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SHIFT FROM COPPER-RESISTANCE TO ZINC-RESISTANCE IN BARBADIAN POPULATIONS OF *Xanthomonas campestris* pv. *vesicatoria*, CAUSAL AGENT OF BACTERIAL SPOT OF PEPPER AND TOMATO

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ABSTRACT

Research monitoring bacterial spot of pepper and tomato (caused by *Xanthomonas campestris* pv. *vesicatoria*) in Barbados over the past 11 years has shown a shift from copper-resistance to zinc-resistance in the pathogen population. This observation parallels a change from copper to zinc formulations as the main chemical control agents of bacterial spot. This paper addresses a strategy for chemical control of bacterial spot.

INTRODUCTION

Tomato (Lyco*persicon esculentum* Mill.) and pepper (*Capsicum annuum* L.) output in the Caribbean is greatly limited by bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye. The disease is caused by races 1,2,3 and 4 of the pathogen (Ward and O'Garro, 1992; O'Garro and Tudor, 1994), each capable of overcoming specific disease resistance genes in the host (Minsavage et al., 1990; O'Garro and Tudor, 1994).

From inception, standard methods of bacterial spot control in Barbados utilized sprays of copper and/or zinc formulations (O'Garro and Ward, 1989; Ward and O'Garro, 1992) and over the last 11 years, the response of the bacterium was investigated. In 1984 the bacterium was overwhelmingly sensitive to zinc but copper-resistant (O'Garro and Ward, 1988). This observation immediately led to widespread use of zinc-based bactericides for bacterial spot control and by 1989 the frequency of zinc-resistance in the pathogen population had increased significantly while the incidence of copper-resistance decreased from 100 to 60% (Ward and O'Garro, 1992).

Studies on the genetic basis of copper resistance in *X. c.* pv. *vesicatoria* have shown that resistance is plasmid-borne (Stall et al., 1986; Cooksey, 1990; O'Garro and Durant, 1993). Presently the genetic basis of zinc resistance in the bacterium is unknown. The present study reports further on the response of *X. c.* pv. *vesicatoria* to copper and zinc and proposes a strategy for chemical control of bacterial spot.

MATERIALS AND METHODS

Isolation and identification of pathogen

Typical disease lesions were excised from bacterial spot-infected fruits and leaves of pepper and tomato plants obtained from various farms in Barbados. Lesions were surface-disinfected by dipping in solutions of ethanol (95%) followed by sodium hypochlorite (1.25%) for 2 and 10 sec, respectively. Surface-disinfected lesions were then rinsed in sterile distilled water (SDW) and homogenized in SDW (100 µl) using an ethanol-sterilized mortar and pestle. Loopfuls of the homogenate were streaked onto Tween 80 agar plates for selection of *X. c.* pv. *vesicatoria*. Presumptive colonies of *X. c.* pv. *vesicatoria* obtained were subcultured onto nutrient yeast-extract glycerol agar (NYGA) and later stored at -20 °C suspended in nutrient yeast-extract glycerol broth (NYGB) amended with 20% glycerol until required. A random sample comprising 10% of strains of presumptive *X. c.* pv. *vesicatoria* was tested for xanthomonadins (Schaad and Stall, 1988).

Test for sensitivity to bactericides

X. c. pv. *vesicatoria* was tested for sensitivity to copper and zinc by preparing a bacterial suspension (10^8 cells/ml) in aqueous solutions of $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$, respectively, and then plating aliquots (3 µl) in duplicate onto NA or NYGA after 2 h incubation at 25 °C. Solutions of $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ were used at concentrations of 50, 200, 300, 400 and 500 µg/ml. Strains of *X. c.* pv. *vesicatoria* producing confluent growth on NA or NYGA within 5 d after treatment with the copper and zinc salts at concentrations of 200 µg/ml or greater were considered resistant to copper and zinc, respectively.

RESULTS

Five widely distributed pepper and tomato farms infested with bacterial spot were surveyed and an average of 353 strains from each were randomly selected and tested. All strains tested were positive for the presence of xanthomonadins. The frequency of resistance to zinc or copper in the pathogen population varied with the farm. The frequency of copper-resistance varied from 22.9 to 67.6% while the incidence of resistance to zinc ranged from 97.1 to 100%. Of the strains of the bacterium tested, 53.5% and 97.8% were resistant to copper and zinc, respectively (Figure 1) and 53.5% were unrestricted by both compounds when combined.

DISCUSSION

The switch from almost exclusive dependence on copper to frequent use of zinc for bacterial spot control in pepper and tomato in Barbados has resulted in changes in the response of the pathogen to these two chemicals (Figure 1). Reduction in use of copper-based bactericides is associated with a considerable decrease in the frequency of copper-resistant strains of *X. c.* pv. *vesicatoria*. Studies have shown that in the absence of copper in growth media, copper-resistant strains of *X. c.* pv. *vesicatoria* reverted to copper-sensitivity at high frequencies (O'Garro and Durant, 1993). The reversion was linked to loss of plasmids encoding copper-resistance (O'Garro and Durant, 1993).

Widespread use of zinc-based bactericides over the past decade to control bacterial spot induced by copper-resistant strains of *X. c.* pv. *vesicatoria* in Barbados has led to a prevalence of zinc-resistance in the pathogen population.

A typical farm, National Hatcheries, is a case in point. Over the past decade, this farm has been constantly monitored for changes in response of *X. c.* pv. *vesicatoria* to sprays of copper and zinc bactericides. In 1985, strains tested showed a 100% incidence of copper resistance. In contrast all strains were sensitive to zinc (O'Garro and Ward, 1988). By 1991 the incidence of copper-resistance declined to 61% while the frequency of zinc-resistance increased to 82% (O'Garro and Ward, 1991; Ward and O'Garro, 1992). Presently copper-resistant strains of the bacterium account for 68% of the pathogen population while the frequency of zinc-resistance is 98%.

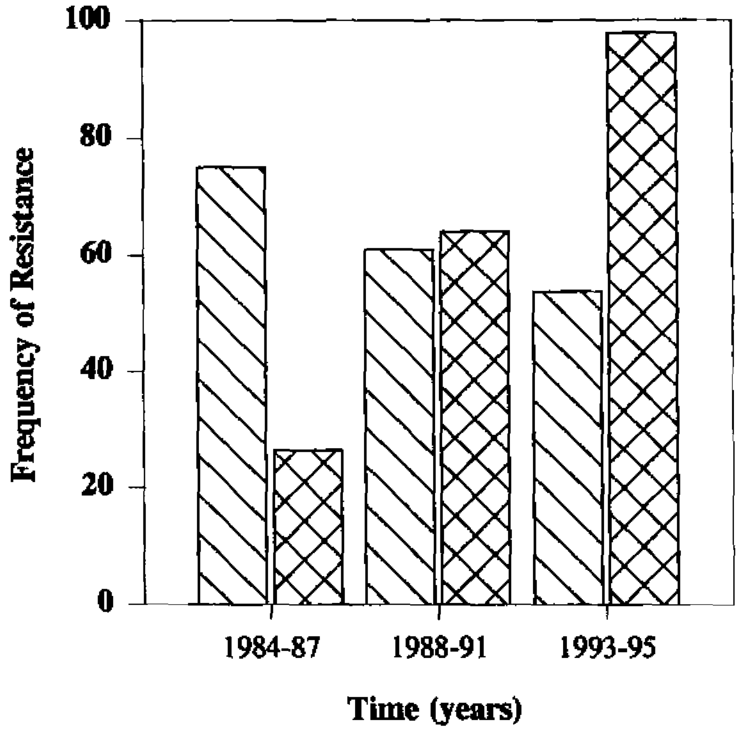

Figure 1 Frequency of resistance of copper  and zinc  in samples of *Xanthomonas campestris* pv. *vesicatoria* in Barbados tested over an 11yr period

Copper-based bactericides appear to have utility for bacterial spot control on account of the instability in the copper-resistant trait observed in *X. c.* pv. *vesicatoria* (O'Garro and Durant, 1993). It may be possible to exploit this instability for disease control. In this approach, pepper and tomato fields infested predominantly with copper-resistant strains of the bacterium will be removed from treatment with copper bactericides thus allowing strains to revert to copper-sensitivity. Copper bactericides could then be used for control of bacterial spot caused by copper-sensitive strains of *X. c.* pv. *vesicatoria*. This approach to disease control is a novel one and promises to enhance the effectiveness of chemical control and reduce the cost of chemical control and the release of toxic compounds to the environment. We are presently implementing this approach as a strategy for bacterial spot control. We are also investigating the genetic basis of zinc-resistance in *X. c.* pv. *vesicatoria* to determine whether similar instability in zinc-resistance exists.

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