Infiltration of Tomatoes by Aqueous Bacterial Suspensions

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ABSTRACT

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Tomato fruit immersed in bacterial suspensions with negative suspension/fruit temperature differentials (fruit warmer than suspensions) became infiltrated by water and bacteria. Increased weight of the fruit was evidence for water uptake. If the increase exceeded approximately 3% of the original weight, cracking and water-soaking of the fruit surface often developed. Isolation of *Serratia marcesens* (an easily recognizable red bacterium) from internal tissues of fruit previously immersed in suspensions of that organism or the development of bacterial decay in fruit previously immersed in suspensions of *Erwinia carotovora* subsp. *carotovora* (Ecc), *Pseudomonas marginalis*, or *P. aeruginosa* were evidence for bacterial infiltration. In general, the weight increase of treated fruit was correlated with bacterial infiltration. However, disease occurred in fruit that did not have a measurable weight increase (at least 10 mg). Fruits held 1–2 cm below the surface of suspensions of *S. marcesens* under zero or +2 degree differentials were not infiltrated by *S. marcesens* and, in similar tests with decay causing bacteria, rarely became diseased. Fruit with fresh stem scars was more vulnerable to infiltration than was fruit with old stem scars. Green and pink fruits immersed in suspensions of Ecc absorbed more water and became diseased earlier than did similarly treated red fruits. Slight weight increases were noted in fruit held 8 cm below the surface of water baths under zero to +2 degree differential conditions, evidence that hydrostatic forces also cause infiltration. These relatively small weight increases were smaller if +11 to +33 degree differentials were used.

Additional key words: Lycopersicon esculentum, postharvest decay.

Evidence from an investigation into the loss of a fresh-market tomato shipment from Florida led to the conclusion that the fruits were inoculated by being infiltrated in the packinghouse dump tank (1).

Infiltration of tomatoes by water was reported (14,19). Kasmire (14) found that tomatoes at 29 C immersed for 10 min in water at 2, 10, 29, and 41 C increased in weight by 0.37, 0.17, 0.00, and 0.05%, respectively. Studer and Kader (19) reported that immersion of freshly harvested tomatoes in water for 15–120 min resulted in a substantial number of cracked fruits due to water uptake. The fruit cracking was accentuated when warm fruits were immersed in cold water but did not occur if harvested fruits were held overnight before treatment. Showalter (18) observed weight increases up to 4% for tomatoes immersed 8 cm in a water bath that was 18 degrees colder than the incoming fruit. Thus, the infiltration of tomato fruit by water was associated with a negative temperature difference (or differential) between the water and the fruit (water temperature − fruit temperature = differential).

Although tomato fruits are infiltrated with water during the immersion of warm fruit into cooler water baths, the fate of bacteria that might be suspended in the water has not been demonstrated. Segall et al (17) found more decay in fruit previously immersed in a suspension of *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al (Ecc) under positive temperature differential conditions than in fruit that had been immersed with a negative temperature differential. However, in two different reports, tomato fruits were inoculated with bacteria by being immersed in a suspension and then subjected to a vacuum (1,12). Thus, in the experimental conditions used in those reports, the external tissues of tomato fruit did not prevent bacterial ingress. Moreover, Haines and Moran (11) reported 40 yr ago that high precentages of chicken eggs rotted when dipped in bacterial suspensions cooler than the incoming eggs (negative temperature differential). However, if the immersion occurred under positive temperature differentials, the subsequent decay incidence was significantly lower. This report provided the basis for subsequent commercial egg cleaning recommendations (4,5).

In the following, bacterial ingress into tomato fruit was shown to occur as a result of negative temperature differentials and was associated with the uptake of water by the fruit. Portions of this report were previously published in an abstract (18).

MATERIALS AND METHODS

Tomato fruits used for these tests were hand-harvested from several different breeding lines and cultivars grown in Florida. Some fruits were removed from the plants conventionally; the stems (pedicels) remained on the plant. Others were removed from the plants with a pruning shears so that the pedicel remained on the fruit. Fruit ripeness/maturity varied among the tests, but was similar within the different treatments of a given test. All fruits were at least mature green when harvested. All fruits were gently wiped with a towel before treatment to remove sand, debris, spray residues, etc.

Bacteria used included the red bacterium, *Serratia marcesens* Bizio (Sm), and three nomenspecies known to cause decay of tomato fruit: Ecc (Florida isolate SR-12 [2]), *Pseudomonas marginalis* (Brown) Stapp (Pm, isolated from tomato fruits in Florida [1]), and *P. aeruginosa* (Schroeter) (Pa). Inocula used in the various tests, usually at a concentration of 1×10^7 colony forming units (cfu) per milliliter, were prepared from 20- to 28-hr nutrient broth shake cultures as described previously (1–3).

Bacterial suspension, water, and fruit temperatures were measured with a mercury-in-glass thermometer. Weight increases expressed as percentages of the pretreatment fruit weights were determined by weighing fruit to the nearest 10 mg before and after treatments. The fruits were wiped with a towel to remove free water before the final weight measurement.

The infiltration of tomato fruit by water during a 30 min immersion 8 cm below the surface of water baths with different water/fruit temperature differentials was determined with lots of 10 fruits. Bath temperatures ranged from 10 to 43 C; fruit temperatures from 4 to 43 C. The water/fruit temperature differentials ranged from +33 to −35 degrees.

Tests on bacterial infiltration of tomato fruit involved aqueous bacterial suspensions instead of water baths. In preliminary tests with one to four fruits per treatment, suspensions of the red bacterium, Sm, were used. In two of these tests, fruits were warmed to 34 C or cooled to 18 C, then immersed for 30 min in a suspension

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at 18 C. In four other tests, the fruits were 36 and 20 C, the suspension was 22 C and the immersion time was 15 min. After being treated, the fruits were dipped in 0.5% NaOCl for 30 sec, wiped with a cloth wetted with 95% ethanol, and cut into transverse slices 1 cm thick with a sterile knife beginning at the shoulder. A square piece of tissue, 1 cm on each side, was removed from the edge and center of each slice and crushed in 0.5-ml volumes of a buffered saline solution (1–3). A loopful of the resulting macerated tissue was streaked on a nutrient agar plate. The development of red bacterial colonies on the plate within 24 hr was presumptive evidence for infiltration of the fruit with Sm.

Suspensions of the fruit rot bacteria, Ecc, Pm, or Pa, were substituted for the Sm in the remaining tests. Fruit temperatures used ranged from 20 to 40 C. The suspensions were 20, 22, or 40 C. Either eight or 10 fruits were used in each treatment. Treated fruits were rinsed in 50 ppm NaClO, wiped dry, and then stored at 26 C and >80% relative humidity. The development of bacterial lesions around the stem or blossom scar of the fruit was presumptive evidence for infiltration.

Some of the data were analyzed with the one-way analysis of variance, Duncan's new multiple range test or the linear regression/correlation coefficient test.

The usage of temperature differential here is consistent with that in two reports on bacterial uptake during the washing of chicken eggs (4,5). The fruit (or egg) temperature was subtracted from the water or suspension temperature.

RESULTS

Water uptake and the water/fruit temperature differential. Tomato fruit held 8 cm below the surface of a water bath for 30 min increased in weight due to water ingress (Table 1). Weight increases above approximately 3% of the original weight of the fruit were associated with cracking and water-soaking about the stem scar of the fruit. The green fruit gained much more weight than did similarly treated pink fruit. The average weight increases for each grouping of differentials were inversely correlated with the average temperature differential for the respective groups, $r = -0.94$. Thus, fruit treated with a negative differential absorbed more water than did that treated with a neutral or a positive differential. Whereas, fruit treated with the largest negative differential gained the most weight; fruit treated with the largest positive differential gained the least. However, the amount of uptake varied among individual fruit lots treated with roughly the same temperature differential. This variation was not associated with the water or fruit temperature.

Bacterial infiltration. The red marker bacterium, Sm, was isolated from the internal tissues of fruit immersed under negative, but not zero or positive, temperature differential conditions. Tissues immediately below the stem scar were more likely to contain Sm than were other tissues. More Sm was isolated from fruit that gained greater amounts of weight during immersion. For example, one of two fruits treated with a −16 degree differential gained only 0.02% in weight. The marker bacterium was not isolated from that fruit. The second fruit gained 0.52%; Sm was isolated from each of the five slices of that fruit. The weight increase associated with immersion at a given differential appeared to be variable among fruits treated identically and infiltration appeared proportional to the weight increase.

In tests with the pathogenic bacteria, fruits treated under positive differentials were usually free from disease during storage while fruits treated with negative differentials suffered extensive decay. In preliminary tests, seven of eight green fruits and three of eight pink fruits immersed in a suspension of Ecc with a −14 C differential were diseased after 3 days in storage. No disease was noted in the corresponding −2 C differential treatment even after 8 days. Eight green fruits similarly treated with Pm and eight with Pa also were free of disease after 8 days. In the −14 C differential Pm treatment two and four of the eight fruits were diseased at 3 and 5 days, respectively. With Pa the corresponding values were one of eight and five of eight. The fruits in this test were not weighed.

Red fruits treated with a suspension of Ecc and a −14 C differential (22 C suspension/36 C fruit) gained little weight but developed a high incidence of bacterial soft rot during storage. In the first of two tests, 63% of the fruit was diseased at the 3-day observation; in the second, 50% was diseased at the 2-day observation. Slight weight increases, 0.04 and 0.05%, respectively, were recorded for the negative differential treatments. Fruits treated with the positive differentials did not increase in weight or become diseased.

The susceptibility of fruit to infiltration as ripeness/maturity increased was compared in two separate tests. Red fruits imbibed significantly less water than did pink or green fruits (Table 2). In fact, red fruits treated with the −14 degree differential did not gain significantly ($P = 0.05$) more weight than did those that received any of the positive differential treatments. However, over 80% of the red fruits had bacterial soft rot after 5 days in storage. The green fruits and the pink fruits gained significantly more weight than did the red fruits. In addition, those that received the former treatments had a higher incidence of disease after 1 day than did those that received the latter treatment. Although fruits in the +2 degree differential treatments did not increase in weight, some decay was noted after 5 days in storage. The lesions began at wounds on the surface of the fruit rather than beside or beneath the stem scar as did most of the lesions that usually occurred in fruit treated with negative differentials. However, in this test, many of the diseased fruits in the negative differential treatments were literally speckled with lesions. At the apparent origin of each lesion was a small puncture similar to that usually attributed to insect feeding. Yellow to white tissues were noted below disease-free punctures, which was additional evidence for insect feeding.

The −14 degree differential used in previous tests proved larger

TABLE 1. The effect of various temperature differentials on the weight increase of pink and green fruit held 8 cm below the surface of a water bath for 30 min[a]

Range of Differentials[b] (C)	Green fruit		Pink fruit	
	No. samples	Weight increase[c] (%)	No. samples	Weight increase[c] (%)
−30 to −36	3	3.63	3	2.35
−20 to −28	5	2.64	–	...
−5 to −13	1	1.30	2	0.70
0 to +2	2	0.25	–	...
+11 to +33	4	0.08	1	0.00

[a] Average weight of 10 fruit measured to the nearest 10 mg after the fruit were dried with a towel.
[b] Temperature differential = water temperature − fruit temperature.
[c] Weight increase = (final weight − initial weight) × (initial weight)$^{-1}$ × 100.

TABLE 2. The disease and weight increase in tomatoes that resulted from immersion of freshly destemmed fruit at various combinations of two temperatures and three ripeness/maturity classes in a 22 C suspension of *Erwinia carotovora* subsp. *carotovora*[a–d]

Fruit temp. (C)	Temperature differential[e]	Fruit color	Weight increase (%)	Decay (%)	
				1 day[f]	5 days[f]
36	−14	Red	0.05 a[g]	6	81
		Pink	0.22 b	38	88
		Green	0.31 b	31	94
				Avg 25	88
20	+2	Red	0.00 a	0	0
		Pink	0.00 a	0	13
		Green	0.00 a	0	19
				Avg 0	10

[a] Weight increase = (final weight − initial weight) × (initial weight)$^{-1}$ × 100.
[b] Average of two separate tests—eight fruits per treatment in each test.
[c] Held just under the surface of the suspension for 15 min, rinsed in 50 ppm NaClO, dried with a towel, weighed to the nearest 10 mg, and finally stored at 26 C and ≥ 80% relative humidity.
[d] Florida isolate SR-12 at 1×10^7 cells per milliliter.
[e] Suspension temperature − fruit temperature.
[f] Days after immersion.
[g] Values not followed by the same letter were different, $P = 0.05$.

than the minimum needed to cause a significant infiltration in two different tests (Table 3). Immersion of red fruit for 15 min with a −4 degree differential did not cause a measurable weight increase but did result in a substantial number of diseased fruits. Measurable weight increases and an increased percentage of decay were noted in the −8 and −14 C treatments. The incidence of bacterial soft rot was inversely correlated, r = −0.97, with the negative temperature differential.

In the preceding experiments, the temperature of the bacterial suspensions was either 18 or 22 C. These temperatures were selected because they approximated that of water from wells in Florida. The use of warmer, 40 C, suspensions along with a shorter, 10 min, immersion time still resulted in no rot in the zero or positive differential treatments and substantial disease in the negative differential treatments (Table 4). A slight weight increase was recorded in the 40 C suspension/40 C fruit treatment but this was not followed by a development of bacterial soft rot in storage. The fruits used for this test had 2-day-old stem scars. A shorter immersion time was used because in previous tests with 40 C suspensions of Ecc, the bacterium did not survive well in sterile tap water, distilled water, or buffered saline (Bartz, *unpublished*). However, most of the Ecc in suspension survived a 10 min exposure to 40 C; the 40 C treatment did not noticeably affect its virulence.

DISCUSSION

The infiltration of tomato fruit described herein could be predicted from the general gas law that states that any change in pressure of an ideal gas in a closed container of constant volume is directly proportional to a change in temperature of the gas. Tomato fruits are a type of container. They float in water, they have a relatively constant volume, and they contain gases in numerous airspaces. However, they are not completely closed containers. Thus, if they cool, the decrease in internal gas pressures leads to a combination of partial vacuums inside the fruit and an influx from the external environment. An influx could also result from an increased external pressure such as would result from immersing fruit well below the surface of water. Generally the extent of the influx would be regulated by such considerations as length of exposure, amount of cooling, deepness of the immersion, viscosity of the external environment, and size and number of pores leading to internal airspaces.

Since living cells inside plant storage organs need access to external air in order to carry out normal respiratory processes, all fruits and vegetables are permeated with airspaces that are connected in some manner with the external environment. Brooks (6) and Clendenning (9,10) reported that gas exchange in tomatoes occurred through the region of the attachment of the pedicel (stem) with the fruit. Gas exchange in potato tubers that sink in water and contain approximately 1% of their volume in airspaces occurs primarily through lenticels (7,8). The continuity of airspaces inside tomato fruits and potato tubers with the external environment is easily demonstrated with a technique used on pear fruit by Mitchell et al (15). Air is injected into fruits or tubers immersed in water. Bubbles form at and/or stream out of the stem scar of the tomatoes or lenticels of the tubers (J. A. Bartz, *unpublished*).

The portals of exit for injected air should be portals of entrance for water and bacteria if partial vacuums form inside storage organs immersed in bacterial suspensions. Indeed, in this report tomato fruits were infiltrated primarily through the stem attachment area. Most of the successful isolations of Sm were from the tissues beneath the stem scar. Most lesions were located beneath or adjacent to the stem scar. Earlier, Hall et al (12) demonstrated that bacteria could be forced into fruit around an attached pedicel by atmospheric pressures when an artificially induced partial vacuum inside the fruit was released. Samish and Etinger-Tulzcynska (15) concluded that *S. marcesens* applied to the sepals of young fruit enter the developing fruit in the area of the stem attachment.

The low incidence of disease in control treatments (0 or +2 degree differentials) provided evidence that stem scar tissues (fresh or 2 days old) were not susceptible. To infect tomato fruit, Ecc had to penetrate through the surface of stem scar tissues. Penetration was not effected simply by contact of the stem scar with a suspension of Ecc for 10 or 15 min. However, fruit held 8 cm below the water's surface for 30 min did gain weight under conditions of 0 to +33 degree temperature differentials. This uptake of water, also predicted by the general gas law, would result from increased pressure due to the 8 cm head of water on the fruit. The weight increases in the positive differential treatments became smaller as the differential increased. This too was predicted by the general gas law. Water infiltration was prevented or reversed when warmed gases inside the fruit expanded. Somewhat similarly in the tests with chicken eggs, immersion at a zero differential led to some decay (4,5,11). As a result, the final recommendations for the commercial washing of eggs included the suggestion that the wash water be at least 11 degrees warmer than the incoming eggs (5).

Recommendations that tomato dump tank and wash water be at least 11 degrees C warmer than the incoming fruit will not be made at this time. Since decay was seldom noted in fruit held just under the surface of suspensions with 0 or +2 degree differentials, water temperatures 11 degrees warmer than incoming fruit may be not only unnecessary but also expensive. However, the following suggestions concerning the postharvest handling of fruit and vegetables are offered. The water used in the handling or washing of fruit and vegetables should always be warmer than the warmest incoming fruit or vegetable unless: the fruit or vegetable is not vulnerable to infiltration; the fruit or vegetable and the water are free from postharvest decay organisms (or undesirable organisms or substances); and/or even if infiltrated, the shelf life and quality of the fruit or vegetable would not be appreciably affected.

Finally, the authors are aware of three different postharvest losses of tomato shipments from Florida due to bacterial soft rot where the packinghouse managers reported that the dump-tank and wash water were chlorinated. One such incident was reported in the literature (1). The water was not warmed in any of the three

TABLE 3. Weight increase and disease of red tomatoes after immersion of freshly destemmed fruit at four different temperatures in a 22 C suspension of *Erwinia carotovora* subsp. *carotovora*[a-d]

Fruit temp. (C)	Temperature differential[e]	Weight increase (%)	Postimmersion decay (%)		
			1 day	2 days	5 days
20	+2	0.00	0	0	0
26	−4	0.00	0	6	25
30	−8	0.03	6	31	63
36	−14	0.04	13	50	75

[a] Weight increase = (final weight − initial weight) × (initial weight)$^{-1}$ × 100.
[b] Average of 16 fruit—eight fruit per treatment for each of two separate tests.
[c] Held just under the surface of the suspension for 15 min, rinsed in 50 ppm NaClO, dried with a towel, weighed to the nearest 10 mg, and then stored at 26 C and ≥80% relative humidity.
[d] Florida SR-12 at 1×10^7 cells per milliliter.
[e] Suspension temperature − fruit temperature.

TABLE 4. Weight increase and bacterial soft rot that resulted from immersion of mature green tomato fruit at two temperatures in suspensions of *Erwinia carotovora* subsp. *carotovora* at two temperatures

Fruit temp. (C)	Water temp. (C)	Temperature differential[e]	Weight increase (%)	Decay[f] (%)
20	20	0	0.00	0
40	20	−20	0.44	100
40	40	0	0.02	0
20	40	+20	0.01	0

[a] Weight increase = (final weight − initial weight) × (initial weight)$^{-1}$ × 100.
[b] Average of 10 fruit for each treatment.
[c] After being immersed, the fruits were rinsed with 50 ppm NaClO, dried with a towel, weighed to the nearest 10 mg, and then stored at 26 C and ≥80% relative humidity.
[d] Florida isolate SR-12 at 1×10^7 cells per milliliter.
[e] Suspension temperature − fruit temperature.
[f] Percentage of fruit with bacterial soft rot after 2 days in storage.

packinghouses and an automatic chlorinator was used in one. Fruit from two of the three losses was examined and found to have mostly internal decays. Thus, in commercial practice, if the environment favors outbreaks of bacterial soft rot, chlorination of the wash and dump-tank water (13) without also warming that water may not be sufficient to prevent fruit losses.

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