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A POSSIBLE ROLE OF CYANOGENESIS IN THE ONSET OF TAPPING PANEL DRYNESS OF RUBBER (*Hevea brasiliensis*)

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The primary cause of Tapping Panel Dryness (TPD) of rubber trees is still unknown. In a preliminary assay of the role of cyanogenesis as a cause of TPD, hydrocyanic acid (HCN) was released from open punctures and retained in the bark in sealed punctures, which were reported to induce bark dryness. More HCN was released after refoliation, at the leaf stage C, than during leaf senescence. This is in accordance with reports of high TPD incidence after refoliation. Moreover, a role of cyanogenesis could explain TPD occurrence in trees only recently brought into tapping, or its induction by bark compression. The induction of dryness by punctures was only incipient, but the symptoms were similar to the described for Brown Bast. The possible role of cyanogenesis in the onset of TPD deserves therefore further research.

Key words: Brown Bast, linamarin, linustatin, β glycosidase.

Possível papel da cianogênese na indução do secamento do painel de sangria da seringueira (*Hevea brasiliensis).* A causa primária do secamento do painel de sangria da seringueira é ainda desconhecida. Em teste preliminar do papel da cianogênese na indução do secamento, o ácido cianídrico (HCN) foi liberado de puncturas deixadas abertas e retido na casca em puncturas fechadas, relatadas como capazes de induzir secamento do painel. Houve maior liberação de HCN após o reenfolhamento, no estádio foliar C, o que está de acordo com os registros de maior incidência de secamento após o reenfolhamento. Além disso, o envolvimento da cianogênese explicaria a ocorrência de secamento em plantas logo após o início da sangria, ou a indução de secamento por compressão da casca. A indução de secamento por puncturas foi apenas incipiente, mas os sintomas foram similares aos descritos para o Brown Bast. O possível papel da cianogênese na indução do secamento do painel de sangria da seringueira merece portanto ser pesquisado com maior profundidade.

Palavras - chave: Brown Bast, linamarina, linustatina, β glicosidase.

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Tapping Panel Dryness (TPD) of rubber (*Hevea brasiliensis*) is reported since the beginning of its commercial cultivation in South East Asia and still causes heavy yield losses wherever rubber is grown, as its underlying cause remains unclear.The purpose of this work is to draw the attention for a posible role of cyanogenesis in the onset of TPD.

Many controversial results of TPD research could have been avoided if the differences between TPD caused by excessive etephon stimulation and the typical Brown Bast, caused by overtapping, were considered (Faÿ, 1988; Gohet et al., 1994; Jacob, Prévot and Lacrotte, 1994), or by monitoring the developmental stage of TPD for the assessment of biochemical variables (Eschbach, Lacrotte and Serres, 1989). Nevertheless, it still remains to be explained why the general acceptance of a close association of TPD with overexploitation is weakened by the reports of bark dryness from the start of exploitation, or even in untapped trees (Faÿ and Jacob, 1989)

The involvement of cyanogenesis on TPD was conceived in a study of graft incompatibility. Clones of high cyanogenic potential (HCNp) of different *Hevea* species, whose cyanogenic glucosides are translocated from young leaves to the stem, are incompatible when top budded onto the clone IPA 1 (*H. brasiliensis*) due to the very low β cyanoalanine synthase detoxyfying activity of this clone (Moraes, Moraes and Moreira, 2002). The symptoms developed in the stem bark of IPA 1 under these crowns are very similar to the described for Brown Bast (Moraes, Moraes and Castro, 2001). Low doses of KCN or linamarin (the main cyanogenic glucoside of *Hevea*) cause the same symptoms. The increase of cyanide-resistant respiration in trees with TPD (Krishmakumar, Cornish and Jacob, 2001) reinforced the conception of a role for cyanogenesis on TPD.

The induction of bark dryness by sealed punctures and not by open punctures (Sivakumaran and Pakianathan, 1983) suggested a simple procedure for a preliminary test of the involvement of cyanogenesis on TPD.

In a first trial the release of HCN was compared in six trees without previous TPD, of the clones Fx 3899 and Fx 4098, each with sealed and open punctures. The HCN was detected with a small piece of Feigl Anger paper attached to the inner side of a shallow 3 cm diameter cylindrical plastic chamber tightly adjusted to the slightly scraped bark with modelling clay. Six punctures were made to the wood with 1,2 mm diameter nickel coated steel pins on a area of bark to be covered by the chambers. The puncutres were made at 20 to 30 minutes after tapping, to avoid flooding the chambers with latex from the open punctures. The areas of the punctures were located at approximately 5 cm below the cut surface. After fixing the chambers, a thin layer of fresh latex was spread over the modelling clay, to verify bubble formation indicating air leakage. The color developed by the Feigl Anger paper was observed after 60 minutes exposure.

For a rough semiquantitative comparison of HCN release, a series of six color standards was obtained from one to six 0,7 cm diameter leaf disks, at the stage C, after Djikman (1951), of the clone Fx 4098. The disks were quickly ground with sand and a glass rod and immediately put in a closed vial with a piece of Feigl Anger paper. The colors developed after 20 minutes exposure were reproduced with gouache and scored from 1 to 6.

The same procedure described above , but only with open punctures, was employed in a second trial, for the evaluation of HCN release in 10 Fx 3899 and 10 Fx 4098 trees, when most of the plants had senescent leaves and after refoliation, when the leaves of most of the trees were fully expanded and semihardened, at the end of stage C. The colors of the Feigl Anger paper were scored after 20 minutes exposure and the averages for clones and leaf stages were compared by the t test at 5%.

On the stem bark of the same 10 trees of Fx 3899 and Fx 4098, four vertical strips 1 cm wide were slightly scraped from 1 cm below the cut surface to 12 cm downward. The strips were about 2 cm apart from each other and placed below the middle of the extension of the tapping cut. The trees were tapped in ½ S, d/3 6d/7 without stimulation during three weeks from the beginning of the leaf senescence. The tapping was discontinued during three weeks after the start of the new leaf flushes and resumed for another three weeks from the start of the stage of full expanded, semihardened new leaves. On each tapping day, two punctures 1 cm vertically apart were made on each scraped strip, starting downward from the upper end in two adjacent strips and upward, from the lower end in the other two. The occurrence of dry cut was observed visually and its length was measured and its position along the cut recorded, when they became conspicuous. Latex exsudation near the punctures was also verified with short cuts made with the tapping knife. The anatomical study of the dry bark was made according to Faÿ and Hébant (1980), in samples collected three months after the last tapping.

The dark blue color of the Feigl Anger paper showed that HCN was release to the air only from the open punctures. Only a faint, almost imperceptible color was found in two of the six replications of sealed punctures.Therefore, the small quantity of tissue injured by the punctures was sufficient to produce a detectable amount of HCN.

In intact tissue, the cyanogenic glucosides are stored

in the vacuoles and the linamarase (a β glucosidase) is located in the apoplast, as in other cyanogenic species (Gruhnert, Biehl and Selmar,1994). When the tissue is injured, the cyanogenic glucosides are put in contact with the enzyme and after hydrolysis, the cyanohydrine formed may release HCN under the action of a hyroxynitrile lyase, or non-enzymatically in alkaline medium (Conn, 1980). Tapping causes a much more extensive injury than punctures, and most of the HCN is probably lost to the air, but, since it is readily translocated, a presumable fraction could be retained. HCN release by tissue injure could also explain the induction of dryness by local compression of the bark reported by Faÿ and Jacob (1989).

The HCN released by open punctures after refoliation was siginificantly higher than at the senescent stage, with no siginificant difference between Fx 3899 and Fx 4098 at both leaf stages (Table 1). The higher HCN release after refoliation might be related to the high incidence of dryness rported to occur during this period by Compagnon, Tixier and Roujanski (1953) and Parajonthy, Gomez and Yeang (1975), though a high incidence is also reported in rainy season (Eschbach, Lacrotte and Serres, 1989).

The incidence of TPD during refoliation has been interpreted as due exclusively to the transfer of carbohydrate reserves from the stem to the growing leaves (Eschbach, Lacrotte and Serres, 1989) but it is necessary to reconcile the contradictory results related to nutrient depletion and TPD, to ascertain whether cyanogenesis and reduction of nutrient supply can act complementary. The occurrence of TPD in trees brought recently into tapping may indicate that the HCN release by traumatism would be sufficient to cause dryness.

A high HCN release in the rubber tree stem bark is presumed to be dependent on linamarin content and on linamarase activity. According to Selmar (1993) the linamarin is previously transformed into the diglucoside linustatin, which escapes from the linamarin action, during the translocation from cotyledons to leaves of young seedlings. The assumption that this is also true for a postulated transport from leaves to stems of adult plants raises many questions, concerning the relative importance of linamarin and linustatin as the substrate for HCN release, the reconversion of linustatin to linamarin, the porportion of linamarin derived from local synthesis in the stem bark and the effects of different leaf phenological stages on the bark HCNp and its hydrolysing enzyme activities, which may determine seasonal fluctuations of the HCN releasing capacity. Furthermore, a final conclusion about the role of cyanogenesis in the onset of TPD depends on critical experiments for the establishment of clear causal relationships.

In the senescent leaf stage, particularly in the Fx 4098, the latex became viscous, or even with a pasty consistency, and stopped to flow soon after tapping. In this stage the punctures in the bark induced only a small dry area with a radius of 6 mm to 15 mm around the punctures. These areas coalesced along the punctured bark strips and appeared first at the cut surface above the strips with the punctures started near the cut. In only three trees of Fx 3899 the dryness induced by the punctures was more extensive, correspondig from 11.3% to 21,4% of the cut length. In the semihardened leaf stage, the area of dry bark around the punctures was wider, but still not forming a continuous extension of dry cut. Portions of continuous dry cuts occurred in three trees of Fx 4098 and two of Fx 3899, but not immediately above the punctured strips.

According to Faÿ and Jacob (l989) the dryness induced by punctures in an experiment in Ivory Coast was reversible and the symptoms were not the same of Brown Bast. Though not having caused extensive dryness as reported by Sivakumaran and Pakianathan (l983), there was no recovery of latex exsudation by the punctured strips that showed high incidence of dryness in the present study and the anatomical study revealed the same symptoms described for Brown Bast, including the occurrence of tylosoids. Considering the arguments presented, the possibilty of a role of cyanogenesis on TPD deserves further research.

Table 1 – Semiquantitative comparison of HCN released from open punctures in trees at senescent and semihardened leaf stages. Averages of 10 color scores of Feigl Anger paper.

Clones	Leaf stage	
	Semihardened	Senescent
Fx 3899	4,32 a	1,48 b
Fx 4098	4,28 a	1,73 b

The different letters on the rows stand for significant difference between the values of the two leaf stages of each clone, by the t test at 5%. The differences of the column values are not significant.

Literature cited

COMPAGNON, P.; TIXIER, P.; ROUJANSKI, G. 1953. Contribuition a l'étude des accidents physiologiques de saignée. Archives vor der Rubercultuur in Nederlandische Indie. nº extra, maio. pp.4-69.

CONN, E. E. 1980. Cyanogenic compounds. Annual Review of Plant Physiology 3l: 433-439.

DJIKMAN, M., J. 1951. *Hevea.* Thirty years of research in the far-east. Coral Gables, University of Miami Press. 329p.

ESCHBACH, J. M.; LACROTTE, R.; SERRES, E. 1989. Conditions which favor the onset of brown bast. *In* d'Auzac, J.; Jacob, J.L. and Chrestin, H. Physiology of rubber tree latex. Boca Raton, CRC Press. pp 444-483.

FAŸ, E. de. 1988. Ethylene stimulation of *Hevea*, bark dryness and brown bast. Journal of Natural Rubber Research 3(4): 201-209.

FAŸ, E. de; HÉBANT, H. Étude histologique des écorces d'*Hevea brasiliensis* atteintes de la maladie des encoches sèches. Compte Rendu a l'Academie de Sciences de Paris. Serie D. Sciences Naturelles 3(4): 201-209.

FAŸ, E. de; JACOB, J. L. 1989. Symptomatology, histological and cytololgical aspects. *In* d'Auzac, J.; Jacob. J. L.; Chrestin, H. Physiology of rubber tree latex. Boca Raton, CRC Press. pp. 408-428.

GOHET, E. et al. 1997. Relation between clone type, latex sucrose content and the occurrence of tapping panel dryness in *Hevea brasiliensis. In* Workshop on Tappping Panel Dryness, Hainan, 1997. Proceedings. Hertford., IRRDB. pp. 21-27.

GRUHNERT, C.; BIEHL, B.; SELMAR, D. Compartimentation of cyanogenic glucosides and their degrading enzymes. Planta 195(1): 36-42.

JACOB, J. L.; PRÉVOT, J. C.; LACROTTE, R. 1994. The different types of tapping panel dryness. *In* Workshop on tapping panel dryness, Hainan, 1994. Proceedings. Hertford, IRRDB. pp2-9.

KRISHMAKUMAR, R.; CORNISH, K.; JACOB, J. 2001. Rubber biosynthesis in tapping panel dryness affected *Hevea* trees. Journal of Rubber Research 4(2): 131-139.

LIEBEREI, R. 1988. Relationship of cyanogenic capacity (HCNc) of the rubber tree *Hevea brasiliensis* to susceptibility to *Microcyclus ulei*, the agent causing South American Leaf Blight. Journal of Phytopathology 122: 54-67.

MORAES, L. A. C.; MORAES, V. H.. de F.; CASTRO, P. R. C. 2001. Aplicação de KCN e linamarina e a incompatibilidade de enxertia por translocação no clone de seringueira IPA 1 . Scientia Agricola 58(4): 717-729.

MORAES, L. A C. ; MORAES, V. H. de F.; MOREIRA, A. 2002. Efeito da cianogênese na incompatibilidade entre clones de copa de seringueira e o clone de painel IPA 1. Pesquisa Agropecuária Brasileira 37(7): 925-932

PARAJONTHY, K.; GOMEZ, J.B.; YEANG, H.Y. Physiological aspects of brown bast development. *In* International Rubber Conference, Kuala Lumpur, 1975. Proceedings, Kuala Lumpur, RRIM. pp. 181-202

SELMAR, D. 1993. Transport of cyanogenic glucosides: linustatin uptake by *Hevea* cotyledons. Planta 191: 191-198.

SIMAKUMARAN, S; PAKIANATHAN, W. 1983. Studies on tree dryness.I. A simple and rapid method of inducing dryness in *Hevea* tree. Journal of the Rubber Research Institute of Malaysia 31(2): 88-101.

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