metabolized by diseased leaves from solution was 16 times that of the potential formamide (converted from HCN potential) available in those leaves. Since surface-sterilization significantly reduced the formamide metabolized by diseased leaves, a microorganism associated with the lesion may be involved. *Gloeocercospora sorghi* apparently does not metabolize formamide (W. E. Fry, *unpublished*). However, a *Pseudomonas* sp. isolated from lesions on sorghum caused by *G. sorghi* converted formamide to carbon dioxide and ammonia and used formamide as a sole carbon and nitrogen source (27).

*Gloeocercospora sorghi* apparently is exposed to HCN upon infection of sorghum leaves of moderate to high levels of HCN potential. No formamide hydro-lyase was detected in diseased leaves that had no detectable HCN potential at inoculation, but high levels of this enzyme were detected in diseased leaves that had high levels of HCN potential. Although *G. sorghi* produces a low constitutive level of formamide hydro-lyase, exposure to HCN enhances its activity 50-fold to 100-fold (9).

On the basis of this and other studies (9, 11, 22), it seems unlikely that HCN deters *G. sorghi* during the pathogenesis of sorghum. The ability of *G. sorghi* to cope with HCN may in part be due to HCN detoxification by formamide hydro-lyase. One can estimate the potential for formamide hydro-lyase to detoxify HCN in vivo. For example, the decline in HCN potential between 60 and 84 hr after inoculation was about 100 μmoles/g of dry weight, but 14% of the HCN was volatilized. Therefore, formamide hydro-lyase may encounter HCN at a rate of about 3.6 μmoles of HCN per hour per gram of dry weight. The activity of formamide hydro-lyase in extracts of diseased leaves 60–84 hr after inoculation was about 300 μmoles of formamide per hour per milligram of protein, which is equivalent to 13,500 μmoles of formamide per hour per gram of dry weight. Even if steady state HCN concentrations were as low as 0.1 mM ($K_m$ = 27 mM) (10), formamide hydro-lyase could theoretically catalyze HCN metabolism at the rate of 50 μmoles per hour per gram of dry weight. This rate is still

TABLE 2. Metabolism of formamide by healthy or disased sorghum leaves

| Leaf treatment[a] | Formamide metabolized | |
|---|---|---|
| | Healthy μmoles/flask (%) | Diseased μmoles/flask (%) |
| No treatment | 17 (7) B[b] | 80 (32) C |
| Surface-sterilized[c] | 15 (6) B | 25 (10) B |
| Autoclaved[d] | 0 (0) A | 0 ( 0) A |

[a]Ten primary leaves (.0125 g dry wt) were incubated in 10-ml aliquots of a solution containing 250 μmoles of formamide and salts. Flasks were incubated 8 days under fluorescent light at 25 C on a rotary shaker at 100 rev/min. Formamide in these solutions was measured colorimetrically on the eighth day of incubation after forming the hydroxamic acid derivative.

[b]Numbers followed by the same letter in a column are not significantly different (Duncan's multiple range test, $P = 0.05$).

[c]Leaves were surface sterilized for 2 min in 10% bleach in 0.15% Tween-20 prior to incubation.

[d]Leaves were autoclaved for 20 min at 1 atmosphere gauge pressure (16 psi) prior to incubation.

14 times the rate of dhurrin degradation measured. Therefore, the activity of formamide hydro-lyase measured in extracts of diseased leaves appears sufficient to account for the disappearance of HCN produced from degraded dhurrin during pathogenesis.

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