Translocation of cyanogenic glycosides in rubber tree crown clones resistant to South American leaf blight

Larissa Alexandra Cardoso Moraes[1], Adônis Moreira[1], Everton Rabelo Cordeiro[2], and Vicente Haroldo de Figueiredo Moraes[3]

[1]Embrapa Soja, Rodovia Carlos João Strass, Distrito de Warta, Caixa Postal 231, CEP 86001-970 Londrina, PR, Brazil. E-mail: larissa.moraes@cnpso.embrapa.br, adonis@cnpso.embrapa.br [2]Embrapa Amazônia Ocidental, Rodovia AM 010, Km 29, Zona Rural, Caixa Postal 319, CEP 69010-970 Manaus, AM, Brazil. E-mail: everton.cordeiro@cpaa.embrapa.br [3]*In memoriam*

Abstract – The objective of this work was to assess the possible transport of cyanogenic glycosides from leaves of rubber tree crown clones (*Hevea* spp.) resistant to South American leaf blight to the trunk of the panel clones in which they are grafted. The cyanogenic potential (HCNp) of the crown clones was determined in the trunk bark, at different distances from the cambium, and its gradient was evaluated along the trunk. The correlation between the HCNp of the crown leaves and that of the trunk bark was also evaluated. HCNp determined in leaves showed a wide range variation in the species studied as crown clones, with the lowest values registered in *H. nitida* clones, and the highest ones in *H. rigidifolia*. In the trunk bark, the tissue layer nearer the cambium showed higher HCNp values. A positive basipetal gradient was observed along the trunk, i.e., there was an increase in HCNp from the apex toward the base. Although the grafted crowns influence the cyanogenic potential of the trunk bark of panel clones, the absence of correlation between the HCNp of the leaves and trunk bark indicates that the crown is not the main source of the cyanogenic glycosides found in the trunk.

Index terms: *Hevea*, *Microcyclus ulei*, crown budding, HCNp, linamarin.

Translocação de glicosídeos cianogênicos em clones de copa de seringueira resistentes ao mal-das-folhas

Resumo – O objetivo deste trabalho foi avaliar o possível transporte de glicosídeos cianogênicos de folhas de clones de copa de seringueira (*Hevea* spp.) resistentes ao mal-das-folhas para o tronco dos clones de painel em que estão enxertados. O potencial cianogênico (HCNp) dos clones de copa foi determinado na casca do tronco, a diferentes distâncias do câmbio, e o seu gradiente foi avaliado ao longo do tronco. Avaliou-se, também, a correlação entre o HCNp das folhas das copas e o da casca do tronco. O HCNp determinado nas folhas apresentou ampla faixa de variação nas espécies estudadas como clones de copa, com os menores valores registrados em clones de *H. nitida* e os maiores em *H. rigidifolia*. Na casca do tronco, a camada de tecido mais próxima ao câmbio apresentou valores mais elevados de HCNp. Um gradiente basípeto positivo foi observado ao longo do tronco, ou seja, houve aumento do HCNp do ápice para a base. Apesar de os clones de copa influenciarem o potencial cianogênico na casca do tronco dos clones de painel, a ausência de correlação entre o HCNp das folhas e o do tronco indica que a copa não é a fonte principal dos glicosídeos cianogênicos encontrados no tronco.

Termos para indexação: *Hevea*, *Microcyclus ulei*, enxertia de copa, HCNp, linamarina.

Introduction

South American leaf blight (Salb) is a disease endemic to the Amazon Region, caused by the fungus *Microcyclus ulei* (P. Henn.) v. Arx., which mainly affects young leaflets of the rubber tree (*Hevea* spp.). For decades, Salb has hindered the establishment of the crop in this region, despite all the efforts of the scientific community regarding breeding and chemical control programs (Moraes et al., 2008).

After failing to cultivate clones with their own crowns in that region, Embrapa Amazônia Ocidental (Manaus, AM) carried out studies on crown budding using clones resistant to the disease (Embrapa, 1993). Initially, the research focused on elucidating the mechanisms involved in the depressing effect that crown clones of other *Hevea* species (*H. pauciflora, H. rigidifolia*, and *H. guianensis* var. marginata) had on the yield of dry rubber in panel clones (Moraes &

Moraes, 2008; Moraes et al., 2011). It was concluded that the decrease in latex stability expressively reduced the latex flow, an obstacle which was mostly removed by ethephon stimulation, prolonging latex flow and accelerating of sucrose metabolism (Moraes & Moraes, 1995; Tungngoen et al., 2009; Zhu & Zhang, 2009; Dusotoit-Coucaud et al., 2010). The reduction in latex stability occurs mainly due to ruptured lutoids, particles found in the bottom fraction of centrifuged latex, whose contents may cause gelation, followed by flocculation or formation of microclogs (D'Auzac, 1989; Wititsuwannakul et al., 2008).

The possible involvement of cyanogenesis in this process was registered by Moraes et al. (2001), when studying the incompatibility of IPA 1 (*H. brasiliensis*) panel clone, which was crown-budded with *H. pauciflora* x *H. guianensis* var. *marginata* (CPAA C 13 and CPAA C 14), established in clonal nurseries. Under these conditions, the authors concluded that this incompatibility occurred due to the transport of cyanogenic glycosides, mainly linamarin, from the budded crown to the stem. The first observed symptom was latex instability, which restricted latex flow, followed by coagulation in laticiferous vessels and by the onset of necrotic bands in the trunk of three-to four-leaf seedlings of crown-budded panel clones.

In adult plants, no reports on the transport of cyanogenic glycosides from the crown to the stem have been found in the literature. The demonstration of this occurrence may strengthen the evidence of cyanogenesis involvement in crown clone depressing effects on productive panel clones, thus indicating the need to select materials with low capacity to synthesize these compounds.

The objective of this work was to assess the possible transport of cyanogenic glycosides from leaves of rubber tree crown clones (*Hevea* spp.) resistant to South American leaf blight to the trunk of the panel clones in wich they are grafted.

Materials and Methods

This work was carried out in the experimental area of Embrapa Amazônia Ocidental, in the municipality of Manaus, AM (2°53'29"S and 59°58'40"W), Brazil. The soil is a clayey (790 g kg^{-1} clay) Xanthic Ferralsol (Oxisol) with high concentrations of exchangeable aluminum (Al), low base saturation and low concentrations of available phosphorus (P), and 83% of the soil has available P (Mehlich 1 extractant) below 5.4 mg kg^{-1} (Moreira & Fageria, 2009). The climate is tropical moist (Afi, according to Köppen climate classification), with relatively abundant rainfall throughout the year (Antonio, 2010).

The HCNp was determined in the extract obtained from young and mature leaves of crown clones previously selected for high rate of graft fixation and resistance to target leaf spot caused by *Thanatephorus cucumeris*. These determinations were performed in field conditions with six-year-old plants and in ten-month-old plants from clonal nursery. A correspondence between the HCNp of plants of the same clone under these two studied conditions was expected, which is going to allow the selection of clones with low HCNp, in plants still in the clonal nursery phase, before they are used as crown buds.

The species used and the respective crown clones of adult plants were *H. pauciflora* (CBA 2), *H. nitida* (CPAA C 65, and 64), and hybrid clones obtained by crossing *H. pauciflora* × *H. guianensis* var. *marginata* (CPAA C 01, 11, 13, 14, 15, 17, and 20), *H. guianensis* var. *marginata* × *H. pauciflora* (CPAA C 18), and *H. pauciflora* × *H. rigidifolia* (CPAA C 06, and 33). The plants were used for crown-bud panel clone CNS AM 7905, which was grafted upon rootstocks from illegitimate seeds, forming a tricomposed plant.

In the clonal nursery, the crowns budded upon rootstocks from illegitimate seeds belong to the same clones mentioned for adult plants, except for CPAA C 01, with the addition of *H. nitida* (CPAA C 67, and 69) and *H. rigidifolia* (CPAA C 71, 72, 79, and 80) clones.

The HCNp was quantified on five replicates of each clone, according to the method described by Lieberei (1986), adopting the modifications proposed by Moraes et al. (2002), in extracts obtained from 1.0 g of fresh tissue of mature leaflets or leaflets at growth stage C – leaves at the final phase of blade expansion, still hanging, and light green –, macerated using a mortar and pestle with 4.0 mL NaH_2PO_4 (0.067 mol L^{-1}), both pre-cooled. The homogenized product, obtained in duplicate for each treatment, was centrifuged at 20,000 *g* for 14 min, between 0 and 6°C, and the supernatant was transferred to capped vials.

For total release of cyanide, the reaction media, composed of 0.05 mL supernatant and 0.45 mL NaH_2PO_4 (0.067 mol L^{-1}), received 0.1 mL of the enzyme linamarase (β-glycosidase). The enzyme was

extracted from young leaflets (growth stage C) in phosphate buffer pH 6.5, 10 mmol L^{-1}, precipitated with ammonium sulfate, desalinated by washing with phosphate buffer, and successively filtered in Millipore membranes (type PTGC), at a 2.5-bar constant pressure. The activity of the partially purified enzyme so obtained was determined based on the release of ρ-nitrophenol (ρ-NP), using ρ-nitrophenyl-glucopyranoside as substrate (Selmar et al., 1987).

The absorbance was measured at 585 nm, between 5 and 15 min after adding the reagents, which is the time necessary for color development. The amount of cyanide released per gram of rubber tree fresh young leaves was calculated based on the standard curve of free cyanide concentration, at 0.1, 0.2, 0.3, 0.4, and 0.5 mg L^{-1}.

Data were analyzed using the normal distribution, analysis of variance, and F-test, and the means were compared using cluster analysis (Scott & Knott, 1974), at 5% probability. To test the correspondence of the values obtained under each of the tested conditions (adult and clonal nursery plants), the correlation (5%) between both groups of HCNp values was also determined.

To determine HCNp in the trunk bark at different distances from the cambium, one sample per plant was collected at 1.2 m of five plants of each clone, using an iron punch (3.0 cm diameter). For this evaluation, 18-year-old plants of the clones CNS G 124/Fx 4098 and PA 31/Fx 4098 (*H. pauciflora* crown and *H. brasiliensis* panel) were used. As it is impossible to obtain adult plants of clone Fx 4098 with its own crown, due to its high susceptibility to *M. ulei*, 26-year-old plants of clone IAN 6323 with its own crown were used. Each crown/panel combination, as well as the clone with its own crown, was represented by five plants.

In the laboratory, the samples were washed with tap water and dried with paper towels. The crushed margins of the trunk disks were cut out using a sharp penknife, and the disks were then subdivided into three layers of 2.0 mm width each, in order to obtain subsamples of 0 to 2.0 mm, 2.1 to 4.0 mm, and 4.1 to 6.0 mm, from the inner to the outer layers of cambium. HCNp was determined in 1.0 g of fresh tissue of each subsample using the same procedure described for the leaves. All individual data per trunk bark layer of crown/panel combinations or clones were analyzed using the normal distribution, analysis of variance (ANOVA), and F-test, and the means were determined by the Tukey´s test, at 5% probability.

In order to study the longitudinal gradient of HCNp along the trunk in grafting conditions with crown clones resistant to Salb, and in the absence of this procedure by keeping their own crowns, a procedure analogous to that of HCNp determination in the trunk bark at different distances from the cambium was adopted. The samples, consisting of 1.0 g of fresh tissue, were collected from five plants in each predetermined height in the stem, exclusively in the 2.0 mm layer closer to the cambium. For IAN 6323 clone with its own crown, samples were collected at 2.0, 1.2, and 0.4 m aboveground level, and ±3.0 cm above and below the base graft. The crown/panel combinations were the same as described above with the addition of combination CNS G 112/Fx 4098; the samples were collected at 1.2 and 0.4 m aboveground level and ±3.0 cm above and below the crown graft.

All individual data per point of trunk bark collection or crown/panel combinations or clones were analyzed using analysis of variance (ANOVA) and F-test, and the means were compared by the Tukey´s test, at 5% probability.

For the correlation between HCNp of the crown clone leaves and that of the trunk bark under them, the determination was performed in the trunk bark (panel clone CNS AM 7905) of 6-year-old plants (13 crown clones). Five trunk bark samples were used per crown clone, collected at 1.2 m, each sample corresponding to one plant. These results were also analyzed by ANOVA and F-test, and the means were compared by the Tukey´s test, at 5% probability.

Results and Discussion

Significant variations were observed in the results of cyanogenic potential (HCNp) among crown clones (Table 1). Among the grafted 6-year-old crown clones, only 31% showed HCNp values below 200 μg g^{-1}, represented mainly by *H. nitida*, a species in which low HCNp values were already observed by Lieberei (1988); 54% had their HCNp ranging from 200 to 400 μg g^{-1}, and 15% showed results above 400 μg g^{-1}, represented mostly by *H. rigidifolia*. In clonal nursery, most of *H. nitida* individuals had mean and low HCNp values, and *H. rigidifolia* had high and low values. This result indicates that this characteristic is still not well defined in some *Hevea* species.

The clones showing correspondence between adult and clonal nursery plants (CBA 2, CPAA C 06, 11, 13, 14, 15, 17, 18, 20, 33, 64, and 65) exhibited a highly significant correlation coefficient (r = 0.97), which indicates the possibility of early selection of rubber tree clones with low HCNp.

The highest values of HCNp determined in the trunk bark, at different distances from the cambium, were found in the 2.0 mm layer closer to the cambium (Figure 1), both in the clone with its own crown (IAN 6323) and in the crown-budded clones (combinations crown/panel PA 31/Fx 4098 and CNS G 124/Fx 4098). This finding confirms that cyanogenic glycosides concentrate in the layer closer to the tissue that shows active growth (cambium), as observed by Conn (1980), a result that defined the usage of this layer as a standard in experimental procedures involving quantification of HCNp in the trunk bark. In the present study, it was possible to observe that the crown influenced HCNp values in the trunk bark of panel clone Fx 4098 crown-budded with CNS G 124 and PA 31, which showed statistically significant differences among them.

The quantification of HCNp in the trunk bark along the panel of crown-budded clones and the clone with its own crown did not show the expected negative basipetal gradient, with gradual dilution of cyanogenic glycoside from the apex toward the base. The results showed an increase in HCNp from the apex toward the base (positive basipetal gradient), reaching high values above the base graft, both in Fx 4098 clone under the three crown clones used (PA 31, CNS G 124, and CNS G 112) and in IAN 6323 clone with its own crown (Table 2).

The influence of the crown was also observed for HCNp in the panel, and the highest values were registered under PA 31 crown, which were statistically different from the others. The absence of a negative basipetal gradient is an important evidence that such an influence is not due to the transport of cyanogenic glycosides from the crown toward the panel.

High values of HCNp in the tissue above the base graft were also reported by Nandris et al. (2005), who interpreted this accumulation as a result of an obstruction to the transport of cyanogenic glycosides across the graft union tissue. Regarding photoassimilates, the transport across the graft union tissue is free, and no reports were

Table 1. Cyanogenic potential (HCNp) determined in the growth stage C leaves of crown clones resistant to South American leaf blight kept in clonal nursery, and in 6-year-old plants of crown clones grafted on panel clone CNS AM 7905[(1)].

Clone	Species	HCNp (μg g^{-1})	
		Nursery plants	6-year-old plants
CPAA C 01	*H. pauciflora* x *H. guianensis* var. *marginata*	-	652.17a
CPAA C 79	*H. rigidifolia*	780.07a	-
CPAA C 72	*H. rigidifolia*	638.86b	-
CPAA C 71	*H. rigidifolia*	503.51c	-
CPAA C 80	*H. rigidifolia*	500.43c	-
CPAA C 06	*H. pauciflora* x *H. rigidifolia*	417.08d	410.63b
CPAA C 33	*H. pauciflora* x *H. rigidifolia*	381.01e	383.77b
CBA 2	*H. pauciflora*	318.59f	328.22c
CPAA C 69	*H. nitida*	313.58f	-
CPAA C 20	*H. pauciflora* x *H. guianensis* var. *marginata*	303.31f	309.77c
CPAA C 67	*H. nitida*	291.56g	-
CPAA C 17	*H. pauciflora* x *H. guianensis* var. *marginata*	270.56g	273.76d
CPAA C 14	*H. pauciflora* x *H. guianensis* var. *marginata*	221.66h	220.62e
CPAA C 15	*H. pauciflora* x *H. guianensis* var. *marginata*	217.52h	209.72e
CPAA C 11	*H. pauciflora* x *H. guianensis* var. *marginata*	209.78h	206.09e
CPAA C 77	*H. rigidifolia*	196.53h	-
CPAA C 13	*H. pauciflora* x *H. guianensis* var. *marginata*	164.92i	152.43f
CPAA C 18	*H. guianensis* var. *marginata* x *H. pauciflora*	96.78j	109.42g
CPAA C 64	*H. nitida*	71.26j	75.85h
CPAA C 65	*H. nitida*	66.60j	72.71h
CV (%)		8.14	7.83
LSD 5%		30.18	26.88

[(1)]Means followed by equal letters do not differ by Scott Knott test, at 5% probability.

found in the literature about rootstock low-radial growth rate. However, the opposite frequently occurs, resulting in a pronounced radial growth of the rootstock formation which physically separates the scion from the rootstock in two distinct sections. This deformation, known as "elephant's foot", is a consequence of fast growth below the graft union tissue (Gasparotto et al., 1997).

In the studied combinations and in the clone with its own crown, HCNp values measured below the graft union tissue, i.e., in the bark of the rootstock trunk, were the same or slightly higher than the results obtained when measuring this parameter ±3.0 cm above the graft union tissue, indicating no blockage of the cyanogenic glycosides transport, as suggested by Nandris et al. (2005).

A plausible explanation for the increase in HCNp in the base of the trunk could be the presence of compounds furnished by the rootstocks, which are able to promote the synthesis of cyanogenic glycosides. In this case, valine would be the most appropriate compound, taking into consideration that this amino acid is the substrate for the synthesis of linamarin (Jørgensen et al., 2005), and that the transport of nitrogen in the form of amino acid in plants has been observed in several species (Rentsch et al., 2007).

Therefore, the rootstocks that could furnish more valine would promote an increase in the synthesis of this cyanogenic glycoside, which could reach higher amounts in the trunk of the panel clone if its capacity to synthesize linamarin exceeded the capacity of the rootstock – an alternative hypothesis to the transport blockage presented by Nandris et al. (2005).

Comparing HCNp values determined in the trunk bark of panel clone CNS AM 7905 under different crown clones (Table 3) with those presented in Table 1, determined in the leaves at growth stage C, the correlation between HCNp in the crown (leaves) and in the trunk bark was nonsignificant. This result indicates that the crowns are not the main source of cyanogenic glycosides found in the trunk bark. In rubber tree seedlings, the transport of cyanogenic glycosides from the seeds (endosperm) to the leaflets was shown in experiments performed by Selmar (1993). Also, Moraes et al. (2001, 2002) evidenced that the cyanogenic glycosides can be transported in the other way around, from the grafted crown towards the stem of the rootstock, as these authors observed the incompatibility of young panel clone IPA 1 (*H. brasiliensis*) individuals crown-budded with clones showing high to medium HCNp.

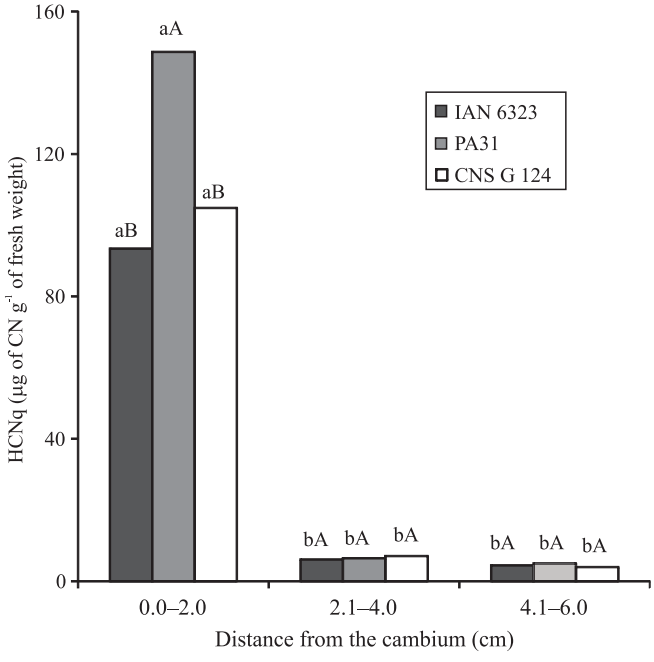

Figure 1. Cyanogenic potential (HCNp) determined at different distances from the cambium in the trunk bark of panel clones IAN 6323 with its own crown, and in Fx 4098 under crowns CNS G 124 and CNS G 112. Samples collected at 1.2 m aboveground level. Means followed by equal letters, lower case between distances and upper case between crown clones, do not differ by the Tukey´s test, at the 5% probability. CV(%) = 20.87.

Table 2. Longitudinal gradient of cyanogenic potential, determined along the trunk of panel clone Fx 4098 under crowns PA 31, CNS G 112, and CNS G 124 (18-year-old plants), and along the trunk of panel clone Fx 6323 with its own crown IAN 6323 (26-year-old plants)[1].

Sampling height	PA 31	CNS G 112	CNS G 124	IAN 6323
	----------HCNp (µg g^{-1})----------			
3 cm above crown graft	266.38bA	254.88aAB	232.75aB	-
3 cm below crown graft	154.98cA	172.76cA	156.98bA	99.44dB[2]
120 cm	144.16cA	100.98dB	96.90cB	125.45dAB
40 cm	172.43cA	148.67cB	118.47bcB	192.09cA
3 cm above base graft	257.72bA	212.90bB	202.59aB	274.93bA
3 cm below base graft	324.77aB	292.17aC	210.75aD	398.20aA
CV (%)	10.69			
LSD 5%	Lines, 31.60; columns, 38.60			

[1]Means followed by equal letters, lower case in columns and upper case in lines, do not differ by the Tukey´s test, at 5% probability. [2] Samples collected at 2.0 m aboveground level.

Such incompatibility may have been caused by not very high amounts of cyanide, not only because of the extreme sensitivity of IPA 1, due to the low activity of the detoxifying enzyme β-cyanoalanine synthase (Moraes et al., 2001, 2002), but also because stems are small in diameter in young plants. This would prevent a dilution effect, which can take place in the trunk of adult individuals. Furthermore, leaf emission in adult crowns of clones resistant to Salb is sporadic and, therefore, the amount of leaves able to export their cyanogenic glycosides is not massive, which reduces the importance of the contribution of cyanogenic glycosides translocation from the crown to the trunk.

Lack of correlation between HCNp in the crown and in the panel (Figure 1) does not eliminate the possibility that in adult plants the transport of cyanogenic glycosides from the crown toward the panel also occurs; however, it shows that the crown is not the main source of cyanogenic glycosides found in the trunk bark.

The mechanisms used by crown clones showing different capacities to promote variations in the HCNp found in the trunk bark, when grafted on the same panel clone, remain unknown. To elucidate them, it is necessary to carry out further studies based on new hypotheses involving chemical substances or signs, which are able to induce or inhibit the in situ synthesis of cyanogenic glycosides in the trunk bark when released by the crown.

Table 3. Cyanogenic potential (HCNp) determined in the trunk bark of panel clone CNS AM 7905 under different crown clones, at 1.2 m aboveground level(1).

Crown clone	HCNp (μg g^{-1})
CPAA C 17	445.24a
CPAA C 13	228.87b
CPAA C 11	200.39c
CBA 2	145.76d
CPAA C 20	132.13e
CPAA C 06	115.45f
CPAA C 01	109.09f
CPAA C 18	93.12g
CPAA C 65	92.75g
CPAA C 33	82.83h
CPAA C 14	79.64h
CPAA C 64	78.38h
CPAA C 15	77.72h
CV (%)	9.90
LSD 5%	7.68

(1)Means followed by equal letters do not differ by the Scott & Knott test, at 5% probability.

Conclusions

1. The cyanogenic potential shows a broad range in the species studied as crown clones, and the lowest values are registered in *Hevea nitida* clones, whereas the highest ones are found in *H. rigidifolia*.

2. Grafted crowns influence the cyanogenic potential of the trunk bark of panel clones.

3. Crown is not the main source of cyanogenic glycosides found in the trunk bark, and the usage of the crown cyanogenic potential, as a criterion to select clones, is not valid.

Acknowledgements

To Renaldo Silva de Araújo, Francisco Exgidras Leite Magalhães, and Sérgio de Araújo Silva, from Embrapa Amazônia Ocidental (CPAA), for data collection and support in laboratory analyses.

References

ANTONIO, I.C. **Boletim agrometeorológico 2009**: estação agroclimatológica da Embrapa Amazônia Ocidental, no Km 29 da Rodovia AM 010. Manaus: Embrapa Amazônia Ocidental, 2010. 26p. (Embrapa Amazônia Ocidental. Documentos, 83).

CONN, E.E. Cyanogenic compounds. **Annual Review of Plant Physiology and Plant Molecular Biology**, v.31, p.433-451, 1980.

D'AUZAC, J. Factors involved in the stopping of flow after tapping. In: D'AUZAC, J.; JACOB, J.L.; CHRESTIN, H. (Ed.). **Physiology of rubber tree latex**. Boca Raton: CRC Press, 1989. p.257-280.

DUSOTOIT-COUCAUD, A.; KONGSAWADWORAKUL, P.; UNSHIRAVIBOONJUN,L.M.;BRUNEL,U.;PUJADE-RENAUD, V.; CHRESTIN, H.; SAKR, S. Ethylene stimulation of latex yield depends on the expression of a sucrose transporter (HbSUT1B) in rubber tree (*Hevea brasiliensis*). **Tree Physiology**, v.30, p.1586-1598, 2010.

EMBRAPA. Centro de Pesquisa Agroflorestal da Amazônia Ocidental. **Plano Diretor do Centro de Pesquisa Agroflorestal da Amazônia Ocidental - CPAA**. Manaus: Embrapa-CPAA; Brasília: Embrapa-SPI, 1993. 35p.

GASPAROTTO, L.; SANTOS, A.F. dos; PEREIRA, J.C.R.; FERREIRA, F.A. **Doenças da seringueira no Brasil**. Brasília: Embrapa-CPAA, 1997. 168p.

JØRGENSEN, K.; BAK, S.; BUSK, P.K.; SØRENSEN, C.; OLSEN, C.E.; PUONTI-KAERLAS, J.; MØLLER, B.L. Cassava plants with a depleted cyanogenic glucoside content in leaves and tubers. Distribution of cyanogenic glucosides, their site of synthesis and transport, and blockage of the biosynthesis by RNA interference technology. **Plant Physiology**, v.139, p.363-374, 2005.

LIEBEREI, R. Cyanogenesis of *Hevea brasiliensis* during infection with *Mycrocyclus ulei*. **Journal of Phytopathology**, v.115, p.134-146, 1986.

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

MORAES, L.A.C.; MORAES, V.H. de F.; CASTRO, P.R. de C. Aplicação de KCN e linamarina e a incompatibilidade de enxertia por translocação no clone de seringueira IPA 1. **Scientia Agricola**, v.58, p.717-723, 2001.

MORAES, L.A.C.; MORAES, V.H. de F.; MOREIRA, A. Efeito da cianogênese na incompatibilidade entre clones de copa de seringueira e o clone de painel IPA 1. **Pesquisa Agropecuária Brasileira**, v.37, p.925-932, 2002.

MORAES, L.A.C.; MOREIRA, A.; FONTES, J.R.A.; CORDEIRO, E.R.; MORAES, V.H. de F. Assessment of rubber tree panels under crowns resistant to South American leaf blight. **Pesquisa Agropecuária Brasileira**, v.46, p.466-473, 2011.

MORAES, V.H. de F.; MORAES, L.A.C. Desempenho de clones de copa de seringueira resistentes ao mal-das-folhas. **Pesquisa Agropecuária Brasileira**, v.43, p.1495-1500, 2008.

MORAES, V.H. de F.; MORAES, L.A.C. Diagnóstico do látex em sangria precoce de seringueiras com copas enxertadas: possibilidades de emprego na seleção precoce de clones de copa e de painel. **Agrotrópica**, v.7, p.49-62, 1995.

MORAES, V.H. de F.; MORAES, L.A.C.; MOREIRA, A. **Cultivo da seringueira com copas enxertadas resistentes ao mal-das-folhas**. Manaus: Embrapa Amazônia Ocidental, 2008. 44p. (Embrapa Amazônia Ocidental. Documentos, 63).

MOREIRA, A.; FAGERIA, N.K. Soil chemical attributes of Amazonas state, Brazil. **Communications in Soil Science and Plant Analysis**, v.40, p.2912-2925, 2009.

NANDRIS, D.; MOREAU, R.; PELLEGRIN, F.; CHRESTIN, J. Rubber tree bark necrosis: advances in symptomatology, etiology, epidemiology and causal factors of a physiological trunk disease. **Tropical Agricultural Science and Technology**, v.28, p.1-9, 2005.

RENTSCH, D.; SCHMIDT, S.; TEGEDER, M. Transporters for uptake and allocation of organic nitrogen compounds in plants. **FEBS Letters**, v.581, p.2281-2289, 2007.

SCOTT, A.J.; KNOTT, M.A. A cluster analysis method for grouping means in the analysis of variance. **Biometrics**, v.30, p.507-512, 1974.

SELMAR, D. Transport of cyanogenic glucosides: linustatin uptake by *Hevea* cotyledons. **Planta**, v.191, p.191-199, 1993.

SELMAR, D.; LIEBEREI, R.; BIEHL, B.; VOIGT, J. *Hevea* linamarase: a nonspecific β-glycosidase. **Plant Physiology**, v.83, p.557-563, 1987.

TUNGNGOEN, K.; KONGSAWADWORAKUL, P.; VIBOONJUN, U.; KATSUHARA, M.; BRUNEL, N.; SAKR, S.; NARANGAJAVANA, J.; CHRESTIN, H. Involvement of *HbPIP2;1* and *HbTIP1;1* aquaporins in ethylene stimulation of latex yield through regulation of water exchanges between inner liber and latex cells in *Hevea brasiliensis*. **Plant Physiology**, v.151, p.843-856, 2009.

WITITSUWANNAKUL, R.; RUKSEREE, K.; KANOKWIROON, K.; WITITSUWANNAKUL, D. A rubber particle protein specific for *Hevea* latex lectin binding involved in latex coagulation. **Phytochemistr**y, v.69, p.1111-1118, 2008.

ZHU, J.; ZHANG, Z. Ethylene stimulation of latex production in *Hevea brasiliensis*. **Plant Signaling and Behavior**, v.4, p.1072-1074, 2009.

Received on March 7, 2011 and accepted on July 24, 2012