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EPIDEMIOLOGY OF BACTERIAL SPOT OF PEPPERS IN BARBADOS*

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ABSTRACT

This study reports on the incidence and severity of bacterial spot of pepper in Barbados and the variation among the isolates of *X. campestris* pv. *vesicatoria* with respect to pathogenecity and sensitivity to copper and zinc. The genetic basis of copper resistance and avirulence is also investigated.

RESUME

EPIDEMIOLOGIE DE LA GALE BACTERIENNE DU POIVRON A LA BARBADE

Cette étude aborde l'incidence et la sévérité de la gale bactérienne du poivron à la Barbade et la variation des isolants de *X. campestris* pv *vesicatoria* en ce qui concerne la pathogénicité et la sensibilité au cuivre et au zinc. La base génétique de la résistance au cuivre et de l'avirulence est aussi étudiée.

INTRODUCTION

Bacterial spot, caused by Xanthomonas campestris pv. *vesicatoria* (Doidge) Dye, is an important disease of pepper (*Caspicum annuum* L.) and tomato (*Lycopersion esculentum* Mill.) in tropical areas (Cook and Stall, 1960 ; Adamson and Sowell, 1983 ; Marco and Stall, 1983). Three races or pathotypes of the bacterium are usually distinguished based on their reactions with differential tomato and pepper cultivars or breeding lines. The

*** Table of results discarded under editorial constraints (the Editor)**

pepper breeding lines PI271322 and PI163192 possess monogenic resistance to race-1 and race-2 isolates of the pathogen respectively. Single genes in the breeding line PI 260435 of *Caspicum chacoense* also confer resistance to race-1 and race-2 isolates. The tomato strain or race of the pathogen gives the susceptible reaction with tomato and a hypersensitive response with PI163192. It was generally believed that all tomato cultivars are susceptible to isolates representing the three common race of *X. campestris* pv. *vesicatoria* but a tomato variety, Hawaii 7998, has recently shown high levels of resistance to the pathogen in laboratory tests (Scott and Jones, 1986).

Bacterial spot is usually controlled by the use of copper bactericides, mancozeb and streptomycin (Conover and Genhold, 1981 ; Marco and Stall, 1983 ; Sharma et al, 1981). However, high levels of resistance to copper have been reported in populations of *X. campestris* pv. *vesicatoria* from pepper growing areas of the USA (Marco and Stall, 1983 ; Adaskaveg and Hine, 1985). Resistance in the pathogen to copper is controlled by plasmid PXvCu. This plasmid is also associated with a specific avirulence gene in race-2 isolates of the pathogen (Stall et al, 1986).

MATERIALS AND METHODS

Pathogen and Host Cultures

The isolates of *X. campestris* pv. *vesicatoria* used in this study are shown in Table 1. The Barbadian isolates were obtained from diseased pepper or tomato plants. All isolates were identified by standard procedures. NYGA consists of oxoid bacteriological peptone (5 g/l), yeast extract (3 g/l), glycerol (20 g/l) and Lab MNO2 agar (10 g/l). NYGB contained the constituents of NYGA except agar.

The tomato cultivar Gardener's Delight and the pepper lines PI163192 and Early Cal Wonder served as hosts. Seeds were germinated in seed trays and grown for 2-3 weeks under light intensity of 4.7 w/m2 and a 12-hour photoperiod in a plant growth chamber maintained at 25°C.

Field Survey

The incidence and severity of bacterial spot of pepper were determined in seven districts of Barbados during the dry and wet seasons of 1988 and 1989 respectively. Each pepper field was sampled twice monthly and at each sample time four upper-most leaves were chosen from each of 10 randomly selected permanent sites per hectare. The proportion of the leaf area covered by typical symptoms induced by *X. campestris* pv. *vesicatoria* per

TABLES

Table 1 : Isolates of X. campestris pv. vesicatoria used in this study

Isolate	Date Isolated	Origin
81 - 23	-	Florida, USA
2595	1960	Brazil
813	July, 1985	Barbados
NAP6	April, 1987	Barbados
NAP5	April, 1987	Barbados
XV3	December, 1984	Barbados
NAP2	April, 1987	Barbados
NAPC2	April, 1987	Barbados
NAPC1	April, 1987	Barbados
NAP4	April, 1987	Barbados
XV6	December, 1984	Barbados
824	-	Florida, USA
NH4	July, 1985	Barbados
NAT2	April, 1987	Barbados
NAP9	April, 1987	Barbados
NAPC3	April, 1987	Barbados
NAP3	April, 1987	Barbados
2446-B	April, 1987	Florida, USA
NAT1	April, 1987	Barbados
NAP1	April, 1987	Barbados
NAP4	April, 1987	Barbados
NAP5	April, 1987	Barbados
NAP6	April, 1987	Barbados
NAP7	April, 1987	Barbados

Isolates 81-23, 824 and 2446-B are from Florida, USA and isolate 2595 is from Brazil (R. Stall, University of Florida). The Brazilien isolate is reported as a copper sensitive tomato strain and 2446-B are copper resistant isolates of the pepper race-2 pathotype.

site was recorded for each pepper field. All fields were cropped with the hybrid variety Bell Tower.

The presumptive *X. campestris* pv. *vesicatoria* in each field was also estimated at each sampling time from 10g of top soil representing pooled 5g samples of each site. A soil suspension was prepared in 100 ml sterile distilled water and 0.1 ml portions of appropriate dilutions plated onto NYGA amended with streptomycin (10 µg/ml). Plates were incubated at 25°C for 3 days during which time colonies of presumptive *X. campestris* pv. *vesicatoria* emerged. The number of presumptive *X. campestris* pv. *vesicatoria* per gram of field soil was determined.

Screening of isolates for sensitivity to copper and zinc

Bacteria representing single colonies of *X. campestris* pv *vesicatoria* were streaked onto NYGA amended with different concentrations of CuSo4.5H2O, or ZnSo4.7H2o. Plates were assessed for bacterial growth after three days incubation at 25°C. Five replicates of each treatment were used for each test of viability.

Screening of isolates for sensitivity to antibiotics

Cells from single colonies of *X. campestris* pv. *vesicatoria* were streaked on to NYGA amended with streptomycin (250 µg/ml), ampicillin (50 µg/ml), kanamycin (50 µg/ml), tetracycline (10 µg/ml), chrorampheniçol (10 µg/ml), spectinomycin (100 µg/ml), rifampicin (50 µg/ml) or gentamycin (5 µg/ml). Plates were assessed for bacterial growth after 3 days incubation at 30°C. Five replicates of each treatment were also used in each test of viability.

Conjugation Experiments

The following pairs of isolates of *X. campestris* pv. *vesicatoria* were mated :

NH4CuR strepS ampS x 2595 CuS strepR ampR

NH4CuR strepS ampS x XV3 CuS strepR ampR

81-23 CuR strepR genS x 824 CuS StrepS genR

These isolates were previously characterized for sensitivity to antibiotics and copper. The superscripts R and S indicate resistance and sensitivity respectively to copper (Cu) streptomycin (strep), ampicillin (amp) and gentamycin (gen).

Single colonies of each isolate of a pair were cultured separately overnight in NYGB at 25°C in an incubator equipped with an orbital shaker. Two milliliters of cell suspension (108-109 cells ml-1) of each isolate of a mating pair were mixed and aliquots from this mixture placed onto sterile membrane filters placed on NYGA medium. The isolates were mated for 16-20 hours and then washed from the filters with sterile distilled water. The resulting bacterial suspensions were diluted appropriately and 0.1 ml aliquots spread onto selective NYGA media to obtain transconjugants. The selective media contained CuSO-4 5H2O (0.02 %) and streptomycin (250 µg/ml), ampicillin (50 µg/ml) or gentamycin (5 µg/ml). Plates were incubated at 25°C for 4-5 days during which time copper transconjugants emerged. A conjugation frequency for each mating was estimated as the number of transconjugants per recipient cell.

Race classification

Two to three week-old host seedlings were stab-inoculated in the stem with a sterile pin previously inserted in a colony of the pathogen grown on NYGA. Approximately 10 bacterial cells are transferred to the host using this inoculations procedure. Seed trays containing the inoculated seedlings were covered with a transparent lid to maintain a humid chamber under the host culture conditions described before. The plants were examined for disease symptoms 24-72 hours following infection.

The race characterization of the pathogen is based on the nature of the host response (Cook and Stall, 1969) and is as follows :

Race of pathogen	Host Lines		
	Tomato (Gardener's Delight)	Pepper (PI163192)	Pepper(Early Cal. Wonder)
Tomato Strain	S	HS	HS
Pepper Strain race 1	S	S	S
Pepper Strain race 2	S	H	S

Abbreviations : S - Susceptible response, HS - Hypersensitive response

Pathogenicity of *X. campestris* pv. *vesicatoria* with differential sensitivity to copper on pepper and tomato treated with copper

Bacterial suspension (106 cells ml-1) of copper-resistant or susceptible isolates was sprayed onto 2-3 week-old seedlings of pepper (Early Calwonder) and tomato (Gardener's Dlight) which were previously treated with 0.02, 0.05 or 0.1 % CuSO4.5H2O solution. Pathogenicity was assessed 7-10 days following inoculation. Each leaf was scored on a 0-4 scale where 0, 1, 3 and 4 represented 0%, >0 ≤ 25 %, > 25 % ≤ 50 %, > 50 % ≤ 75 % and > 75 % ≤ 100 % of the leaf area covered by lesions respectively. A disease index for each plant was then calculated as the mean of ratings for individual leaves.

Pathogenicity of transconjugants on pepper

Ten transconjugants which showed resistance to copper (0.020 % CuSO4 5H2O) were obtained from mating the following pair of isolates of *X. campestris* pv. *vesicatoria* : NH4 CuR StrepS ampS / Xv3 CuS, StrepR, ampR. Bacterial suspensions (106 cells ml-1) of the transconjugants were sprayed to "run-off" onto 2-3 week-old seedlings of the pepper cultivar (PI163192). Seedings were also inoculated with bacterial suspensions of the parental isolates. Foliar disease symptoms were assessed 7-10 days after infection and scored on a 0-4 scale as previously described.

In the foregoing pathogenicity investigations all inoculated seedlings were incubated in a humid chamber under host culture conditions described before and 10 tomato or pepper seedlings were used to test the pathogenicity of each isolate of *X. campestris* pv. *vesicatoria* or transconjugant.

RESULTS (*)

Field Survey

The severity of the disease bacterial spot of peppers caused by *X. campestris* pv. *vesicatoria* and the number of presumptive soil-borne cells of the bacterium were generally much higher during the rainy season of the survey period. The disease was most severe in the pepper fields of districts 4, 6 and 7 .

Zinc and copper sensitivity

All isolates grew in the presence of 0.01 % CuSO4.5H2O. Two isolates, NAP2 and NAPC2 remained viable on medium amended with 0.05 % CuSO4.5H2O. Twelve of 16 isolates tested were copper resistant. In this summary isolates which were viable in the presence of 0.02 % or greater CuSO4.5H2O were considered copper-tolerant.

Only 5 of 19 isolates grew in the presence of 0.01 % ZnSO4.7H2O and all but two failed to grow at 0.015 %

Sensitivity to antibiotics

None of the isolates grew on NYGA amended with tetracycline, spectinomycin or kanamycin. Generally resistance to the other antibiotics varied with the isolate. Five out of 18 isolates grew in the presence of streptomycin.

Bacterial Conjugations

Copper resistance in isolates of *X. campestris* pv. *vesicatoria* was transferred from copper-resistant (CuR) isolates to their copper-sensitive (CuS) mating partners. The high frequencies of CuR transconjugants obtained (2.8 x 10-2 and 3.7 x 10-3) are unlikely to be accounted for by mutation to copper resistance. The presence of two or more antibiotic markers in each pair of mating isolates made it possible to determine the recipient cells in these experiments.

Race Classification

The races or pathotypes of *X. campestris* pv. *vesicatoria* generally give characteristic hypersensitive or susceptible reactions with differential pepper or tomato cultivars. The hypersensitive response usually develops by 24 hours following infection with the pathogen and it is characterized by localized necrotic lesion at the point of inoculation. The susceptible response which may take up to 72 hours to become well defined, appears as a small water-soaked lesion which develops into a large necrotic area. The stem is often girdled or split.

(*) Editorial contraints led to suppress tables of results in this proceedings, the paper out passing the recommanded length.

Of 16 Barbadian isolates investigated only two are of the pepper race-2 pathotype.
Pathogenicity of CuR and CuS isolates on copper-treated pepper and tomato

The copper-sensitive isolates Xv3, 824, NAP1 and 2595 showed significant reduction in pathogenicity on tomato (Gardener's Delight) and/or pepper (Early Calwonder) sprayed with 0.02 % CuSO4.5H4O. 0.05 % CuSO4.5H2O reduced the pathogenicity of the copper-resistant isolates (NAP2 and NAPC2) on tomato but higher levels were required to reduce the disease on

pepper.

Pathogenicity of transconjugants

The parental isolate and the transconjugants induce the hypersensitive response in the pepper line PI163192.

DISCUSSION

This disease bacterial spot of peppers caused by *X. campestris* pv. *vesicatoria* is prevalent in Barbados. The severity of the disease varied with the site surveyed but it was most severe during the wet season. It is interesting that the disease persits at such high levels during the rainy season despite the intensive use of the copper-based pesticides. The efficacy of these compounds in the control of bacterial spot of pepper and other foliar diseases during the wet season needs further investigation. It is also of interest that the amount of presumptive soil-borne *X. campestris* pv. *vesicatoria* increase under moist soil conditions. This bacterium is reported to possess poor saprophytic ability and it would be of interest to investigate the factors which contribute to this increase. Rain splash from infected plants is a likely contributing factor.

The differential sensitivity of Barbadian isolates of *X. campestris* pv. *vesicatoria* to copper and zinc has been illustrated. Ten out of 13 isolates tested were copper-tolerant. This high frequency of copper-tolerant isolates reflect the almost exclusive use of copper bactericides for the control of bacterial spot of pepper and many other foliar diseases. The data presented indicate that much higher copper dosages than the ones currently in use may be required to control the disease caused by the copper-resistant isolates of *X. campestris* pv. *vesicatoria* in the field.

Zinc is a component of the mancozed formulation and it was therefore of interest to determine the inhibitory effects of zinc on the isolates of *X. campestris* pv. *vesicatoria* . All of the isolates were extremely sensitive to zinc as zinc sulphate . This suggest that formulations of zinc compounds may be an effective control of bacterial spot caused by the coppert-resistant isolates. It has been reported however, that zinc tolerance in *X. campestris* pv. *vesicatoria* can be induced
at a high frequency in the laboratory (Adaskaveg and Hine, 1985) and this may imply that the field isolates may develop tolerance to zinc if zinc formulations are used often.

Streptomycin, either singly or in combination with copper bactericides is used to control bacterial spot of pepper in some parts of the world (Adaskaveg and Hine, 1985). It is interesting that 5 of 18 of the isolates of *X. campestris* pv. *vesicatoria* tested in this study were streptomycin resistant, suggesting a high frequency of naturally occurring resistance to this antibiotic in the field. The use of streptomycin for the control of bacterial spot should therefore not be encouraged.

The pathogenic variation among isolates of *X. campestris* pv. *vesicatoria* has been shown using a series of differential tomato and pepper cultivars or lines. The BS-1 gene present in the pepper line PI163192 controls the hypersensitive response to race-2 isolates of the pathogen but not race-1 isolates. These two races can therefore be distinguished by reaction with the BS-1 gene. Most of the Barbadian isolates investigated in this study are of the race-1 pathotype. It is generally believed that race-2 isolated of the pathogen are endemic to Florida, USA, while race-1 isolates predominate elsewhere (Hibbered et al, 1987).

The acquisition of copper resistance by copper-sensitive isolates of *X. campestris* pv. *vesicatoria* following conjugation indicates that copper resistance is a transmissible property which is plasmid mediated. The transmissibility of this trait was previously demonstrated and the transmissible plasmid pXvCu is 190-250 Kb in size (Stall et al, 1984).

Stall, et al (1986) has also previously demonstrated that the hypersensitive reaction between race-2 isolates of *X. campestris* pv. *vesicatoria* and the BS-1 locus in pepper is linked to the transmissible pXVcu plasmid. This observation has been reconfirmed in this study. A copper-sensitive isolate Xv3 (race 1) which gives a susceptible or compatible reaction with the BS-1 locus can be made to give a hypersensitive response with BS-1 on acquisition of the copper-resistance trait following conjugation with NH4 which is copper-resistant and incompatible with BS-1. The genetics of copper resistance and hypersensitivity in this pathogen is still under investigation.

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