

Processing Section

MICROBIOLOGICAL EXAMINATION OF JAFFA ORANGES WITH STYLAR END SPOT

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Citrus fruits are susceptible to the parasitic disease, *black rot*, caused by the fungus, *Alternaria citri* Ellis and Pierce. Suit (4) reported that in Florida black rot has been observed on Hamlin, Jaffa, Parson Brown, Pineapple, Ruby Blood, and Valencia oranges and on tangerines; also that the disease appears to be most prevalent on the early varieties. Fawcett (2) and Christie (1) also have presented information on the occurrence, cause, and control of black rot in oranges.

Jaffa oranges with black rot cannot be used for the manufacture of frozen concentrate because the use of such fruit will result in a product having an off-flavor and a high microbiological count. Complete separation of infected fruit from good fruit by visual inspection is not possible, even though black rot in oranges is often accompanied by a brown spot of varying size at the stylar end of the fruit. Since stylar end spot often occurs in the absence of black rot (Figure 1), this investigation was undertaken to determine if other microorganisms were responsible for this blemish and if any correlation existed between the occurrence of stylar end spot and internal deterioration in Jaffa oranges. Also, it was of interest and importance to find out if other molds, yeasts, or bacteria could gain entrance into the fruit through the stylar end and cause deterioration. Hill and Faville (3) found that acid-tolerant bacteria could grow in fruit on the tree and cause spoilage without producing, to any great extent, visible external evidence of deterioration.

EXPERIMENTAL PROCEDURE AND RESULTS

The fruit used in this investigation was obtained from a 40-acre Jaffa grove containing

approximately 2700 trees. They were heavily bearing trees of medium height, and with healthy foliage.

Preliminary Examination of Fruit during 1951-1952 Season. At the time the grove was first inspected, in January 1952, the fruit was bright orange in color and very mature. Many of the fruit had small brown or black spots at the stylar ends. Approximately three hundred oranges with these external symptoms were cut and only three had internal evidence of *Alternaria* infection. The discoloration of the remaining oranges went only to the juice sacs, sometimes not even through the albedo. Most of the oranges infected with *Alternaria* had apparently dropped from the trees prior to this inspection of the grove.

Three of the trees were stripped of their fruit and the oranges were separated into two groups. One group contained oranges which would ordinarily be considered to have pro-

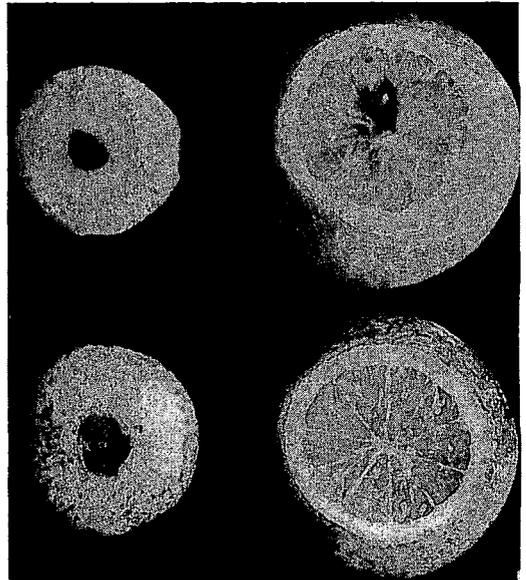


Fig. 1. Jaffa oranges showing stylar end spot with (top) and without (bottom) internal deterioration.

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nounced symptoms of "black rot"; the other group had either doubtful symptoms or none. The juice from the two groups of oranges was extracted separately in the pilot plant. The total microorganism count on orange serum agar, pH 5.8, for the juice from oranges with pronounced symptoms was 195,000 per ml. The colony count for the juice from oranges with doubtful or no symptoms of "black rot" was 183,000 per ml. The juice from the two groups of oranges was combined and concentrated in the pilot plant evaporator. The Brix was held at 25° for 6 hr. by the addition of distilled water. There was no significant increase in count over this period of time, possibly due to the relatively low pH of 3.5 of the juice.

Examination of Fruit during 1952-53 Season. To determine whether the external symptom, namely the styler end spot, was an indication of infection by bacteria, yeast, or molds, visual inspection of the grove and microbiological examination of fruit was begun in October 1952. Ten trees were tagged at regular intervals diagonally across the grove. In the event the fruit was picked for commercial use, these trees were to remain undisturbed.

The entire grove was inspected at various times and between 40 and 50 oranges with external symptoms of infection were brought to the laboratory for a more extensive examination. Each orange was bathed in a bichloride of mercury solution and then rinsed with sterile water. A notation was made of the external symptoms and the orange was opened aseptically. The interior of the orange near the styler end was examined for visible deterioration and evidence of black rot as shown in Figure 1. Potato dextrose agar plates, pH 3.5, and orange serum agar plates, pH 5.8, were streaked with juice of the orange from near the central cylinder. Juice was transferred to tubes of orange serum, pH 4.0, and of dextrose broth, pH 7.0. A portion of the central cylinder was excised and transferred to a tube of orange serum. Bacteria and yeast developing in the tubes of broth and on the agar plates were isolated and the plates incubated for a sufficient period of time to permit development of the mold. The genera of molds which developed were identified by microscopic examination. Examinations of the Jaffa grove and of the fruit collected were made once in October, twice in November,

Table 1
Examination of Jaffa oranges with styler end spot

Date picked	Visible internal deterioration %	Visible internal black rot %	<i>Alternaria</i> isolated %
10-29-52	51.3	27.0	43.2
11-12-52	18.2	9.1	24.2
11-25-52	10.3	10.3	12.8
12- 3-52	30.8	12.8	25.6
12-10-52	15.0	10.0	12.5
1-29-53	5.0	5.0	12.5

twice in December, and once in January (Table 1).

The grove early in the season contained large numbers of oranges with styler end spot. In October, only about 5 to 10 percent of the fruit was colored and most of these had symptoms of deterioration. *Alternaria* was isolated from many oranges which were still of a greenish color or only partly colored. Towards the end of the observation period in January, it became increasingly difficult to locate oranges with styler end spot.

Tables 1 and 2 are tabulations of the results obtained from the visible and microbiological examinations of the oranges conducted over a period of three months. In October, 27.0 percent of the oranges picked had visible internal *Alternaria*-like infection although *Alternaria* was isolated from 43.2 percent of the fruit. Fifty-one percent of the oranges had visible internal deterioration of one type or another. In January, only 5 percent of the oranges with styler end spot had visible internal black rot. *Alternaria* was isolated from only 12.5 percent of the oranges and visible internal deterioration of any kind had dropped to 5 percent. Information in Table 2 shows the isolations of *Alternaria* from Jaffa oranges with and without visible internal deterioration.

Table 2
Occurrence of *Alternaria* in Jaffa oranges with styler end spot

Date picked	Isolations from all fruit %	Isolations from fruit with internal deterioration %	Isolations from fruit without internal deterioration %
10-29-52	43.2	40.5	2.7
11-12-52	24.2	18.2	6.0
11-25-52	12.8	10.2	2.6
12- 3-52	25.6	20.5	5.1
12-10-52	12.5	7.5	5.0
1-29-53	12.5	5.0	7.5

Molds other than *Alternaria* isolated from the oranges represented the genera *Penicillium*, *Diplodia*, *Aspergillus*, *Colletotrichum*, *Tetro-*

sporangia, *Hormodendrum*, *Nigrospora*, and *Fusarium*. *Nigrospora* has not been previously reported as occurring in citrus fruits. Isolations of bacteria and yeast capable of growing in orange serum were made from these oranges. However, bacteria or yeast in high enough numbers to be considered the predominating organism were present in less than 2 percent of all the oranges examined.

In February, fruit was carefully and completely removed from two trees. This fruit was sorted into two groups; those which were apparently sound and those which had external symptoms of deterioration. The oranges were then counted, opened, and visually examined for internal deterioration. No attempt was made to isolate microorganisms, the purpose being to obtain an actual count of the number of deteriorated oranges remaining on the trees. This procedure was repeated one month later in early March.

Of the 3,093 Jaffa oranges examined from the two trees picked in February, 0.5 percent showed visible internal deterioration. Furthermore, although 3.3 percent of the oranges had external symptoms of internal deterioration, only 13.5 percent of these had visible internal deterioration, as compared to 51.3 percent of oranges with comparable symptoms in October, 14.3 percent in November, 22.9 percent in December and 5.0 percent in January.

Of the 3,062 Jaffa oranges examined in March, 4.8 percent had visible external symptoms of deterioration of which 8.1 percent were visibly deteriorated internally. In March, of the total number of oranges examined only 0.4 percent had visible internal deterioration.

Juice from fruit from the remaining tagged trees was extracted, finished, and made into frozen orange concentrate on March 3 in the pilot plant. Analysis of the juice indicated 10.3° Brix, 0.69 percent acid, and a Brix/acid ratio of 14.9. Microbiological counts on dextrose agar, pH 7.0, and potato dextrose agar, pH 3.5, for the freshly extracted juice were 25,000 and 8,000 per ml., respectively; the counts for the concentrate, using the same media, were 21,500 and 12,000 per ml. No off-flavor was detectable in the concentrate and the over-all quality of the product was good.

DISCUSSION AND SUMMARY

The results obtained in this investigation indicated that the incidence of internal deterioration in Jaffa oranges was very great in the early part of the season but decreased as the season progressed. In October, 51 percent of the fruit with stylar end spot had visible internal deterioration but this decreased to 5 percent in January. Also, less than 0.5 percent of all fruit from four trees examined in February and March showed internal deterioration. Therefore, one method for the elimination of infected fruit prior to its use as fresh fruit or for processing would be to pick the fruit late in the season after most of the infected fruit has dropped. Such late season fruit should be satisfactory for either frozen concentrate or canned juice. Infected fruit often colors prematurely; therefore, the removal of such fruit from the trees very early in the season would provide another method for the elimination of undesirable fruit.

Alternaria was the principal microorganism isolated from Jaffa oranges with stylar end spot and other molds, yeast, and bacteria were very seldom found to be the predominating organism present.

No correlation was found between the occurrence of stylar end spot and visible internal deterioration since such spots were found on both internally decayed and good fruit.

ACKNOWLEDGEMENT

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