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Physiological characteristics of *Microcyclus ulei* (P. Henn.) V.ARX. – a fungal pathogen of the cyanogenic host *Hevea brasiliensis*

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Summary

The ascomycete *Microcyclus ulei* causes premature leaf shedding of the rubber tree, *Hevea brasiliensis*. During the development of this biotrophic fungus in the tissues of infected leaves HCN is liberated from the lesions. Despite high HCN concentrations in the leaf tissue the pathogenic fungus develops infection hyphae. The reaction of *M. ulei* to hydrogen cyanide and the biochemical properties of a fungal β-glucosidase which is active on the cyanogenic precursors of the host plant are reported.

Introduction

Microcyclus ulei is the causative agent of South American leaf blight (SALB), the most serious disease of rubber tree *Hevea brasiliensis* in South America. The biotrophic pathogenic ascomycete is characterized by its narrow host range, attacking genotypes of four (*H. brasiliensis*, *H. benthamiana*, *H. spruceana*, *H. guianensis* (Chee, 1976) out of eleven species of the genus Hevea (Schultes, 1970). Within the species *Hevea brasiliensis* and H. *benthamiana* a distinct race-cultivar relationship was described (Junqueira et al., 1986), additionally first reports on the attack of genotypes of *H. pauciflora* were reported.

The host-pathogen relations varied strongly with the fungal isolates, underlining the fact that *M. ulei* reveals a highly variable pathogen race structure with a range of obviously freely combining virulence factors. Using molecular approaches, recently new contributions to the genetic polymorphism of the pathogen were given by Le Guen et al. (2004). In an enlarged field study Mattos et al. (2003) analysed the variability of *M. ulei* isolates from Southeast Bahia. Fifty isolates taken from 29 rubber tree clones were tested with respect to their qualitative and quantitative development on young leaf stages of twelve *Hevea* sp. clones. These clones were either pure *H. brasiliensis*, *H. pauciflora*, F1 hybrids of various crossings of the species *H. brasiliensis* and *H. benthamiana* or backcrossings of these F1 hybrids with *H. brasiliensis*. The authors evaluated virulence profiles of the isolates tested and concluded, that at least 36 isolates turned out to be new races of the pathogen, besides the description of 11 other races by Rivano (1997). Only two isolates of these 36 new races revealed similar properties to the isolate profiles described earlier by Junqueira et al. (1986). The importance of this high variability and the high potential to adapt to genotype properties of the host species is discussed by Mattos et al. (2003) with special view on the difficulties in resistance breeding. Under this aspect of virulence variability it is interesting to point out that the pathogenic range of *M. ulei* still is restricted to *Hevea* spp. First steps of host range amplification by this fungus have recently been reported by Hagen et al. (2003) who reported the physiological interaction of *M. ulei* with *Manihot esculenta* Crantz leaves.

It is assumed that the narrow host range of this pathogen might be due to typical physiological properties of *Hevea* species which facilitate the attack by *M. ulei*. The ability to accumulate cyanogenic compounds and to store them in considerable amounts is a characteristic biochemical feature of both, the genus *Hevea* and the genus *Manihot*. In both genus the cyanogenic storage compounds are the β-glucosides linamarin and lotaustralin. The compounds are accumulated in vacuoles and after cell destruction these glucosides are cleaved and HCN is liberated. The β-glucosidases cleaving these compounds (linamarase) are of apoplastic origin (Selmar et al., 1987).

Cultivars of *H. brasiliensis* with a high capacity to liberate HCN are readily susceptible to *M. ulei* (Lieberei, 1988, 1989), whereas low cyanogenic genotypes of *H. brasiliensis* and genotypes of *H. viridis* and *H. pauciflora*, characterized by a reduced ability to liberate HCN (Lieberei, 1988), reveal the typical host-pathogen cultivar specific resistance pattern (e.g. PA31, Mattos et al., 2003; Chee and Wastie, 1980).

HCN is liberated from *Hevea* leaves infected by *M. ulei* throughout the entire process of pathogenesis from penetration of host tissues to conidium formation. The time course of HCN liberation has been quantified, and these results also allow to calculate the cyanide concentration inside the leaf tissue (Lieberei, 1986). This volatile and readily soluble compound diffuses directly after liberation from precursors through the tissues surrounding the infected area, thus both the plants and the pathogen are in contact with the HCN formed. A more detailed description on the physicochemical properties of HCN in leaf tissue is provided by Lieberei et al. (1996).
Studies of the influence of HCN on *M. ulei* isolates in pure culture revealed that the pathogens are tolerant to HCN. Vegetative mycelial development is enhanced in pure culture, but spore formation is inhibited by the presence of HCN (Lieberei et al., 1983). This tolerance to HCN and the stage specific promotion of mycelial growth by HCN suggest a strong physiological adaptation of the pathogen to the cyanogenic properties of the host plant. This adaptation is of considerable interest for the understanding of biochemical coevolution between host and pathogen. The knowledge of these biochemical interactions will contribute important aspects to the practical development of resistance screening steps and for planning of molecular biological approaches to modify host preconditions against the pathogens. Molecular approaches to change the biochemical qualities of the host plants with respect to the cyanogenesis is underway in the important crop plant cassava (*M. esculenta*) and it must be taken into consideration to transfer this work to rubber tree germplasm.

The focus of this paper is to analyse the metabolic adaptation of *M. ulei,* the specialized biotrophic fungal plant pathogen to its cyanogenic host plant, *Hevea* species. The analysis of cyanide resistant respiration of the fungus, the substrate specificity of the fungal-β-glycosidase to the cyanogenic precursor of the host and the reaction of the pathogen to β-cyanoalanine, a detoxification product of HCN, formed by the plant, is presented and the consequences of these findings for breeding and selection are discussed.

Abbreviations
CR = cyanide resistant respiration, β-CA = β-cyanoalanine, PSA = potato sucrose agar.

Materials and methods

Fungus culture

M. ulei was cultivated on potato sucrose agar (PSA 0.5 %) pH 5.0 at 23°C and 90 min of light (Osram W/30, 40 cm distance) according to CHEE (1978). It was subcultured every 14 days using spores and mycelium. For spore mass production cultures were kept dark for 15 days, followed by light induction for three days. Mass cultures were run in 300 ml Erlenmeyer flasks with 80 ml PSA 0.5 % (LIEBEREI et al., 1983).

Spore isolation

Spore forming cultures after light induction were thoroughly shaken with 6 ml sterile distilled water (2 x). The spores were liberated easily and normally contained about 90 % of two celled spores.
Suspension density was adjusted using an „Improved Double Neubauer-Chamber".

Cultivation of mycelia in liquid culture

5 ml of a freshly isolated spore suspension (1.2 x 10^5 spores l^{-1}) were mixed with 25 ml of potato-sucrose-medium (0.5 %). The resulting spore density was 2.0 x 10^4 spores l^{-1}. The suspensions were cultured at 23° C in the dark in a 3 mm liquid layer.
After 5 days the mycelia were harvested by filtration on Schleicher and Schuell filters (0.2 µm), suspended in phosphate buffer 0.067 mmol l^{-1} pH 5.0 amended with 0.5 % sucrose. These samples were taken to respiration measurements in a YSI Yellow Springs Instruments Model 53 Biological Oxygen Monitor equipped with Clark Electrode YSI 5331.

Spore exsudates

Freshly isolated conidia were adjusted to 8 x 10^4 spores ml^{-1} and left in sterile Petri dishes in sterile tap water at 23°C for 24 hrs. The liquid layer did not exceed 3 mm, the germination rate was counted at onset of the experiment and after 24 hrs. The exsudates were isolated by filtration using filters of Schleicher and Schuell 0.2 µm pore size. Concentration was done by freeze drying.

β-glucosidase

Enzyme activity was estimated according to SELMAR et al. (1987).

Results

Cyanide resistant respiration of the pathogen

The mitochondrial electron transport chain is very sensitive to HCN because that substance strongly inhibits the cytochrome a/a3 dependent oxidase, however, in all cases so far studied, residual, cyanide-resistant O_2 consumption in the presence of HCN is seen. The pathway can be determined using specific inhibitors. HCN inhibits the main respiratory pathway, whereas salicylhydroxamic acid inhibits the alternative respiratory path. The time course of inhibition of respiration and the concentration of inhibitors vary and must be identified experimentally for the organisms under study. Six minutes after addition of HCN to mycelia of *M. ulei* liquid cultures, maximal inhibition was reached (Tab. 1). At a HCN concentration of 1 mmol l^{-1}, the O_2 consumption of the samples was inhibited by about 67.4 %. Enhancement of the HCN concentration up to 5 mmol l^{-1} did not lead to higher rate of inhibition. The alternative path of respiration was inhibited by salicylhydroxamic acid (SHAM) (SCHÖNBAUM et al., 1971). Incubation with 2.5 mmol l^{-1} for 30 minutes was sufficient to reach maximum inhibitory action (Tab. 1). The total amount of inhibition of mycelial respiration observed depended on the developmental status of the fungal samples used (Fig. 1): oxygen consumption of fresh spores at the onset of germination is inhibited by HCN to less than 50 % of the control, whereas that of 5 days old cultures was inhibited up to almost 80 %. The conidia of *M. ulei* show large constitutive amount of cyanide resistant O_2 consumption. The absolute amount of cyanide resistant respiration rises during mycelial development, but due to the enhance-

Tab. 1: Inhibition pattern of respiration and cyanide resistant respiration of *M. ulei*.

Inhibitory compound	Final concentration of inhibitor [mol/l]	Inhibition of respiration in [%] of control
KCN	5.0 C 10 $^{-4}$	41.3
	7.5 C 10 $^{-4}$	59.6
	1.0 C 10 $^{-3}$	67.4
	5.0 C 10 $^{-3}$	67.4
SHAM	1.0 C 10 $^{-3}$	44.4
	2.5 C 10 $^{-3}$	52.4
	5.0 C 10 $^{-3}$	52.5

The KCN solution for inhibitory action was prepared in the measuring buffer and was adjusted to the physiological pH of the cultivation media directly in the moment of addition in order to avoid interferences by other factors like pH-changes.

SHAM, salicyl hydroxamic acid was solubilized in DMSO solution in such concentrations, that 1% (vol/vol) of DMSO was never exceeded.

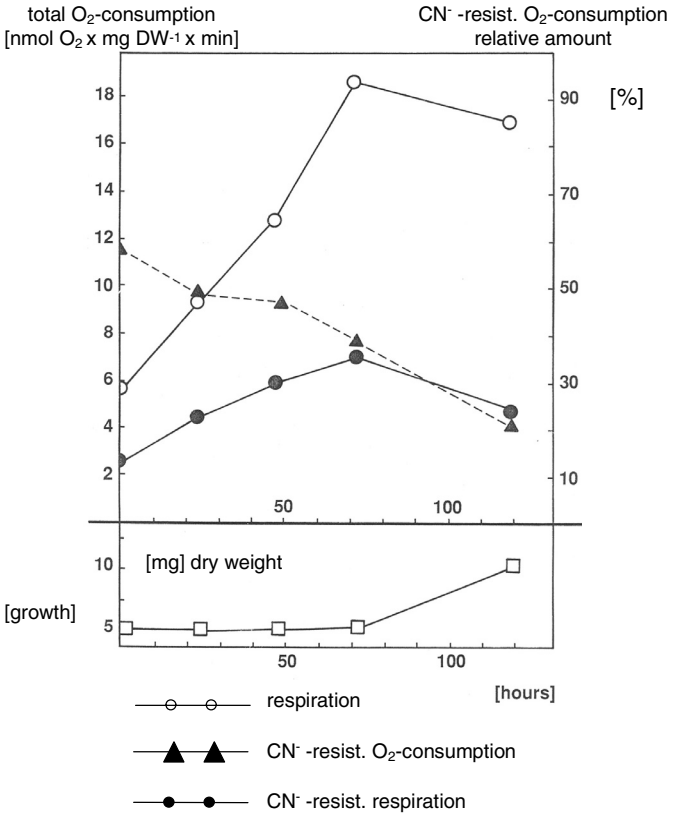

Fig. 1: Oxygen consumption and growth pattern of *M. ulei* with respect to sensitivity to HCN

ment of total O_2 consumption of the culture, the relative amount of cyanide resistant respiration is diminished. The cyanide resistant O_2 consumption of *M. ulei* is inhibited to a great extent by SHAM, but residual O_2 consumption persists. This low residual level of oxygen consumption is inhibited neither by HCN, nor by SHAM. This type of non-inhibited O_2 consumption is also known for other fungi (RISSLER and MILLER, 1977). In general this finding supports the view that *M. ulei* has a high biochemical potential to grow in the presence of HCN.

Induction of cyanide resistant respiration in mycelia by HCN

When mycelia of *M. ulei* growing in liquid culture media are treated with small concentration of KCN, the inhibition pattern of O_2 consumption is considerably changed. Overall O_2 consumption in cyanide treated cultures is significantly higher than in control cultures without addition of KCN (1 mmol l^{-1}). The additional O_2 consumption found in cyanide-treated cultures is completely due to enhanced activities in the cyanide resistant pathway (Tab. 2).

β-Glucosidase production

As is known from many other fungi, *M. ulei* produces soluble exoenzymes during germination and early fungal growth. The first colonization phase of the host tissue by this biotrophic pathogen is leading to HCN liberation due to breakdown of linamarin from plant origin. HCN liberated is able to promote fungal development. In order to know if the fungus is able to interact with HCN liberation which promotes fungal development and impairs plant resistance reactions, it was studied if the fungus itself produces enzymes able to catalyse the cleavage of cyanogenic glucosides present in the host plant tissue.
Ungerminated spores of *M. ulei* contain an active β-glucosidase (Tab. 3). About 30 % of this activity can be washed off from the spores with distilled water, about 70 % are attached to the walls. Both, spores and supernatants (30,000 g) from spore homogenates or from spores ultrasonicates contain the same β-glucosidase activity, thus the β-glucosidase present in spores is wall bound and is located in the periphery, easily accessible to aqueous treatment (Tab. 3).

In the course of germination, the extracellular level of β-glucosidase activity is strongly enhanced and is partially soluble. However, there is also a variable amount of the β-glucosidase activity still bound to fungal cell walls.

The amount of β-glucosidase produced during germination is mostly soluble. The higher the amount of germinated spores the higher is the amount of β-glucosidase in the germination medium (Tab. 3).

β-glucosidase characteristics

The β-glucosidase found in the germination medium reveals a broad pH optimum from pH 5.0 to 5.6 with half maximal rates at pH 4.0 and 6.5 (Fig. 2). The soluble enzyme exhibits β-glucosidase activity, but also catalyses the cleavage of α-glucosides, β-galactosides and cellobiosides (cellobiose, amygdalin) to a very limited extent (Tab. 4).

Of the natural substrates, the cyanogenic glucoside linamarin, the main cyanogenic compound in tissues of the rubber tree, is readily cleaved. The K_M of *M. ulei* β-glucosidase for linamarin is 4.2 mmol l^{-1} for linamarin in contrast to 0.82 for the artificial model substrate p-nitrophenol-β-D-glucopyranoside (p-NPG). The Vmax for linamarin is very high and about twice that of p-NPG, i.e. the turn over is high.
The fungal enzyme reveals a higher affinity for the cyanogenic substrate of the host plant than the enzyme of host plant itself. The linamarin cleaving β-glucosidase of *Hevea brasiliensis* has a K_M for

Tab. 2: Influence of culture treatment with HCN on the oxygen consumption

HCN preculture (15 hours)	O_2-consumption	O_2-consumption under 1 x 10^{-3} mol HCN/l	
	nmol O_2 min x mg DW	nmol O_2 min x mg DW	% of O_2-consumption without cyanide
no cyanide	49.2	23.4	47.6
Cyanide (2 x 10^{-4} mol/l)	112.9	95.1	84.9

Tab. 3: β-Glucosidase activity in spores and germination medium

SampleNo.	Germination	Time of Germination	β-Glucosidase activity in germination medium (free β-Glucosidase)	β-Glucosidase activity attached to germinated spores	Relation of free to bound β-Glucosidase
	[%]	[hours]	[nmol x $s^{-1}l^{-1}$]	[nmol x $s^{-1}l^{-1}$]	[%]
1	15	24	0.3	6.3	4.8
2	16	24	0.3	5.5	5.5
3	18	24	0.2	3.3	6.1
4	2	0	2.6	6.2	41.9
	91	24	93.4	6.6	1415.1
5	74	24	73.2	5.2	1407.7

Spore isolates 1, 2, 3 were isolated from undefined *H. brasiliensis* genotypes, Mato Grosso
Spore isolates 4 and 5 were isolated from *H. brasiliensis* IAN 717, Amazonas, Manaus
Germination was run at 25 °C in the dark in sterile tap water at 3mm liquid layer

linamarin of 7.6 mmol l^{-1} (SELMAR et al., 1982). The relative cleavage rates (Vmax Linamarin: Vmax pNPG) are 1.56 for *M. ulei* β-glucosidase and 2.8 for Hevea β-glucosidase. Thus, the soluble extracellular β-glucosidase of the pathogen produced during early germination stages can contribute to linamarin cleavage and HCN liberation. In other words, the HCN-liberation capacity, as defined in LIEBEREI (1988) is influenced by the pathogen.

Influence of β-cyanoalanine on germination and mycelial development of *M. ulei*

β-cyanoalanine (β-CA) is a detoxification product of cyanide in plants. The enzyme β-cyanoalanine synthase, which is present in cyanogenic as well as in non-cyanogenic plants (e.g. MILLER and CONN, 1980), produces β-cyanoalanine. It catalyzes the reaction of cysteine with HCN to form β-cyanoalanine and H_2S. β-CA is neurotoxic to mammals. It has been suggested that this compound causes disturbances in biomembranes.

The presence of β-cyanoalanine in tissues of cyanogenic plants during HCN liberation has been established (CASTRIC et al., 1972). β-cyanoalanine synthase activity of *Hevea brasiliensis* is high (KÖRNER, 1994) and during cyanogenesis caused by fungal attack, β-cyanoalanine is assumed to be present in the tissues. In order to evaluate the influence of β-CA on *M. ulei*, in vitro germination tests with β-CA were carried out.

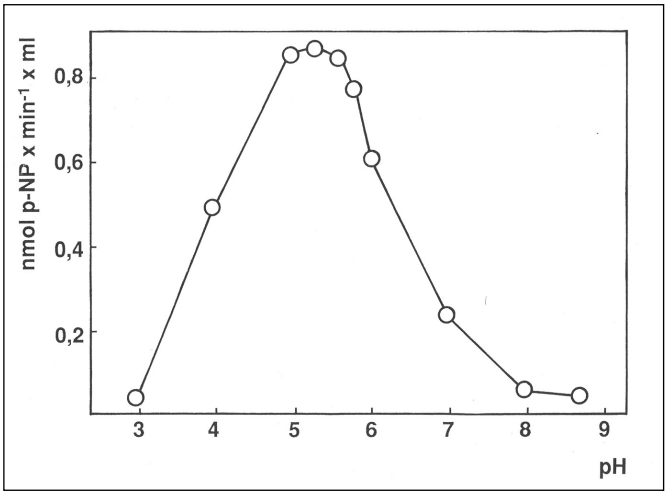

Fig. 2: Activity of *M. ulei* β-glucosidase with respect to pH
Substrate: p-NP-glucoside

Spore germination and mycelial development of *M. ulei* are significantly inhibited by β-CA (Fig. 3.). At 90 µmol l^{-1} β-CA in the germination medium, the ungerminated spores develop very fragile germ tubes and do not grow out to form normal hyphae. Less than 1 % of spores form new germination buds. When β-CA is added to germinating spores after a 20 hours period of dark incubation, a distinct inhibition of spore formation takes place. At 90 µmol β-CA less than 2 % of dark incubated spores react with new spore formation after light induction. In Fig. 4 the general spore formation pattern is shown which can be reached after light induction in standard assays.

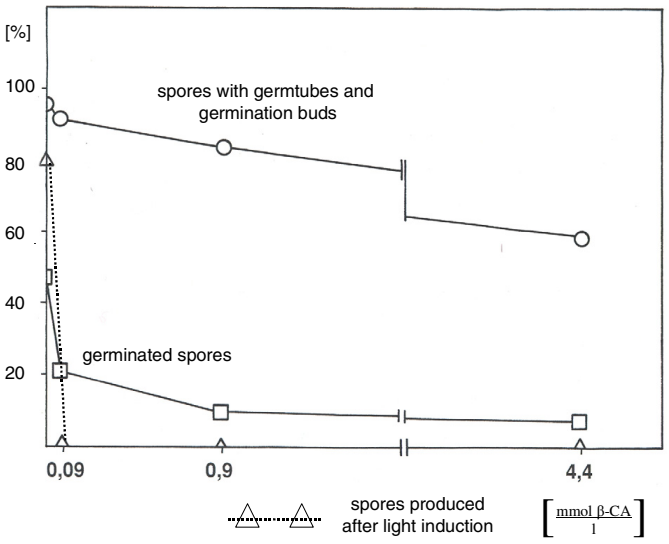

Fig. 3: Influence of β-cyanoalaine on spore germination and mycelium development

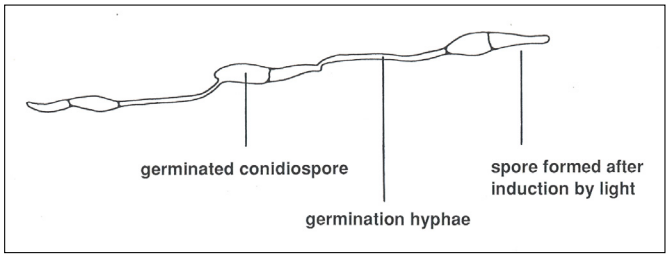

Fig. 4: Spore formation in germination tests after artificial induction of sporulation by light

Tab. 4: Kinetic properties of β-Glucosidase from *M. ulei*

Substrate	K_m mmol/l	V_{max} mol p-NP min $^{-1}$	activity per hour µmol/h nkat
p-Nitrophenyl-β-D-glucopyranoside	0.82	1.4	
p-Nitrophenyl-β-D-galactopyranoside	minimal activity	minimal activity	≤ 0.006
p-Nitrophenyl-α-D-glucopyranoside	minimal activity	minimal activity	≤ 0.39
p-Methylumbelliferyl-β-D-glucopyranoside	0.81	0.89	
Cellobiose	minimal activity	minimal activity	≤ 0.0005
Salicin	minimal activity	minimal activity	≤ 0.006
Linamarin	4.2	2.27	
Prunasin	minimal activity	minimal activity	≤ 0.34
Amygdalin	minimal activity	minimal activity	≤ 0.02

Discussion

Young mycelia of *M. ulei* reveal a high potential of cyanide resistant respiration. Addition of small amounts of cyanide to mycelial suspension in culture media, enhances respiratory O_2 consumption. The additional O_2 consumption appears to be completely due to enhanced cyanide resistant respiration. As shown previously (LIEBEREI et al., 1983), the growth of young mycelia of *M. ulei* in pure culture in the presence of HCN is higher than in cultures without added HCN. The changes in energy metabolism that are expressed by enhanced cyanide resistant respiration obviously do not impair the vegetative development of the fungus.

The flexibility of fungi to use alternative pathways of energy metabolism when the cytochrome dependent respiration pathway is inhibited also is known from *Neurospora crassa* (STURANI et al., 1977) and other fungi. *N. crassa* develops enhanced fermentative metabolism and enhanced cyanide resistant respiration. The cyanide tolerant fungal pathogen *Stemphylium loti* of the cyanogenic host plant *Lotus corniculatus* also responds with high cyanide resistant respiration when the cytochrome dependent respiration is blocked by HCN (RISSLER and MILLER, 1977). The role of cyanide resistant respiration in higher plants is still under discussion and its primary role to produce ATP is not proven (e.g., LAMBERS, 1982). For microorganisms, the cyanide resistant respiratory path is assumed to be a metabolic way to form ATP (PALMER, 1981). The amount of ATP produced per glucose in the cyanide resistant pathway is far less than in the cytochrome dependent pathway, but it is more than in fermentative processes (DAY et al., 1980).
M. ulei is a very slow growing fungus in culture (BLASQUEZ and OWEN, 1957; CHEE, 1978) and it does not undergo sexual stages in pure culture. In the vegetative growth phase, it is cyanide insensitive, but morphogenetic changes such as sporophore formation and production of conidia are inhibited by cyanide (LIEBEREI et al., 1983). Spore formation of *M. ulei* is correlated with a significant rise in cyanide sensitive respiration (Fig. 1), after more than 70 hrs. of incubation and with a considerable rise in dry weight. This metabolic pattern is in accordance with the general theory of TURIAN (1977), that for the onset of morphogenetic processes in fungi, the fermentative pathway has to be diminished and cytochrome dependent energy metabolism is needed. According to this idea, the growth of *M. ulei* can be described in the following way: energy metabolism of germinating conidia and young mycelia is realized predominantly via fermentation and/or cyanide resistant respiration. The hyphae are produced by spending the storage compounds of the conidia. Due to this fact there is no essential gain in dry weight in this developmental phase. The cyanide sensitive respiratory path is correlated with the ability of the fungus to form spores. The amount of ATP produced per reduced carbon substrate rises. The amount of dry matter produced also rises. When these aspects are transferred to the infection process, the following scheme arises: during penetration and early colonization of rubber tree leaf tissue by *M. ulei* there is liberation of HCN: *M. ulei* can adapt its energy metabolism to the presence of HCN. In contrast, the early active resistance responses of the host plant are impaired by HCN (LIEBEREI et al., 1989). After four days of colonization the fungus induces conidiophore production, again there is a HCN burst. One day later the conidiophores break through the epidermis, the amount of HCN outside the leaf tissue is low, the morphogenetic process of spore formation takes place.

Another very distinct biochemical adaptation of *M. ulei* to its cyanogenic host plant is seen in the expressed specificity of the fungal β-glucosidase to the cyanogenic substrate of the host tissue. Specificity of enzymes to their substrates is always tested in artificial situations under optimized assay conditions in the laboratory and, therefore, they will not always give good arguments for the discussion of functionality, but specifically this β-glucosidase has greater affinity to the host plant's natural substrate than the host enzyme itself. Furthermore, a series of tests of β-glucosidases of cyanogenic and non-cyanogenic plants to various cyanogenic and non-cyanogenic substrates showed that normally especially high substrate specificity of the plants β-glucosidase co-occurs to the special cyanogenic glucosides present in the plants under study (HÖSEL and NAHRSTEDT, 1975; HÖSEL, 1981).

The sensivity of *M. ulei* to β-CA is striking and shows how a biochemical factor such as HCN which gives rise to sensitivity of a plant to a biochemical adapted fungal pathogen, could be turned biochemically into a potential resistance factor by the plant, but for *Hevea* no free β-CA has been reported in healthy or in infected leaf tissue (e.g. MEVENKAMP, 1986, unpublished). According to further plant selection on biochemical level it would be very interesting to look for Hevea plants revealing low levels of cyanogenic capacity (LIEBEREI, 1988) and to look for plants with a potential for developing transient pools of β-CA in the course of pathogen attack. Biochemical features like these may help to design directed molecular markers and may allow the design of biological approaches to develop rubber tree material tolerant to the most devastating rubber tree disease of the New World.

New selection approaches on molecular level should use the knowledge of resistance physiology in that way, that markers for cyanogenic features and the detoxification processes should be included in the modern DNA-chip technologies for breeding and selection.

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References

BLASQUEZ, C.H., OWEN, J.H., 1957: Physiological studies of *Dothidella ulei*. Phytopathology 47, 727-732.

CASTRIC, P.A., FARNDEN, K.J.F., CONN, E.E., 1972: The formation of asparagine from β-cyanoalanine. Arch. Biochem. Biophys. 152, 62-69.

CHEE, K.H., 1976: Assessing susceptibility of *Hevea* clones to *Microcyclus ulei*. Ann. Appl. Biol. 84, 135-145.

CHEE, K.H., 1978: South American leaf blight of *Hevea brasiliensis*: Culture of *Microyclus ulei*. Trans. Br.mycol. Soc. 70, 341-344.

CHEE, K.H., WASTIE, R.L., 1980: The status and future prospects of rubber diseases in tropical America. Rev. Plant Pathol. 59, 541-548.

DAY, D.A., ARRON, G.P., LATIES, G.G., 1980: Nature and control of respiratory pathway in plants: The interaction of cyanide-resistant with the cyanide sensitive pathway. In: Stumpf, P.K., Conn, E.E. (eds.), The Biochemistry of Plants – A Comprehensive Treatise, Vol. 2, 197-241. Acad. Press New York, London.

HAGEN, J., GASPAROTTO, L., MORAES, V.H.F., LIEBEREI, R., 2003: Reaction of cassava leaves to *Microcyclus ulei*, causal agent of South American leaf blight of rubber tree. Fitopatol. Bras. 28, 477-480.

HÖSEL, W., 1981: Glycosylation and glycosidases. In: Stumpf, P.K., Conn, E.E. (eds.), The Biochemistry of Plants - A Comprehensive Treatise,

Vol. 7, 725-753. Acad. Press New York, London.

HÖSEL, W., NAHRSTEDT, A., 1975: Spezifische Glucosidase für das Cyanoglucosid Triglochinin. Hoppe Seyler's Z. Physiol. Chemie 356, 1265-1275.

JUNQUEIRA, N.T.V., CHAVES, G.M., ZAMBOLIM, L., GASPAROTTO, L., ALFENAS, A.C., 1986: Variabilidade fisiologica de *Microcyclus ulei*. Fitopatol. Bras. 11, 823-833.

JUNQUEIRA, N.T.V., CHAVES, G.M., ZAMBOLIM, L., ALFENAS, A.C., GASPAROTTO, L., 1988: Reação de clones de seringuera a vários isolados de *M. ulei*. Pesquisa Agropecuaria Brasileira 23, 877-893.

KÖRNER, R., 1994: Resistenzkomponenten von Kautschukbäumen (*Hevea* spp.) gegen Blattschadpilze. Ph.D. thesis, Fachbereich Biologie, University of Hamburg.

LAMBERS, H., 1982: Cyanide resistant respiration: A non phosphorylating electron transport pathway acting as an energy overflow. Physiol. Plant. 55, 478-485.

LE GUEN, V., RODIER-GOUD, M., TROISPOUX, V., XIONG, T.-C., BROTTIER, P., BILLOT, C., SEQUIN, M., 2004: Characterization of polymorphic microsatellite markers for *Microcyclus ulei*, causal agent of South American leaf blight of rubber trees. Molecular Ecology Notes 4, 122-124.

LIEBEREI, R., 1984: Cyanogenese und Resistenz – Physiologische Studien zur Wirt-Pathogen Beziehung bei cyanogenen Pflanzen, durchgeführt am Wirt-Pathogen System *Hevea brasiliensis – Microcyclus ulei*. Habilitationsschrift an der Technischen Universität Braunschweig.

LIEBEREI, R., 1986: Cyanogenesis during infection of *Hevea brasiliensis* with *Microcyclus ulei*. J. Phytopathology 115, 134-146.

LIEBEREI, R., 1988: Relationship of cyanogenenic capacity (HCN-c) of the rubber tree *Hevea brasiliensis* to susceptibility to *Microcyclus ulei*, the agent causing South American leaf blight. J. Phytopathology 122, 54-67.

LIEBEREI, R., SCHRADER, A., BIEHL, B., CHEE, K.H., 1983: Effect of cyanide on *Microcyclus ulei* cultures. J. Rubber Res. Inst. Malaysia 31, 227-235.

LIEBEREI, R., BIEHL, B., GIESEMANN, A., JUNQUEIRA, N.T.V., 1989: Cyanogenesis inhibits active defense reactions in plants. Plant Physiology 90, 33-36.

LIEBEREI, R., FOCK, H.P., BIEHL, B., 1996: Cyanogenesis inhibits active pathogen defence in plants: Inhibition by gaseous HCN of photosynthethic CO_2 fixation and respiration in intact leaves. J. Appl. Bot. 70, 230-238.

MATTOS, C.R.R., GARCIA, D., PINARD, F., LE GUEN, V., 2003: Variabilidade de isolados de *Microcyclus ulei* no Sudeste da Bahia. Fitopatol. Bras. 28, 502-507.

MEVENKAMP, G., 1986: Charakterisierung und intrazelluläre Lokalisierung der β-Cyanoalaninsythase in Blättern von *Hevea brasiliensis* (1985/1986). Diploma thesis, Faculty of Natural Sciences, Tech. University Braunschweig.

MC MAHON, J.M., ARTECA, R.N., 2000: Molecular control of ethylene production by cyanide in *Arabidopsis thaliana*. Physiologia Plantarum 109, 180-187.

MILLER, J., CONN, E.E., 1980: Metabolism of hydrogen cyanide by higher plants. Plant Physiol. 65, 1199-1202.

PALMER, J.M., 1981: Cyanide-resistant respiration of eukaryotic cells. In: Vennesland, B., Conn, E.E., Knowles, C.J., Westkey, H., Wissing, F. (eds.), Cyanide in Biology, 437-449. Acad. Press London.

RISSLER, J.F., MILLAR, R.L., 1977: Contribution of a cyanide insensitive alternative respiratory system to increases in formamide hydrolase activity and to growth in *Stemphylium loti*. Plant Physiol. 60, 857-861.

RIVANO, F., 1977: La maladie sud-américaine des feuilles de l'hévéa. I. Variabilité du pouvoir pathogène de *Microcyclus ulei*. Plantations Recherche Développement 4, 104-110.

SCHÖNBAUM, G.R., BONNER, W.D. Jr., STOREY, B.T., BAHR, J.T., 1971: Specific inhibition of the cyanide insensitive pathway in plant mitiochondria by hydroxamic acids. Plant Physiol. 47, 124-128.

SCHULTES, R.E., 1970: The history of taxonomic studies in *Hevea*. Bot. Rev. 36, 197-276.

SELMAR, D., LIEBEREI, R., BIEHL, B., VOIGT, J., 1987: *Hevea linamarase* – a non specific β-glycosidase. Plant Physiol. 83, 557-563.

STURANI, E., CHIMENTI, R., TREZZI, F., MARTEGAN, E., 1977: Effects of chloramphenicol on some aspects of growth in *Neurospora crassa*. J. Submicrosc. Cytol. 9, 83-95.

TURIAN, G., 1977: Fungal differentiation. In: Meyrath, J. Bu'Lock, J.D. (eds.), Biotechnology and fungal differentiation, FEMS-Symposium 4. Acad. Press, London, New York, Toronto, Sydney, San Francisco.

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