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Bacterial Wilt Newsletter

A publication of the *Association M'Baraso*

Number 17 December 2002

From the Editor

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Sponsorship for this issue

We are extremely grateful to CIRAD-FLHOR (and CIRAD's subsidiary VITROPIC), CIP and OEPP for institutional donations to the association M'Baraso which allow this revived issue of the Newsletter and printing of a thousand. This issue to be delivered to 876 people, including 89 libraries in 74 countries.

CIRAD is a French scientific organization specializing in agricultural research for the tropics and subtropics of the world. Its mission is to contribute to rural development in the countries of these regions through research, experiments, training, and dissemination of scientific and technical information. Its work covers agricultural, veterinary, forestry, and food sciences.
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It is very challenging to walk in the footprints of Chris Hayward, who created, and has been the driving force behind the Bacterial Wilt Newsletter for the last 16 issues and who drove the Newsletter to such a quality that it will be difficult to maintain. For the present, I am happy to address this reviving issue of the Newsletter to all previous readers having or not access to Internet and the Web of Bacterial Wilt. An online edition is part of the answer keeping in mind that it is still a minority of subscribers that have access to the Internet (aprox. 25%). Consequently, as far as financial support will be secured through the association, the BWNL will be maintained as a hard copy provided to libraries and anyone not having access to the Internet. People who do have Internet access to the corresponding PDF file and accept to download it rather than receiving the hard copy, are welcomed to notified the Editor by email.

Contributions as short review articles or research reports of from 300 to 1500 words with one photograph, table or graph are invited in particular on the following aspects: inoculation methods and evaluation of resistant germplasm; effect of crop rotation on disease intensity; effects of soil type, site selection and time of planting on bacterial wilt; pathogen diversity, diagnostics and genetics of pathogenicity; new host records including weed hosts; biological control and integrated control; new developments in control strategies.

In addition readers are encouraged to submit material on the following: countrywide or regional status reports on bacterial wilt; notices of forthcoming meetings in plant protection; Letters to the Editor, comment or discussion of recent research findings; notices of new projects on bacterial wilt; notices of useful Websites on plant protection. It must be submitted by e-mail as an attached document to philippe.prior@avignon.inra.fr or by electronic copy on a diskette (IBM-compatible) at the editor address. No hard copy will be accepted. Software : Microsoft Word 4.0 or later, Word Perfect 5.0 or later, or Rich Text Format (.rtf). Copy for the next issue of the Newsletter or address changes should be sent to the following address :

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The association M' Baraso (Maladies Bactériennes causées par *Ralstonia solanacearum / Ralstonia solanacearum* and Bacterial Wilt Information Exchange) is an alternative to the previous ACIAR sponsorship to generate funds which are necessary in the real world for exchanging on diseases caused by *R. solanacearum* and closely related organisms. Many thanks to our first 40 members. Your contributions have been used first to produce and mail the Newsletter, which is a powerful way to provide people from economically less favoured world with an additional access to updated knowledge on these diseases and to publish their finding. Depending on how popular will be the association, aims will be extended to offer some support for attending or/and organizing the organization of the IBW Symposium and to improve the web site dedicated to bacterial wilt. This is the very opportunity offered to survive and maintain alive the BWNL for free, and to modestly contribute to improve communication between people from North and South.. To become a member and to support aims of the association, in particular to maintain the Newsletter, it will be requested a subscription (flyer enclosed).

Please note that by paying your due, you will be supporting the valuable flow of information on Bacterial Wilt to and from researchers in developing countries. The possibility is now offered to people and libraries to subscribe as an active or funding member, and to institutional support to subscribe as a donator member. All members will be provided with a receipt for tax-deductible purpose. Important note - this is not a subscription to the Newsletter as the BWNL is still delivered for free to any people on request to the editor. Your support is strongly needed to establish this "reviving" Newsletter.

Philippe Prior, Chris Hayward, Mark Fegan, and presently 40 members

New Hosts of *Ralstonia solanacearum* in the Brazilian Amazon

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Eryngium foetidum

Bacterial wilt, caused by *Ralstonia solanacearum*, has a wide host range (Hayward, 1995; Kelman, 1953), and new reports of new hosts flourish in the literature. These reports are important to partially explain why the disease suddenly appears in areas apparently free of the pathogen and to recommend crop rotation.

In 2000, plants of *Eryngium foetidum* were found in the green belt of the city of Ananindeua, State of Para, showing chlorosis and wilt symptoms, which later resulted in leaf prostration and plant death. Locally known as "chicória", *E. foetidum* is a non conventional vegetable crop of the Umbeliferae family cultivated in the Brazilian Amazon as a substitute for coriander. It is also cultivated in some countries of Asia, mainly in India, and South and Central Americas.

(Cardoso & Silva Filho, 1997). Bacterial oozing was observed at the base of the cut stem, suggesting that the pathogen was of bacterial origin. Isolation from oozing on nutrient agar and TZC (Kelman, 1954) media yielded pure cultures of a white-colony bacterium which was identified as *Ralstonia solanacearum* based on biochemical tests proposed by Hayward (1991).

Pathogenicity of two isolates of *R. solanacearum* obtained from *E. foetidum* was tested in a greenhouse (20-40°C) by root inoculating 10 plantlets each of tomato 'IPA-5' and *E. foetidum* with a bacterial suspension of ca. 10^8 cfu/ml. Wilt symptoms (Fig. 1) started in the fifth day, only on inoculated plants, from where the same bacterium was reisolated. Biochemical tests proposed by Hayward (1976) indicated that the isolates from *E. foetidum* belong to biovar I, which is the biovar most frequently isolated from non solanaceous plants from the Amazon (non published data).

This is apparently the first report of *R. solanacearum* affecting *E. foetidum* or even a species of the Umbeliferae family.

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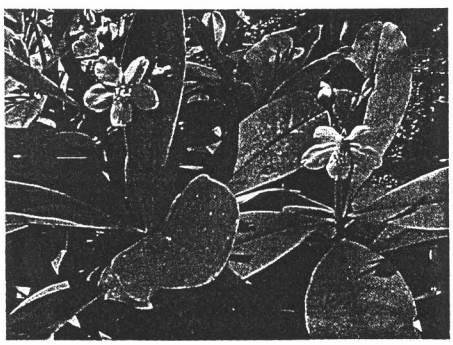
Figure 1. Wilted and healthy plant of *E. foeti*



Talinum triangulare

Talinum triangulare (Jacq.) Wiild, locally known as "cariru", is a non-conventional vegetable crop of the Portulacaceae family cultivated in the margins of the Amazon River. It is consumed mainly in the North Region of

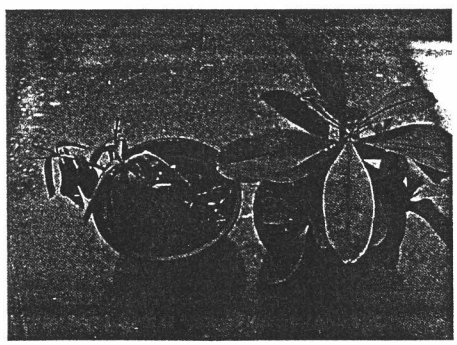


Figure 1. Inoculated plant of "cariru" (left) showing typical wilting and downward leaf rolling.

Brazil, especially in the States of Pará and Amazonas, where its highly nutritious soft leaves make it a reasonable substitute for spinach (*Spinacea oleracea*). It is well adapted to the local hot and humid weather and low

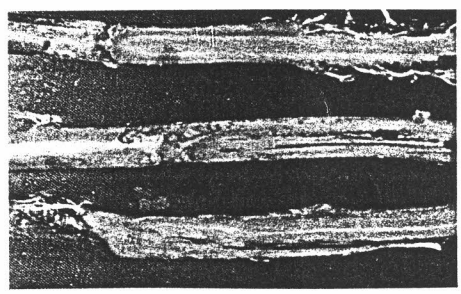


Figure 2. Reddish vascular discoloration (two bottom stems) of "cariru" infected with *Ralstonia solanacearum*

soil fertility, what makes its cultivation an important economical activity for small growers. Worldwide, *T. triangulare* is cultivated in western Africa, Asia (India, Indonesia and Malaysia) and South America (Brazil and Peru) (Cardoso, 1997).

In March of 2000, in the green belt of Belém, State of Pará, plants of "cariru" were found naturally wilted, with leaves usually rolled downward. As a consequence, plant death occurred few days later. Affected plants showed discolored vascular bundles at the base of the stems, with characteristic bacterial oozing, typical of the bacterial wilt disease of solanaceous crops.

The bacterium was isolated in tetrazolium medium (Kelman, 1954), where it yielded irregular-shaped white colonies with pink centers, resembling those of *Ralstonia solanacearum*. This causal agent was confirmed through a series of biochemical tests suggested by Hayward (1964).

The pathogenicity of two isolates from distinct plants was confirmed in 20 plants of "cariru" and 10 plants of tomato 'IPA-5'. Plants of "cariru" were prepared for inoculation either by rooting young stems or sowing seeds in styrofoam trays containing sterile commercial substrate. Rooted plantlets, obtained 15 days after stem rooting or 30 days after seed sowing, were inoculated by dipping their roots for 1 min in bacterial suspensions with approximately 10^8 cfu/ml. Thirty-day tomato plantlets were inoculated the in same manner to check for possible pathogen's specificity. The inoculated plantlets were immediately transplanted to 1 L pots with sterile commercial substrate and thereafter maintained in a glasshouse (20-40 °C). Ten control plants of each species were similarly treated with water for control purpose.

Within eight days after inoculation, all inoculated cariru and tomato plants wilted and died, contrasting with the controls, which remained symptomless (Fig. 1). The xylem region at the base of the stems of infected plants consistently showed reddish discoloration (Fig. 2), from where the bacterium could easily be re-isolated.

Biochemical tests proposed by Hayward (1964) indicated that both isolates from "cariru" belong to biovar I of *R. solanacearum*, which has been the biovar most frequently found in different hosts collected in the same geographic region, where bacterial wilt is endemic in most of the agricultural areas, caused either by biovar I or III..

This is apparently the first report of a member of the Portulacaceae family as a natural host of *R. solanacearum* in Brazil. *Talinum racemosum*, a wild species of the same genus of "cariru", has been reported as a host of this pathogen in Medan, Indonesia (Palm & Fulmek, 1922 - cited by Kelman, 1953). *Portulaca oleraceae*, a weed species of worldwide distribution, is another Portulacaceae reported as a host of *R. solanacearum* (Moffett & Hayward, 1980). *Talinum triangulare*, therefore, increases the host range of *R. solanacearum* indicated by Kelman (1953) and Hayward et al. (1995), who listed about 200 species belonging to more than 50 botanical families infected by this pathogen.

This information is important to define strategies for bacterial wilt control in northern Brazil, as related to crop area selection and rotation. Additionally, since "cariru" is asexually propagated by cuttings, it is necessary to avoid spreading the disease though infected planting material.

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Biocontrol Efficiency of Three PGPR Strains Admixture to Pepper Bacterial Wilt

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Many kinds of strains including avilulent *Ralstonia solanacearum*, *Bacillus* and fluorescent Pseudomonas have been assayed to control bacterial wilt in our lab (Ren *et al*, 1993; Guo et al, 2001). In 1999, we found that two strains with different origins (BB11 and FH17) can make pepper growing better in a biocontrolling plot experiment. After compantional tests and greenhouse tests, an admixture was obtained to give better bioncontrolling efficiency.

The admixture biocontrolling preparation is from J3 (*Pseudomonas* spp.), BB11 and FH17 (*Bacillus* spp.). Seedlings of pepper (Bian Jiao No. 5) without soil-borne pathogens are from Huaian Vegetable Research Institute. All strains were cultured in NA cultural liquid at 30°C for 24h and then be mixed to the admixture with 1:1:1 in final concentrations of about 10^{10}cfu/ml. The suspensions of single bacteria were all in the same concentration. Organic fertilizer has been composted and applied into the test soil in plastic house where the incidence of bacterial wilt last spring is 30%. Then the bacterial preparations were sprayed evenly into them by spraying machine with 1000ml/acre. Covered with plastic film until the pepper seedlings were trans-planted as ever. Pepper seedlings as high as 10-12cm could be trans-planted. There are four treatments, three are with bacterial suspensions of J3, BB11 and FH17 respectively, the other is with admixture of all these strains. All suspensions were used firstly while the seedlings were transplanted, secondly is one month after transplanting. Two control treatments were conducted: (i) agricultural streptomyces (made in the Second Pesticide Factory of Dandong), applying by watering the roots of peppers with 200u/ml, while trans-planting for the first time, and than, once every 10 days later; (ii) water. Each plot repeated four times, and there were 60 seedlings in a plot. The disease incidences were gotten 60 days after transplanting.

Table 1. Biocontrol efficiency of single bacteria and admixture on pepper (Huai-an, 2001)

Treatement	Disease incidence (%)	Biocontrol efficiency (%)	Yield (kg/plot)	Yield increase (%)
Control	29.5±8.71 a[z]	0±29.5 d	18.0±7.65 e	0±42.5 e
Streptomyces	21.0±6.65 b	28.7±22.5 c	21.5±10.7 de	19.4±59.6 de
J3	10.6±2.51 cd	64.0±8.54 ab	38.0±6.34 b	112±37.3 b
BB11	13.0±3.60 c	55.8±12.2 b	32.5±9.84 bc	80.6±54.8 bc
FH17	18.5±3.74 b	37.1±12.7 c	27.0±9.21 cd	50.0±51.1 cd
Admixture	7.29±3.31 d	75.3±11.2 a	54.0±9.43 a	200±52.4 a

[z] Means within a row followed by the same letter are not significantly different as determined by the LSD test ($P = 0.05$)

The yields of all pepper in each plot were calculated. Two years (2000, 2001) plot experiments have been conducted in the farm of Huai-an, Jiangsu province. Average results (Table 1) show that strains J3 and BB11 have evidently decreased the disease incidence and raised the pepper production. An enhanced and more steady biocontrolling efficiency to bacterial wilt and better growth-promoting role in pepper was found in the admixture of three strains of PGPR. The biocontrol efficiency have been increased from 22.6% to 49.4%, and the yield increased from 38.0, 32.5, 27.0 Kg/plot to 54Kg/plot.