SUMMARY

Epiphytic populations of Xanthomonas citri subsp. citri (Xcc) were found on symptomless grapefruit and mandarin fruits for as long as six and five days of incubation under laboratory and orchard conditions in Saudi Arabia, respectively. No differences were noticed between populations of the bacteria in the susceptible cultivar of grapefruit and the moderately susceptible cultivar of mandarin under both conditions. The occurrence of epiphytic populations was not related to subsequent appearance of symptoms on branches or leaves directly below contaminated fruits. A single chlorine treatment at 200 ppm without prewashing in water did not reduce total Xcc recovery compared to the control. However, prewashing fruit and treating with chlorine significantly reduced total bacterial recovery compared to chlorine treatment alone with both varieties.

Key words: population dynamics, Xanthomonas citri subsp. citri, Saudi Arabia, postharvest treatments.

INTRODUCTION

Citrus (Citrus spp.) has been cultivated as a commercial crop in Saudi Arabia since 1983. The total area of citrus in Saudi Arabia reaches 14,884 ha and the yield may reach approximately 150,000 tonnes per year (Ministry of Agriculture, Central Administration of Economic Studies and Statistics, 2008). Citrus canker is one of the most feared diseases of citrus in Saudi Arabia, affecting all important types of citrus. The disease causes extensive damage and severity varies with different species and varieties and the environmental conditions. The disease is endemic in Saudi Arabia and eradication has been attempted and failed (Whiteside et al., 1988; Schubert and Miller, 2000).

Xanthomonas citri subsp. citri (Xcc) can survive in high numbers within foliar, stem and (green) fruit lesions. Survival of the bacterium within lesions or on the fruit surface is a key to fruits acting as disseminators of inoculum (Schubert et al., 2001; Gottwald et al., 2002; Shiotani et al., 2009). Various regulatory risk analyses have been conducted to evaluate the potential of fruits to act as a pathway for Xcc dissemination and establishment in new previously unaffected citrus-producing areas (Anonymous, 2007).

These analyses are based on existing published data for Xcc spread, transmission and disease establishment. However, critical information gaps remain, that if bridged, would more clearly establish potential risk from these sources (Shiotani et al., 2009). The epidemiological potential of harvested fruits to produce inoculum and act as source of infection has only recently been considered, in a single study on Satsuma mandarin from Japan (Shiotani et al., 2009). They found no evidence for prolonged survival of epiphytic bacteria on healthy fruit surfaces or on discarded fruits within an orchard after three days. These results, while indicative, are not necessarily directly comparable to the situations in Saudi Arabia where different hosts and environmental factors are involved.

The effectiveness of chlorine immersion (200 ppm for 2 min at pH 7.0) of fruits, which is used for exports from citrus canker-infested areas in the world, was mandated by the US Department of Agriculture as a prerequisite for exports of Mexican citrus into the United States. Current and packinghouse measures which are used for exports from citrus canker-infested areas in the world are not completely effective: the authors (Ibrahim and Al-Saleh, 2009) were able to detect viable Xcc on symptomatic fresh citrus fruits in shipments from Pakistan and China to Saudi Arabia in 2009. Shipments, from which Xcc-infected citrus fruits were sampled, were certified to have been treated with bactericides (chlorine or sodium orthophenylphenate (SOPP). Samples were positive in PCR and isolation/pathogenicity tests. These results indicate that the disinfection protocols used were not completely effective. Similar results were obtained from tests on shipments from Argentina and Uruguay to Spain. In 11 out of 15 shipments Xcc
was isolated from fruits. Again in this case, shipments from which Xcc-infected citrus fruits were sampled were certified to have been treated with bactericides chlorine or SOPP (Golmohammadi et al., 2007).

There is no information on the epidemiology of citrus canker disease under Saudi Arabian conditions. Considering these facts, the overall objective of this work was to determine if symptomless fruits that have not undergone post-harvest treatments can transmit Xcc under Saudi Arabian conditions.

First, we examined the length of time epiphytic Xcc can survive on contaminated grapefruit and mandarin fruits after incubation under laboratory and orchard conditions. Secondly, we evaluated the potential transmission of this bacterium to susceptible host fruits under orchard conditions. Thirdly, we evaluated the effects of some post-harvest treatments of fruits on survival of the bacteria.

MATERIALS AND METHODS

Bacterial strain and plant material. X. citri subsp. citri, (Xcc-A), strain N3 recovered from a commercial navel orange fruit and identified with the primer pair 4/7 and Box PCR to Xcc in 2008 (Ibrahim and Al-Saleh, 2009) was used in this study. Fruits of Duncan grapefruit (Citrus paradisi) as a highly susceptible variety, and mandarin (C. reticulata cv. Kinnnow) as moderately resistant variety were obtained from a commercial orchard in Riyadh city.

Survival of Xcc on the surface of citrus fruits under laboratory and orchard conditions. The bacterial inoculum was prepared from a 16-h culture at 28°C on potato semisynthetic agar (PSA) medium (Wakimoto, 1967). Plates were flooded with sterile phosphate-buffered saline [(PBS, (3.0 g KH₂PO₄, 7.0 g Na₂HPO₄·7H₂O, 0.4 g NaCl per litre of distilled water, pH 7.2)] (Leben et al., 1968) and resulting suspensions were adjusted turbidimetrically to approximately 10⁶ CFU/ml⁻¹. Two drops of Tween 80 were added per 200 ml of inoculum suspension to enhance wetting of the fruit surfaces. One hundred mature fruits each of grapefruit and mandarin were soaked in the bacterial suspension for 5 min in 200 ml sterile PBS and the suspension was diluted to 10⁻¹ and 10⁻² and 100 ml spread in triplicate on KCB medium [nutrient agar plus 16.0 mg l⁻¹ kasugamycin, 16.0 mg l⁻¹cephalexin, and 12.0 mg l⁻¹ chlorothalonil, according to Graham and Leite (2004)]. Plates were incubated at 28°C for 3-7 days. Numerous yellow, mucoid bacterial colonies were counted and expressed as CFU ml⁻¹.

Potential spread of citrus canker disease under orchard conditions. In April 2007 and 2008, 100 mature fruits of grapefruit and of mandarin were soaked in the bacterial suspension for 5 min as described above. The contaminated fruits were kept dry at room temperature for 24 h. Four fruits per tree were distributed randomly beneath lemon trees in an orchard in Riyadh city, at Dirab Research Station. Disease incidence was assigned to each tree based on lesion abundance.

Effect of post-harvest treatments on survival of Xcc on grapefruit and mandarin fruits. To determine the effect of chlorine as a disinfectant alone or following pre-washing fruits with tap water on bacterial survival on grapefruit and mandarin fruits, three replications of five contaminated fruits as described above were treated as follows: (1) untreated control, (2) chlorine immersion (200 ppm for 2 min at pH 7.0) and (3) prewash with tap water followed by chlorine immersion. The water washes were performed for 1 min on rotating, soft bristled brushes with an overhead sprayer. To determine the population of Xcc on fruits, individual fruits from each plant variety were assayed for total surface bacteria as described above.

RESULTS

Survival of Xcc on the surface of citrus fruits under laboratory and orchard conditions. From symptomless grapefruit and mandarin fruits treated with the bacterial suspension, the bacteria were not detected five days post-inoculation (dpi) under orchard conditions (Fig. 1 and 2). The Xcc population was statistically similar at 0 and 1 dpi on both plant varieties and then decreased with increasing duration post-incubation. No differences were observed between bacterial populations on the susceptible grapefruit and the moderately susceptible mandarin cultivars. Under laboratory conditions
Bacterial populations were detected on both cultivars up to 6 dpi.

**Potential spread of citrus canker disease under orchard conditions.** During the two experiments started on April 2007 and June 2008, no canker symptoms were observed on any branches or leaves of lemon trees below which the contaminated fruits were placed. This may be because weather conditions were unfavorable for disease spread during this period.

**NaOCl treatment of grapefruit and mandarin fruits.** Numbers of recoverable bacteria varied among treatments ($F = 114.1, F < 0.0001$) based on SAS analysis. The chlorine treatment alone for 2 min in 200 ppm Cl (as NaOCl), did not reduce recovery of total bacteria ($Xcc$ plus other bacteria) compared to the control (Fig. 3) based on Tukey’s means separations. No differences were observed between populations of bacteria on grapefruit or mandarin fruits. Prewashing fruit and treating with chlorine significantly reduced the total bacterial count compared to the chlorine treatment alone, with both plant varieties. No apparent economic damage to the fruits was observed after a 2-min treatment in NaOCl at 200 ppm especially for mandarin.

**DISCUSSION**

The increase of a pathogen population on plants in the absence of symptoms may be of epidemiological importance by building up inoculum, serving as a source of inoculum for secondary spread, and providing pathogen cells that may survive in unfavorable condi-

---

![Fig. 1. Epiphytic survival of *X. citri* subsp. *citri* strain N3 on the surface of grapefruit fruits under orchard and laboratory conditions. Data are mean of three replications repeated twice, ± standard error.](image1)

![Fig. 2. Epiphytic survival of *X. citri* subsp. *citri* strain N3 on surface of mandarin fruits under orchard and laboratory conditions. Data are mean of three replications repeated twice ± standard error.](image2)

![Fig. 3. Effects of chlorine treatment at 200 ppm and prewashed followed by chlorine (200 ppm) on the population of total bacteria of grapefruit (A) and mandarin (B) fruits. Fruits were soaked in a $10^6$ CFU/ml bacterial suspension of *Xcc* strain N3 for 5 min and treated as follows: 1) untreated control, 2) immersed in chlorine (200 ppm) and 3) prewashed with tap water followed by immersion in chlorine. Bars with the same letters are not significantly different from each other based on Tukey’s means separation.](image3)
tions (Cafati and Saettler, 1980).

Epiphytic populations of Xcc were found on symptomless fruits of grapefruit and mandarin for as long as 6 dpi at room temperature under laboratory conditions. These results corroborate previous research that Xcc only survives on symptomless fruits sprayed with bacterial suspension of 10^6 CFU/ml^1 for five days at room temperature under laboratory conditions (Belasque and Rodriguez Neto, 2000). However, under orchard conditions, populations of Xcc were no longer detected on both fruits by the fifth dpi. Goto (1962, 1968) found that Xcc only survives for a limited time (8-72 h) in the sun and shade on fruit. The shorter survival of epiphytic Xcc populations on symptomless fruits under natural conditions would be expected due to natural environmental conditions being harsher than controlled laboratory conditions. In addition, the differences between the survival of Xcc duration reported in the two studies might be due to differences in plant cultivars used.

Shiotani et al. (2009) examined the duration of epiphytic Xcc survival on fruit surfaces under natural conditions in Japan. They reported that inoculated fruit sampled on day 0 yielded viable Xcc, but thereafter, up to 21 days, no viable Xcc were recovered. Variation in epiphytic populations from leaf to leaf on the same plant species can be significant even if plants are grown in similar environmental conditions and it has been shown that different plant species can support different populations of epiphytic bacteria (O’Brien and Lindow, 1989). Factors that affect development and growth of epiphytic bacterial populations are moisture, ultraviolet radiation and nutrients (O’Brien and Lindow, 1989; Mercier and Lindow, 2000).

No canker symptoms were observed on any branches or leaves that were directly below the contaminated fruits. This might be because weather conditions were unfavorable for disease spread during this period. As a condition for successful establishment of Xcc in amounts sufficient to cause infection, the bacterium must encounter not only an environment with a conducive temperature, relative humidity, moisture, and wind events for infection, but also enter a host plant tissue that is either at a susceptible growth stage or wounded, and then must successfully enter this tissue. In addition, fruits discarded in orchards begin to rot within nine days and this is not favorable for Xcc survival (Fulton and Bowman, 1929). Shiotani et al. (2009) reported that contaminated fruits which were harvested from trees with citrus canker symptoms did not give rise to infection in the field under natural conditions nor was viable Xcc detectable on contaminated fruits after brief field exposure to nearby inoculum sources. Our data indicate that mature fruits are not a significant risk as a source of bacteria for infection of citrus, and the epidemiological significance of surface contamination on mature fruits in the spread of bacteria of Xcc is probably minimal.

Our results show that treatment for 2 min in 200 ppm chlorine (as NaOCl) did not reduce recovery of total numbers of bacteria (Xcc plus other bacteria) as compared to the control. These results are in contrast to those obtained by Canteros et al. (2000), who stated that post-harvest treatments, such as chlorine or SOPP, guarantee the complete eradication of epiphytic Xcc on fruits without symptoms. These differing results could be attributed to different bacterial loads on the fruit being treated. Results of a previous study showed that four strains of Xcc did not survive a 2-min exposure to more than 10 ppm chlorine in aqueous suspension (Stapleton, 1986), suggesting that bacteria would not survive in packing shed dip-tank NaOCl solutions. Both intact and wounded fruit surfaces may provide bacteria with protected sites, allowing them to evade the effects of chlorine treatment. In addition, fruits enter dip-tank solutions with some associated detritus and organic matter, which significantly reduces the bactericidal properties of chlorine (McCulloch, 1936). The failure of NaOCl to completely disinfect fruit allows the possibility of pathogen dissemination on chlorine-treated fruits. Prewashing fruits followed by treating with chlorine significantly reduced total bacterial counts compared to chlorine treatment alone with both varieties, but did not completely eradicate surface bacteria from clean fruit. Gottwald et al. (2009) showed that prewashing fruit and treating with chlorine significantly reduced total bacterial recovery compared to chlorine treatment alone, and prewashing fruit followed by chlorine and detergent was the most effective method for killing bacteria on the fruit surface.

We conclude that that symptomless, commercially produced fruits are not a likely pathway for the transmission of Xcc in the natural environment in Saudi Arabia, and the occurrence of epiphytic populations of Xcc is not related to subsequent appearance of citrus canker symptoms. Post-harvest treatments (including a prewash) substantially reduce bacterial populations on fruits. However, the effects of disinfesting agents more active than NaOCl should be tested.

**REFERENCES**


