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ALTERNATE HOSTS TO *Xanthomonas campestris*, THE CAUSAL AGENT OF A LEAF BLIGHT OF ONION

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ABSTRACT

The response of pepper, tomato, corn, French bean, soybean and winged bean to strains of *Xanthomonas campestris* causing a leaf blight of onion was assessed. French bean, soybean and winged bean were identified as alternate hosts of the bacterium. This is the first known report on alternate hosts to the bacterium.

INTRODUCTION

A leaf blight of onion (*Allium cepa*. L.), caused by *Xanthomonas campestris* is a serious disease in Barbados (Paulraj, 1991; Paulraj and O'Garro, 1993). The disease has recently appeared on the neighbouring islands of St Kitts and Nevis, where the onion industry is expanding. Onion output is also expanding on several other Eastern Caribbean islands and there is concern that the leaf blight will eventually constrain the expansion.

Efforts to control the leaf blight utilize sprays of copper and/or zinc-based bactericides including Cuprasan, Manzate, Champion and Vandozeb. The bactericides are generally ineffective in Barbados because of widespread resistance to them in the pathogen population (Paulraj and O'Garro, 1992). Failure of chemical control of the leaf blight has focused attention on alternative disease control measures, notably the use of resistant onion genotypes and crop rotation.

The present study reports on alternate hosts and resistance in onion to onion strains of X. camp*estris*.

MATERIALS AND METHODS

Pathogen culture

X. campestris strains G6, F58A and W4 were used in this study. The strains were isolated from diseased onion and characterized based on cellular fatty acid composition and other biochemical and physiological tests (Paulraj and O'Garro, 1993). The bacterium was routinely cultured on nutrient agar or in nutrient broth (Difco). Suspensions for inoculations were prepared by growing the bacterium overnight in nutrient broth at 28–30 °C on a rotary shaker set at 220 oscillations per min. Bacterial pellets were collected following centrifugation at 10,000 *g* for 10 min, washed twice and then resuspended in sterile distilled water (SDW). The suspension was adjusted to 106 cells/ml and used as inoculum.

Culture and inoculation of potential hosts

(a) Laboratory studies

Tomato (*Lycopersicon esculentum* Mill.) cv. Walter, pepper (*Capsicum annuum*. L) cv. Calwonder 300 TMR, corn (*Zea mays* L.) cv. Pioneer, French bean (*Phaseolus vulgaris* L.) cv. Green Crop, Soybean (*Glycine max*. (L.) Merr.) cv. Davis and an unknown cultivar of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) served as potential hosts of onion strains of *X. campestris* in this study. Plants were grown, one per pot (10 x 10 cm) in sterilized potting compost (Yates, New Zealand) and inoculated when 7 or 8 weeks old. All plants were maintained in a plant growth chamber set at 26–28 °C, 85-95% relative humidity and a 12 h photoperiod maintained by fluorescent lamps of total light intensity of 1,020 μE/s per m^2.

Plants were inoculated by spraying abaxial and adaxial surfaces of leaves to run-off with a bacterial suspension (10^6 cells/ml). Control plants were sprayed with SDW. All treated plants were kept under the host culture conditions described above and misted at 6-h intervals for 10 min with tap water to provide high humidity. Three plants of each genotype were treated with each strain of the bacterium and observed daily for symptoms. The experiment was repeated twice.

(b) Field studies

Onion, cv. Yellow Granex, was grown from seed sown directly in three raised soil beds 80 cm wide by 30 cm apart. After germination, 4-week-old seedlings were sprayed with a suspension (106 cells/ml) of onion strains of *X. campestris* to induce the leaf blight and used to infest soil with the bacterium by ploughing affected plants into soil beds. Seeds of potential host plants of the bacterium were sown directly into infested soil and the ensuing plants misted regularly with water over a 6-week period and observed for the appearance of disease. Each plant genotype was arranged in a random design with three replicates of six plants and tested in soil beds each infested with a different strain of the bacterium. Plants serving as controls were grown in an uninfected soil bed. The experiment was repeated twice.

Leaf sections, incorporating disease lesions were excised and processed for the isolation of presumptive onion strains of X. *campestris* as described above, except that nutrient agar was amended with the appropriate amounts of relevant antibiotics required to select each strain of the bacterium (Table 1).

Table 1 Characteristics of onion strains of *Xanthomonas campestris* used in the study

Strain	Antibiotic resistance*
F58A	Strep. Rif. Amp
G6	Amp. Rif. Srep
W4	Tet. Rif. Strep

*The minimum concentrations of streptomycin (Strep), rifampicin (Rif), ampicillin (Amp) and tetracycline (Tet) to which strains are insensitive are 100, 30, 50 and 10 μg/ml respectively.

Identification of pathogen

Presumptive xanthomonads were tested for the presence of xanthomonadins (Schaad and Stall, 1988). Five strains of presumptive *X. campestris* obtained from lesions on each suspect alternate host tested with strain F58A, G6 or W4 were also tested on Yellow Granex by a pin-prick method previously described (Paulraj and O'Garro, 1993). Treated plants were kept under humid conditions in a plant growth chamber and observed for symptoms.

RESULTS

Disease symptoms on plants tested in the laboratory appeared on French bean, soybean and winged bean 6–8 days after inoculation. Symptoms on French bean initially appeared as watersoaked lesions on the abaxial surface of leaves. Lesions were later surrounded by yellow halos, which gave leaves an overall yellowish appearance. As the disease advanced, lesions became necrotic and many coalesced to form large areas of brown tissue.

On winged bean, symptoms were first observed on the abaxial surface of leaf tips as watersoaked lesions that advanced rapidly to entire leaves and young stems within 9 days. Advanced stages of the disease were associated with extensive necrosis and/or chlorosis of tissues.

Symptoms on soybean appeared as yellowing of the margin of leaves. The yellow areas expanded and were replaced by extensively necrotic tissue 2 weeks after infection. Yellowing and necrosis of intercostal sites was also observed on some infected leaves.

Of plants tested in infested soil, only legumes were affected by disease. Visible symptoms appeared within 3.5–4 weeks after planting and were similar to those on plants treated in the laboratory. However, French bean and winged bean were most severely affected by disease in the field.

All bacterial strains obtained from French bean, soybean and winged bean treated with onion strains of X. *campestris* induced watersoaking on Yellow Granex. In contrast, plants of each genotype serving as controls were free of visible disease symptoms.

DISCUSSION

Recent elucidation of X. *campestris* as causal agent of a leaf blight of onion (Paulraj, 1991; Paulraj and O'Garro, 1993) has made it possible to identify alternate hosts and resistance in onion to the bacterium. Three xanthomonads, X. *campestris pv. glycines, X. campestris* pv. phaseoli and X. c*campestris* pv. vignicola, all of which are pathogenic on genotypes in the Leguminosae (Bradbury, 1986), are most closely related to X. campestris from onion on the basis of fatty acid analysis (Paulraj, 1991; Paulraj and O'Garro, 1993). The possibility that the relatedness between onion strains of X. *campestris* and these other xanthomonads might also be indicated by their host range was investigated in the present study. French bean, soybean and winged bean have been selected for study as members of the Leguminosae. Moreover, the two former genotypes are also natural hosts of X. *campestris* pv. glycines and X. *campestris* pv. phaseoli (Bradbury, 1986). Tomato, pepper and corn were chosen as non-leguminous hosts. They also represent hosts of xanthomonads (Bradbury, 1986) less closely related to onion strains of *X. campestris*. The study shows that French bean, soybean and winged bean are hosts of *X. campestris* from onion. The use of fatty acid composition to predict the host range of a phytopathogenic bacterium has not been previously reported. It would be of interest to test other legumes for susceptibility to the bacterium. Onion should also be tested with X. *campestris* pv. phaseoli and X. *campestris* pv. glycines to determine if it is a host to these xanthomonads.

The present study provides useful information for the control of the leaf blight of onion caused by *X. campestris*. French bean, soybean and winged bean should not be rotated with onion in fields infested with the leaf blight as each has the potential to maintain inoculum of the pathogen. Onion cultivars, H-942 and H-502 appear resistant to the leaf blight and should be introduced in Barbados.

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REFERENCES

Bradbury, J.F. 1986. Guide to plant pathogenic bacteria. Slough, UK: CAB International Mycological Institute, 332 pp.

Collinge, D.B. and Sluzarenko, A.J. 1987. Plant gene expression in response to pathogens. Plant Mol. Biol. 9:389–410.

Paulraj, L. 1991. Epidemiology of a leaf blight of onion in Barbados. Ph.D. Thesis, The University of the West Indies, Cave Hill, Barbados.

Paulraj, L. and O'Garro, L.W. 1992. Aspects of the epidemiology of a leaf blight of onion in Barbados. Proc. Barbados Soc. Technol. Agric. 10:89–96.

Paulraj, L. and O'Garro, L.W. 1993. Leaf blight of onions in Barbados caused by Xanthomonas campestris. Plant Dis. 77:198–201 .

Schaad, N.W. and Stall, R.E. 1988 *Xanthomonas*. Pages 81-94. In: Schaad, N. W.(ed.) Laboratory guide for identification of plant pathogenic bacteria, 2nd edn. St Paul, MN, USA: American Phytopathology Society.