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Resistance of *Xanthomonas campestris* pv. *vesicatoria* isolates from Tanzania to copper and implications for bacterial spot management

Kenneth C. Shenge[1,5]*, Robert B. Mabagala[2], Carmen N. Mortensen[3] and Kerstin Wydra[4]

[1]The Ohio State University, OARDC Food Animal Health Research Program, 1680 Madison Avenue, Wooster, OH 44691, USA.
[2]Department of Crop Science and Production, Sokoine University of Agriculture, P.O. Box 3005, Morogoro, Tanzania.
[3]Danish Seed Health Centre for Developing Countries, Department of Plant Biology, Plant Pathology Section, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, Frederiksberg C, DK-1871, Copenhagen, Denmark.
[4]Georg-August-University Göttingen, Centre for Tropical and Subtropical Agriculture and Forestry (CeTSAF), Buesgenweg 1, D-37077 Gottingen, Germany.
[5]Department of Crop Protection, Faculty of Agriculture/Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria.

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Thirty isolates of *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) from Tanzania were evaluated for sensitivity to copper sulphate ($CuSO_4$:$5H_20$) using dilution plate counts. Ninety-three percent (28/30) of the isolates produced countable colonies on nutrient glucose agar amended with 0.8 mM $CuSO_4$. All the *Xcv* isolates (7/7) from Arusha (northern Tanzania) grew on the copper medium. Isolates of the pathogen from Iringa (southern Tanzania) produced variable results (15/21). However, neither of the two *Xcv* isolates from Morogoro (Eastern Tanzania) grew on the copper medium. These results indicate long-term exposure of *Xcv* isolates from Tanzania to selection pressure for copper tolerance.

Key words: *Xanthomonas campestris* pv. *vesicatoria*, copper resistance, Tanzania.

INTRODUCTION

Bacterial leaf spot (BLS) of tomato (*Solanum lycopersicum* L.), caused by *Xanthomonas campestris* pv. *vesicatoria* (Syn. *Xanthomonas axonopodis* pv. *vesicatoria* (Vauterin et al., 2000) or *Xanthomonas euvesicatoria* (Jones et al., 2004)) is one of the most destructive and widely distributed diseases of tomatoes (Hovarth et al., 2012; Yu et al., 1995). The disease affects every above-ground part of the tomato plant. Attack on leaves causes defoliation, resulting in exposure of fruits to sun scald (Dougherty, 1978; Pohronezny and Volin, 1983). However, the main economic effect of the disease is the reduction in fruit weight and quality. Bacterial spots on the fruits have been reported to account for up to 52% weight loss in infected fruits (Jones et al., 1986). Disease control is exceedingly difficult to achieve when environmental and weather conditions are conducive for pathogen proliferation (Jones et al., 1986).

It is not clear when tomato bacterial spot was first

*Corresponding author. E-mail: shenge.1@osu.edu. Tel: +1(330) 466-2316. Fax: +1(330) 319-6634.

identified and recorded in Tanzania, but Black et al. (2001) reported that the disease was widespread in tomato and pepper fields in all the vegetable-growing regions in Northern and Southern mainland Tanzania. Other surveys by Kaaya et al. (2003) confirmed wide-spread occurrence of bacterial spot in the country. Although most tomato growers in Tanzania rely on fixed copper chemicals to manage tomato bacterial spot, the disease has continued to spread in spite of the chemical application (Shenge et al., 2007). In spite of these reports, the long-term impact of these chemicals on the disease, the environment and agricultural production in Tanzania has not been assessed. The present study, therefore, aimed at assessing isolates of *Xcv* from Tanzania for resistance to copper, and determining if copper-based chemicals were an effective way of managing the disease in Tanzania.

MATERIALS AND METHODS

Seven samples were collected from Arusha, 2 from Morogoro, and 21 from Iringa region (Figure 1). Presumptive *Xanthomonas campestris* pv. *vesicatoria* isolates from tomato fruit lesions, and one reference *Xcv* strain from The Göttingen Collection of Phytopathogenic Bacteria (GSPB), GSPB 2043 were tested for *Xcv* determinative characteristics, including Gram reaction, nitrate reduction, Kovac's oxidase reaction, starch hydrolysis, pectin degradation, Biolog and hypersensitive reaction on tobacco leaves, following methods and classifications outlined in Schaad et al. (1988), Holt et al., (2000), O'Garro et al. (2003) and Woodland, (2004). Pathogenicity of the isolates was confirmed by misting an inoculum suspension of the bacteria adjusted to 10^8 cfu ml^{-1} onto 35-day-old tomato (cv. Tanya) plants and scoring disease symptoms 14 days after inoculation. Suspensions of 48 h old bacterial isolates were prepared in phosphate buffered saline (PBS) and adjusted to an optical density (OD) of 0.06 at 620 nm, corresponding to ca 10^8 cfu ml^{-1}. Twenty microliters of each suspension were spread evenly onto the 0.8 mM copper-containing medium (200 mg $CuSO_4.5H_2O$/1000 ml of NGA). NGA served as the untreated control. Inoculated plates were incubated at 28°C and enumerated 36 to 48 h later. Counts on the copper-amended medium and NGA were compared statistically using t-tests at $P \leq 0.05$.

RESULTS AND DISCUSSION

Yellow-pigmented *Xcv* isolates from lesions on tomato fruit samples were rod-shaped, unipolar flagellated, Gram-negative, oxidase-negative and catalase-positive. All the isolates hydrolyzed starch and degraded pectin, but were unable to reduce nitrates. Tobacco leaves inoculated with suspensions of the isolates showed a hypersensitive reaction within 24 h, and typical bacterial spot symptoms that were similar to natural symptoms developed on inoculated tomato (cv. Tanya) plants following inoculation. The isolates differed widely in their carbon source utilization profiles in Biolog; the dendrogram showed three distinct clusters that confirmed a strong relationship with geographic origin (Shenge et al., 2007).

The response of Xcv isolates from Tanzania to 0.8 mM CuSO4 is presented in Figure 2. Seventy-three percent of the isolates (22/30) produced countable colonies on the copper-amended medium, with all the isolates from Arusha Region (7/7) showing resistance to copper. Out of 21 isolates from Iringa Region, 15 grew on the copper-amended medium; neither of the isolates from Morogoro produced colonies on the copper medium.

The findings of this study demonstrated that resistance to copper-based chemicals was widespread within *Xcv* populations from the northern and southern highlands of Tanzania. These regions are also the leading tomato production areas in Tanzania, indicating that long-term use of copper-based chemicals in tomato production inadvertently led to *Xcv* selection for resistance to copper in the regions. In addition to tomato production, Arusha Region also has a long history of coffee production, which also receives heavy applications of copper compounds for coffee disease management.

Copper is required in trace quantities by many bacteria for their structural composition, as a co-factor in enzymatic functions (Bai et al., 2007), and electron transport and redox reactions (Cervantes and Guitierrex-Corona, 1994). However, at high concentrations, copper molecules become toxic to bacteria, interfering with the energy transport system and disrupting enzyme active sites, as well as the integrity of cell membranes (Cervantes and Gutierrez-Corona, 1994; Garcia-Horsman et al., 1994). High cellular copper concentrations have also been shown to damage lipids, proteins and DNA (Bai et al., 2007). Owing to these toxic properties, copper formulations have been used against a wide range of bacterial and fungal plant pathogens for more than 100 years (Cooksey and Azad, 1992). However, copper ions are not degraded in soil and can accumulate to high levels at locations with a history of intensive copper application (Koller, 1998). Long-term microbial exposure to sub-lethal concentrations of the chemical leads to adaptation through the development of plasmid/chromo-somally-borne cellular copper sequestration (Cooksey, 1990) and detoxification systems that protect the bacteria from toxic concentration levels of copper, while at the same time ensuring that their nutritional copper require-ments are met (Voloudakis et al., 2005).

In general, the ability of *Xcv* strains to tolerate copper in artificial growth media is used to measure copper resistance in the field. Several instances of this approach have been documented in literature (Cooksey et al., 1990; Gore and O'Garro, 1999; Tesoriero et al., 1997; Zevenhuizen et al., 1979; Martin and Hamilton, 2004) with similar levels of effectiveness. In studies by Zevenhuizen et al. (1979), strains of bacteria were considered to be resistant to $CuSO_4.5H_20$ if they were able to survive in a medium containing 1.0 mM of the compound. In other studies, Gore and O'Garro (1999) reported that ability of *Xcv* strains to express confluent growth on NA amended with copper sulphate at a concentration of 200 µg ml^{-1} (0.80 mM) was an expression of resistance to the

Figure 1. Geopolitical map of Tanzania. Red stars indicate locations where *Xanthomonas campestris* pv. *vesicatoria* isolates were collected. The locations were selected based on a history of tomato production, and also to cover a wide range of ecological conditions.

chemical. In the current study, 73% of *Xcv* isolates from Tanzania produced countable colonies in NGA amended with 0.8 mM $CuSO_4$. Based on the conclusions of previous studies, the *Xcv* strains from Tanzania can, therefore, be classified as resistant to copper.

The findings of this study are in agreement with other reports, which showed that increasing occurrence of copper resistance within populations of *Xcv* was becoming a serious problem in many tomato production areas (Gore and O' Garro, 1999; Martin and Hamilton, 2004; Mirik et al., 2007). These findings highlight the negative environmental impact of long-term use of copper-based chemicals as a plant disease management option. With particular reference to Tanzania, these results demonstrated that copper pesticides are no longer an effective means for tomato bacterial spot management. Identification of alternative natural and synthetic antimicrobial agents against *Xcv* is therefore, exigent. Recent reports by Mbega et al. (2012) indicated that some plant extracts were effective in reducing seed-borne xanthomonads associated with bacterial leaf spot. It seems that tomato bacterial spot management approaches that

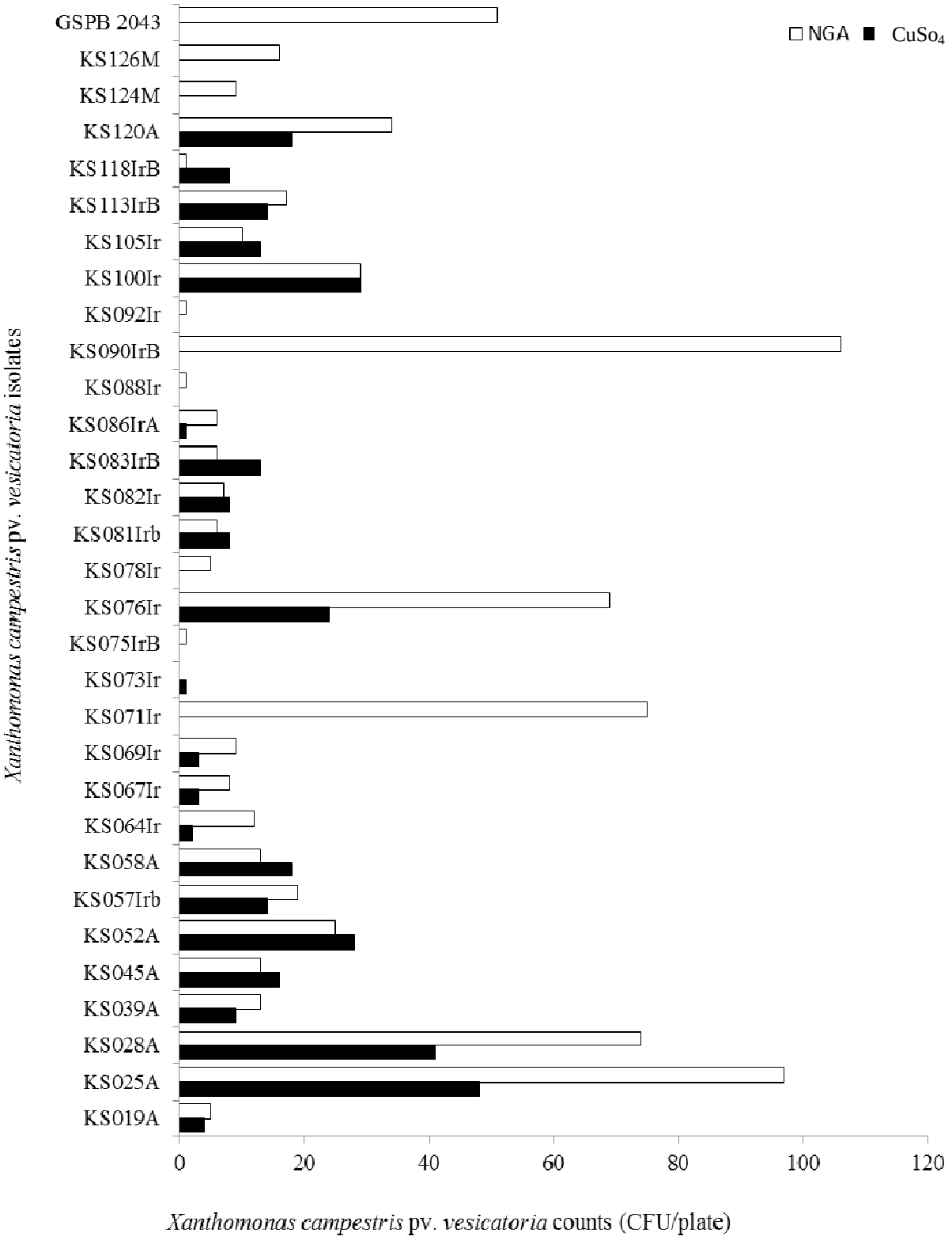

Figure 2. Resistance of *Xanthomonas campestris* pv. *vesicatoria* to $CuSO_4$. Solid, black bars indicate the number of countable *Xcv* colony forming units (CFU) on nutrient glucose agar (NGA: Nutrient agar + 5% glucose) medium amended with 0.8 mM $CuSO_4$:$5H_2O$ while unshaded bars indicate the number of *Xcv* CFU on NGA medium without $CuSO_4$. Labels on the vertical axis represent isolates used in the study. Isolate names ending with A indicates that the isolates were collected from Arusha Region; Ir, IrA and IrB were collected from Iringa Region, while those ending with M were collected from Morogoro region. IrA and IrB indicate that the diseased tomato fruit samples from which the pathogens were isolated came from the same field.

integrate the use of such plant products, with synthetic chemicals, disease-free seeds, field sanitation and resistant tomato varieties would be an effective way of managing the disease in a sustainable manner. In general, successful management of bacterial leaf spot in Tanzania would require the design of spray programs that accommodate the pesticide sensitivity status of pathogen populations. One component of such a program should consist of regular field surveys to determine the likelihood that farmers will encounter resistant strains of the pathogens, and a system that combines one or two antimicrobial compounds to eliminate the likelihood of the pathogens developing resistance to any one of them.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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