

TOMATO FRUIT ROT INFECTION CYCLE IN A FRESH MARKET PACKING OPERATION¹

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Abstract. Tomato fruit rot in pallet boxes in a ripening room was due to *Rhizopus stolonifer*, *Mucor hiemalis*, and *Geotrichum candidum*. *R. stolonifer* was the most damaging pathogen. It infected green and ripe tomato fruit. *M. hiemalis* and *G. candidum* infected only ripe fruit. Most of the rotted fruit in the boxes had been damaged by wood stringers buttressing the bottom of the stacked boxes. The pathogens affecting fruit were also isolated from soil near the packing house and from the wood stringers. Surface treatment with NaOCl did not rid wood of *R. stolonifer*.

Most tomatoes grown in south Florida are harvested mature-green for fresh market consumption. Fruit are generally hand-harvested into buckets then dumped into wooden pallet boxes for transport to a packing house. Most fruit are placed in ripening rooms and exposed to ethylene gas to promote uniform ripening (4). Fruit rots in pallet boxes following storage in a ripening room at a Florida packing house were brought to the attention of the authors in January 1981. Most of the rotted fruit were on the top layer of tomatoes in each pallet box and most had been in contact with or were damaged by the three 3 inch x 4 inch wood stringers buttressing the bottom of the pallet boxes stacked above. Following dumping of the stored fruit, the inside of the pallet boxes were washed with water and sprayed with 250 ppm NaOCl if time permitted, or they were sent back unwashed to the field. In many cases the washed and unwashed pallet boxes were placed on soil adjacent to the packing house before being sent back to the field for re-filling. The pallet boxes were not removed from trucks in the field.

The following is a report of investigations made to determine the manner in which infection of tomato fruit was occurring and the effect of several treatments on the pathogens.

Materials and Methods

Damaged and rotted fruit were obtained from pallet boxes that had been stored in ripening rooms. Small pieces of tissue from the leading edge of lesions were cut out and plated on Difco potato dextrose agar acidified with lactic acid (Difco APDA) with 20 µg per ml dichloran, and a selective medium (RS medium) (5) developed for isolation of *Rhizopus stolonifer* (Ehrensberg ex Fr.) Lind. Mucoraceous fungi growing out from lesions were transferred to fresh RS medium. Hyphal tips of these fungi were transferred to Difco PDA. The mucoraceous fungi were identified as *R. stolonifer* and *Mucor hiemalis* Wehmer by E. Bose and T. Michaelaides, University of California, Davis, CA. Non-mucoraceous fungi were transferred to Difco APDA. The most consistently isolated non-mucoraceous fungus was *Geotrichum candidum* Link.

Soil samples were collected from areas where pallet boxes were stacked before or after the fruit were dumped or after the pallet boxes were washed. The soil was mixed thoroughly and one gm aliquots of each sample was suspended in 100 ml of autoclaved 0.25% agar. The suspensions were shaken for one minute and 0.5 ml dispensed onto RS medium in 9 cm petri plates. The suspension was spread over the surface of the medium with a bent glass rod. Ten plates per sample was incubated at 22 ± 2 C for 72 hr. Mycelia from margins of some colonies developing on these plates were transferred to fresh RS medium to remove microbial contaminants. Following growth on RS medium, mycelia were transferred to Difco PDA and the fungi identified after growth on this medium.

Green and ripe 'Duke' tomato fruit were obtained from a field in Delray Beach, FL. The fruit were dipped in 200 ppm NaOCl for three minutes, allowed to air-dry, injured with a sharp point of a 2 mm dia glass rod, and inoculated with a 4 mm disk from the margin of two- to five-day-old cultures of *R. stolonifer*, *M. hiemalis* and *G. candidum*. Two isolates of each fungus were used. Three green and three ripe fruit were inoculated per isolate. The fruit were placed in a randomized block design in covered plastic boxes with 300 ml tap water and a wax-coated wire screen serving as a platform for the fruit. The plastic boxes were incubated at room temperature (22-25C). The diameter of lesions was measured 72 hr after inoculation.

Shavings of wood were obtained from the wooden stringers on the base of pallet boxes. The shavings were dipped into NaOCl at 200, 400, and 1600 µg per ml, air-dried and plated on RS medium. In another experiment, 3-6 cm long slivers of stringer wood were dipped into copper-8-quinolinolate 90% (obtained from the Southland Corporation, Great Meadows, NJ) at 1000 µg per ml for one minute; Botran 75W (dichloran, obtained from TUCO, Division of The Upjohn Co., Kalamazoo, Mich.) at 900 µg per ml for one minute; or 5250 µg per ml NaOCl for three minutes. The slivers were inserted in small punctures made in green tomato fruit. The fruit were incubated in plastic boxes at room temperature (22-25C) as described above. A randomized block design with three replications was used. The incidence of *R. stolonifer* in sliver-inoculated fruit was determined four days after slivers were inserted.

Results

R. stolonifer was consistently isolated on Difco APDA from water-soaked areas on infected fruit and from fruit that had obvious signs of a *Rhizopus*-like organism. On Difco APDA and RS medium, the slower growing *M. hiemalis* and *G. candidum* were overgrown by *R. stolonifer*. On Difco PDA plus dichloran growth of *R. stolonifer* was inhibited. *M. hiemalis* and *G. candidum* were not affected by 20 ppm dichloran and were thus isolated on Difco PDA plus 20 ppm dichloran.

R. stolonifer was pathogenic to green and ripe fruit. There was no difference in amount of lesion development of *R. stolonifer* on green or ripe fruit (Table 1). *M. hiemalis* and *G. candidum* only infected ripe fruit (Table 1).

R. stolonifer, *M. hiemalis*, and *G. candidum* were recovered from soil using the RS medium. The population of *R. stolonifer* and *G. candidum* was greater in the area where the pallet boxes were being washed, immediately adjacent to the packing house, than in areas further from the pack-

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Table 1. Growth of fungi isolated from damaged and rotted tomatoes on green and ripe Duke tomato.

Fungi	Isolate	Lesion radius (cm) ^a	
		Ripe fruit	Green fruit
Rhizopus stolonifer	R-1	3.5	3.8
	C-1	3.7	3.5
Geotrichum candidum	T-9	0.3	0
	T-14	0.1	0
Mucor hiemalis	T-11	0.5	0
	T-12	0.6	0

^aAfter 72 hr incubation at 22-24° C.

ing house (Table 2). The soil obtained from areas 50 to 100 feet from the packing house was relatively dry, while the area immediately around the packing house was moist and usually muddy.

R. stolonifer was consistently isolated from shavings of wood from stringers surface-sterilized with 200, 400, and 1600 µg per ml NaOCl. Occasionally, *M. hiemalis* and *G. candidum* were obtained from wood. Green tomato fruit in which dichloran-dip-treated wood slivers were inserted had fewer *R. stolonifer* infections than tomato fruit with slivers of wood dip-treated with copper-8-quinolinolate or sodium hypochlorite (Table 3). No infection occurred on unpunctured fruit.

Table 2. Incidence of *R. stolonifer*, *M. hiemalis* and *G. candidum* in soil from two areas where pallet boxes were stored before and after dumping of tomato fruit.

	Fungal colonies obtained per gm soil ^a		
	<i>R. stolonifer</i>	<i>M. hiemalis</i>	<i>G. candidum</i>
Holding area ^b	173	133	533
Washing area ^c	460	80	4360
	*	NS ^x	* ^x

^aDry area, 50-100 ft from packing house.

^bMuddy area, immediately around packing house.

*Significant at .05 level of probability, NS = nonsignificant.

Table 3. Effect of fungicide treatments on *Rhizopus stolonifer* infected wood slivers from pallet box stringers.

Fungicide	Concentration	Fruit infected (%)
Control—no treatment		100.0 a ^z
Copper 8-quinolinolate	1000 µg/ml	87.6 a
Sodium hypochlorite	5250 µg/ml	66.7 a
Dichloran	900 µg/ml	22.3 b

^aMean separation by Duncan's multiple range test, 5% level.

Discussion

R. stolonifer appeared to be the most damaging pathogen in the disease situation investigated. Although *M. hiemalis* and *G. candidum* were also present, *R. stolonifer* grew much more rapidly and was able to move to uninjured fruit by mycelial extension during the time fruit were in the ripening room. *R. stolonifer* infected green fruit while the other two organisms did not. *G. candidum* is reported to infect green tomato fruit exposed to low temperatures before inoculation (1).

The temperature in ripening rooms, about 20-25 C, is

ideal for growth of *R. stolonifer*. The temperature for optimum growth of *R. stolonifer* in culture is reported to be between 23 and 26 C. (6).

R. stolonifer appears to be only a ripe fruit pathogen on tomatoes in California (2), while it is reported to be a green and ripe fruit pathogen in the northeast U.S. (3). In the present study, there was no difference in amount of *R. stolonifer* lesion development on green and ripe tomato fruit.

The most significant initiator of the disease problem in the situation investigated appeared to be the damage caused by the wood stringers on the base of the pallet boxes. In addition, the stringers were serving as a source of inoculum.

The initial inoculum of *R. stolonifer* may have been soil- or air-borne. A scenario for disease development and inoculum increase can be: stringers damage fruit in overfull pallet boxes; *R. stolonifer* from soil on stringers or from the air infects damaged fruit; *R. stolonifer* from infected fruit grows into uninjured fruit, into stringers or into wooden walls of the pallet boxes; conidia produced on rotted fruit or on wood are washed into soil around packing house or released into air; and stringers on pallet boxes placed on soil where wash water has been released are reinfested with *R. stolonifer* conidia. The next batch of damaged fruit are then exposed to higher levels of inoculum from within the stringer, from soil on the stringers and conidia in the air.

There are several places where the disease cycle can be broken. Avoiding the over-filling of pallet boxes with fruit, which results in injury by stringers, may be the most important measure, as *R. stolonifer* usually requires injury for infection. Another measure is to prevent contact of stringers with soil. In addition to transmission of the pathogen by direct contact of stringer with fruit, soil loosely held on stringers can fall on injured fruit not in contact with stringers and initiate infection. The inside walls of pallet boxes and stringers should be surface-sterilized with NaOCl, this should reduce inoculum present on the surface. Since the fungus appears to grow into the wood stringers and the wooden walls of the pallet boxes, it may be necessary to sterilize or fumigate the pallet boxes if the fungus inside the stringers or walls serve as important sources of inoculum. If inoculum in the air of ripening rooms is high, it may be necessary to fumigate ripening rooms.

Although dichloran is registered for use in post-harvest protection against *R. stolonifer* in other crops, it is not currently registered for use as a post-harvest treatment for tomatoes. Registration of its use as a post-harvest treatment of tomatoes has been requested and is being processed. If registered, it may have some use in controlling *R. stolonifer* in ripening rooms if the procedures suggested above are not effective.

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