

deepest soils. This suggests that factors other than soil depth are involved. It may be that plants on well drained soils with depths >75 cm require more water than was supplied by precipitation or irrigation. It also may be that water and nutrients in shallower soils are closer to the roots and plants due to the presence of the hardpan. If this is the case plants may not have to expend as much energy in the production of roots to reach the pool of nutrients. In deeper soils the nutrients may have been leached lower in the soil profile. The optimum amount of water, and the distribution of nutrients and/or water in soils of various depths, required for optimum bean production needs further study.

Although conservation management can cause problems with dry bean germination on cool soils (Hardwick, 1988), and disease and moisture retention in soils in some parts of the United States (Webber et al., 1987), the soil types in southeastern Oklahoma tend to dry and warm quickly in the spring. It does not appear that the lower yields for beans with reduced-tillage were due to wet, cold soil.

Dry bean yields under conservation tillage are reported to be less than under plow and rotary-till systems (Smith and Yonts, 1988). This was the case for both bean cultivars in this study. Black bean yields approached United States yield averages only under conventional tillage, and in soils shallower than 75 cm. The input reduction due to the lack of disking in soil preparation and field maintenance under reduced-tillage would not provide sufficient financial incentive to use this tillage method for plants grown on the most productive soil depths. More work is necessary to clarify the optimum conditions for dry bean production in the Southern Plains. This is especially necessary to better understand effects that soil depths in the region have on plant development and yield.

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Peel Injury on 'Marsh' Grapefruit from Quaternary Ammonia

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ADDITIONAL INDEX WORDS. phytotoxic chemical injury, chlorine, surfactant, Triton N-101, *Citrus paradisi*

SUMMARY. Quaternary ammonia (QA) has been used on equipment and fruit bins in Florida to reduce the risk of spreading citrus canker. This study was initiated to understand the cause of a previously unknown peel injury believed to be associated with QA residues. Symptoms of QA injury on 'Marsh' grapefruit (*Citrus paradisi*) usually developed within 24 to 36 h of contact with QA and ranged in severity from very slight discoloration to severe, dark brown, necrotic peel tissue that collapsed to form large sunken areas. Placing fruit in 10 mL (0.34 fl oz) of ≥ 100 mg·L⁻¹ (ppm) fresh QA solution caused moderate to severe peel injury. Drying the QA solutions on polystyrene petri dishes and then redissolving the residue with 10 mL deionized water before fruit contact resulted in essentially the same degree of peel injury as contact with fresh QA solutions. Peel injury on early (November) or late-season (April) grapefruit also occurred when fruit were placed on a thin film of QA solution left on polystyrene petri dishes after dipping the dishes in ≥ 300 mg·L⁻¹ QA solutions or if fruit themselves were dipped in QA solutions ≥ 500 mg·L⁻¹. No significant peel injury occurred when dipping solutions contained only water with 200 mg·L⁻¹ chlorine, 0.025% (v/v) Triton N-101, or a combination of both.

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Peel injury can significantly reduce the marketability of fresh citrus (*Citrus* spp.) fruit. Early season fruit in particular appear more sensitive to various disorders including phytotoxic injury from common postharvest chemicals (Grierson and Newhall, 1960; Ritenour and Dou, 2000). Early in the 2000-01 season, there were scattered reports of a previously unreported peel injury on Florida citrus (Fig. 1). Unlike the previously reported Green Ring disorder of early season fruit (Ritenour and Dou, 2000), this peel disorder developed in the absence of any postharvest fungicide drench treatment.

Immediately before these reports, widespread use of 2000 mg·L⁻¹ quaternary ammonia was initiated to sanitize equipment and fruit bins to prevent the spread of citrus canker (*Xanthomonas axonopodis* pv. *citri*). QA is not registered for direct fruit contact and a water rinse is required if ≥ 200 mg·L⁻¹ QA is applied to equipment or brush surfaces that directly contact fruits and vegetables (Office of the Federal Register, 2000). It is unclear what concentrations of QA result in peel injury to fresh grapefruit. Commercially, injured fruit often appeared at the bottom of bins or on fruit in contact with the bin sides. These observations suggested that QA residues on bins, spray drift from grove equipment sanitation stations, or other means of inadvertent fruit contact with QA might be involved in the development of this peel injury. The present study was conducted to determine if exposure to QA results in the development of this new peel injury on 'Marsh' grapefruit and, if it is involved, to determine which concentrations result in peel injury.

Materials and methods

'Marsh' grapefruit were used for all experiments. QA solutions of 0 (water alone), 100, 300, 500, 1000, and 2000 mg·L⁻¹ were prepared from a commercial QA product (CS-170-C; 21.7 % ammonium chloride forms of QA; Chemical Systems of Florida, Inc., Zellwood, Fla.). The pH of the QA solutions was not adjusted but increased as QA concentrations increased from 100 mg·L⁻¹ (pH 7.8) to 2000 mg·L⁻¹ (pH 10.3). Citrus peel is commonly exposed to solutions of pH 8.5 to 12 without peel injury in commercial packinghouse operations (Pao et al., 1999; Wardowski and Brown, 1993). However, QA compounds have been reported to be more

active at higher pHs (Schmidt, 1997). All current experiments were conducted in a completely randomized design with 10 replicates (fruit) per treatment. Peel injury was evaluated on a scale of 1 (no injury) to 5 (severe injury) (Fig. 1).

EXPERIMENT 1. In the first experiment (17 Nov. 2000), fruit were obtained from a local packinghouse after they had been degreened with ethylene [standard conditions of ≈ 2 to 5 $\mu\text{L}\cdot\text{L}^{-1}$ (ppm) at 29 °C (84.2 °F) for 2 to 3 d], but had not received fungicide or any other postharvest treatments. Fruit were transported to the laboratory where they were placed in 100 mm diameter polystyrene petri dishes containing either 10 mL of fresh QA solution, QA residue left after forced-air-drying 10 mL of QA solution (which formed a greasy film on the bottom of the petri dish), or redissolved QA residue using 10 mL of deionized water. In all experiments, fruit were placed in the petri dishes on their sides so that the equatorial region contacted the dish. Peel injury was evaluated after 3 d at ambient laboratory conditions [21 °C (69.8 °F) with about 50% relative humidity (RH)]. At the end of the experiment, QA concentrations remaining on the dishes were measured using pHydration test paper (QT-10; Micro Essential Laboratory, Inc., Brooklyn, N.Y.) and QACQR test strips (code 2951; LaMotte, Chestertown, Md.) and both fresh and redissolved QA solutions were found to have maintained their approximate original concentration levels up to 500 mg·L⁻¹ (the limit of the testing strips).

EXPERIMENT 2. To better simulate QA-fruit contact that might occur commercially from bin surfaces or on fruit that received inadvertent QA spray drift, a second experiment was conducted on 22 Nov. 2000. Fruit were obtained from a local packinghouse where they had been degreened as before, but had not received fungicide or any other postharvest treatments. In the laboratory, polystyrene petri dishes were dipped into one of the QA solutions (0 to 2000 mg·L⁻¹) and the adhering solutions were allowed to drip off for 5 s. In this case, only a thin film (≈ 0.3 mL) of solution remained on the dishes. Untreated, dry fruit were placed on the moist QA residue remaining on dipped petri dishes. An additional set of petri dishes was dipped in 2000 mg·L⁻¹ and then forced-air dried before placing a dry, untreated fruit on each dish. In this case, the dry residue was not greasy

because only a thin film of QA was dried instead of the 10 mL previously used. Finally, whole fruit were lowered half way into the QA solution for about 2 s and then, with the treated side facing down, were placed on untreated, dry petri dishes. Weighing fruit before and after dipping revealed that about 0.8 mL of solution adhered to the fruit. Peel injury was evaluated after 5 d at 29 °C with 95% RH (simulated commercial degreening conditions).

EXPERIMENT 3. The above experiment was repeated 5 Apr. 2001 on late-season fruit harvested from a local grove and transported directly to the laboratory without degreening (fruit color was already well developed). Additional treatments for the Spring experiments included 200 mg·L⁻¹ sodium hypochlorite (pH 7.5; FMC Corp., Lakeland, Fla.), 0.025% (v/v) Triton N-101 (nonionic surfactant; FMC Corp.), or a combination of both. The chlorine treatment is an approved alternative sanitation method for citrus canker. Test of a surfactant was included because previous work suggested that surfactants may play an important role in the development of phytotoxic peel injuries of citrus (Albrigo and Grosser, 1996; Coggins and Henning, 1988; Ritenour and Dou, 2000). Peel injury was evaluated after 4 d at 20 °C (68.0 °F) with 80% RH. The fruit were then washed and waxed and placed back in storage for an additional 7 d under the same conditions before a final evaluation to determine if simulated commercial packingline treatments exacerbated the injury.

Data were analyzed by ANOVA using SAS (PROC GLM) for PC (SAS Institute Inc, Cary, N.C.). When differences were significant ($P \leq 0.05$), least significant differences (LSD) were calculated at the 0.05 level.

Results and discussion

Preliminary and current studies show that symptoms of QA injury usually develop within 24 to 36 h after QA contact (data not shown) and range in severity from very light peel discoloration to severe, dark brown, necrotic peel tissue that collapses to form sunken areas (Fig. 1). Intermediate symptoms often are manifest as small, necrotic specks that increase in density and size as symptoms progress and eventually begin to coalesce. Even the most severe injury appears to only affect the flavedo and outer albedo tissue

of the peel. Injured areas of the peel were limited to those areas in contact with the QA compounds. This agrees with

other published reports stating that QA compounds are surface active and break down the cell walls of microorganisms

(Petrocci, 1983). In preliminary tests, three commercial QA products from different companies resulted in similar peel injury to navel oranges (*Citrus sinensis*; data not shown).

EXPERIMENT 1. Placing whole grapefruit in 10 mL of QA solution caused moderate to severe injury at concentrations as low as 100 mg·L⁻¹ (Fig. 2A). Peel injury increased as QA concentrations increased to 1000 mg·L⁻¹ QA, at which concentration all fruit developed severe injury. The average peel injury on fruit exposed to between 300 and 2000 mg·L⁻¹ of fresh QA did not differ significantly. Control (water alone) treatments resulted in no injury.

Exposure of fruit to redissolved QA residues resulted in similar peel injury as exposure to fresh QA (Fig. 2B). Thus, even dry QA residue on plastic surfaces (e.g., bins) can injure fruit if redissolved (e.g., by condensation or wet fruit). While the residual QA antimicrobial film can be an advantage for sanitation purposes (Schmidt, 1997), it can also contribute to the development of this peel disorder.

Though levels of peel injury tended to be lower from contact with redissolved solution than from contact with fresh solution, differences were not significant. The consistent trend suggests that some QA activity might have been lost during drying and redissolving. Our measurements of QA activity after drying did not indicate a loss of activity, however, the measurement techniques lacked the precision needed to accurately detect small possible losses. Dried residue from 2000 mg·L⁻¹ QA that was not redissolved but left as a greasy film caused moderate injury that was not significantly different in severity from injury from redissolved QA residue (Fig. 2C).

EXPERIMENT 2. Placing early-season (November) fruit on petri dishes containing a thin film of 100 or 300 mg·L⁻¹ QA resulted in no significant peel injury (Fig. 3A). Likewise, partially dipping whole fruit in 100 or 300 mg·L⁻¹ QA solutions resulted in no peel injury (Fig. 3B). However, peel injury significantly increased when fruit were placed on dishes dipped in 500 mg·L⁻¹ QA and injury increased significantly as QA concentration to 2000 mg·L⁻¹ (Fig. 3A). Peel injury also increased significantly when whole fruit were dipped in 500 mg·L⁻¹ QA, but injury did not significantly increase further until 2000 mg·L⁻¹ QA was used (Fig.

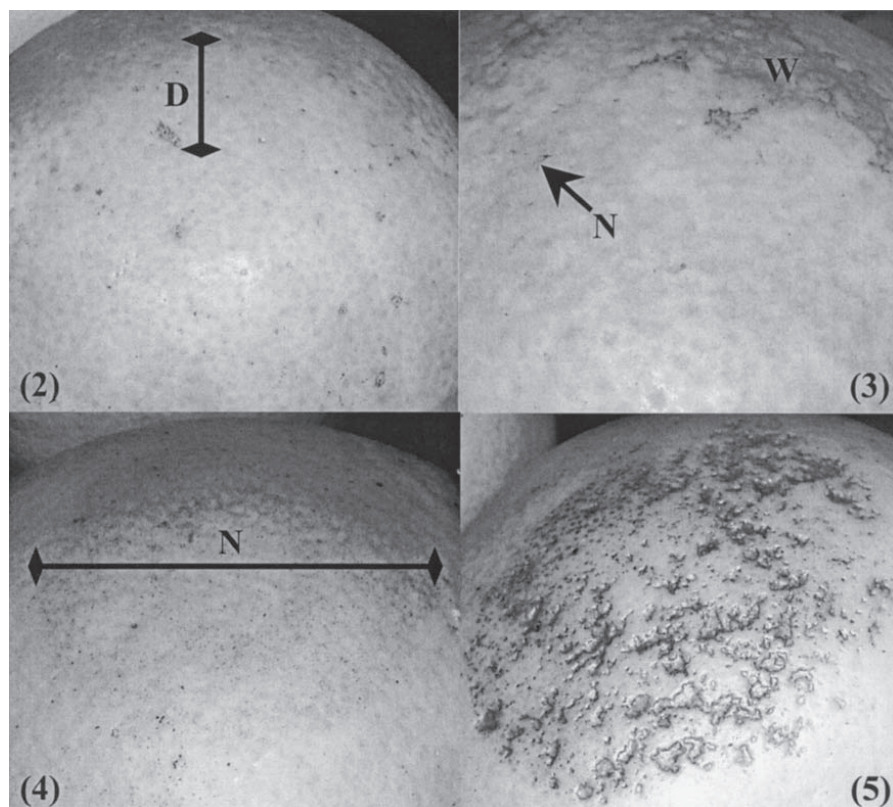


Fig. 1. Quaternary ammonia (QA) injury on 'Marsh' grapefruit. Injury was rated on a scale of 1 (no injury) to 5 (severe injury). Numbers in parentheses indicate corresponding injury rating (fruit with no injury not shown). Early QA injury symptoms exhibited light peel discoloration (D), with small necrotic spots (N) coalescing as injury progressed into larger depressions. Wind scarring (W) was not related to the QA injury.

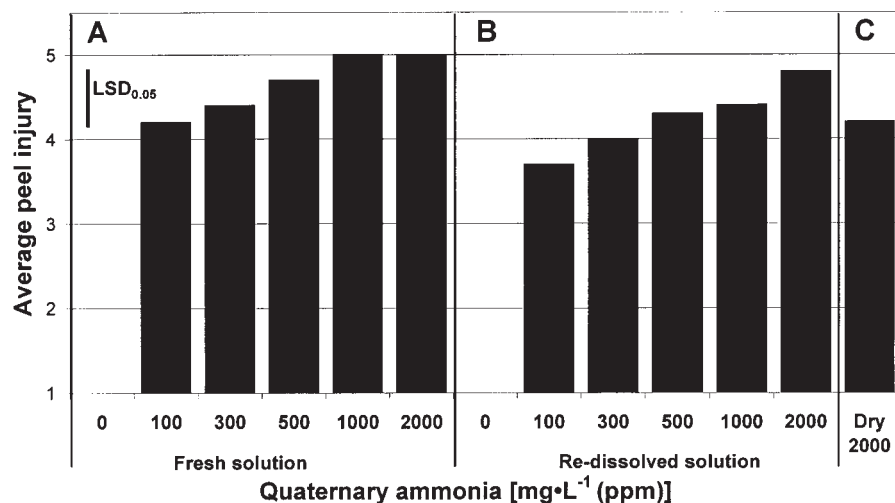


Fig. 2. Average peel injury of early-season (November) 'Marsh' grapefruit after exposure to 10 mL of quaternary ammonia (QA) solution (A) or QA solutions that were forced-air dried and then redissolved with 10 mL (0.34 fl oz) deionized water (B) or QA solutions that were forced-air dried and left as a greasy film on the dish (C). QA concentrations (0 to 2000) are expressed as mg·L⁻¹ (ppm). Fruit were evaluated after 3 d at 21 °C (69.8 °F) with about 50% relative humidity. Peel injury was scored visually using a scale of 1 (none) to 5 (severe). The vertical bar represents the 5% least significant difference (LSD) value.

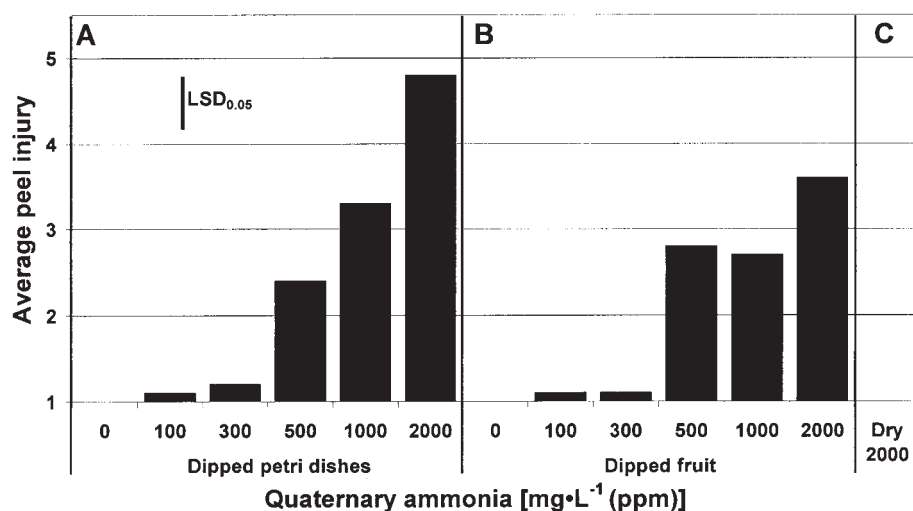


Fig. 3. Average peel injury of early-season (November) 'Marsh' grapefruit that were placed on polystyrene petri dishes dipped in quaternary ammonia (QA) solutions (A), or of whole fruit dipped in QA solutions (B). For the dry treatment (C), remaining 2000 mg·L⁻¹ (ppm) QA solution on the dipped petri dishes was dried before fruit were placed on the dish. QA concentrations (0 to 2000) are expressed as mg·L⁻¹. Peel injury was evaluated after 5 d at 29 °C (84.2 °F) with 95% relative humidity (simulated commercial degreening conditions). Peel injury was scored visually using a scale of 1 (none) to 5 (severe). The vertical bar represents the 5% least significant difference (LSD) value.

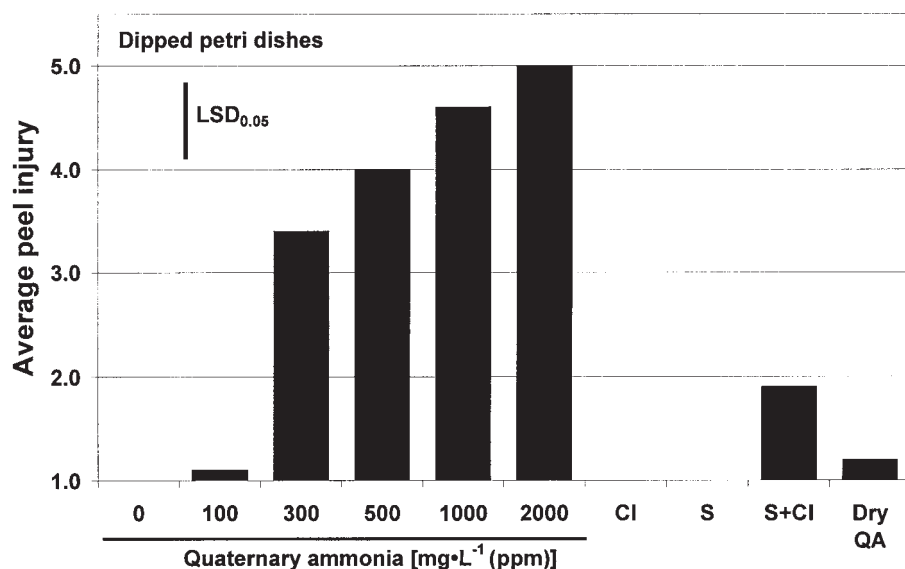


Fig. 4. Average peel injury of late-season (April) 'Marsh' grapefruit that were placed on polystyrene petri dishes dipped in quaternary ammonia (QA) solutions. QA concentrations (0 to 2000) are expressed as mg·L⁻¹ (ppm). For the dry treatment, remaining 2000 mg·L⁻¹ QA solution on the dipped petri dishes was dried before fruit were placed on the dish. CI = dishes were dipped in water with 200 mg·L⁻¹ sodium hypochlorite (pH 7.5). S = dishes were dipped in water with 0.025% (v/v) Triton N-101. Fruit were washed and waxed after 4 d storage at 20 °C (68.0 °F) with 80% relative humidity and then stored an additional 7 d under the same conditions before being evaluated for injury. Peel injury was scored visually using a scale of 1 (none) to 5 (severe). The vertical bar represents the 5% least significant difference (LSD) value.

3B). Peel injury tended to be more severe when fruit were placed on QA solutions adhering to petri dishes compared to when whole fruit were dipped in the QA solutions; Only at the 2000 mg·L⁻¹ concentration were differences

significant. As was expected, peel injury was more severe on fruit placed in 10 mL of fresh or redissolved QA (Fig. 2) than on fruit dipped in QA or placed on petri dishes dipped in QA (Fig. 3A and B).

Peel injury on early- (Fig. 3C) and late-season (Fig. 4) grapefruit that had been placed on dry QA residue remaining after dipping petri dishes in 2000 mg·L⁻¹ QA were not significantly different from controls. Unlike the 10 mL of QA solution that dried to leave a greasy film, the small amount of QA residue left on dipped petri dishes dried completely.

EXPERIMENT 3. As with early-season fruit, placing late-season (April) fruit on petri dishes with a thin film of 100 mg·L⁻¹ QA solution resulted in no significant peel injury (Fig. 4). However, peel injury increased sharply when fruit were placed on petri dishes dipped in 300 mg·L⁻¹ QA and injury increased significantly as QA concentrations increased to 2000 mg·L⁻¹. Compared to the evaluation 4 d after QA exposure (data not shown), average peel injury tended to be slightly greater (0.2 units) after fruit were washed and waxed and then stored for an additional 7 d. The increase may have been related to an enhanced ability to observe peel injury after washing and waxing the fruit.

When late-season whole fruit were dipped in different QA solutions, significant injury was again not observed until QA concentrations reached 500 mg·L⁻¹ (Fig. 5). At 300 mg·L⁻¹ QA, some fruit exhibited peel discoloration indicative of early injury, but levels of injury were not significantly different from the control. Peel injury increased as QA concentrations increased from 500 to 2000 mg·L⁻¹ at which point all fruit were severely injured. As was observed with early-season fruit, peel injury tended to be more severe when fruit were placed on QA solutions adhering to petri dishes compared to when whole fruit were dipped in the QA solutions; In this case, only at the 300 mg·L⁻¹ concentration were differences significant.

Although QA-induced citrus peel injury tended to be lower on the early-season fruit compared to the late-season fruit, these fruit came from different groves and such slight differences in susceptibility to QA injury could just as well be related to grove location, cultural practices, weather conditions, etc. Overall, it is clear that the development of peel injury from QA exposure can occur in both early- and late-season grapefruit and that the severity of injury increases as the level of QA exposure increases. Peel injury from 2000 mg·L⁻¹ QA has also been observed in midsea-

son (December and early January) navel oranges and 'Dancy' tangerines (*Citrus reticulata*; data not shown).

Dipping petri dishes or fruit in water with 200 mg·L⁻¹ chlorine, 0.025% (v/v) Triton N-101, or a combination of both did not result in significant peel injury (Figs. 4 and 5). Though not significantly different from the control, we did observe peel discoloration on some fruit placed on petri dishes dipped in water with 200 mg·L⁻¹ chlorine plus surfactant, and fruit dipped in surfactant alone. None of the treatments containing only chlorine resulted in peel injury or discoloration. Ritenour and Dou (2000) found that incidence of peel injury from compounds potentially found in commercial fungicidal drench operations only occurred when a surfactant (Triton N-101) was included. Use of Triton N-101 has declined in postharvest citrus fungicidal drenches not because of issues with peel disorders, but because manufacturers of high density polyethylene bins believe that it weakens the bin strength over time. Taken together, these data suggest that the role of surfactants should be investigated further to determine their potential role in enhancing phytotoxic peel injury.

The deposition of epicuticular waxes on the fruit surface likely has little effect on the sensitivity of citrus peel to injury from QA. Wax content of citrus peel generally increases during fruit growth and development (Albrigo, 1972b) and even after harvest (Schulman and Monselise, 1970), but we found that late-season fruit remained very susceptible to QA injury. In addition, Albrigo (1972a) found thicker epicuticular wax and no stomata within an approximate 3-mm-ring around the button of oranges. However, personal observations of injured fruit from commercial operations revealed that peel injury was not excluded from any portion of the peel. The surfactant properties of QA compounds (Schmidt, 1997) likely facilitate penetration of QA through the fruit cuticle.

The study reported here demonstrates that quaternary ammonia can cause the peel malady observed in the field and describes the concentrations of QA that can result in peel injury of citrus. Since the initial field observations where QA was suspected of inducing peel injury, packinghouses have largely switched to alternative bin sanitizers such as chlorine and peroxyacetic acid

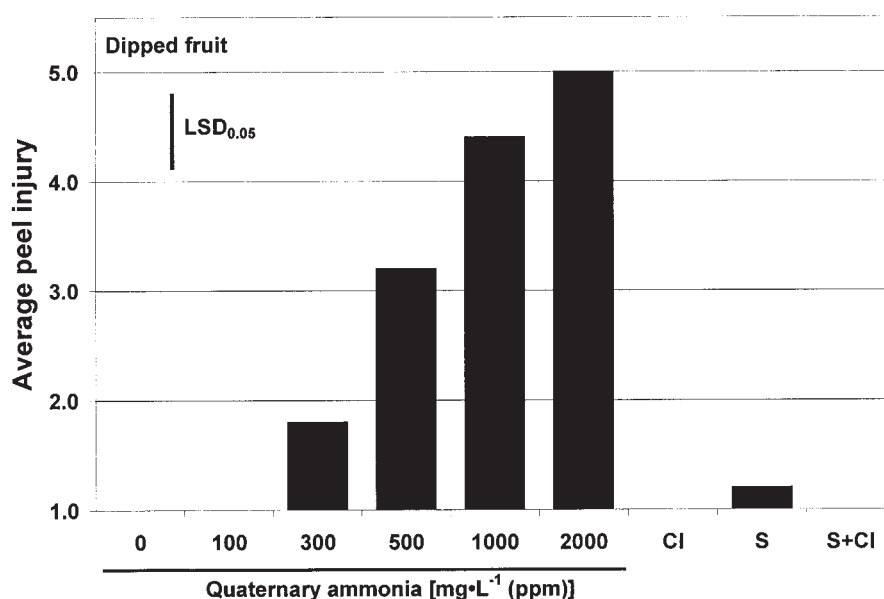


Fig. 5. Average peel injury of late-season (April) 'Marsh' grapefruit that were dipped in quaternary ammonia (QA) solutions and placed on dry, untreated polystyrene petri dishes. QA concentrations (0 to 2000) are expressed as mg·L⁻¹ (ppm). Cl = fruit dipped in water with 200 mg·L⁻¹ sodium hypochlorite (pH 7.5). S = fruit dipped in water with 0.025% (v/v) Triton N-101. Fruit were washed and waxed after 4 d storage at 20 °C (68.0 °F) with 80% relative humidity and then stored an additional 7 d under the same conditions before being evaluated for injury. Peel injury was scored visually using a scale of 1 (none) to 5 (severe). The vertical bar represents the 5% least significant difference (LSD) value.

compounds. Furthermore, in the field, steps have been taken to minimize the possibility of inadvertent QA-fruit contact by turning off upper manifolds of spray stations when trucks with fruit or bins pass through. As a result of these precautions, virtually no reports of QA injury have been reported within the past (2002-03) season.

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