centrate was passed through the colloid mill before reconstituting and centrifuging.

A flotation method for the rapid estimation of the quantity of coarse pulp in frozen concentrated citrus juices was found to be satisfactory and is described. No relationship existed between the coarse pulp content and the total pulp content in 11 samples of commercial concentrates examined.

STUDIES ON THE ARTIFICIAL INFECTION OF ORANGES WITH ACID TOLERANT BACTERIA[1]

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A recent study by the authors (2) has shown that several species of bacteria occur and grow readily in orange juice. Indications were that some of these acid-tolerant bacteria may be in the juice because they were present in the fruit when it was on the tree. It would seem logical to assume that these same organisms would proliferate in citrus fruit when provided with a means of entry and if the pH of the juice is not below the limiting pH of the organisms. On the other hand, factors such as oxygen tension within the fruit and the natural physiological processes of the fruit itself might act as deterrents to the growth of bacteria. The studies reported here were conducted to provide information on the infection of citrus fruit by bacteria.

Bacteria pathogenic to citrus and known to cause diseases of concern to the citrus grower have been studied by several workers. Hasse (3) first isolated and described *Xanthomonas citri*, the casual organism of citrus canker. Black pit on citrus fruits was first described by Smith (5), who showed by inoculation experiments that the disease was due to *Pseudomonas syringae*.

Bacterial spot of South Africa resembles black pit of California but is caused by *Erwinia citrimaculans*. The disease was found in South Africa on lemon, tangerine, and mandarin and navel oranges; it was also induced by inoculation in shaddock, grapefruit, citron, and sour and sweet limes. The characteristics of the organism responsible for this disease were described by Doidge (1). Passalacqua (4) isolated and described a yellow-pigmented rod which he called *Bacterium citri deliciosae*, said to cause a disease on mandarins called "mal della terra."

These organisms usually infect the tissues of the tree itself or the surface of the fruit and, while the diseases caused by these organisms are important from the production viewpoint, external blemishes on the fruit are of little concern to the canner or concentrator. However, the internal quality of fruit used for production of frozen concentrate is of the utmost importance, and future investigations dealing with factors contributing to bacterial infection of citrus while the fruit is still on the tree may have important practical aspects for the canner.

There are several ways in which a citrus fruit conceivably could become inoculated with microorganisms while it is still attached to the tree. In the case of insect transmission the inoculation would be shallow and probably would not penetrate into the juice sacs, although a few insects might be able to penetrate a thin peel. Thorn injury, on the other hand, could result in a much deeper inoculation of the fruit. Microorganisms possibly may enter the fruit through other openings in the peel, such as those caused by hail, which do not extend into the juice.

INITIAL FRUIT INOCULATION EXPERIMENT

To determine which type of inoculation was required to infect citrus fruit with bacteria successfully very mature oranges were inoculated with several species of bacteria known to be capable of growing in sterile orange juice at a pH of 4.0 or less. The organisms used in this initial experiment were four of the acid-tolerant species described previously by Faville and Hill (2), namely, *Leuconostoc mesenteroides*, *Achromobacter* sp., *Xanthomonas* sp. "A," and an unidentified Gram-negative rod which grew at pH 3.23 in orange juice *in vitro*.

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2—Florida Citrus Commission
3—Florida Citrus Experiment Station.

Since the pH of juice from Pineapple and Valencia oranges was too low to permit growth of these organisms when this investigation was started, a Parson Brown tree was chosen as the source of fruit. Spot checks indicated that pH of the juice of oranges from this tree ranged from 3.90 to 4.17, values considered to be sufficiently high for the growth of all of the organisms used.

Sound oranges were first surface sterilized with a mercuric chloride solution at the point of inoculation and then injected with 24 hour cultures of the appropriate organisms. Three types of inoculation were employed:

(1) Peel—a drop of culture was placed on the surface of the orange and then inoculated into the peel by means of a sterile needle protruding about 1/16 inch through a cork;

(2) Juice—a small amount of culture was injected at a depth of approximately one inch with a sterile No. 26 hypodermic needle; and

(3) Core—the organisms were injected into the core at a depth of approximately one inch by means of a sterile No. 26 hypodermic needle inserted through the stylar end.

For each orange inoculated on the tree, a duplicate orange was picked, inoculated and stored in the laboratory. A representative number of oranges showing all three types of inoculation in both laboratory and tree fruit, were selected and examined at weekly intervals for a period of three weeks. Each orange was examined for external signs of deterioration, washed with detergent and water and then sliced through the point of inoculation with a sterile knife. Juice from the area immediately below the point of inoculation was examined microscopically for the presence of large numbers of the organisms.

In general there was very little or no difference between the results obtained in the fruit inoculated and stored in the laboratory and those inoculated on the tree. In both cases, the organisms apparently had to come in contact with the juice before infection could occur. The few instances where infection occurred in fruit which had been inoculated into the peel or the core were evidently due to a puncturing of the juice sacs as a result of a very thin peel or failure to inoculate directly down the core of the fruit.

In the majority of instances, there was very little or no external evidence of decomposition of the fruit even though the internal portions were highly infested with microorganisms. This was particularly true of the fruit which had been inoculated with *Leuconostoc mesenteroides*. The most obvious external symptom in any of the fruit was a small soft depression at the site of inoculation, this depression usually being not more than one-half inch in diameter and still rather inconspicuous three weeks after inoculation.

Growth of Microorganisms in Valencia Oranges on Trees

After Valencia oranges had matured to such an extent that the pH of the juice was high enough to permit growth of the organisms, a second experiment was set up to determine the length of time after inoculation that was required for the organisms to reach maximum numbers in the fruit. The organisms used were *Aerobacter aerogenes*, *Xanthomonas* sp. "A" and *Achromobacter* sp. All oranges were inoculated on the tree by placing a drop of 24 hour broth culture on the sterilized portion of the orange surface and then injecting a sterile needle through the drop of culture to a depth of approximately one inch. Sufficient numbers of fruit were inoculated to permit examination of ten at each sampling. Samples were picked immediately after inoculation and at weekly intervals for a period of five weeks. Ten uninoculated control fruit also were picked and examined at each weekly sampling. Each fruit was washed, sliced and juiced aseptically, and then plated on dextrose agar. The pH of the juice of each orange was determined using a Beckman pH meter.

From the data presented in Table 1 it is apparent that all three organisms used in this inoculation experiment are able to grow in oranges on the tree. With the exception of one orange, all fruit sampled immediately after inoculation showed low bacterial counts (less than 1000 microorganisms per ml.). In general, the fruit inoculated with these three organisms reached a maximum count in approximately three weeks and maintained high counts throughout the observation period of five weeks.

The maximum pH at which the acid-tolerant organisms described by Faville and Hill (2) will grow were: *Leuconostoc mesenteroides*, 3.6; *Aerobacter aerogenes*, 3.8; *Lactobacillus*

TABLE 1.
MICROORGANISM COUNTS AND pH OF VALENCIA ORANGE JUICE FROM FRUIT ARTIFICIALLY INOCULATED ON THE TREE WITH CULTURES OF ACID TOLERANT BACTERIA.

Initial		1st Week		2nd Week		3rd Week		4th Week		5th Week	
Count/ml.	pH	Count/ml.	pH	Count/ml.	pH	Count/ml.	pH	Count/ml.	pH	Count/ml.	pH
					Aerobacter aerogenes						
0-9	3.6	60,000	3.6	3,400	3.8	163,000	3.8	700	3.8	1,800,000	3.8
210	3.8	450,000	4.0	0-9	3.7	530	4.0	920,000	3.9	1,240,000	4.3
60	3.7	10	3.7	5,600,000	4.0	11,000	4.0	1,800	4.1	9,400,000	4.2
10	3.6	0-9	3.7	10	4.0	62,000,000	4.1	0-9	4.1	2,400,000	4.0
510	3.8	15,000,000	4.1	4,000,000	4.0	70,000	3.9	23,000,000	4.2	990,000	3.9
20	3.6	460,000	4.0	420,000	3.8	640,000	3.9	77,000	4.3	0-9	3.8
20	3.6	68,000	3.7	105,000	3.7	150,000	3.9	310,000	4.0	75,000	3.7
10	3.7	54,000	3.7	800	4.0	27,000	3.7	0-9	4.0		
20	3.7	1,000	3.9	5,300	3.9	40	3.8	300,000	3.8		
10	4.1	210,000	4.1	33,000	3.7			9,900,000	4.2		
					Xanthomonas sp. "A"						
10	3.5	1,180,000	3.6	3,900,000	4.0	16,000,000	3.6	6,000,000	3.9	11,000	4.1
0-9	3.7	1,700,000	3.5	360,000	3.8	7,800,000	3.7	1,020,000	3.9	1,300,000	3.9
77,000	3.5	30	3.6	2,100,000	3.6	9,000,000	3.8	2,900,000	3.9	780,000	3.6
10	3.6	3,000,000	3.5	8,300,000	3.6	1,430,000	3.8	270,000	3.9	28,000,000	4.2
20	3.6	130	3.6	500,000	3.8	116,000,000	4.0	11,000,000	3.9	430,000	4.3
0-9	3.6	2,400,000	3.7	150,000	3.7	810,000	3.6	4,000,000	4.0	157,000,000	4.1
10	3.6	510,000	3.8	0-9	3.5	250,000	3.6	1,710,000	4.1	640,000	3.9
200	3.8	1,100,000	3.8	210,000	3.9			430,000	4.7	420,000	3.7
0-9	3.7	8,100,000	3.6	3,100,000	3.7			1,940,000	3.9		
230	3.9	460,000	3.6	4,100,000	3.6						
					Achromobacter sp.						
500	3.7	109,000	3.6	109,000	3.6	900,000	3.9	1,510,000	3.9	176,000	4.0
30	3.6	430,000	3.9	46,000	3.8	2,120,000	3.9	440,000	3.9	650,000	3.9
10	3.6	370,000	3.8	162,000	3.9	10	4.0	1,800,000	4.0	20	4.0
0-9	3.8	460,000	3.8	19,000	3.8	54,000	4.0	225,000	4.0	71,000,000	3.8
0-9	3.7	75,000	3.7	210,000	3.8	2,300,000	4.0	1,310,000	4.1		
0-9	3.8	135,000	3.8	128,000	3.9	650,000	3.8	193,000	4.0		
20	3.6	1,390,000	3.9	88,000	3.7	0-9	3.6	236,000	3.7		
0-9	3.7	670,000	3.7	5,100	4.1	10,000,000	3.8	0-9	4.1		
0-9	3.7	70,000	3.7	280,000	3.9	6,600,000	3.8	720,000,000	4.2		
700	3.9	234,000	3.7	870,000	3.8	18,000	3.9	237,000	4.1		
					Uninoculated Fruit						
Range	Range	Range	Range	Range	Range	Range	Range	Range	Range	Range	Range
0-80	3.5-4.1	0-70*	3.5-4.1	0-60	3.6-4.0	0-150	3.6-4.1	0-150	3.6-4.3	0-40	3.6-4.2

*—One orange in this group had a microorganism count of 7,600,000 organisms per ml. and was not included in the range as given above.

sp. 4.0; *Xanthomonas* sp. "A" 3.8; *Xanthomonas* "B," 4.0; and *Achromobacter* sp., 3.4. A correlation between the minimum pH values at which these organisms grew in the laboratory and the minimum pH at which they will grow in fruit is not evident. In certain cases growth did not occur when the pH of the fruit was considerably above the limiting pH of the organism. Similarly, there is apparently no correlation between the magnitude of the count obtained in the inoculated fruit and the pH of the juice of that fruit.

Only one of the 151 uninoculated control fruit had a microorganism count above 150 organisms per ml. However, this one fruit was very highly infected, having a count of 7,600,000 organisms per ml. Assuming that each fruit contains approximately the same amount of juice (about 100 ml.), a composited juice from these 151 oranges would theoretically have a microorganism count of approximately 50,000 organisms per ml. This count is significant when it is realized that these control fruit were selected carefully as being apparently sound fruit.

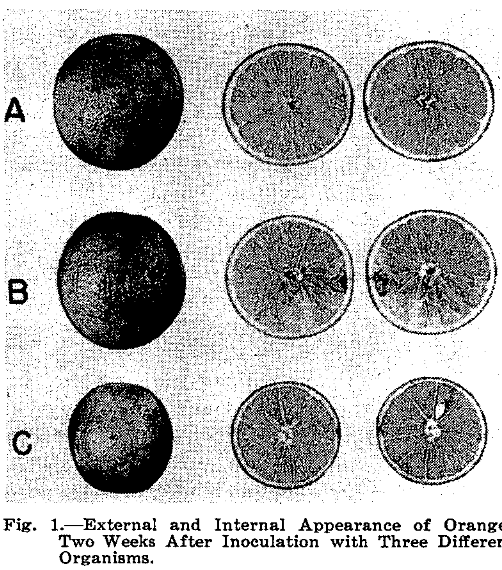

Fig. 1.—External and Internal Appearance of Oranges Two Weeks After Inoculation with Three Different Organisms.

Microorganism	Total count per ml. of juice
(A) Lactobacillus sp.	86,000,000
(B) Xanthomonas sp. "A"	500,000,000
(C) Aerobacter aerogenes	4,500,000

Appearance of Infected Fruit

Examples of the external and internal appearance of oranges inoculated with *Lactobacillus* sp., *Xanthomonas* sp. "A" and *Aerobacter aerogenes* are shown in Figure 1. These oranges were picked and examined two weeks after inoculation. The point of inoculation can be seen near the center of the uncut orange. Deterioration of the peel and juice sacs can be seen inside of the fruit, the sides through which the needle passed being directly opposite each other. The internal deterioration and the external appearance give but slight indication of the high counts that were present.

Summary

Acid-tolerant bacteria isolated from orange juice produced deterioration of tissue and high counts only when inoculated into the juice of the fruit. This fact suggests that natural infection of citrus fruit could be caused either by thorn injury or by insects capable of piercing the peel.

The organisms, after being inoculated into the fruit on the tree, produced maximum counts within approximately three weeks and maintained high counts throughout the observation period of five weeks.

There was little indication in the external appearance of the fruit that high counts were present. Experienced graders would seldom, if ever, be able to recognize and reject fruit infected in this manner. Oranges growing on the tree were found to have a range in bacterial count from 0 to 150 organisms per ml. of juice, although an occasional apparently sound fruit may harbor high numbers of bacteria.

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