Causes of Postharvest Losses in a Florida Tomato Shipment

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ABSTRACT

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At least six different postharvest plant pathogens were isolated from lesions on tomatoes in a commercial shipment that was rejected at the receiving point because of excessive decay. Sixty percent of the fruit from a representative 30-lb box were diseased. The lesions were light to dark, slightly sunken, and water-soaked. Based on symptoms, 2% of the lesions were associated with wounds, 10% were beneath or beside the blossom scar, 59% were adjacent to the stem scar, and 29% were internal. Pectolytic or decay bacteria were isolated from 90% of 50 randomly selected lesions. *Erwinia* spp. comprised 66% of the isolates, *Pseudomonas marginalis* 17%, and *P. aeruginosa* 17%. Representative isolates of the two pseudomonads, though considerably less virulent on tomatoes than a known isolate of *Erwinia carotovora* var. *carotovora*, were capable of causing lesions that contained the macerated tissues, juices, and bacteria needed for secondary spread. The pseudomonads could function as primary rather than secondary organisms in naturally occurring outbreaks of bacterial decay in tomatoes.

Bacterial soft rot of solanaceous crops has been attributed to three pectolytic *Erwinia* spp. (pE): *E. carotovora* var. *carotovora* (Ecc), *E. carotovora* var. *atroseptica*, and *E. chrysanthemi*. According to Dowson (11), other bacterial species isolated from macerated tissues were frequently disregarded because 1) pE colonies were found and assumed to cause the problem, or 2) colonies of fluorescent bacteria were noted but were thought to be the ubiquitous but saprophytic *Pseudomonas fluorescens*. But pectolytic nomenspecies other than pE have been shown to be pathogens of potato tubers (4,18) and tomato fruit (23). Many vegetables reportedly became soft rotted after inoculation with pectolytic fluorescent *Pseudomonas* spp. (3,10,11).

Two pectolytic nomenspecies that resemble the fluorescent bacterium *P. fluorescens* are *P. marginalis* and *P. aeruginosa*. Although both have been reported to cause plant disease (3,4,7,10,11), the causal association of *P. marginalis* with soft rots has been substantiated much more thoroughly than that of *P. aeruginosa*. In fact, Misaghi and Grogan (20) stated that "it seems more likely that the reputed plant pathogenicity of *P. aeruginosa* is a 'laboratory artifact,' and that it would rarely, if ever, function as a natural plant pathogen." On the other hand, Cho et al (6) considered *P. aeruginosa* a "quasi-pathogen" intermediate between saprophytism and parasitism. Elrod and Braun (10) presented evidence that under proper environmental conditions, *P. aeruginosa* isolated from humans and animals caused lesions in tobacco leaves, a wilt and rot of lettuce, and a soft rot of potato, cucumber, and onion. Cother et al (7) proved a causal relationship between *P. aeruginosa* and an internal brown rot of onion. Green et al (13) showed that after inoculation by vacuum infiltration, *P. aeruginosa* grew in lettuce and pinto bean leaves at 27 C and 85–95% relative humidity but not at 16 C. They suggested that humid, rainy climates would be ideal for invasion of plants by *P. aeruginosa*.

The following report concerns the phytopathogens isolated from lesions on tomatoes from a shipment that was rejected at the receiving point because of excessive decay. The experimental approach was to isolate and identify the causal organism from each of 50 representative lesions.

MATERIALS AND METHODS

In July 1978, a 30-lb box of 'Improved Walter' tomatoes from a rejected shipment was examined in the laboratory. The grower-packer was interviewed to determine how the fruit had been handled. The percentage of tomatoes with primary lesions was calculated, and the locations of lesions on the fruit were listed. Lesions that obviously resulted from secondary spread—ie, those that originated on healthy tissues at points of intimate contact with lesions on adjacent fruit or with "juices" composed of bacteria and macerated tissue from

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nearby lesions—were disregarded.

Fifty primary lesions were examined for consistency (soft to firm), color (light to dark), pH (Duotest pH Indicator Paper, Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY), and fluorescence in long-wave (366 nm) ultraviolet light in a CHROMATO-VUE (Ultra-Violet Products Inc., San Gabriel, CA). Isolations from lesions with pH of 5.6 or higher were made on crystal violet polypectate medium (CVP) (8) and nutrient agar. Potato-dextrose agar was used for isolations from lesions with pH below 5.0. Bacteria were isolated on nutrient agar from colonies at the bottom of depressions in CVP, from the edge of lesions in inoculated tomatoes, or from single translucent colonies on nutrient agar. Pure cultures were tested for causing soft rot of tomatoes; those found to be pathogenic were stored in sterile distilled water in screw-cap vials.

Both red and green tomatoes were inoculated by stabbing the surface with a transfer needle that had been dipped into a colony of the isolate being tested. The inoculated fruit, which also contained wounds made with a flamed needle, were stored at 25–27 C in high relative humidity (above 80%) for up to 7 days.

Physiologic tests for identifying bacterial isolates were considered presumptive for the known soft rot bacteria (5,12,14). First, the isolates were separated into two groups according to their ability to ferment glucose in Hugh and Leifson's medium (15). Hugh-Leifson negative isolates were then tested for pigment production on King's medium B (KB) and King's medium A (KA) (16), fluorescence of pigments produced on KB, growth on nutrient agar plates at 42 C, production of a grapelike odor, oxidase production with Taxon N disks (to identify *Neisseria* spp. and *Pseudomonas* spp.) (BBL Microbiology Systems, Cockeysville, MD 21030), and an antibiotic sensitivity pattern (this test performed at the Clinical Microbiology Laboratory, Shands Teaching Hospital, University of Florida). Hugh-Leifson positive isolates were tested for growth in nutrient broth with 5% NaCl or in 1% peptone at 37 C, acid production from alpha methyl D-glucoside, reducing sugars from filter-sterilized sucrose, and production of phosphatase in nutrient agar with 0.05% phenolphthalein diphosphate.

The relationship of the pseudomonads to the original lesion was estimated by wound-inoculating red tomatoes with mixed inocula containing equal numbers (1×10^7 colony forming units [CFU]/ml) of Ecc, *P. aeruginosa*, and *P. marginalis* (1,2). The inoculated fruit were stored at 26 C for 2 days, then examined in visible and ultraviolet light. Isolations were made from the center and advancing edge of several representative lesions, using KA, KB, and CVP. Colonies at the base of depressions in CVP were examined with oblique light under a dissecting microscope for the typical internal markings described by Cuppels and Kelman (8).

The pseudomonads' ability to multiply in fruit held at normal commercial transit and storage temperatures was investigated by dilution-plate analysis of bacterial populations in inoculated wounds. Fruit wound-inoculated with *P. aeruginosa* were stored at 12, 18, and 24 C, and those inoculated with *P. marginalis* were stored at 22 C. Tissues were removed from the wound area after 0, 24, and 48 hr and crushed in buffered saline (2). Dilutions were made as needed, and 0.05 ml of the final suspension was plated on KB. Fluorescent colonies were counted after 24 hr at 30 C.

The relative virulence of *P. aeruginosa* and *P. marginalis* compared with Ecc was studied by vacuum-infiltration inoculation of green tomatoes with a representative isolate of each of the three bacteria and with a mixture of all three isolates. Vacuum infiltration was used to produce internal lesions similar to those noted in the original fruit. Five fruit were used for each of three replicates for each treatment. The fruit were immersed in a suspension of bacteria (1×10^7 CFU/ml) and then subjected to a vacuum of 51 cm Hg for 3 min. When the vacuum was released, the fruit were removed from the suspension and stored at 25 C.

RESULTS

The disease outbreak. At the first examination, 60% of the fruit had primary lesions, which were water-soaked, light to dark, and slightly sunken. Within 1 wk, the percentage of decayed fruit had risen to 65%. At that time, the survivors were presumed to be ripe, healthy, and marketable and were discarded.

Fifty-nine percent of the lesions were next to the stem scar, 10% were adjacent to or beneath the blossom scar, and 29% were inside the fruit about midway between the blossom and stem scars; only 2% were directly connected to a surface wound. Many lesions seemed relatively firm and were somewhat darker than those usually associated with Ecc. Exactly half of the 50 lesions fluoresced bright yellow- and blue-green in ultraviolet light. Most of the fluorescence came from inside the lesions and was not readily apparent unless the lesions were cut or had broken open.

Isolation tests. Each isolate caused a soft rot of tomatoes. Soft rot bacteria were isolated from 90% of the lesions and *Geotrichum candidum* Link ex Pers. emend. Carmichael from 14%. Four percent contained both pectolytic bacteria and *G. candidum*. *G. candidum*, a yeastlike fungus that causes sour rot, was present when the pH of the contents of a lesion was 4.0–4.9; lesions that yielded only pectolytic bacteria had a pH range of 5.6–8.4. In lesions with both *G. candidum* and pectolytic bacteria, *G. candidum* was in a mat at the surface; the pH was less than 5.0 at the mat but more than 5.5 below the mat.

Thirty of the 45 bacterial isolates fermented glucose within 48 hr and were presumed to be *Erwinia* sp. based on this test and on their ability to cause soft rot in tomatoes and pits in CVP. The remaining isolates oxidatively utilized glucose; produced a fluorescent, water-soluble green pigment in KB; and were oxidase positive, characteristic of fluorescent *Pseudomonas* sp. Eight grew at 42 C on nutrient agar; produced a grapelike odor; and produced pyocyanin, a chloroform-soluble blue pigment that diffused into KA, presumptive for *P. aeruginosa*. The remaining seven isolates did not produce a pigment in KA or grow at 42 C, presumptive for *P. marginalis*. The *P. aeruginosa* isolates had an antibiotic sensitivity pattern identical to that of normal (no R factors) clinical isolates of *P. aeruginosa*. The *P. marginalis* isolates had different patterns. Thus, the two *Pseudomonas* spp. differed in growth at 42 C, production of grapelike odor, production of pyocyanin on KA, and antibiotic sensitivity pattern, but both could cause decay in tomatoes.

The pE were apparently derived from more than one population. At least one isolate each of Ecc, *E. carotovora* var. *atroseptica*, and *E. chrysanthemi* was identified by the appropriate tests. In addition, two isolates of the pE did not fit the presumptive tests: they produced acid from alpha methyl D-glucoside, produced reducing substances from sucrose, grew in nutrient broth with 5% NaCl, and did not produce phosphatase, but grew well in nutrient broth at 37 C.

Importance of the pectolytic bacteria. In the original isolation procedure, the most pectolytic organisms on CVP or the most prevalent translucent colony type on nutrient agar was selected. Many isolates were obtained by inoculating a healthy tomato with a mass of bacteria from a pit on CVP and then reisolating on CVP or nutrient agar from the margin of the lesion that developed. The most prevalent, pectolytic, and/or pathogenic organism isolated may not have been the only soft rot bacterium present in the lesion. Just as two lesions had both *G. candidum* and pectolytic bacteria, at least two other lesions contained two different types of pectolytic bacteria. One contained an atypical pE and *P. aeruginosa*; the other had an Ecc and an atypical pE. Almost half of the pE came from fluorescent lesions. Although the soft rot pseudomonads isolated from streaks on CVP came from shallow depressions, pE were also sometimes isolated from shallow depressions. Thus, neither fluorescent pigments in the macerated tissues nor the depths of the

depressions on CVP were presumptive evidence for the presence or absence of pE.

The lesions that developed on fruit inoculated with equal numbers of Ecc, *P. marginalis*, and *P. aeruginosa* or with *P. marginalis* or *P. aeruginosa* alone were always fluorescent. None of the lesions in fruit inoculated with Ecc alone were fluorescent. Fluorescent soft rot pseudomonads were consistently isolated from fluorescent lesions. Lesions in red fruit inoculated with *P. aeruginosa* became blue with age, and the odor of grapes was quite distinct above the usual aroma of ripe tomatoes. In the wound-inoculation treatments, *P. aeruginosa* was never isolated from the margin of lesions caused by the mixture of bacteria, while *P. marginalis* was occasionally isolated. *P. aeruginosa* was always isolated from the center of lesions and could survive in the presence of Ecc and *P. marginalis*. On the other hand, Ecc was isolated from the center and edge of every lesion sampled. Deep depressions characteristic of Ecc developed on all CVP plates. Finally, colonies bearing the characteristic markings of Ecc were noted at the bottom of each deep depression.

Both *P. aeruginosa* and *P. marginalis* multiplied in tomatoes at ripening room temperatures (12–24 C) (Table 1). Both survived and multiplied in wounds after being introduced at relatively low numbers (300–1,000 CFU/1.6 mm^3 hole). Thus, both would be able to multiply under commercial handling conditions. However, they did not multiply as rapidly as Ecc (2;Bartz, *unpublished*) and did not cause lesions as rapidly as Ecc after inoculation by vacuum infiltration (Table 2). Lesions caused by *P. marginalis* appeared within 24 hr of inoculation but only on 53% of the fruit, compared with 93% in the Ecc treatment. *P. aeruginosa* was much less virulent than either Ecc or *P. marginalis*; external symptoms were not visible until 6 days after inoculation and then only on 30% of the fruit. Nonetheless, both *P. aeruginosa* and *P. marginalis* caused lesions in fruit harvested from field-grown plants, were reisolated from diseased fruit in nearly pure cultures, and produced the inocula needed for secondary spread.

DISCUSSION

In many previous accounts of naturally occurring bacterial soft rot, one nomenspecies has been isolated, characterized, shown to be pathogenic, and assumed to be the causal organism (3,11,23,24). The outbreak described here, however, could not be attributed to one plant-pathogenic organism. Three pE and two *Pseudomonas* spp. capable of causing a bacterial rot in tomatoes were isolated.

Because the pE appeared more virulent, the role of the two pseudomonads may be questioned. The primary evidence supporting a direct causal role for the pseudomonads was the fact that they were isolated. If pE were present in all lesions, they should have been isolated by the techniques employed. A pE was always isolated from the lesions resulting from artificial inoculation with a mixture of Ecc, *P. marginalis*, and *P. aeruginosa*. However, in the original isolations, some pE came from shallow pits. De Ceara (9) noted that a strain of the saprophytic bacterium *E. herbicola* prevented Ecc from deeply pitting CVP. Thus, the absence of deep pits is not presumptive evidence for the absence of pE.

On the other hand, both *P. marginalis* and *P. aeruginosa* produced lesions in tomatoes inoculated with pure cultures by either of two methods and stored at 25 C. Apparently, *P. aeruginosa* did not produce lesions rapidly enough (within 4 days) to qualify as a primary pathogen in the outbreak. However, *P. aeruginosa* causes lesions more rapidly in vegetables, including tomatoes, at 30 and 35 C than at 25 C (13;Bartz, *unpublished*). The tomatoes in this outbreak were harvested during a season when afternoon temperatures normally equal or exceed 35 C. If the packed fruit were not promptly cooled to 20–25 C (and the evidence suggests that they were not), then environmental conditions would have been ideal for growth of *P. aeruginosa* (13). Thus, the pseudomonads' role in the outbreak cannot be lightly dismissed as that of secondary invaders.

The ability of *P. aeruginosa* to multiply in tomatoes at normal storage temperatures has implications outside plant pathology. Kominos et al (17) investigated the origin of clinical isolates of *P. aeruginosa* and found up to 2.2×10^3 CFU of *P. aeruginosa* per milliliter of tomato homogenate prepared from fruit from a hospital kitchen. Other fresh salad vegetables purchased (originating in Mexico, southern California, and Louisiana) also occasionally yielded *P. aeruginosa*. Considerably higher populations may occur naturally in tomatoes: de Ceara (9) reported that wounds approximately 1.6 mm^3 in volume on the surface of a symptom-free tomato may contain nearly 1×10^9 CFU of *P. aeruginosa*. Since several such sites or a few much larger sites may exist on a single fruit, it is fortunate that, according to Kominos et al (17), "Generally, the organism does not infect the healthy human."

The etiology of the outbreak reported here contains a major nonbiological postharvest plant disease determinant, suggesting that produce may be infiltrated by postharvest pathogens. Tomatoes are generally thought to be inoculated by soft rot bacteria through wounds or weakened tissues during harvesting or packing (19) or by fluorescent pseudomonads and other bacteria while still attached to the plant, probably through connective tissue at the stem attachment (21,22). Wingard (24) noted that bacterial soft rot lesions frequently developed when macerated tissues were placed at the edge of the stem scar.

The fruit examined in this study, however, were not exposed to macerated tissues and did not have many lesions adjacent to wounds. Furthermore, the high percentage of fruit that developed primary lesions caused by one or more of six different pathogens within 4 days of harvest supports the hypothesis of nearly simultaneous inoculation involving all six pathogens. The absence of such losses in unharvested tomato fields argues

Table 1. Numbers[a] of *Pseudomonas aeruginosa* and *P. marginalis* in pinhole wounds in tomatoes during storage after inoculation with needles dipped into 1×10^7 CFU/ml suspension

Pseudomonas sp.	Storage temperature (C)	Hours after inoculation		
		0	24	48
P. aeruginosa	12	1.2	9.7	1.2
	18	9.2	21	200
	24	1.2	98	300
P. marginalis	22	3.2	14	200

[a] CFU/ml $\times 10^3$; average of six punctures.

Table 2. Relative virulence of *Erwinia carotovora* var. *carotovora* (Ecc), *Pseudomonas marginalis* (Pm), and *P. aeruginosa* (Pa) on harvested tomato fruit after vacuum infiltration inoculation[a]

Organism	Percentage of fruit diseased			
	1 day	2 days	6 days	9 days
Ecc	93[b]	100	...	...
Pm	53	93	...	...
Pa	0	0	30	70
Ecc + Pm + Pa	87	100	...	...

[a] Green fruit from an experimental F_1 hybrid were immersed in suspensions of 1×10^7 CFU/ml of each bacterium and subjected to 51 cm Hg for 3 min. Fruit were stored at 25 C.
[b] Average of three five-fruit replicates.