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Communication

Cyanogenesis Inhibits Active Defense Reactions in Plants[1]

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ABSTRACT

In the course of fungal attack on the cyanogenic rubber tree (*Hevea brasiliensis* Muell.-Arg.) HCN is liberated from infected tissue. The HCN interferes with plant host and fungal pathogen. It becomes inhibitory to active defense responses which are dependent on biosynthetic processes as far as a threshold concentration is transgressed.

Many plants liberate HCN after mechanical or chemical injury to their cells (2) or in the course of fungal infection (20). The role of this cyanogenic process in plants has been widely discussed but still remains unclear. There is considerable evidence that HCN liberated during tissue destruction by grazing animals acts as a feeding deterrent (10), but the data on cyanogenesis are quite controversial in the case of fungal diseases. Fry's working group (4) provided excellent evidence that fungal pathogens of cyanogenic plants generally are highly tolerant to hydrocyanic acid. From these findings it was deduced that plant pathogenic fungi which lack a mechanism to cope with HCN are incapable of infecting cyanogenic plants. Therefore, cyanogenic compounds were generally regarded to be preformed resistance factors (8).

In contrast to this conclusion, Lüdtke and Hahn (18) reported that highly cyanogenic flax varieties are susceptible to *Colletotrichum lini*, the causal agent of flax anthracnose, whereas moderately cyanogenic varieties are resistant to the same isolates of the fungus. In addition, Lieberei (15) reported a distinct correlation between a high capacity for HCN liberation and the high susceptibility of rubber tree leaves to *Microcyclus ulei*, the causal agent of rubber tree leaf blight. *M. ulei* is tolerant to HCN, it even grows better in HCN-containing atmosphere (17). Obviously in flax and the rubber plant, HCN is not a simple resistance factor which can be overcome by pathogens adapted to it (16, 17); instead the HCN is impairing the defense response of these plants. Screening studies revealed that all strongly cyanogenic *Hevea* varieties were susceptible to all existing isolates of *M. ulei*, whereas low cyanogenic plants respond in typical race-cultivar specific reaction patterns (11, 15).

The objective of this study was to test directly, at the molecular level, to what extent the host plant's resistance is influenced by cyanogenesis. It could be shown that the active metabolic defense response characterized by the production of the *Hevea* phytoalexin scopoletin (6), is considerably impaired by HCN confirming its role on a molecular level as a susceptibility factor.

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MATERIALS AND METHODS

Defined *Hevea* clones were grown in standard garden soil in a glass house at 85 to 90% RH and 20 to 27°C, under normal daylight with additional artificial illumination from HQI lamps (800 μE m^{-2} s^{-1}). Liquid fertiliser (Hoagland solution [9]) was applied once weekly. For the infection studies, young leaves of the defined developmental stage B (3) were chosen.

The cyanogen content was measured indirectly by analyzing HCN after the enzymatic breakdown of the cyanogenic glycosides (14). Scopoletin was extracted in ethanol, partially purified by TLC, and quantified by fluorometry in a Hitachi F-3000, according to Giesemann *et al.* (6). Culture of the pathogen *Microcyclus ulei* has been described by Lieberei *et al.* (17) as have the conditions for inoculation and incubation (14).

RESULTS

Plant-Pathogen Combinations

Microcyclus ulei occurs as a variety of physiological races which can be divided into two main groups according to their virulence for *Hevea benthamiana* and *Hevea brasiliensis* (11). Isolates of virulence group I infect *Hevea* plants carrying genes of *H. benthamiana*. They normally do not infect *H. brasiliensis*, whereas the isolates of virulence group II infect pure *H. brasiliensis* especially but normally react incompatibly with crossbreeds carrying genes of *H. benthamiana*. This general relationship is broken, however, by those varieties of *H. brasiliensis* which are characterized by a high HCN-potential (Table I).

HCN-Liberation from Infected Leaves

The amount of HCN liberated in the course of leaf infection depends on the *Hevea* clone and the respective host reaction (14). In a comparative assay the amount of HCN liberated, as well as the amount of the phytoalexin scopoletin (6) produced were estimated (Table II). The low cyanogenic, highly resistant plants liberated only small amounts of HCN but

Table I. *Host Reaction to Four Isolates of the Different Virulence Groups of M. ulei*

Hevea Clones	Species	HCN-Potential in Leaves	Host Reaction to			
			Virulence group I		Virulence group II	
			Isolate 1	Isolate 2	Isolate 3	Isolate 4
		mg CN^-/g dry weight				
Fx 3899	*H. benthamiana* × *H. brasiliensis*	3.4 ± 0.4	S[a]	S	R	R
F 4542	*H. benthamiana*	2.6 ± 0.7	S	S	R	S/R
F 985	*H. brasiliensis*	1.2 ± 0.3	R	R	S	S
MDF 180	*H. brasiliensis*	1.3 ± 0.4	R	R	S	S
RRIM 600	*H. brasiliensis*	13.7 ± 1.3	S	S	S	S
IAN 717	*H. brasiliensis* × *H. benthamiana*	12.4 ± 2.8	S	S	[b]	S

[a] S, leaves susceptible or highly susceptible to *M. ulei*; R, Resistance reaction; S/R, intermediate. [b] IAN 717 was not tested with this isolate 3, but was susceptible to 14 out of a series of 16 isolates of *M. ulei*, collected from all growing areas of Brasil (11). For description of symptoms, see Junqueira *et al.* (11).

Table II. *HCN Liberation and Scopoletin Accumulation in the Course of Leaf Infection of Hevea by M. ulei*

Infection was done with isolate 42 (virulence group II) of the collection of N. T. V. Junqueira. Quantification of scopoletin was performed by fluorometry of TLC-purified samples using scopoletin as standard. Although scopoletin accumulation was restricted to the border of the infection site, the content is calculated per leaf weight. Since less than 10% of the leaf area was infected, the scopoletin concentration at the infection site was at least 10 times larger than given here.

Hevea Clone	Species	Reaction to Virulence Group II	HCN Liberation within 48 h	Scopoletin Accumulation within 48 h
			μg CN^-/g dry weight	*μg/g dry weight*
GT 1	*H. brasiliensis*	Susceptible	33.2 ± 7.6	0.880
RRIM 600	*H. brasiliensis*	Susceptible	38.6 ± 2.4	0.480
Fx 25	*H. brasiliensis*	Intermediate	2.2 ± 0.2	1.375
F 4542	*H. benthamiana*	Resistant	2.3 ± 0.1	3.175

accumulated large amounts of scopoletin around the penetration points. The concentration of scopoletin around the lesions on resistant reacting plants is high enough to cause inhibition of spore germination and of hyphal growth of *M. ulei* (6). In contrast, highly cyanogenic, susceptible plants liberated relatively large amounts of HCN and revealed a greatly reduced accumulation of scopoletin (Table II).

Enhancement of Scopoletin Production under Low Cyanide Conditions

The concentration of HCN in the atmosphere around the infected leaf, liberated during pathogenesis, can be reduced by passing a continuous stream of moistened air through the incubation vessel which contains the inoculated leaves or by adding an alkaline trap for HCN to the incubation system. Both treatments lead to a significant reduction of the size and number of the lesions (14). Correlated with this enhanced resistance is the accumulation of scopoletin, impaired in the presence of HCN, which takes place in the tissue adjacent to the infection sites. This reaction was checked in a number of clones (Fig. 1). Under conditions of HCN depletion the highly cyanogenic, susceptible clones GT1 and RRIM 600 (both pure *H. brasiliensis*) accumulated increased amounts of scopoletin. The low cyanogenic, resistant clone F 4542 (*H. benthamiana*) did not respond in this manner but did show a significant decrease in scopoletin accumulation, when HCN was artificially added to the incubation vessel containing the inoculated leaves. The artificial addition of HCN to the tissue corresponded in time (48 h) and in concentration to the amount of HCN which is liberated by infected, strongly cyanogenic plants. The liberation pattern of HCN from infected leaves has been described in detail (14). Apparently, in weakly cyanogenic plants the defense response, as measured by scopoletin production, is not inhibited by the low amounts of HCN liberated during infection, whereas in highly cyanogenic plants the resistance phenomenon can only occur when the concentration of endogenously produced HCN is artificially lowered.

DISCUSSION

In contrast to a wide range of other fungitoxic substances produced by plants, HCN does not accumulate around infected cells but easily diffuses throughout the host tissue. As a volatile, highly soluble gas, it passes through a leaf blade in less than 30 s (13). Therefore, after liberation from its precursor, HCN rapidly becomes distributed throughout the neighboring tissue and comes into contact with the pathogen as well as with the surrounding host tissue. This suggests that both the pathogen and the host will be poisoned by cyanide unless they are capable of effective detoxification reactions.

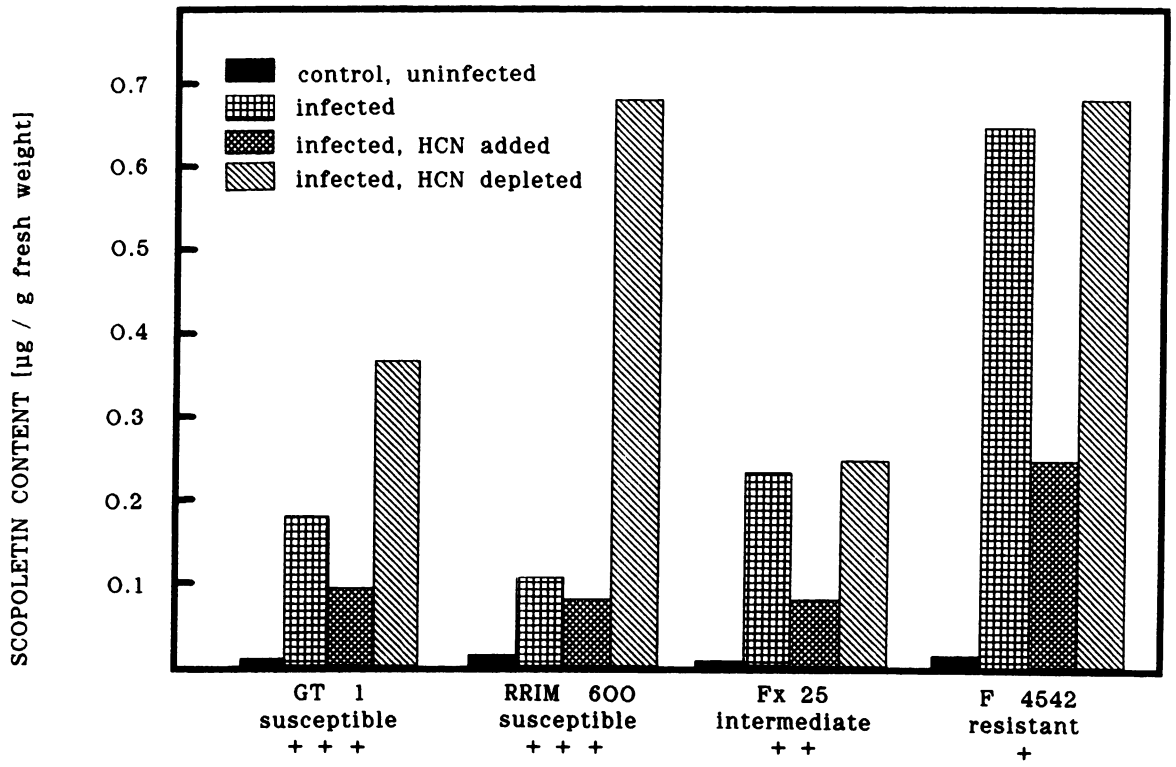

Figure 1. Enhancement of scopoletin production under low cyanide conditions. Inoculated leaves of developmental stage B2 (3) were incubated in Lips-Conway diffusion plates, containing an inner chamber of 4 cm diameter and a concentric outer chamber of 8 cm diameter. The leaves were located at the borders of the inner chamber. The outer chamber was filled with 5.0 mL of water (control and infected leaves) with 5.0 mL of 0.04 mM sodium cyanide solution (HCN added) or with 5.0 mL of 0.1 M NaOH (HCN depleted). In a second method, HCN depletion was effected by flushing a stream of prewashed, moistened air over the surface of the incubating leaves. Both methods for HCN depletion led to the same results. The control leaves often contained small amounts of scopoletin, which was induced by stress conditions in the greenhouse. For every clone leaves without scopoletin always occurred, when the plants were grown under semisterile conditions in growth chambers (6). (+++), Highly cyanogenic clones; (++), intermediate cyanogenic clones; (+), weakly cyanogenic clones.

The pathogen *M. ulei* is highly tolerant to HCN (17) but, in contrast, in leaves of the plant host, HCN concentrations lower than 0.025 mmol/L are sufficient to inhibit the photosynthetic CO_2 fixation in intact tissue; as a consequence of this inhibition, many synthesis associated reactions are stopped (R Lieberei, unpublished data).

Successful pathogens on cyanogenic plants have been shown to produce the enzyme formamide hydrolyase (FHL) which catalyses the hydration of HCN to produce formamide (5). HCN metabolism in plants differs from that of fungi; FHL has not been detected in plants which contain instead rhodanese and β-cyanoalanine synthase. These enzymes are thought to be involved in HCN detoxification (1, 19).

Weakly cyanogenic plants liberate only small amounts of HCN against which their detoxification reactions provide sufficient protection. Thus, the resistance reactions and the amounts of scopoletin produced in the course of these reactions are not inhibited. Therefore, scopoletin production will not be enhanced when HCN in the atmosphere surrounding the leaf is diminished experimentally. However, in strongly cyanogenic plants the liberation of HCN is rapid and the resulting HCN concentration in the leaves is too large to be detoxified immediately by the plant. Consequently, the plant's defense responses will be hampered by HCN. This becomes evident when comparing the amount of scopoletin produced in highly cyanogenic plants under standard incubation conditions with the same plants under conditions of HCN-depletion (Fig. 1). Additionally, these data suggest that the susceptible, highly cyanogenic plants possess the same genetic potential for defense reactions as the weakly cyanogenic plants but are inhibited by HCN produced endogenously in the course of tissue colonization. These findings do not rule out a role for HCN as a defense factor against noncyanide adapted microorganisms. However, the fact that most of the microorganisms involved are able to detoxify HCN (12) suggests that HCN *per se* can hardly be regarded as a resistance factor against them. It would seem that the HCN produced by the plant beyond a certain threshold level inhibits its own defense mechanisms, thus acting as a susceptibility factor in the plant. Like in many other plant-pathogen interactions (7), this example also supports the view that the speed of a resistance reaction is far more decisive for the outcome of an infection than whether it occurs or not. Considering the system described in this paper, it is not just the velocity of HCN liberation which is decisive, but rather the combination of HCN liberation and HCN detoxification. The velocity and extent of HCN-liberation in cyanogenic plants are known to be governed by several factors recently described by Selmar

et al. (21) and Lieberei (15). However, little is known about the rate of HCN detoxification in plants.

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