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Survey of Bacterial wilt of Solanaceous crops in French West Indies and in French Guiana — B. Digat

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I. INTRODUCTION

The bacterial wilt caused by **Pseudomonas solanacearum** E.F. Sm. is always recognized to be the most important bacterial disease in the tropical zone of the world (1, 2).

Although it was identified since 1896 in U.S.A. by SMITH E. F. its economic importance only began to be suspected only about fifty years ago in the Caribbean area.

In the French West Indies, **Pseudomonas solanacearum** E.F.Sm. was isolated in 1964 in Guadelope from tomato by ESCUDIE. In 1965 and in 1966 we have isolated the bacterium from Tomato, Pepper, Eggplant and Irish Potato in Guadeloupe, in Martinique and in French Guiana.

The study of the disease became necessary not only economically in so far as a limiting factor of the Tomato, Pepper and Potato culture, but also scientifically because of hosts and strain diversity of the pathogen.

The object of this work is to specificy host range, symptoms and distribution of that important disease in French West Indies and French Guiana.

Moreover, the emphasis is on the interest of serological methods, of the study of pathotypes, of biotypes and antibiotypes in order to contribute to a better recognition of the bacterium of its strains in the prospected zones.

II. DISTRIBUTION

Most soils in Guadeloupe and Martinique are infected by **Pseudomonas solanancearum** E. F. Sm.

It is of interest of note that, in uninfected areas, annual rainfall is less than 60 inches. In those areas where rainfall is relatively moderate it is likely that the bacterium, subjected the soil to periods of dryness during

several months, cannot survive.

On the other hand, in areas where anual rainfall is over 60 inches, Solanaceous crops are very frequently endangered by damages caused by the bacterial wilt, whatever the soil type.

The problem remains entire because irrigations of solanaceous crops is necessary in uninfected areas with moderate rainfall. Then the experience shows the continuous irrigation induces sooner or later the infecton by the bacterial wilt.

III. HOST RANGE and SYPMTOMS

A) Host range

In solanaceous crops, in Guadeloupe as in Martinique, the presence of the pathogen is very frequently detected in Tomato (**Lycopersicon esculentum** Mill.) Pepper (**Capsicum frutescens** L.) and Tobacco „Kentucky 16" variety. In Peanut (**Arachis hypogaea** L.) no wilt caused by **Pseudomonas solanacearum** E. F. Sm. has been observed.

In Musuceae the presence in Guadeloupe or Martinique of the **Pseudomonas solanacearum** strain virulent for Banana* and isolated in Guadeloupe by ADVIER then by LANGUILLON, respectively in 1935 and 1950, is questioned by BUDDENHAGEN.

B) SYMPTOMS

In Tomato, before the wilt advantitious** roots are formed. Production of adventitious roots coincides with an accumulation of Indol-Acetic-Acid in the plant.

Then the first symptoms of the wilt appear some leaflets become flaccid, roll, the petioles slope down (epinasty), and the plant begins to wilt.
In Potato, the development of symptoms is very similar but there are no adventitious roots.

In Eggplant and Pepper, the infectious process is slower. A yellowing of the oldest leaves precedes the wilt. There are no adventitious roots at the basis of the stem.

In Tobacco, in the beginning of the wilt, only one or two leaves (sometimes only half of a leave) become flaccid, then turn yellow wilt and die.

For every species, the cross-section or longitudinal section of the

* No experimental pathogenicity of "Pseudomonas" isolated by these bacteriologists from necrosed tissue of Banana was demonstrated and it was too premature to attribute for these "Pseudomonas" the species name "Solanacearum".

** These adventitious roots (like small tumours) are also present in plants infected by *Fusarium oxysporum*.

It is easy to obtain a production of these adventitious roos by immerging the basis of an uninfected cutting in a solution of Indol-Acetic-Acid.

stem shows the brown necrosis of vascular tissue which could be mistaken with the necrosis caused by **Fusarium oxysporum.**

C) **Point out of bacterial exudates**

However, the mistake is avoided by placing the root system of the infected plant in water and keeping the cross-section of the stem above the surface of the water. If the plant is infected by **Pseudomonas solanacearum** E. F. Sm. the formation of the dirty-white exudates, induced by the root-pressure, occurs without delay specially at the level of big vessels. The flow of bacerial exudates is quicker in weakly woody plants (as Tomato, Potato, Tobacco) than in strongly woody plants (as Pepper and Eggplant).

Only a few minutes are sufficient to obtain exudates from the first. a longer time is necessary for Pepper and Eggplant.

IV. SEROLOGICAL DIAGNOSIS

As diagnostical methods, immunological techniques possess a sepcificity and a sensibilitiy rarely attained by the other chemical techniques.

Therefore, between serological reactions, we have selected **sero-agglutination** in order to make a simple, quick and practical diagnosis, useful in the field to distinguish the **species "solanacearum".**

1°) **Quick diagnosis by sero-agglutination test (5)**

a) **Material**

Antigens are constituted by parts of bacterial cell-wall. (Bacterial cells are inclused in bacterial exudates obtained as in III C).

Antiserum with agglutinins is obtained by three intravenous injections into rabbit every fourth day with a bacterial suspension containing two milliards of living bacteria in one millilitre. Eight days after the last injection, agglutinins in the antiserum obtained by taking a little blood of the rabbit are titrated. If concentration in agglutinins is considered satisfactory, then heart puncture technique is used in order to obtain 40—50 ml of blood. Generally, obtained antiserum has a $^1/_{320}$e limit dilution point. The usual dilution is $^1/_{10}$e or $^1/_{20}$e in physiological serum containing 1‰ merthiolate solution as antiseptic.

One rabbit from one series of bleeding gives about 100 ml of antiserum. If for one test 0,5 ml of diluate antiserum is necessary, about two thousand plants can be tested with one rabbit.

Prepared antiserum must be kept at 4 C°.

b) **Method**

To realize the sero-agglutination test, a little bacterial exudate is collected. With collected exudate a smear is made on the left side of a microscopic blade and then an identical smear on the right side of blade

is made. Two or three antiserum drops are put on the left smear and an equal quantity of physiological serum on the right smear. Mixing of each smear with serum is finished by a slow rolling movement.

c) **Results**

Sero-agglutination reaction is positive if there appears after three minutes:

— in antiserum drop, on the left side of blade, white, small in the beginning, then more and more numerous and bigger agglutinates. Those agglutinates are made by connection of **Pseudomonas solanacearum** cells with specifical antibodies of antiserum.

— in physiological serum drop, on the right side of blade, only a slight cloudiness.

d) **Value of the method and antiserum specificity**

We have never obtained mistaken positive sero-agglutinations. However, among several thousands of tests made in the field, some exudates (about 5%) gave us negative sero-agglutination reaction. Those exudatese contained however **Pseudomonas solanacearum** E.F. Sm. as laboratory isolations have shown. Every time, culture from those exudates was not pure and several saprophytic bacteria were found with the **Pseudomonas solanacearum** E.F. Sm. Are those saprophytes in exudate of infected plant inhibitors for normal fixation of antibodies on Pseudomonas solanacearum E.F. Sm., since the latter isolated in pure culture agglutinated normally?

Antiserums made from isolates of **Pseudomonas solanacearum** E.F. Sm' from Guadeloupe and French Guiana are very specific since they have agglutinated all the isolates from Caribbean zone and from other countries* (see Table 1).

Because of its simplicity and its quickness, the technique can be easily applied in the field and allows anybody to detect the pathogen within few minutes in the field.

Laboratory test have confirmed the value of the method applied in in the field.

This method has allowed the determination of approximate boundaries of areas infested by the bacterium in Guadeloupe and Martinique; owing to this sero-agglutination test, the bacterium was detected in French Guiana.

Applicable to all solanaceous crops, this method could be adjusted to Bacterial wilt of Musuceae (BUDDENHAGEN, personal communication).

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* We admit that agglutinins of our antiserums, made from Guadeloupe and French Guiana isolates, were unable to be fixed on some strains from other countries, strains which could possess very different antigenic sites.

Its utilization for other bacterial wilts with vascular origin in other host-plants may be possible.

V. SPECIFICAL CHARACTERS OF ISOLATES

Isolates from Guadeloupe, Martinique and French Guiana possess specific characters. By grouping those characters for each isolate it is possible to define strain and race of each isolate.

Isolates from Guadeloupe have been studied more specially. For isolates from Martinique and French Guiana work is in progress.

Typical characters*, defined as the most important by BUDDENHAGEN and KELMAN (2) are:

— Pathotype,

— Colony type,

— Biochemical type,

— Lysotype,

— Serotype,

— Bacteriocinotype.

Among the typical characters we believe that Antibiotype may be added because it is typical for sensibility of each isolate to antibiotics.

1. PATHOTYPE

Among typical characters, pathotype of an isolate or pathogenicity of an isolate for host plants seems to be the most important character.

Material and Method

Exudates are extracted as in III C. From those exudates **Pseudomonas solanacearum** cells are isolated on Tetrazolium medium of KELMAN (4). Purified virulent isolates are then inoculated (5). Cross-inoculations are made. Inoculations are made on a definite host range including Solanaceae (Tomato, Potato, Pepper, Eggplant, Tobacco), Musaceae (Musa sp., **Heliconia psittacorum**), and Peanut.

Twenty isolates from Tomato ("Floralou, Marglobe, Indian River") variety and two isolates from Tobacco "Kentucky 16" variety have been used.

Results

Results are summarized in Table 2. All isolates from infested Tomato plants from several parts of Guadeloupe possess homogeneous characters of pathogenicity.

However experimental results show that there are marked differences in relative pathogenicity among isolates from Tomato and Tobacco.

In the same area (Capesterre — Guadeloupe) we have found the two pathotypes that support work of OKABE and GOTO (6). Concluding:

1°) Host plant would „select out" the pathotype to which it is most susceptible.

2°) More than one pathotype could be present in the same field.

TABLE 2

PATHOGENICITY for SOLANACEAE and MUSACEAE of ISOLATES of **PSEUDOMONAS SOLANACEARUM** E. F. Sm. from TOMATO and from TOBACCO from GUADELOUPE

Isolates from	**Tomato**	**Potato**	**Pepper**	**Eggplant**	**Peanut**	**Tobacco**	**Banana**	**Heliconia psittacorum**
Tomato	+++	+++	+++	++	+	—	—	—
Tobacco	+	++	+	+	+	++	—	—

Degrees of virulence:: +++ **strong**
++ **medium**
+ **weak**
— **avirulence.**

2. BIOCHEMICAL TYPE (BIOTYPE)

Biotype of an isolate may be restricted to "the ability or failure to utilize the three disaccharides (Maltose, Lactose and Cellobiose) and the three hexose alcohols (Manitol, sorbitol and Dulcitol)" (7).

Material and Method used are the same as in (6)

Results show that isolates from Guadeloupe have biotype I or biotype III.

3. ANTIBIOTYPES

Antibiotype shows the sensitivity of each isolate to usual antibiotics.

Material and Method (8)

The diffusion method gives us the best results.

1 ml of bacterial suspension (one loopful of each isolate in 5 ml of sterile distilled water) is poured into each Petri dish containing 10 ml of medium (Papaïnic Digest of Bovine Heart 100 ml, Pancreatic Peptone SC 6 g, Glucose 2 g, Bacto-Agar 15 g, distilled water 900 ml,).

After the excess of bacterial suspension was removed, six "Institut Pasteur" disks for antibiotic sensitivity tests were chosen* and placed on the medium in each Petri dish. Plates are turned over and incubated at 30°C. Antibiotics diffuse from disks and, if the bacterium is sensitive, an inhibition zone of growth appears round the disk.

* It is necessary to chose one disk of each antibitic family.

Rseults

Results are obtained after three days of incubation at 30°C. They are summarized in Table 3.

TABLE 3

SENSITIVITY of ISOLATES from GUADELOUPE, MARTINIQUE and FRENCH GUIANA to USUAL ANTIBIOTICS

ANTIBIOTICS	ISOLATES FROM		
	Guadeloupe	Martinique	French Guiana
Streptomycin	+++	+++	+++
Tetracyclin	+++	+++	+++
Oléandomycin	—	—	—
Chloromycetin	+	+	+
Polymyxin	—	—	—

Degrees of sensitivity:

+++ sensitive = diameter of inhibition zone more than 20 millimeters.

\+ slighty sensitive = diameter of inhibition zone less than 20 millimeters.

— resistant = no inhibition zone.

It is evedent that all isolates from Guadeloupe, Martinique and French Guiana have the same antibiotype.

VI. CONCLUSIONS

The very broad host range and also the wide distribution of **Pseudomonas solanacearum** E.F.Sm. shows the easiness of adaptation of the pathogen in the Caribbean area.

As yet, strains of Guadeloupe, Martinique and French Guiana that we are studying are virulent for Solanaceae and avirulent for Musaceae. However, existence of other strains in a latent state or very weakly virulent is possible.

Moreover emphasis must be on the interest of systematic prospections and, obviously, on the importance of diagnostical methods allowing the early detection of new infection focuses and the presence of new virulent strains.

The soil-borne bacterium cannot be attained and destroyed directly in the soil, The best methods of control are in my opinion, to give to the plant serious possibilities of resistance either by selection or resistant varieties (9) or by grafting susceptible varieties on resistant rootstocks (10).

Results obtained in Guadeloupe and French Guiana by those two methods of control are very hopeful.

Nevertheless the future of Solanaceae in the Caribbean zone in connected with continual improvement of possibilities of control against this destructive disease.

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SUMMARY

In the French West Indies and in French Guiana, the Bacterial wilt of Solanaceous crops caused by **Pseudomonas solanacearum** E.F.Sm. was identified first in Tomato in 1964, then in 1965 in Pepper, Eggplant and Potato and in 1967 in Tobacco.

As yet, the host range seems to be limited to those plants but the most part of Guadeloupe and Martinique is invaded by the pathogen.

The emphasis is on diagnostical methods and specially on serological methods. A sero-agglutination test allows to distinguish the bacterial wilt and the Fusarium wilt.

The importance of the disease in the French West Indies and in French Guiana is so great that the future of Solanaceous crops is connected with possibilities of control.

RESUMEN

Reconocimiento de la Marchitez bacteriana de las solanacea en las Antilles Frances y en Guyana Frances.

En las Antilles Frances, la marchitez bacteriana de las solanaceas causada por **Pseudomonas solanacearum** E.F. Sm. fue reconocida en primer lugar en la Tomate en 1964, despues en 1965 en el Pimiento, en la Berengena y en la Patata, y en 1967 en el Tabacco.

Hasta ahora, el serie de plantas hospedadoras parece limitade a estas plantas, pero el parasito ha invidado la mas grande parte de la Guadeloupe y de la Martinique.

El acento esta en las metodos del diagnostico y, particularimente, en las metodas serologicas. Una prueba de sero-agglutinacion permite de distinguar la marchitez bacteriana de la enfermeded al Fusarium y de diagnosticar rapidamente el agente causal.

La importancia de la marchitez bacteriana en las Antillas Franceses esta tan grande que la posteridade de la solanaceas esta ligada a las posibilidades de lucha contra esta enfermedad.