Report on the second *Phytomonas* workshop

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K. VICKERMAN[1], M. DOLLET[2]

INTRODUCTION

In 1987 a workshop was held in Cayenne to discuss work on the flagellate parasites of plants and their relationship to plant disease (1). These flagellates belong to a family -the Trypanosomatidae- which includes the aetiological agents of sleeping sickness, Chagas' disease and leishmaniasis. The trypanosomatids of plants are relatively poorly known in comparison and have tentatively been assigned to a single genus -*Phytomonas*-. Most described *Phytomonas* spp. live in the latex vessels of their hosts and appear to cause no harm. The discovery of intraphloemic flagellates associated with wilt diseases first in coffee and then in oil and coconut palms, however, raised interest in these parasites among a variety of research workers in different countries.

The second workshop was organized by IRHO/CIRAD (France) and CENIPALMA (Colombia) in North Colombia, with financial help of the DG XII of EEC. It paid particular attention ot the *Phytomonas*-associated diseases marchitez of oil palm and hartrot of coconut. It also demonstrated increasing awareness of the widespread occurrence of trypanosomatids in plants, especially in their fruits, and raised the questions to whether the damaging effects of these parasites may threaten other plant-based industries.

The following report attempts to summarise the major advances in our knowledge of *Phytomonas* and our understanding of its relationship to disease as discussed at the second workshop.

❑ Cultivation of parasites *in vitro*

An important development since 1987 has been the isolation in axenic culture of *Phytomonas* from coconut and oil palms, where the flagellates inhabit the mature sieve tubes of the phloem tissue. After several passages of isolates in modified Grace's medium with insect cells, the CIRAD team in Montpellier has been able to dispense with the feeder cells; twelve isolates from hartrot-affected coconut palms and four from marchitez-diseased oil palms have been obtained.

Although parasitization of the phloem is associated with plant disease, the fulfilment of Koch's postulates has not yet been achieved. Difficulties in injecting material into the sieve tubes have so far proved insuperable. A further problem may lie in the lack of infectivity of the culture forms.

It is at present, presumed however, that the multiplicative *in vitro* flagellates correspond to those observed in the plant. The non-pathogenic *Phytomonas* of lactiferous plants from all continents grow well in Grace's medium with 10 % foetal calf serum without feeder cells. Changes in morphology of the culture promastigotes (e.g. decrease in length and in spiralling of the body) have been observed (M. Dollet). SDM79 semi-defined medium, developed for the procyclic (vector gut) forms of *Trypanosoma brucei*, has also been utilised for bulk growth of latex *Phytomonas* by Sanchez-Moreno and associates. A complex medium (glucose, yeast extract, peptone, hamin, meat infusion and salts) has proved satisfactory for isolating trypanosomatids from a variety of fruits and seeds by A.M. Lorenzi and colleagues.

The growth *in vitro* of *Phytomonas* spp. has enabled comparison of isolates for purposes of identification, metabolic studies on the parasites and clarification of the epidemiology of the parasitoses.

❑ Taxonomic identification

• The genus

The morphology of the parasite and the number of hosts necessary for it to complete its life cycle are considered of paramount importance for the classification of trypanosomatids. Thus *Phytomonas* is currently defined as :

– "A genus of trypanosomatid flagellates whose members have a two-host life cycle involving a plant and a heteropteran hemipteran host, and whose morphology always exhibits the promastigote form".

It is possible that from the plant pathologist's viewpoint, three different groups of trypanosomatids have been referred to the genus *Phytomonas* :

– parasites living in the latex of laticiferous plants. With the important exception of *P. francai* of manioc, these species are not associated with a wilt of the host plant.

– parasites living in the phloem sieve tubes of non-laticiferous plants These species are pathogenic.

– parasites retricted to fruits and unable to live in other parts of the plant. Damage to the host is localized to the fruit.

At the meeting M.M.G. Teixeira and E.P. Camargo reported that DNA fingerprinting of ribosomal genes (18, 24S Pi and B subunits) has shown that members of the genus *Phytomonas* display restriction enzyme digest patterns for Pvu II and Eco RI enzymes which distinguish them from *Crithidia*, *Herpetomonas* and *Leptomonas* -the genera most frequently confused with *Phytomonas*-. They suggest that the utilization of synthetic oligonucleotide probes will eventually make possible the identification of flagellates *in situ* without previous culturing. S. Marche and co-workers, also using restriction analysis of 16SrDNA PCR fragments, have found that the pathogenic phloem-dwelling *Phytomonas* show a profile quite distinct from the nonpathogenic isolates

(1) Department of Zoology, the University, Glasgow G12 8QQ, Scotland

(2) Division Virologie CIRAD/IRHO - Laboratoire de Phytovirologie des Regions Chaudes - BP 5035, 34032 Montpellier cedex 1, France

of lactiferous plants. Profiles of the various isolates from coconut and oil palms are identical.

As demonstrated by Conchon and associates trypanosomatids isolated from fruits may belong to different genera (2). M.L. Almeida and co-workers reported that isolates from tomatoes showed variation in their arginase and citrulline hydrolase activities suggesting that they were not all *Phytomonas* spp. It is possible that the pericarp provides an ideal culture medium for a variety of trypanosomatids: Catarino and co-workers have shown that inhibitory substances in other tissues of the host plant may prevent spread of the parasite within the plant.

The conclusion to date is that there is a need for more satisfactory generic identification of plant trypanosomatids and definition of the genus *Phytomonas* in particular.

- **Species and clones**

The designation of species within the genus *Phytomonas* is at present extremely cumbersome and confusing and some sort of nomenclature to enable easy references in relation to disease is needed. A tendency has grown up to refer to a particular isolate initially using its host's name (e.g. *Phytomonas* from *Euphorbia characias*).

Guerrini and co-workers presented evidence from isoenzyme variability among 31 stocks that *Phytomonas* zymodemes (populations characterised by their isoenzyme profile) behave as natural clones; they maintain that the clone should be considered the natural taxonomic unit.

S. Marche and co-workers produced evidence from polyclonal and monoclonal antibody studies and SDS-PAGE electrophoresis of total and surface proteins as well as restriction analysis of ribosomal RNA genes, that the pathogenic stocks from diseased palms are closely related, differ susbtantially from the latex parasites and could justifiably be united under a single species.

The fast-sedimenting kinetoplast (mitochondrial) DNA of *Phytomonas*, as in other trypanosomatids, consists of a large network of interlocked minicircles with relatively few maxicircles. Minicircle size and the electrophoretic analysis of minicircles digested with restriction endonucleases can be used to distinguish isolates. Ahomadegbe and colleagues reported how minicircles from different isolates have been used as probes in cros hybridization experiments; a high sequence homology has been found between minicircles of *Phytomonas* from coconut trees afflicted with hartrot, suggesting close relationship of the isolates. Interestingly, there appears to be no sequence homology between minicircles of *Phytomonas* (*Euphorbia* and palm isolates) and the minicircles of other trypanosomatids.

❑ Pathogenicity

The association of parasitization of the phloem sieve tubes by *Phytomonas* with fatal wilt diseases may be due to blockage of transport of photoassimilates. To date, pathogenicity of phloem *Phytomonas* has been recorded from plants of the Arecaceae, Rubiaceae and Zingiberaceae: parasitization of the latex vessels in Asclepiadiaceae, Euphorbiaceae and Apocynaceae does not normally appear to result in disease.

The native South American oil palm (*Elaeis oleifera*) appears to be resistant to marchitez, in that symptoms of the disease have not been observed in this tree, though J.F. Llosa reported the presence of insects of the genus *Lincus*, genus of the vectors of marchitez, on native oil palms. Hybrids between *Elaeis guineensis* and *E. oleifera* are apparently susceptible to infection.

The pathogenicity of trypanosomatids from fruits is controversial. Damage appears to be local and intercurrent fungal or bacterial infections may be in part responsible. The fruit pericarp or seed aril may serve as a culture medium for the flagellates.

Phytomonas from *Euphorbia characias* in culture has been shown by Sanchez-Moreno and co-workers to secrete the cellulose-degrading enzymes amylase, invertase and carboxymethylcellulase, also the pectin-degrading enzymes polygalacturonase and oligo-D-galactosiduronate lyase. These enzymes may assist the flagellates in finding their way into the latex vessels of the host plant or in releasing the major substrates of energy metabolism (glucose, fructose, mannose) from higher polysaccharides. Enzymes produced by the pathogenic phloem parasites have yet to be studied.

❑ Transmission

Five species of *Lincus* have been implicated in the transmission of marchitez. *Lincus tumidifrons* has been reared in the laboratory from egg to egg by F.A. Alvarez and H Calbache, and has a 5 1/2 month life cycle. *Ochlerus* sp. by Dollet and colleagues, with a 5 month cycle. In the laboratory, *Lincus* has the disadvantage that it is gregarious; the five larval instars congregate together under the influence of a pheromone. J.F. Llosa has studied the ecology of *Lincus* spp. on a variety of palms: eggs are found on fertile plants only, nymphs on the flowers and fruit of *Elaeis*, while the adults are found on the leaves.

Lincus tumidifrons and *L lethifer* collected from marchitez-infected oil palms, transmitted the infection to coconut palms (Dollet and co-workers).

The developmental cycle of the phloem-restricted parasites in the bug has not been studied, but infection of the gut and salivary glands probably occurs.

Invasion of the salivary glands obviously implies cyclical transmission, probably with adaptive metabolic change during development in the vector. It is possible that *in vitro* manipulation of the culture medium may produce these different stages. Thus Sanchez-Moreno and colleagues have found that *Phytomonas* from *Euphorbia characias* produces promastigotes in SDM79 medium and these excrete ethanol, pyruvate, glycine and glycerol: in Grace's medium, however, large numbers of amastigotes are produced and these excrete succinate, but not pyruvate.

It is possible, however, that mechanical (contaminative) transmission of *Phytomonas* via the mouthparts of the vector also occurs, at least for some clones. The discovery that the kinetoplast minicircles of *Phytomonas* from *Euphorbia pinea* were homogeneous with respect to their base sequence (while minicircles of all other isolates examined were heterogeneous) by Ahomadegbe and colleagues invites comparison with the mechanically transmitted trypanosomes (*Trypanosoma evansi, T. equiperdum*) which also have homogeneous minicircles. The striking parallel between the energy metabolism of bloodstream *T brucei, T. evansi* and *E equiperdum* on the one hand and *Phytomonas* from *E. characias* (culture forms presumed to correspond to latex forms) invites further comparison.

The demonstration of specific assocations between particular clones and their host plants has now ruled out that *Euphorbia* weeds on palm plantations may serve as reservoir hosts for palm *Phytomonas*. Whether wild palms (e.g. *Roystonea, Bentinkia*) which harbour *Phytomonas* are reservoir hosts is unknown.

❑ Control

The most practical method of preventing spread of marchitez and hartrot is probably by attacking the insect vector with insecticides, but long term use is of course not desirable. A hymenopteran, *Hexacladia linci*, parasitizing *Lincus* spp. may prove useful in biological control of the vector (J.F. Llosa).

Speculation on possible treatment of infected palms centres around the inhibition of specific biochemical pathways in *Phytomonas*, insofar as these have been investigated for

culture forms. Sanchez-Moreno and colleagues have found that the culture forms of *Phytomonas* from *Euphorbia characias* have an energy metabolism similar to the bloodstream form *Trypanosoma brucei*, that is carbohydrates are catabolised by aerobic glycolysis. The Embden Meyerhof pathway enzymes are largely located in a special organelle, the glycosome, characteristic of kinetoplastid protozoa. Glucose is degraded to pyruvate and acetate principally, though some further fermentation to glycerol, succinate and ethanol occurs. The mitochondrion is inactive in that it is incapable of oxidising succinate, 2-oxoglutarate, pyruvate, malate or proline but has a high capacity to oxidise glycerol-3-phosphate. produced in the glycosome, utilising oxygen. This last pathway is susceptible to inhibition by salicyl hydroxamic acid and so hydroxamic acids may prove of value in eradicating the parasite from plants.

Sterol biosynthesis was suggested by J. Goad as another target for drug therapy. The sterol metabolism of *Phytomonas* resembles that of fungi rather than that of the host plant. Of the available antifungal compounds, triazole derivatives were not appropriate for phloem parasites, but morphalline may prove useful in treating palms to isolate infected trees.

CONCLUSION

Progress registered since the first *Phytomonas* workshop in Cayenne is significant. Research workers have now in their hands all the elements to conduct basic and applied research programmes. They have at their disposal cultures of several isolates of *Phytomonas* including the most important ones the phloem-restricted *Phytomonas* associated with hartrot- and marchitez diseases. They know the vectors and they have access to different techniques of characterization like electrophoresis of isoenzymes or restriction profiles of kDNA or DNA fingerprinting of rDNA, etc.

The field excursion which formed part of the workshop was particularly useful to the laboratory-based participants. By venturing into the oil palm plantations and palm oil extraction factory they were able to obtain a clearer picture of the relationship of marchitez to the palm oil industry. They were able to see, in addition the problems of working under hot and humid conditions, of detecting diseased trees and of identifying the ideal stage for sampling for *Phytomonas*. They were also shown how to dissect the infected palm, leaf by leaf, to find the vector.

From the knowledge of *Phytomonas* and its vectors acquired over the last five years, we are confident that new vistas will shortly be opened up for integrated control of the diseases caused by these organisms. At the same time research on plant trypanosomatids will contribute much useful information to protistology, molecular and cell biology, and greatly enhance understanding of the host parasite relationship.

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Retour au menu

Abstracts of the communications presented during the second *Phytomas* workshop

IN VITRO CULTURE

DETECTION, ISOLATION AND GROWTH OF *PHYTOMONAS* IN A NEW LIQUID COMPLEX MEDIUM

ALMEIDA MARIS LORENZONI ; JANKEVICIUS I. ; CAVAZZANA M. ; JANKEVICIUS J.V.; CATARINOS L.

University Londrina, Brésil

Phytomonas were detected in several plants such as Euphorbiaceae, tomatoes, common bean, soybean, guava, maize, urucum and promegranate in southern Brazil. In this work, twenty strains isolated from plants were tested in a new liquid medium containing glucose yeast extract, KCl, NaCl, peptone, hemin and meat infusion, pH 7.0, autoclaved for 20 min./120°C (GYPMI). The cells grew at pH 5.5-9 0, preferably at pH 6.5 and temperature range of 20-34°C, except for 4 clones that did not grow at 34°C, strain 274 Tc (from tomatoes) was the unique to grow at 37°C. The optimum temperature was around 28°C. Generation time was determined for all strains as 12-16 h. Growth rate in different media (biphasic medium, FYTS, MDR and GYPMI) was evaluated by cells production and in phosphate buffered GYPMI medium all strains grew up to five times better than in other media.

IN VITRO CULTIVATION OF PHYTOMONAS FROM LATEX AND PHLOEM-RESTRICTED *PHYTOMONAS*

GARGANI D. ; MENARA A. ; SEGUR C. ; DOLLET M.

IRHO, LPRC/CIRAD, Montpellier, France

The *in vitro* culture of *Phytomonas* was the main obstacle to their study. In and after 1982 we were able to grow *Phytomonas* from laticiferous plants : *Euphorbia pinea, E. hirta, E. hyssopifolia, Mandevilla scabra,* from different areas of the world. But phloem-restricted *Phytomonas* remained difficult to grow and it was not until 1986 we obtained the first primoculture of *Phytomonas* associated with hartrot disease of coconut, in a medium containing insect cells. After several passages axenic cultures have been obtained. Today we are growing 9 isolates of hartrot of French Guiana, 1 from Brazil and 2 from Venezuela. *Phytomonas* associated with marchitez disease of oil palm were cultured for the first time in 1989. Four isolates from Colombia are now being cultured. Four of these phloem-restricted isolates have been cloned (37 clones).

Recently *Phytomonas* -apparently phloem-restricted- were discovered in Grenada by P. Hunt in *Alpinia purpurata*. Two isolates were obtained in insect cell cultures in the laboratory.

AISLAMIENTO DE *PHYTOMONAS* SP. EN EXPLOTACIONES AGRARIAS DEL SUR DE ESPANA

MONESTIER A. ; SANCHEZ-MORENO M. ; FERNANDEZ-BECERRA M.C.; OSUNA A.

University of Grenada, Spain

En un estudio llevado a cabo en las explotaciones hortofrutícolas de la costa Mediterranea española en las que se han muestreado plantaciones de tomates, melones, pepinos, pimientos, chirimollas y mangos, se ha aislado y cultivado una especie de *Phytomonas* obtenida de plantaciones de tomates ; el cultivo se mantiene desde hace cinco meses en medio SDM-79. La observación bajo microscopía óptica "*in vivo*" y bajo tinción, así como la efectuada bajo microscopia electronica nos confirman : una morfología de los protozoos trypanosomatidos cultivados como formas flageladas de *Phytomonas* pudiendo considerar este aislamiento como el primero que se realiza en explotaciones agrarias españolas y en la región Mediterránea.

INTERACTION OF CULTURE FORMS OF *PHYTOMONAS* WITH PLANT CELL EXTRACTS

CATARINO L.M.G.M. ; NOGUEIRA D. ; JANKEVICIUS J.V. ; JANKEVICIUS I.

University of Londrina, Brazil

In order to understand why some strains of *Phytomonas* of fruits and seeds are not demonstrable in other parts of the plants, we tested the action of plant extracts on culture forms of the parasite The culture forms were put together in equal parts of crude extracts of different parts of the plants and microscopically observed. With tomatoes and *Phytomonas serpens*, we observed that except for mature tomato pericarp, all other extracts immediatelly paralised the promastigote form, indicating harmful action of the parasite. On the other hand, the pericarp extract alone did not maintain the cultures. With few exceptions, the results are essentialy the same with extracts of corn, soybean and various edible fruits and other strains of *Phytomonas*. This action of plant extracts can be used as a criterion to exclude strains of *Phytomonas* as a parasite of a specific plant or part of it.

Retour au menu

ACTION OF RABBIT NORMAL SERA ON CULTURE FORM OF *PHYTOMONAS*

CARRARA F.E. ; JANKEVICIUS J.V. ; JANKEVICIUS I.

University of Londrina, Brazil

The behaviour of protozoa of the genus *Phytomonas*, defined as plant parasites, has not been carefully studied in animals. We investigated the action of sera from normal rabbits against culture forms of various strains from tomatoes, corn and "urucum" (*Bixa orellana*). The culture forms of *Phytomonas* were placed in presence of fresh and inactivated sera and observed microscopically. In presence of inactivated sera, it was observed an intense agglutination and normal motility, with somatic agglutination pattern. All strains showed a strong lysis in presence of fresh serum or inactivated serum plus complement. These results suggest the existence of natural antibodies in normal rabbits that should interact with culture forms of *Phytomonas* that in the presence of complement lead to a total lysis, impairing the development of *Phytomonas* in rabbits.

ESTUDIO DEL DESARROLLO DE *PHYTOMONAS* EN DIFERENTES MEDIOS DE CULTIVO

FERNANDEZ-BECERRA M.C. ; MONESTIER A. ; OSUNA A. ; SANCHEZ MORENO M.

University of Grenada, Spain

En el presente trabajo hemos estudiado la evolución de *Phytomonas* aisladas de *Euphorbia characias* (Dollet, 1982) en diversos medios de cultivo. Al comparar el medio SDM-79, LIT, MTL y Grace se observa como el mayor rendimiento de formas flageladas se logra en el medio SDM-79 con el que se obtiene 5.10^7 flagelados/ml a los seis días de cultivo , si bien el medio LIT y MTL muestran una curva de crecimiento similar, las densidades de protozoos/ml obtenidos son ligeramente inferiores al medio SDM-79. En medio Grace se observaron en un 70 % formas "like" amastigotas y esferomastigotas con las formas obtenidas en los diferentes medios se han llevada estudios a microscopía electrónica al objeto de poder caracterizar las diferentes formas en medios de cultivo.

DETERMINACION DE CATABOLITOS FINALES DE *PHYTOMONAS* SP. AISLADAS DE *E.CHARACIAS*

SANCHEZ-MORENO M. ; MONESTIER A. ; FERNANDEZ-BECERRA M.C. ; LOPEZ-GAY J. ; OPPERDOES F. ; OSUNA A.

University of Grenada, Spain

Se ha llevado a cabo un exhaustivo estudio metabolico cualitativo por NMR y cuantitativo (por métodos enzimáticos) de los metabolitos excretados al medio de cultivo por las diferentes formas del parásito. Por un lado formas promastigotes cultivadas *"in vitro"* en medio SDM-79 excretan mayoritariamente acetato, etanol, piruvato, glicina y glicerol, mientras que por otro lado las formas amastigotas obtenidas en medio Grace modificado presentan como metabolitos mayoritarios etanol y glicina y cantidades inferiores de acetato y piruvato. Sin embargo, estas formas "like" amastigotas no excretan piruvato pero sí succinato. La ausencia y presencia de estos dos últimos metabolitos se ha visto que está relacionado con el proceso de transformación de las formas promastigotes del parásito a formas amastigotas.

EPIDEMIOLOGY AND CONTROL

THE STEROLS OF *PHYTOMONAS* SPECIES

GOAD L.J.

University of Liverpool, United Kingdom

The production of sterols by *Phytomonas* species has been examined to determine if the sterol biosynthetic pathway is a possible target for anti-protozoal drugs. The results of sterol analysis of several *Phytomonas* species and the effects of anti-fungal sterol biosynthesis inhibiting compounds on growth and the sterol compositions of the protozoa will be described

SITUACION ACTUAL DE LA "MARCHITEZ SORPRESIVA" DE LA PALMA ACEITERA (*ELAEIS GUINEENSIS* JACQ.) Y "HARTROT" DEL COCOTERO (*COCOS NUCIFERA* L.) EN VENEZUELA

DIAZ QUINTANA A.

FONAIAP, Maturin, Venezuela

Se presenta la distribución e incidencia de la enfermedad en las áreas productoras de cocotero y palma aceitera de Venezuela en los últimos años Asimismo se discuten aspectos de la presencia de la misma en las jovenes plantaciones de palma aceitera. Se reporta la presencia de insectos asociados

a plantaciones afectadas por la enfermedad, principalmentede la familia Pentatomidae, *Lincus tumidifrons*, *Proxys victor*, *Oncopeltus* sp. y *Berecynthus* sp., con los cuales se realizan trabajos de cría y las pruebas de transmisión en jaulas. Se mencionan las principales malezas asociadas a las plantaciones afectadas por "hartrot" en el estado Sucre, haciendo énfasis en las malezas laticíferas, que son reservorios de flagelados. Ademas, se informa sobre el comportamiento de una parcela de variedades e híbridos de cocotero en donde se observa alta variabilidad del material genético con respecto a la enfermedad.

ESTUDIOS BIOLOGICOS DE *LINCUS TUMIDIFRONS* (HEMIPTERA : PENTATOMIDAE) VECTOR DE LA MARCHITEZ SORPRESIVA

ALVANIL ALVAREZ F. ;
CALVACHE GUERRERO H.

CENIPALMA, Colombia

Las chinches del género *Lincus* han sido registradas como vectores del protozoario *Phytomona*, agente causal de la marchitez sorpresiva. En Colombia se han identificado las especies : *L.tumidifrons* y *L.stylinger* Breddin. *L.tumidifrons* se ha encontrado en los Llanos Orientales y en el Zulia (Norte de Santander) en palmas de aceite afectadas con marchitez sorpresiva.

El insecto, en los estados de huevo, ninfa y adulto, se localiza generalmente en las bases peciolares de los hojas 12 a 33 de palmas cuyas edades oscilan entre los 5 y 20 años y se encuentran asociadas con una hormiga del género *Camponotus* sp. La relación de sexos es 1:1, lo cual es más o menos constante en los diferentes muestreos.

Bajo condiciones controladas laboratorio y de campo, se han probado varios métodos de cria del insecto. Las mejores condiciones para su desarrollo son : temperatura 23°centígrados y humedad dentro del rango 70-80 %, utilizando dietas alimenticias artificiales y naturales. Hasta el momento se ha logrado establecer el número promedio de huevos por postura ; la duración del periodo de incubacion es de 7 a 9 dias, y del primer instar ninfal de 8 a 9 dias. La longevidad a nivel de laboratorio fue de aproximadamente dos meses.

Se ha logrado establecer un promedio de 7 huevos por postura, un periódo de incubación promedio de 7.86 y un período ninfal, a través de 5 instares, de 142 días. La longevidad en el laboratorio ha sido de 2 meses aproximadamente.

TRIALS OF REARING VECTORS OF PHLOEM-RESTRICTED PHYTOMONASEXPERIMENTAL TRANSMISSIONS, AND REARING OF *OCHLERUS* SP. (PENTATOMIDAE)

DOLLET M. ; DAMBIER D. ; GARGANI D. ; SEGUR C.

IRHO, LPRC/CIRAD, Montpellier, France

In order to prove the pathogenicity of cultured *Phytomonas*, we needed to rear healthy vectors. We tried to rear two species of Pentatomids : *Lincus* spp., vectors of hartrot or marchitez in Guiana, Brazil, Venezuela, Colombia and Peru, and *Ochlerus* sp., a presumed vector of hartrot in Para state, Brazil. During these trials we were able to transmit *Phytomonas* to coconut through bugs collected from marchitez infected oil palms. This demonstrated that 1°) the vectors are able to infect both coconut and oil palm, 2°) one isolate of *Phytomonas* can parasitize both coconut and oil palm.

We reared *Ochlerus* from adult to adult through the eggs and 5 larval stages in a cycle of about 140 days.

ECOPHYSIOLOGY AND POPULATION DYNAMICS OF THE GENUS *LINCUS* (HEMIPTERA; PENTATOMIDAE; DISCOCEPHALINAE), PRESUMED INSECT VECTOR OF "MARCHITEZ SORPRESIVA", IN THE PERUVIAN AMAZONIA

JULIO F. LLOSA F.

Lima, Peru

Since 1988, two species of Amazonic *Lincus* have been studied in Peru, on both, different native palm species and introduced African oil palms, all of the palm group Arecoidae. The number of bugs found is always higher on fertile palms than on sterile ones. On *Astrocaryum* spp. adults are more abundant than nymphs, the former being found on leaf sheaths and the latter on reproductive structures. On *Elaeis* spp., the opposite happens and nymphs are found in higher numbers than adults, being all grouped on the reproductive structures. Eggs are only found on fertile plants. Sex ratio in *L spurcus* is 1:1, while in *L.malevolus* adults have been found to be parasited by an Hymenoptera Encyrtidae. *Hexacladia Linci*, gregarinus endoparasite which carries out its whole development in its living host. The effects of this parasitic relationship are initial castration and final death of the host. Future research could prove this parasite of interest as a biological control for *Lincus*. We have not been able to demonstrate the presence of *Phytomonas staheli* (Trypanosomatidae) in *Lincus*, although electron microscopy has been used. We are now studying the contents of their tegumentary defensive glands.

CHARACTERIZATION

IDENTIFICATION OF PATHOGENIC PLANT TRYPANOSOMES BY KINETOPLAST DNA ANALYSIS

AHOMADEGBE J.C.(1) ; DOLLET M. (2) ; GARGANI D.(2) ; RIOU G.(1)

(1) Institut Gustave Roussy, 94800 Villejuif, France
(2) IRHO/CIRAD, 34032 Montpellier Cedex 1, France

We have extracted and compared the kinetoplast DNA (kDNA) of thirtheen *Phytomonas* isolates obtained from different plants originating from various countries and cultured *in vitro*. The kDNA structure from all the isolates appeared as a large network of interlocked minicircles with some maxicircles extruding from the network. The sizes of the minicircles varied from 1.3 to 2.8 kb according to *Phytomonas* isolates. The electrophoretic analysis of the minicircles digested with various restriction endonuclease permitted to charaterize each *Phytomonas* isolate. Cross hybridization experiments were performed by Southern blot techniques using minicircles from different isolates as probes. A high sequence homology was found between minicicles from *Phytomonas* isolates from crude sap of coconut trees affected with hartrot disease in French Guiana and Brazil. The overall results show that kDNA analysis could be used for distinguishing *Phytomonas* isolates and thus for identifying the infection of plant by pathogenic strain.

CARACTERIZACION DEL METABOLISMO DE CARBOHIDRATOS Y DEMOSTRACION DE GLICOSOMAS EN *PHYTOMONAS* SP. AISLADAS DE *E.CHARACIAS*

SANCHEZ-MORENO M.(1) ; F. OPPERDOES(2) ; A. OSUNA(1)

(1) University of Grenada, Grenada, Spain
(2) International Institute of Cellular and Molecular Pathology, Brussels, Belgium

El catabolismo de carbohidratos en *Phytomonas* sp. aisladas de *E characias* ocurre principalmente via glucolisis aeróbica, siendo la glucosa, fructosa y manosa lso principales sustratos energéticos. Todas las enzimas pertenecientes a la via glucolítica han sido detectadas

Las mitocondrias fueron incapaces de oxidar al succinato. 2-oxoglutarato, piruvato, malato y prolina, pero sí tenian una alta capacidad para oxidar al glicerol 3-fosfato y esta oxidación fué inhibida por el ácido salicyl hidroxámico. No se ha podido demostrar la existencia de citocromos, tanto en mitocondrias intactas como en particulas submitocondriales. La respiración mitocondrial no fué inhibida por antimicin, ácido o cyanida. Las enzimas de la glucolisis estaban asociadas a glicosomas, con una densidad de 1.24 g/cm en sacarosa. La enzima citosólica piruvato quinasa fué activada por la fructosa 2,6-bisfosfato.

BIOCHEMICAL TESTS ON *PHYTOMONAS*

LORENZONI A.M. ; JANKEVICIUS I.S. ; KANESHIMA E. ; UENO T. ; JANKEVICIUS J.V. ; NOGUEIRA D.

University of Londrina, Brazil

Some biochemical tests were carried out in twenty strains isolated from plants and maintained in liquid complex medium (GYPMI). the oxidation-fermentation test demonstrated that *Phytomonas* present fermentative pathway being negative for methyl red test and positive for Voges-Proskauer test. When they were submitted to assays of the capacity to utilize 21 different sources of carbon. was not detected growth in tubes containing pentoses (ribose and xylose). only when they were examined for the presence of enzyme of ornithine-arginine metabolism, the enzyme patterns of the isolates were not homogeneous : the majority of strains presented patterns similar to those described by Camargo *et al* (1987) but isolates from tomatoes in 1989 (268Tb, 269Ta, 270Ta and 274Ta) were positive for arginase and negative or traces for citrulline hidrolase. and the clone 274Tc was positive for both.

ISOZYME VARIABILITY OF THE GENUS *PHYTOMONAS* : GENETICAL, TAXONOMICAL AND EPIDEMIOLOGICAL SIGNIFICANCE

GUERRINI F.(1) ; SEGUR C.(2) ; GARGANID. (2) ; TIBAYRENC M.(1) ; DOLLET M.(2)

(1) Laboratoire de génétique des parasites et des vecteurs, ORSTOM, Montpellier, France
(2) Division Virologie, IRHO/CIRAD, Montpellier, France

31 *Phytomonas* stocks isolated from various hosts and a broad geographical range have been studied by isozyme electrophoresis (14 loci) and population genetics analysis. The total variability is considerable since many stocks share no allele. *Phytomonas* zymodemes behave as natural clones, as already proposed by us for several other protozoan species. These clones should be considered as taxonomic unit in all applied studies. Latex plants and "phloemic plants" (coconut and palm tree) harbor distinct sets of clones : hence latex plants studied in this article are probably not a reservoir for parasites of coconut and palm tree.

SEROLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF *PHYTOMONAS* ASSOCIATED WITH COCONUT AND OIL PALM PATHOLOGY

MARCHE S.(1) ; D. BALTZ(1) ; GARGANI D.(2) ; DOLLET M.(2) ; BALTZ T.(1)

(1) Laboratoire d'Immunologie et de Parasitologie Moléculaire, Université Bordeaux II, Bordeaux, France
(2) Division Virologie, IRHO/CIRAD, Montpellier, France

Identification and characterization of *Phytomonas* associated with coconut and oil palm pathology in South America, were undertaken with the aid of :

- polyclonal and monoclonal antibodies
- electrophoresis (SDS-PAGE) of total and surface proteins
- restriction analysis of 16s rRNA PCR fragments

The serological and biochemical studies allowed us to distinguish isolates associated with plant pathology from isolates of laticiferous plant.

The restriction analysis shows a specific profil for isolates associated with pathology : differenciating these isolates from adventitious plant isolates.

The various coconut and oil palm show the same characteristics : the same *Phytomonas* species could be at the origine of both plant diseases : "hartiot" and "marchitez sorpresiva".

r-DNA FINGERPRINT AND *PHYTOMONAS* SPP. IDENTIFICATION

TEIXEIRA M.M.G. ; CAMARGO E.P.

Departamento de Parasitologia, Universidade de Sao Paulo, Brazil

Identification of genera of trypanosomatid parasites of plants and insects is still an unsolved problem. Ribosomal DNA probes may help to solve this problem since they have been shown to be capable to distinguish other genera of trypanosomatids.

The r-DNA organization of several species or isolates from plants and phytophagous insects were examined by analysis of restriction pattern of genomic DNA, utilizing as probes cloned fragments of ribosomal genes containing the 18S, 24S alfa and 24S beta subunits.

Our results have shown that the genus *Phytomonas* displays distincts r-DNA restriction patterns for Pvu II and Eco RI enzymes, which are different from species of *Crithidia*, *Herpetomonas* and *Leptomonas* examined.

Results point to the usefulness of these restriction markers as diagnostic tools for the identification of *Phytomonas* spp.