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Population dynamics of *Xanthomonas campestris* pv. *vitians* on different plant species and management of bacterial leaf spot of lettuce under greenhouse conditions

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ABSTRACT

The population dynamics of *Xanthomonas campestris* pv. *vitians* (*Xcv*) was studied both externally and internally in lettuce, tomato and pepper plants. In addition, the application of bactericides during transplant production period was carried out for the management of bacterial leaf spot of lettuce under greenhouse conditions. Epiphytic populations of *Xcv* were recovered on leaves of lettuce plants (10^5 CFU/g) 5 weeks after sprayed than the other plant species when inoculated with 10^8 CFU/ml of *Xcv*. When plants of each crop species infiltrated with the bacterium at 10^5 CFU/ml, the highest populations were developed in lettuce (10^8 CFU/cm^2) followed by pepper with 10^6 CFU/cm^2 and tomato with 10^5 CFU/cm^2 10-days after infiltration. Application of a mixture of Maneb and Kocide or Kocide alone as a foliar spray on lettuce significantly reduced the incidence and disease severity of bacterial leaf spot by 29 and 27% respectively. Spread of the bacterium and development of the disease may partly be managed by avoiding intercropping of these plants commonly grown in close proximity to lettuce. For the management of leaf-associated populations of *Xcv* in lettuce, use of a mixture of Maneb and Kocide is advocated to minimize the effect of attacks.

1. Introduction

Bacterial leaf spot (BLS) of lettuce caused by *Xcv* was first reported in South Carolina and Virginia in 1918 (Brown, 1918). Since then, it has been reported in many lettuce-growing areas of the world (Toussaint, 1999; Barak et al., 2001; Al-Saleh and Ibrahim, 2009). Contaminated seed has been suggested as a possible source of outbreaks in various lettuce-growing areas around the globe (Sahin and Miller, 1997; Carisse et al., 2000). BLS is seed transmitted and can survive on in seed as well as survive in fields in plant debris and on leaves of symptomless weeds during fallow periods between lettuce crops (Barak et al., 2001) or on lettuce leaf surfaces as an epiphyte under field conditions in Quebec and Florida (Toussaint, 1999; Robinson, 2003). There have been a number of studies on the host range of *Xcv* within crop plants and weed species. Tsuchiya et al. (1981) produced an extensive list of host plants with visible symptoms after inoculation with *Xcv*. They found a number of hosts within *Cruciferae*, *Polygonaceae*, *Tropaeolaceae*, and *Compositae* families. Sahin and Miller (1998) have reported that tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.) are hosts of *Xcv*. Our field observations showed that most producers in Saudi Arabia intercrop lettuce plants in rows between rows of pepper and tomato plants in order to protect them from inclement weather. On commercial farms this cultivation strategy may increase the spread and survival of the bacterial pathogen as these plants grown in close proximity to lettuce may serve as reservoirs of inoculum of *Xcv*. Examination of the length of time *Xcv* can survive on or in leaves of plant species under greenhouse conditions may provide insight which may lead to feed into disease management strategies.

In several lettuce-production areas including Saudi Arabia, seeds are planted in plug seedling trays and grown for 28–35 days in greenhouse, and then transplanted into the field. Wellman-desbians (1999) showed that *Xcv* is disseminated readily from plant to plant via overhead irrigation during lettuce transplant production. Interestingly, it was determined that almost all lettuce plants were contaminated with *Xcv* without showing typical symptoms. It is not

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clear if the absence of symptoms in the greenhouse was due to (1) the low temperature in the greenhouse, (2) a low population of *Xcv* or/and (3) other factors which maintained *Xcv* in an epiphytic rather than in a pathogenic stage (Carisse et al., 2000). The chemical applications of bactericides during transplant production period may reduce inoculum levels of the pathogenic bacteria and fit into a management strategy.

The objectives of this study were to (i) monitor the epiphytic populations of *Xcv* on inoculated leaves of lettuce, tomato and pepper plants under greenhouse conditions, (ii) monitor the population of *Xcv* within leaves of the same plants injected with the pathogen, and (iii) test chemical bactericide applications to gain insight in the host–pathogen interaction to suggest possible management of BLS of lettuce during transplant production under greenhouse conditions.

2. Materials and methods

2.1. Growth of plants

Seeds of Cos lettuce (cv. Darkland), tomato (*L. esculentum* Mill. cv. Farah) and pepper (*Capsicum annuum* L. cv. Cayenne Long Slim) were sown in seedling trays (288 cells per tray – National Company of Riyadh, Saudi Arabia) containing sterilized peat for three weeks. After emergence, plants were fertilized once a week with a 20–20–20 (N–P–K) soluble fertilizer (1 $g1^{-1}$) (NAFCO Co., Saudi Arabia). Four-week-old seedlings were transplanted in 20-cm diameter plastic pots (2 kg soil/pot) filled with a sterilized mixture of clay and sand (4:1 W/W) with one seedling per pot. Each pot received 5 g of a slow-release fertilizer 1% N–P–K (12:4:6) every 4 weeks (NAFCO Co.). The plants were watered every 2–3 days with approximately 100–200 ml water. The experiments were carried out in an air-conditioned greenhouse with temperatures ranging from 18 to 28 °C and 50–70% relative humidity (RH). The plants were exposed to natural light only with a photoperiod of 10–12 h. The light intensity at the plant height was 755 $\mu Em^{-2}s^{-1}$.

2.2. Inoculum preparation and application

Xcv strain L3, recovered from a commercial lettuce field in Saudi Arabia in 2008 (Al-Saleh and Ibrahim, 2009) was used in the studies. The bacterium was grown for 72 h on nutrient agar amended with 0.5% glucose (GNA) (aqueous wt./vol) (Robinson et al., 2006). Plates were flooded with sterile phosphate-buffered saline [PBS, (3.0 g KH_2PO_4, 7.0 g $Na_2HPO_4.7H_2O$, 4.0 g and NaCl per liter of distilled water, pH 7.2)] (Leben et al., 1968) and the resultant suspensions were adjusted turbidimetrically to approximately 1×10^8 CFU/ml (0.15 absorbance at 600 nm, model Jenway 6051 Spectrophotometer, UK). Two drops of Tween 80 from a P10 ml glass pipette were added per 200 ml of inoculum suspension to enhance wetting of the leaf surfaces upon application. The required number of cells ml^{-1} was obtained by diluting the suspension.

2.3. Survival of epiphytic Xcv populations on lettuce, tomato and pepper plants

Five-week-old transplants of lettuce, tomato and pepper (approximately 6 weeks from sowing seed) were sprayed with 1×10^8 CFU/ml of *Xcv* using a backpack (141T/90) sprayer (Gloria Co. Germany) until runoff. Immediately after inoculation, plants were covered with plastic bags. Two days post inoculation, the plastic bags were removed and the inoculated plants were placed on a greenhouse bench under the conditions described above. Immediately following inoculation (0 weeks after inoculation), approximately 1 g randomly of leaf tissue was harvested from each treatment in three replicates for population determinations of *Xcv* on each sampling date. The 1 g of leaf tissue sample consisted from either 6–9 leaves of tomato or pepper or one leaf of lettuce. The leaf tissue sampling was repeated weekly for eight weeks after inoculation. All samples were obtained from asymptomatic leaves. Each sample was immediately placed in a plastic bag in a cooler and transported to the laboratory. The fresh weight of each sample was determined, and the tissue was placed in a 250 ml Erlenmeyer flask containing 10 ml sterilized tap water per gram of plant tissue. Flasks were shaken on KS 500 shaker (Janke & Kunkel Co. Germany) for 30 min at 175 rpm. The wash water from each sample was serially diluted and plated on triplicate GNA plates (Robinson et al., 2006). The plates were incubated at 28 °C for 72 h. The colonies were counted and expressed as colony forming units (CFU) per gram fresh tissue. Each experiment was defined as the inoculation of *Xcv* or PBS to 15 pots (one plant/pot) of lettuce, tomato or pepper with three replications. The experiment was arranged in a randomized complete block design and repeated twice. Disease incidence and severity were assessed for each plant species weekly.

2.4. Population dynamics of Xcv in leaves of lettuce, tomato and pepper plants

Leaves of five-week-old lettuce, tomato and pepper plants were infiltrated with approximately 0.03 ml bacterial suspension at 10^5 CFU/ml per leaf using a hypodermic syringe without needle. Five leaves per plant were infiltrated and marked at the time of inoculation with a white correction pen. Inoculated plants were placed on a greenhouse bench under the same previously described conditions. One leaf from each plant species was removed from each replicate with three replications infiltrated with *Xcv* or PBS. Each leaf was processed individually for *in vivo* population determinations and final populations were reported as an average of 15 plants for each sampling date (0, 3, 5, 8, 10 and 14 days after infiltration) (Robinson et al., 2006). Leaves were collected and surface sterilized by dipping them in a 10% NaOCl for 15 s and rinsed three times in sterile deionized water for 1 min. Leaves were blotted on sterile paper towels until dry. A 1 cm^2 area of leaf tissue surrounding at the inoculation site was cut out, placed in 2 ml PBS and macerated in plastic bag using a ball bearing tissue grinder (Ecomcat Co. Germany). Serial tenfold dilutions were performed in sterile distilled water and 0.1 ml of the appropriate dilutions were spread on GNA plates in triplicate. The concentrations of bacterial populations were calculated based on CFU/cm^2 of leaves. Each inoculation treatment (10^5 CFU/ml or PBS) was applied to fifteen plants per replicate with three replications. The experiment was also arranged in a randomized complete block design and repeated twice. Disease incidence and severity were assessed for each plant species weekly.

2.5. Possible chemical management of BLS of lettuce under greenhouse conditions

The efficacy of Copper hydroxide, Copper sulfate WP and Dithane M-45 with the rate of application at 5.5, 4.5 and 2.5 g per liter respectively, and a combination of Copper hydroxide and Dithane M-45 were evaluated in reducing disease incidence and severity of BLS of lettuce under greenhouse conditions (Table 1). Four-week-old transplants of lettuce were inoculated with *Xcv* at a rate of 1×10^8 CFU/ml as described previously. Bactericide treatments were applied every week for three weeks using a backpack 141T/90 sprayer until runoff starting 24 h prior to inoculation. Disease incidence and severity were assessed for each plant one week before harvest. Every week, 10 plants were selected arbitrarily and evaluated for disease symptoms. Disease severity was rated using a 0 to 5 scale, in which 0 = no visible symptoms, 1 = a few individual lesions (<3 mm), 2 = many individual lesions (>3–5 mm), 3 = small patches of coalesced lesions (>5–8 mm), 4 = medium sized patches of