NEW DISEASES CAUSED BY VIRUS, FUNGI AND ALSO A BACTERIUM ON RUBBER FROM BRAZIL AND THEIR IMPACT ON INTERNATIONAL QUARANTINE

Nilton T.V. Junqueira, Luadir Gasparotto, Vicente H.F. Moraes, Hercules M. Silva and Tow Ming Lim

Abstract

The rubber tree, *Hevea* spp., originated in the Amazon Basin. Attempts since the 1920s to cultivate it in several countries around the Basin failed because of the presence of the destructive leaf blight (SALB) caused by *Microcyclus ulei*, while plantings subsequently established in the East and Africa are prospering in the absence of SALB. Other leaf diseases caused by *Thanatephorus cucumeris*, *Colletotrichum gloeosporioides* and *Phylachora huberi* were well recorded. More recently, leaf diseases attributable to virus, other fungi and possibly a bacterium were detected. In the state of Amazonas, symptoms of a systemic infection caused by virus (being identified), typified by leaf mosaic, little leaf, malformation and tree stunt were confirmed in clonal nurseries and field plantings of clones MDX 15, Fx 590, F 4512 *(H. benthamiana)*, IAN 6158 and other clones of *H. benthamiana* at CNPSD. In tests, the symptoms of virus appeared more distinct at temperatures below 22°C. The new fungal leaf pathogens discovered in the nurseries and fields are *Corynespora cassiicola*, *Periconia manihoticola* (previously of minor importance), and an Ascomycete (Dothideaceae) in association with *P. huberi* yet to be identified. Also discovered were leaf symptoms in the form of tiny lesions, followed by premature leaf yellowing and fall, suggestive of a bacterial infection. These were reproduced in artificial inoculations with a culture of the bacterium repeatedly isolated from fields of clones Fx 3864, PFB 5, IAN 873 and PA 31. Relating these potentially dangerous new diseases to the well-being of the rubber industry worldwide, international quarantine measures adopted today against *Microcyclus* alone must now be reviewed. These should include additional measures for the inspection, identification and preventive pre and postquarantine treatments against the virus, bacterium and hitherto unknown fungi.

Introduction

The cultivation of rubber *(Hevea* spp.) in the Amazonia has, until recently, been unsuccessful due to the presence of *Microcyclus ulei*, the causal agent of the most destructive rubber disease known as South American Leaf Blight (SALB). In the absence of this blight, the natural rubber industry, on the other hand, prospered in the East and Africa. Since species of *Hevea* originated in the Amazonia, germplasm of both high and low resistance is found in this region (11, 12, 17). Thus, physiologic races of *M. ulei* of high virulence (4, 5, 12) as well as various other pathogens have been reported. Besides SALB, diseases of the panel and roots and other leaf diseases such as target leaf spot *(Thanatepgorus cucumeris)*, anthracnose *(Colletotrichum gloeosporioides)* and black crust *(Phyllachora huberi)* were also known. Among these leaf diseases, only *C. gloeosporioides* and *T. cucumeris T. cucumeries* have so far been recorded in the East.

New diseases caused by fungi

At the National Centre of Research on Rubber and Oil Palm (CNPSD), Manaus in the State of Amazonas, recent research revealed the occurrence of several new leaf diseases. A "round spot" caused by *Corynespora cassiicola* was observed in the nurseries, clonal gardens and field plantings (7). On the susceptible clones IAN 717 or its derivatives and Fx 3899, this fungal pathogen causes premature leaf drop, as in Malaysia (2). A "concentric spot" caused by *Periconia manihoticola*, a leaf disease considered by Albuquerque (1) to be of minor economic importance, was found on some clones in the nurseries in the State of Minas Gerais (personal

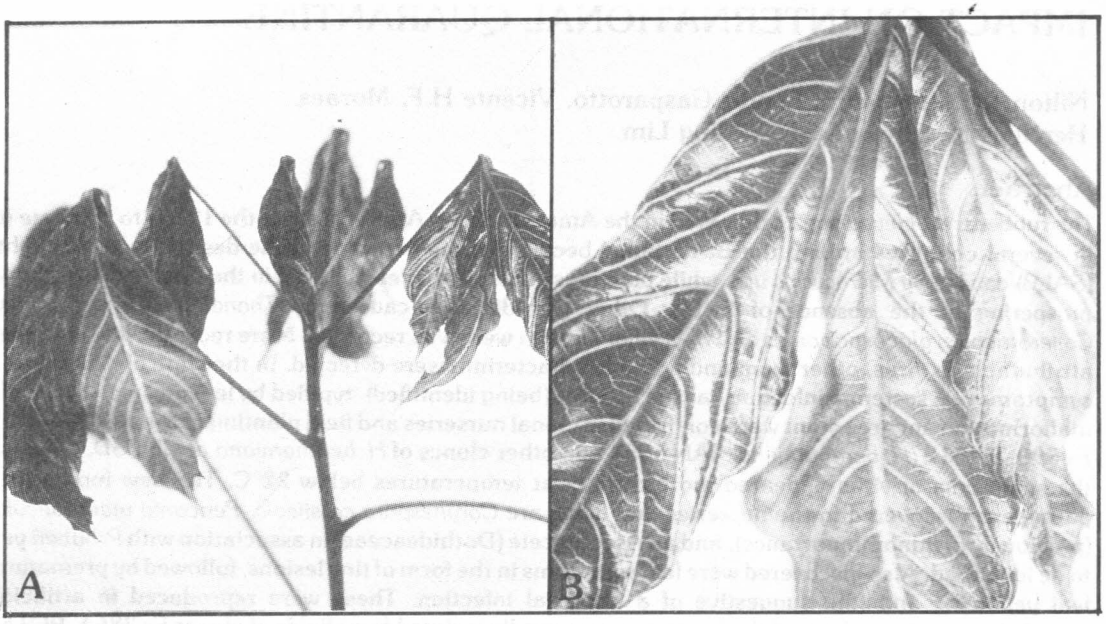

Fig. 1. Leaves showing inter-veinal chlorosis due to Carlavirus, a new disease of *Hevea* in Brazil

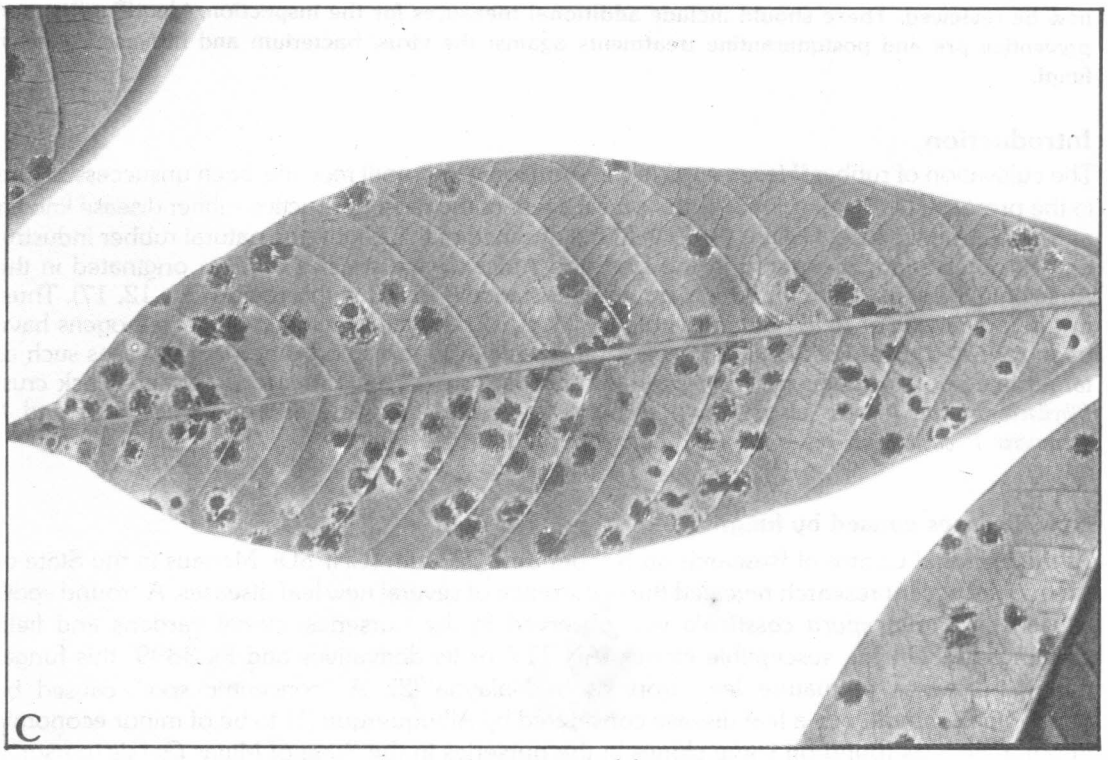

Fig. 2. 'Round spot' symptoms on *Hevea* leaves caused by *Corynespora cassiicola*

communication), a SALB-escape area. The disease was considered severe enough to warrant weekly protective spraying with mancozeb at 4.0g/l or chlorotalonil at 3.0g/l (17). In the Amazonas, *P. manihoticola* was detected in the nurseries, clonal gardens and field plantings of clones IAN 717 and Fx 3899, causing premature leaf drop. This disease has not so far been reported in rubber from the East of Africa.

Also observed recently on the undersurface of mature leaves (at the stage "D") was an Ascomycete fungus (Dothideaceae) identified by Dr. Bezerra J.L. of CEPLAC in Bahia (personal communication) as probably a *Rosenscheldiella* sp. This fungus was observed to always occur on the affected leaf in association with black crust *(P. huberi),* forming an agglomerate of dark, spherical structures, each with a mean diameter of 90-112 microns, frequently around the little stromas of black crust. Within the spherical structure, can be found one pseudothecia (means 57 x 67 microns) containing bitunicates ascus (12 x 62 microns) and ascopores (3 x 12 microns) very similar to those of *M. ulei.*

The conidial stage of this fungus has not yet been observed "in vivo". The culturing and sporulation of this fungus "in vitro" have not been very successful. However, a few conidia have been observed in cultures in the initial stage of development. The low rate of conidial production "in vitro" and the lack of knowledge of the conidial stage "in vivo" and the failure in inoculations with ascopores made the tests of pathogenicity difficult. However, because of its constant association with small stromas of *P. huberi,* this leads to the suspicion that the ascomycete only infects leaf tissues of rubber previously colonized by *P. huberi,* or that it is a case of hyperparasitism on *P. huberi* (Bezerra, 1985-CEPLAC, personal communication) which increases the damage on leaf by the formation of necrotic lesions of 3-5 mm in diameter on the undersurface of the leaves. These necrotic lesions rarely occur when leaves show only infection by *P. huberi.* This ascomycete is frequently found in fields of clone IAN 6158 which shows horizontal or incomplete resistance to *M. ulei* (11), with a mean of 6-15 lesions per leaflet, each of which measuring 3-6mm in diameter. This fungus has so far been found on clones IAN 717, Fx 3899 and IAN 873, the 3 principal clones widely planted since 1972 under the government-assisted rubber planting project known as PROBOR.

The observations of pathologists concerning the damage on rubber caused by *P. huberi* have been at variance, according to Gasparotto et. al (8) and Silva (- EMBRAPA/CNPSD, personal communication) who considered this leaf spot disease to be of minor importance. On the other hand, Lim et. al (13) thought that black crust accelerated the senescence process with a reduction in productive life of the leaflet. In view of this most recent finding of *Rosenscheldiella* sp. occurring in association with *P. huberi,* this probably helped accelerate leaf senescence or leaf-fall, mainly because of the intensive formation of necrosis on the surface of the affected leaflets.

New diseases caused by virus

The first report on the occurrence of Carlavirus on rubber tree was by Gama et al. (6), who based the analysis of leaves of stunted seedlings, with little leaves possessing inter-veinal chlorosis, that were raised from seeds collected from native trees in the Amazonia. During 1985, in a survey carried out on the CNPSD experimental fields, the presence of a virus on clones IAN 6158 {Fx 43-665 Fx 213 (F4542 x AVROS 183) x AVROS 183 Fx590 (F4542 x AVROS 368}, MDX 15 and CNS AM 7901, CNS AM 8218, CNS AM 8224 and CNS AM 8216 which are all primary clones derived from native trees in Amazonas (11). In field plantings, the virus is found to be particularly damaging on clones of F 4512, Fx 590 and CNS AM 8214.

The virus causes a systemic infection, producing characteristic symptoms such as leaf mosaic, inter-veinal chlorosis, leaf deformation, distortion and little leaf and tree stunt. These symptoms appeared more clear on leaf flushes which were produced on trees during the rainy, cooler periods (January-March). On clone IAN 6158, some affected plants showed stem

200ppm or chloranphenicol at 50ppm. The pruning of canopy of the affected young plants, followed by an application of 0.3-0.5 ml of sulphate of streptomicin at 200ppm by stem (0.8-1.5cm diameter) injection have allowed the production and maintenance of healthy and vigorous leaf flushes for 30-45 days. After this period, in most cases, the leaves begin to show the same disease symptoms again, requiring a repeat spraying. In clonal gardens, the disease can be observed on some adult plants of some clones.

This bacterium is under identification, but the process is made difficult due to natural contamination by other bacteria, among which, a *Bacillus* sp. appeared to dominate during biochemical tests. After the first sub-culturing, pathogenicity of the bacterium tended to be lost, resulting in the predominance of *Bacillus*. Also observed in pathogenicity tests was that cultures sub-cultured more than once did not prove injective anymore; various attempts to separate the pathogenic bacterium from the other species were not successful, including by C.M.I. at Kew. More recent work carried out in Brazil and West Germany suggested that this new bacterium is possibly a *Pseudomonas* sp. A principal means of its dissemination known to date is through grafting using contaminated buds. Among the clones most affected are prominently Fx 3864 and other polyploid clones, with also reports from clones PA 31, IAN 873, PFB 873, PFB 5 and Fx 985.

The isolation of a pure culture of the pathogenic bacterium from the field also proved difficult due to the occurrence of many other bacteria within the plant tissues and flow of rubber latex. These occurred also from healthy plants among which were clones PA31, Fx3864, Fx4098, IAN 717, Fx 3899, IAN 3081, Fx 3810 and IAN 873 which showed the presence of many bacteria in the latex and on the inside of the tissues of young stem and petioles. The young stem and petiole fragments analyzed were previously treated with Hg Cl_2 at 0.1% and hypochlorate of calcium at 0.5%.

Impact of new diseases on international quarantine

The great rubber industry in Asia and Africa was established based solely on *H. brasiliensis* which was derived from some 21 seedlings collected from the bank of River Tapajos in Para Brazil. Vigorous breeding and selection from this small population over the years resulted in a great increase in yield. Any further improvement in yield or secondary character conferring disease resistance, especially against *M. ulei* could only be achieved by introduction into this population, new germplasm materials from the Amazon Basin. With this in mind, several collections of *Hevea* plants have been made in Brazil for distribution to rubber producing countries in the East and Africa.

The rubber germplasm materials, periodically sent from Brazil to natural rubber producing countries outside it, are subjected normally to strict pre, intermediate and postquarantine and inspection treatments for avoiding the accidental introduction with them of *M. ulei*.

According to Paranjothy et al. (15), plant materials composed of seeds and budwoods are first treated with fungicides and insecticides in Brazil before being sent to Europe (seeds) or a French Island in the Atlantic Ocean (budwood) for intermediate quarantine following inspection. The seeds are normally re-treated and inspected before being sent to their final destination in Malaysia or Africa. Upon receipt in these countries, the seeds are again subjected to final post-inspection, and treatment procedures have proved effective so far in preventing the entry of *M. ulei* from Brazil. However, such phytosanitary treatment scheme has not provided the required treatment against pathogens other than *M. ulei*, which may be present within or outside the seeds, eg. virus and bacterium. For budwood, this is normally multiplied in the intermediate quarantine station for a period before new wood is cut and sent to their final destinations. Here again, the need for inspection and treatment against the newly discovered virus within the budwood must now be considered for the determination or indexing of viruses on materials during quarantine.

The necessary thorough inspection and treatment are usually made in a glass-house. According to Batista (3), the procedure includes careful observation of materials received through the use of virus indicator plants (3, 9, 14), serological tests (3, 14) and by the direct examination for any viral particles under an electron microscope of preparations made from "leaf dip" and ultra-thin sections.

In the case of rubber, the seeds suspected to be infected by virus must be germinated and the seedlings grown under a lower than normal air temperature between 18-20°C until the production of 2 or more leaf flushings.

The leaf flushings produced and developed under this lower incubation temperature, if containing virus, will certainly show more visible or clearer symptoms, and thus facilitate tests for virus detection or indexing (serology, leaf dip, etc.). These tests must also be made with young buddings made of budwood received suspected to be infected by virus. Any of the seed or budwood materials found to harbour a virus must quickly be confiscated and destroyed. In cases in which the materials are of high importance and difficult to acquire, the plants must be maintained under constant quarantine inspection while undergoing elimination of its virus and then multiplying, if possible. Until now, there is no known treatment procedure recommended or used for virus elimination from budwood or seeds introduced from Brazil to rubber growing countries in the East or Africa. The use of meristem culture represents another possible alternative method for virus elimination, if this technique proves to be possible for use on rubber cultivation.

For the detection of bacteria, the seed or budwood materials must be germinated or multiplied and maintained in growth under the ideal temperature and humidity conditions to facilitate the production of rapid and clear bacterial symptoms on the leaf. To confirm the presence of a pathogenic bacterium on such leaves, isolation and identification may be required.

Conclusion

Until now, rubber producing countries outside the American tropics recognize only SALB as a threatening disease and consequently enforce a strict inspection and treatment scheme against this fungus for *Hevea* materials introduced from the SALB-infected countries.

This report on the discovery of additional leaf-defoliating fungi, viral and bacterial diseases in Brazil means that similar strict treatment measures appropriate to each of these diverse pathogen types must be considered. Further studies in this correction, along with those on the new diseases themselves, will hopefully provide a phytosanitary treatment scheme as effective as those used so far against SALB.

Session discussion

Surachmat: In Indonesia, we have a serious problem with *Corynespora cassiicola*, which was first reported to occur in Central Java early this year. Some rubber plantations are now suffering from the disease. I would like to know whether you have an experience in controlling the disease.

Answer: This disease is new in the sense that its outbreak is incited by the planting of certain clones and prevalence of the right weather, eg. a dry period, followed by rain. For control, RRIM's experience I believe, is spraying with fungicides, eg. Benlate.

C.H. Teoh (Comment): With reference to *Corynespora* leaf disease in Indonesia, I observed a serious outbreak in the Banten district of Java in 1982. This occurred after a prolonged drought. There was very clear clonal response to the fungus. Cultivars PPN 2058 and 2444 were highly susceptible while neighbouring clones GTI, RRIM 600 and LCB 1320 were unaffected. I have in fact sent RRIM recommendations on treatment to the estate concerned. Perhaps the participant from Indonesia could contact PTP XI to find the effectiveness of the treatment.

N.A. Samad: 1) Is the virus mechanically transmitted?

2) How wide is the host range of the virus?

Answers: 1) Preliminary attempts at mechanical transmission so far gave negative results, suggesting grafting and infection transmissions as observed to be the main means of dissemination of the virus.

2) Still to be defined but tests with anti-serum provided by CIAT, Colombia, tentatively placed the new "rubber tree virus" in other "cassava mosaic group".

N. Ganapathi: What is the species of Botryodiplodia?

Answer: We found if to be the commonly occurring species, *B. theobromae,* and maybe Dr. Wheeler can comment on this.

B.E.J. Wheeler: Yes, I think it is *B. theobromae.*

K.G. Singh: Two questions 1). What is the practical and economic importance of virus on *Hevea?*

2) In view of the fact that the virus in observed to be seedborne, should further importation of new rubber germplasm based principally on seeds be stopped for the time being or until treatments against it are worked on.

Answers: 1) Being so recently discovered, and for the first time on rubber anywhere, in fact, the importance of this virus has not yet been assessed. In the Amazonia, at least, we are worried in that our all-round leaf-disease resistant clone, IAN 6158 is highly susceptible to this new pathogens.

2) Many viruses are known to be seedborne in other crops, but these do not stop importation of seeds for crop improvement, while highlighting the occurrence of this non-fungal, dangerous new pathogen. We hope to stimulate awareness on the quarantine people into working out the appropriate phytosanitary treatments so that rubber seeds can still be imported from South America with safety.

O. S. Dharmaputra: 1) Although *Ganoderma* spp. are not specific pathogens on rubber, could you tell me the number of species of *Ganoderma* that attacks rubber trees all over the world?

2) In North Sumatra, Indonesia, some oil palm plantations are converted into rubber plantations because of *Ganoderma* spp. infestations. What is your opinion?

Answer: *Ganoderma phillipi* on *Hevea* is host-specific and so far, only this species is recorded as the main pathogen on rubber.

2) The *Ganoderma* sp. on oil palm is different from that of rubber so there is no fear of virus infection by this fungus during the conversion of oil palm to rubber.

References

1. Albuquerque, F.C. (1980). Doencas da seringueira. *In* Curso de Especializacao em Heveicultura **7** Belem, SUDHEVEA/FCAP, 30pp.
2. Anonymous (1975). Corynespora leaf spot. *Plant Bull. Rubber Res. Inst. Malaya, Kuala Lumpur* **139:** 84-86.
3. Batista, M.F. (1985). Quarentena de material vegetal. *Fitopatologia Brasileira,* **10:** 207.
4. Brasil, S. (1975). Plano Nacional da Borracha, pesquisas fitopatologicas. *In* Pesquisas e experimentacao realizadas com a seringueira, Rio de Janeiro. Pp. 36-107. (Anexo II).
5. Chee, K.H. (1984). Development on the study of physiologic races of *Microcyclus ulei* and clonal susceptibility. Report. S.I., S. ed., 9p.
6. Gama, M.I.C., E.W. Kitajima, A.C. Avila and M.T. Lin (1983). Um carlavirus em seringueira *(Hevea brasiliensis). Fitopatologia Brasileira,* **8:** 621.
7. Gasparotto, L., E.B.C. Pereira and T.M. Lim (1985). Ocorrencia de *Corynespora cassiicola* em seringueira no Estado do Amazonas. EMBRAPA-CNPSD. *Comunicado Tecnico* **39:** 2.

8. Gasparotto, L., D.R. Trindade and H.M. Silva (1984). Doencas da seringueira. EMBRAPA-CNPSD. *Circular Tecnica.* **4**:66.
9. Gibbs, A and B. Harrison (1976). The Principles of Plants Virology. New York, Halsted. 292pp.
10. Goncalves, P.S., J.R. Paiva and R.A. Souva (1983). Retrospectiva e atualidade do melhoramento genetico da seringueira *(Hevea spp.)* no Brasil e em paises asiaticos. *EMBRAPA-CNPSD. Documentos* **2:** 69.
11. Junqueira, N.T.V. (1985). Variabilidade fisiologica do *Microcyclus ulei* (P. Henn.) v. Arx. Vicosa, U.F.V. Tese Doutorado. 135pp.
12. Junqueira, N.T.V., L. Gasparotto, H.M. Silva and T.M. Lim (1985). Complexo de doencas foliares em seringais de cultivo no Estado do Amazons. *Fitopatologia Brasileira,* **10:** 221.
13. Lim, T.M., L. Gasparotto and H.M. Silva (1984). Crosta negra – um problema de queda de folha apos o reenfolhamento da seringueira. *In* Seminario Nacional Da Seringueira, **4,** Salvador. Resumos. Rio de Janeiro., SUDHEVEA. P.7.
14. Noordam, D. (1973). Identification of plant viruses. Methods experiments. Wageningen. 207pp.
15. Paranjothy K., T.M. Lim, R. Othman, I. Hashim, A. Aziz and L.S. Kwai (1981). Transfer of *Hevea* seed germplasm. P. 17 *in* Seminar of Transfer of *Hevea* Seed Germplasm from South America, Kuala Lumpur, Malaysia.
16. Pinheiro, E. and V.E. Libonati (1971). O emprego da *Hevea pauciflora* M.A. como fonte de resistencia ao mal das folhas. *Polimeros* **1(1):** 31-40.
17. Zambolim, L., N.T.V. Junqueira and G.M. Chaves (1985). Doencas que incidem sobre a seringueira em Minas Gerais. *Informe Agropecuario* **11(121):** 36-42.