Survival of *Xanthomonas campestris* pv. *malvacearum* in Soil[1]

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ABSTRACT

The survival of a rifampin-resistant mutant of *Xanthomonas campestris* pv. *malvacearum*, the cause of bacterial blight of cotton, was determined in sterile and non-sterile black earth soil. In non-sterile soil the pathogen was not detectable after 50 days and in sterile soil after 80 days at 20°C (± 2°C). These results confirm that this pathogen has limited capacity for survival in soil free from cotton plant material.

COMPENDIO

En este trabajo se estudió la supervivencia en suelos estériles y no estériles de *Xanthomonas campestris* pv. *malvacearum*, agente causal del tizón bacteriano del algodonero. Se empleó un mutante bacteriano resistente a rifampicina. El patógeno tuvo una supervivencia máxima de 50 días en suelos esterilizados y de 80 días en suelos sin esterilizar, ambos mantenidos a 20°C (± 2°C). Estos resultados confirman que *Xanthomonas campestris* pv. *malvacearum* tiene una capacidad limitada para vivir en suelos carentes de restos del cultivo de algodonero.

INTRODUCTION

Bacterial blight of cotton caused by *Xanthomonas campestris* pv *malvacearum* (6) is considered one of the most serious diseases of cotton in many production areas throughout the world (11). Although much is known about the prolonged survival of the pathogen in plant debris (1, 3, 14, 15), occurrence as a resident on leaf surfaces (13) and the importance of infected seed in the disease cycle (2, 4, 8, 16), very little is known about the survival in the soil of *Xanthomonas campestris* pv *malvacearum*, or of other pathovars of *Xanthomonas campestris*, free of plant material

The purpose of this investigation was to examine the survival in a black earth clay soil of a rifampin-resistant mutant of the bacterial blight pathogen.

Sterile and non-sterile soils were compared in order to evaluate whether the pathogen is capable of surviving with and without the competition of antagonistic microflora. Investigations were undertaken as to whether such populations of the pathogen persisted over winter, thus serving as an inoculum to reestablish new infections in the following season.

MATERIALS AND METHODS

A mutant designated rrm-ma of *Xanthomonas campestris* pv. *malvacearum* (UAM 2702) resistant to 100 μg ml^{-1} of rifampin (Rifampicin®, an antibiotic which specifically inhibits DNA-dependent RNA polymerase of bacterial and chloroplast origin) was isolated by the following procedure. A suspension in sterile distilled water containing 10^{10} cfu ml^{-1} was prepared from a 36 h culture, and 0.1 ml volumes were spread with a glass spreader over the surface of dried plates of sucrose-peptone agar (SPA) (7) containing 0.1 μg ml^{-1} rifampin. Colonies developing on this medium were streaked on progressively higher concentrations (0.5; 1.0; 5.0; 10.0; 15.0; 20.0; 25.0; 50.0 and 75.0 μg ml^{-1}) until a strain resistant to 100 μg ml^{-1} was obtained (5). The rrm-ma was used as a genetically marked strain and its pathogenicity was determined by spray inoculation of cotton plants. The differential cultivars required to determine races (9) were also inoculated.

Samples of a black earth clay soil –Vertisol (17)– were collected at random in a field used for cotton cultivation at Brookstead, Southeastern Queensland. Samples were bulked and broken up and subsamples removed for the determination of field capacity and air dry moisture content.

Three hundred grams of air-dried soil were placed in each of six polythene bags (30 x 42 cm x 0.1 mm). Three of the bags were sterilized in an autoclave at 108°C for 1 h on each of three successive days.

1 Received for publication 8 October 1988
This work was carried out at the Department of Microbiology, University of Queensland, Australia during the term of a CONICET grant. The author is grateful to Dr. A.C. Hayward for his suggestions during this research and help in the preparation of the manuscript.

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Suspensions of rrm-ma were prepared from 48 h SPA medium containing rifampin (100 μg ml^{-1}) in sterile distilled water to a concentration of 10^{10} cfu ml^{-1}

Each sample was inoculated by spraying the soil surface with the bacterial suspension, and the moisture content of the soil was adjusted to 70% of the water holding capacity. The plastic bags were closed by folding three times and tying with a rubber band, in order to minimize moisture loss. The bags were held in a box in the dark at a temperature of 20°C ± 2°C. The moisture content of the soil samples was measured at intervals and adjusted to the original level.

At 10-day intervals two 1 g samples of soil were withdrawn from each bag. One of the 1 g samples was dried overnight at 105°C in order to determine the oven-dried weight, and on this basis the moisture content of the soil was adjusted by addition of sterile distilled water. The second 1 g sample was added to 9 ml of sterile distilled water, shaken vigorously for 30 min and diluted in a tenfold series. Triplicate 0.1 ml volumes of the three highest dilutions were spread over the surface of SPA medium containing rifampin (100 μg ml^{-1}) and cycloheximide (Actidione®) (50 μg ml^{-1}), the latter to supress fungal growth. The plates were incubated for 72 h at 28°C and yellow mucoid colonies of *Xanthomonas* were then counted using a Quebec colony counter.

RESULTS AND DISCUSSION

The mutant, rrm-ma, was found to be as virulent to cotton as the parental wild type and both conformed in character to race 18 of the blight pathogen as described by Hunter *et al.* (9) and Hussain and Brinkerhoff (10).

The results show that *X. campestris* pv. *malvacearum* survived for a longer period in sterile as compared with non-sterile soil (Fig. 1). In non-sterile soil the bacterium was no longer detectable after 50 days, whereas in sterile soil the bacterium was no longer detectable after 80 days.

The bacterial blight pathogen can overwinter in infected plant debris on the soil surface; however, cotton debris buried in moist soil lost infectivity in 40-107 days depending on the extent of microbial descomposition of the debris, and the pathogen survived for eight days when applied in water suspensions in both moist and air dried soil at 21-33°C (3). Similarly, Arnold and Arnold (2) found that infected cotton trash lost infectivity when burried, sooner than trash lying on the ground. They concluded that although the pathogen may survive in trash unitl sowing time, it is unlikely to cause infection of the new crop if the trash is effectively burried during cultivation.

These observation suggest that although the bacterial blight pathogen can survive for periods making dissemination by wind-borne dust possible or movement of surface water, survival in plant debris is likely to be of much greater significance in the epidemiology of the disease, as *Xanthomonas campestris* pv. *malvacearum* appear to be a poor competitor with other Soil organisms under natural situations.

There may be several reasons for the greater survival of free cells of the bacterial blight pathogen in sterile soil compared with non-sterile soil, including the absence from sterile soil of predation by protozoa, bacteriophage lysis, antibiotic production by antagonistic microflora, or of nutrient competition effects (12).

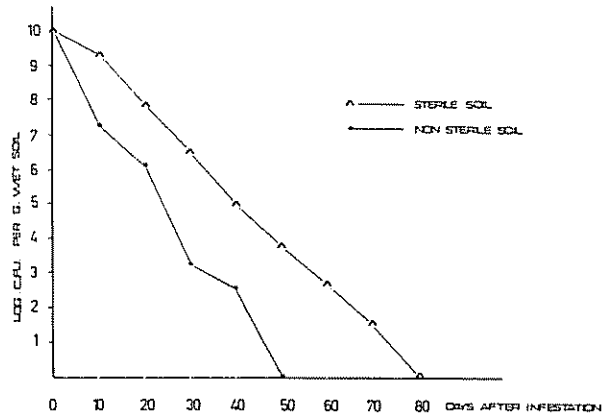

Fig 1 Populations of *X. c.* pv. *malvacearum* (rrm-ma) on sterile and non-sterile soil. Values are average of three replications.

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