Etiology of Watermelon Rind Necrosis

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ABSTRACT

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Bacteria were isolated consistently from healthy watermelons and from melons with watermelon rind necrosis (WRN). No single bacterial species was isolated consistently from WRN tissue. The diversity of the internal bacterial flora of melons with WRN was similar to that of healthy melons. However, enterobacteria were isolated more frequently from melons with WRN than from healthy melons. Most of the bacteria isolated produced localized rind necrosis when injected at high concentrations but at low concentrations some bacteria were more effective than others. With some isolates a systemic rind necrosis was produced which resembled that often observed in natural infections. In field studies, foliar sprays of mineral nutrients did not affect WRN incidence. It is hypothesized that the WRN symptoms result from the multiplication of one of the resident bacteria to a level high enough to induce a necrotic reaction in the watermelon.

In Georgia, an internal browning of watermelon was attributed to prolonged drought conditions during fruit maturation in 1925 (5). A relatively inconspicuous vascular browning of the rind was observed in Hawaii and attributed to physiological causes (11). Bacterial rind necrosis of watermelon also has been reported from Hawaii (11), Texas (18), Florida (8), and California (13). Symptoms of watermelon rind necrosis (WRN) include a brown, dry, and hard necrosis of the rind that rarely extends into the flesh of most watermelon cultivars. The only external symptom is misshapen fruit in a few severely affected melons. Severely affected melons are unmarketable when sliced.

Significant differences have been observed in the incidence and severity of rind necrosis in various watermelon cultivars. Round-fruited cultivars tend to have a higher incidence of WRN than long-fruited ones. Incidence of WRN is high enough in some cultivars to make them unsuited for production in Florida (3, 9).

Watermelon rind necrosis was reported to be caused by a species of *Erwinia* resembling *E. carnegieana* (11, 18). In our preliminary studies, bacteria consistently were isolated from the necrotic areas, and Erwinia-type bacteria were most common. However, the localized necrosis symptoms of WRN were reproduced at the inoculation site when any of several bacterial types were injected into the rinds. Extensive systemic browning of the rind that is often found in natural infections did not occur (8). Symptoms of WRN resemble those that have been reported for boron and calcium deficiencies in various fruits and vegetables. Our objective was to determine whether or not the watermelon rind necrosis disease is caused by a single bacterium and to evaluate the role of nutrition, host resistance, and other factors in the disease syndrome. Preliminary reports have been made on parts of this study (4, 8, 10).

MATERIALS AND METHODS

Isolations from watermelon rinds were made by peeling off the surface of the rind with a sterile scalpel and comminuting a small internal section (8-15 mm^3) of necrotic rind in 1 ml of sterile water. This suspension was streaked on plates of King's Medium B (12) and, after an appropriate period of incubation, stock cultures were prepared from single colonies either directly or after dilution plating. Stock cultures were maintained in sterile, demineralized water at room temperature.

Standard bacteriological tests, conducted as described elsewhere (1, 2, 14), were used to identify the various bacterial types which were isolated from watermelons. Isolates were considered to be members of the Enterobacteriaceae if they were Gram-negative rods, facultatively anaerobic, and either motile by peritrichous flagella or nonmotile. Enterobacteria which grew as yellow colonies on nutrient agar, produced little or no gas from glucose, and did not decarboxylate arginine, lysine, or ornithine were classified as *Erwinia herbicola* types. The fluorescent pseudomonads were isolates which were Gram-negative, aerobic, motile by polar flagella, and produced diffusible yellow-green fluorescent pigments on King's Medium B (12). Isolates were classified as xanthomonads if they were Gram-negative, aerobic, motile by a polar flagellum, and produced a non-water-soluble yellow pigment on nutrient agar. All bacteriological tests were performed at least twice on each isolate. Known isolates were included as standards in the tests.

Watermelon fruits were inoculated by injecting bacterial suspensions into the rind. The inoculum was

adjusted to 10^8 viable cells/ml by measuring OD_{600nm} with a Spectronic 20 colorimeter, and other inoculum concentrations were prepared by dilution of this adjusted suspension. Inoculum suspensions (0.5 ml) were injected into each of three sites per melon. Fruits which were at least 2 wk from maturity were used for inoculations. At maturity, melons were first sliced through the center of the inoculation sites and then sliced into 5 cm wide, or smaller, slices. Localized and systemic symptoms were recorded.

To determine rates of multiplication of bacterial isolates in watermelon furit, one-third to full-sized immature melons were inoculated by injecting 0.5 ml of bacterial suspension, containing either 10^5 or 10^7 viable cells/ml, at three locations on the fruit. Inoculated melons either were stored at 26 ± 5 C or left attached to the vine in the field. Melons were sampled periodically to determine bacterial concentrations at inoculation sites. The surface of the rind was cut from the injected spot with a sterile scalpel and a disk of tissue was removed from the inoculated area with a sterile 6-mm-diameter cork borer. Aseptically, a 1-mm slice was cut transversely from the disk of tissue and placed in 2 ml of sterile water. The slice was crushed with a sterile glass rod and the bacterial concentration per milliliter of suspension was determined by dilution plating. There were at least three replications of each treatment. Bacterial colonies were counted 24-48 hr after plating.

Field experiments were conducted to determine the effect of supplemental boron and calcium on the incidence of WRN. In 1973 the cultivars Charleston Gray (moderately tolerant to WRN) and Summerfield (highly susceptible) were planted. In 1974 the test was repeated with the cultivars Charleston Gray and Klondike Blue Ribbon, highly susceptible to WRN. Test plots consisted of 10 hills in two rows with 1.5 m between hills and 3 m between rows. Each treatment was replicated four times. Boron (0.24 g/liter) and calcium (3.1 g/liter) foliar sprays were applied to runoff five times at 2-wk intervals beginning 13 March in 1973 and 28 March in 1974. Mature fruits were harvested between 28 May and 3 July. All melons were sliced in the field and WRN incidence was recorded.

TABLE 1. Frequency of isolation of various bacterial types from watermelons with and without rind necrosis

Bacterial type	Rind necrosis[a]		Healthy[a]
	1974	1975	1975
Enterobacteriaceae:	61	54	23
Erwinia herbicola	39	12	9
Other enterobacteria	32	42	18
Fluorescent pseudomonads	8	8	5
Xanthomonads	10	8	0
Misc. Gram-negative	8	35	68
Gram-positive	26	50	36

[a]Data are given as percentage of melons from which the bacterial type was isolated. More than one type was isolated from some melons. Isolations were obtained from 51 melons in 1974, 26 melons with rind necrosis in 1975, and 22 symptomless melons in 1975.

RESULTS

Isolations from melons with watermelon rind necrosis.—In 1974, bacteria were isolated from 126 of 132 melons with WRN and 25 of 30 symptomless ones. In 1975, bacteria were isolated from 29 of 30 WRN melons and 27 of 30 symptomless melons.

Bacterial isolates obtained from 51 WRN melons in 1974 and 26 WRN melons in 1975 were characterized in an attempt to determine whether any one bacterium or bacterial type could be associated consistently with the disease (Table 1). Enterobacteria were the most frequently isolated bacterial type. However, in 1974, more than half the enterobacteria were *Erwinia herbicola* types, which are common on plant surfaces and as secondary invaders. Gram-positive bacterial types also were common, but these consisted of at least four or five different bacteria. Pseudomonads, xanthomonads, and other Gram-negative bacteria also were found in melons with WRN. No single bacterium could be isolated consistently.

TABLE 2. Effect of inoculum concentration on watermelon rind necrosis symptom development in two watermelon cultivars after bacterial injection

Cultivar and isolate	Infectivity[a] of various inoculum concentrations (cells/ml)			Systemic necrosis[b]
	10^7	10^5	10^3	
Sweet Princess (tolerant)				
RN71-3	3/3	9/9	5/6	1/6
RN74-10	...	3/6	2/6	0/4
RN74-3	5/5	3/3	3/5	0/5
Klondike Blue Ribbon (highly susceptible)				
RN71-3	9/9	6/6	7/9	5/8
RN74-10	...	...	2/8	0/3
RN74-3	9/9	8/8	8/8	3/9

[a]Data are expressed as number of necrotic sites over the total number of injected sites. There were three inoculated sites per melon.
[b]Data are expressed as number of melons with systemic necrosis over the total number of melons injected. This includes melons injected with all three inoculum concentrations. "..." indicates that data was not obtained for that inoculum concentration.

Since bacteria could be isolated from most watermelons, comparisons were made between the bacterial flora of melons with rind necrosis and that of symptomless melons (Table 1). The most interesting difference was the much more frequent occurrence of enterobacteria in the necrotic tissue than in the symptomless melons. This difference primarily was traceable to enterobacteria other than *E. herbicola*. Based on the arginine, lysine, and ornithine decarboxylase reactions and on gas production in glucose (1, 14), these appeared to be mostly an *Enterobacter* sp. and a few *Klebsiella* and *Erwinia* types. Gram-positive bacteria also occurred slightly more often in the WRN melons. The miscellaneous unidentified Gram-negative bacteria were isolated twice as frequently from symptomless melons as from melons with WRN.

Pathogenicity tests.—In preliminary tests, WRN symptoms were reproduced readily at the inoculation sites by injection with both *Erwinia* and *Pseudomonas* isolates, but the extensive systemic necrosis of the rind that is found often in natural infections was not observed.

The effect of inoculum concentration on symptom development was studied in two cultivars with different levels of susceptibility to WRN (Table 2). Inoculations were made in the field. All three bacterial isolates reproduced the necrotic symptom at the site of injection with inoculum concentrations of only 10^3 cells/ml. Even lower concentrations occasionally gave localized necrosis. Isolates RN 71-3 (an *Enterobacter* sp.) and RN 74-3 (a pseudomonad) were more effective than was RN 74-10 (an *Erwinia herbicola* type) in causing necrosis.

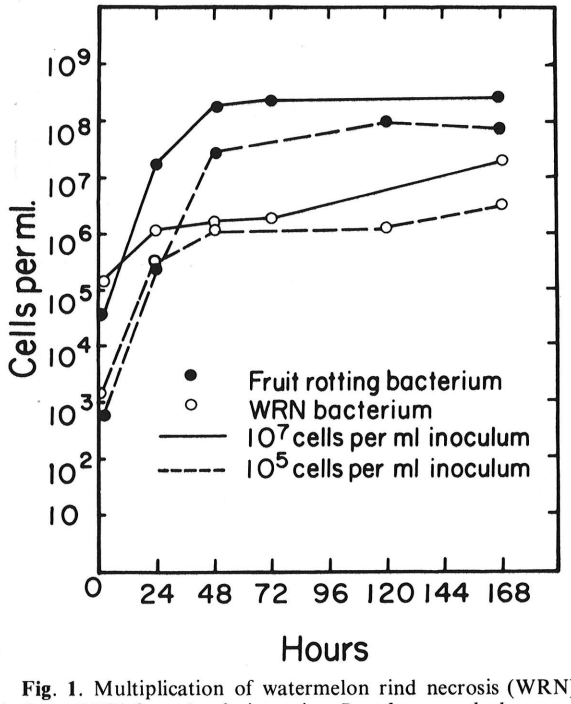

Fig. 1. Multiplication of watermelon rind necrosis (WRN) isolate, RN71-3, and a fruit rotting *Pseudomonas lachrymans* isolate at two inoculum levels in Charleston Gray watermelon tissue. Bacterial concentration is given as viable cells per milliliter, and each ml of extract represents 14 mm^3 of tissue.

Frequency of necrosis at the injection site was similar with the tolerant Sweet Princess and the highly susceptible Klondike Blue Ribbon.

In contrast to earlier tests, systemic necrosis of the rind was observed in these inoculated melons (Table 2). Development of systemic necrosis after inoculation by injection was much more frequent in Klondike Blue Ribbon than in Sweet Princess. Systemic necrosis developed in 1 of 15 inoculated Sweet Princess melons and in 8 of 20 inoculated Klondike Blue Ribbon melons. The bacterial isolates also seemed to vary in ability to cause systemic symptoms. Isolate RN 71-3 was the most effective and isolate RN 74-10 was ineffective.

Multiplication patterns of watermelon rind necrosis bacteria versus fruit-rotting bacteria.—The multiplication pattern in Charleston Gray of WRN isolate RN 71-3 was compared with that of isolate 64-3 [an isolate of *Pseudomonas lachrymans* (E. F. Sm. & Bryan) Carsner] which rots the fruit (Fig. 1). At the lower inoculum level (10^5 cells/ml) both bacteria multiplied at a similar rate, with the WRN isolate reaching a maximum between 24 and 48 hr and the fruit-rotting isolate reaching its maximum 48-72 hr after injection. Both isolates reached maximum population levels approximately 24 hr earlier following injection at the higher (10^7 cells/ml) inoculum concentration. Necrosis first occurred with the WRN bacterium when its maximum population was reached and rotting also occurred when the *P. lachrymans* isolate reached its maximum population. Final populations of the WRN isolate were 20-100 times less than the final populations of the fruit-rotting isolate. The rot from the fruit-rotting bacterium spread throughout the melon, whereas the necrosis from the WRN bacterium was restricted to the rind. This experiment was repeated three times. Three other WRN isolates were found to have similar multiplication patterns in other tests.

Supplemental boron and calcium.—Foliar nutrient sprays of boron and calcium, applied both separately and combined, failed to significantly reduce WRN. The incidence of WRN was slightly lower in the boron and calcium spray treatments in 1973, but was higher in 1974. In no instance did supplemental mineral nutrients eliminate WRN.

Watermelon mosaic virus.—It has been reported that WRN is associated with watermelon mosaic virus (WMV) infection (18). However, we observed that incidence of WRN was not correlated with WMV incidence. In our 1973 tests watermelon mosaic was very severe, but in 1974 WMV infection occurred rarely if at all in our test plots. In contrast, there was a slightly higher overall incidence of WRN in 1974 than in 1973.

DISCUSSION

A mixed bacterial flora commonly is present inside healthy vegetables, but little is known about the significance of this flora (7, 15, 16). Of the cucurbits, cucumbers are known to contain a diverse bacterial flora (7, 15). In this study we consistently isolated bacteria from healthy watermelons and from melons with WRN. Thus, it appears that watermelons also harbor resident bacterial populations internally.

Systemic necrosis of the rind similar to that often observed in natural infections was reproduced in these tests. Frequency of systemic necrosis development seemed to depend both on susceptibility of the watermelon cultivar and on the virulence of the bacterium. This demonstrated that WRN symptoms can be reproduced totally by bacteria, but did not prove that they are the primary incitants in natural infections.

We have not observed any nutritional effect on the disease. The basic fertilizer mix that was used routinely each year contained fritted trace elements which should supply adequate amounts of trace elements, still WRN occurs every year. The failure to reduce WRN with supplemental mineral nutrients indicates that the disease is not simply a deficiency disorder in which the bacteria are secondary invaders.

From these studies, we have concluded that in Florida WRN may be incited by more than one member of the normal internal bacterial flora of watermelon. The WRN disease syndrome appears to result from a general resistance reaction that may be hypersensitivity. The association of more than one bacterial type with the disease and the multiplication patterns of the incitant bacteria are consistent with this hypothesis.

With this hypersensitivity hypothesis, there are several events that must occur for the disease to develop and the probabilities of these events occurring determines the susceptibility of a watermelon cultivar. First, there must be an internal bacterial flora. Although this was demonstrated in healthy melons, there may be differences in populations among cultivars. Second, the bacteria must multiply to a level that initiates the hypersensitive reaction. In most melons the resident bacteria never multiply to levels that cause WRN. Perhaps the resident bacteria are better able to multiply and cause WRN under certain environmental conditions or at times of stress on the plant, such as that of WMV infection or nutritional imbalance. Third, the effectiveness of the hypersensitive response determines the amount of systemic necrosis that occurs and, therefore, the severity of the disease. In very susceptible watermelon cultivars, such as Klondike Blue Ribbon, the necrosis seems to spread through the vascular tissue and results in systemic necrosis around the rind and even extending into the flesh of the melon. Perhaps in more tolerant cultivars, the hypersensitive reaction is more effective in localizing the bacteria.

In many ways WRN is very similar to graywall of tomato. Both diseases apparently are incited by more than one bacterium (17), both show cultivar differences in susceptibility (6), and both exhibit multiplication patterns of the incitant bacteria that indicate hypersensitivity (17). Rind necrosis of watermelon, graywall of tomato, and perhaps others may represent a class of diseases that are incited by bacteria that are normally residents of the healthy host. When the proper predisposing environmental conditions are present, these residents may cause disease.

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