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BACTERIAL SOFT ROT OF AVOCADO FRUIT. II.

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ABSTRACT

A bacterial soft rot disease on avocado fruit was found in Israel for the first time in December 1953. The causal organism was identified as *Erwinia carotovora* var. *aroideae.* Like other organisms of the same group it is a wound parasite and requires relatively high humidity to induce infection.

The pathogenicity of the organism to 4 different varieties of avocado fruits and to several other hosts was tested. It produces lesions similar to those of the original avocado fruits on all 4 varieties. It induces soft rot in tomato and pepper fruits as well as on slices of potato or carrot in the presence of water. On potato plants it causes typical black leg symptoms, followed by collapse, and disintegration of the stems.

Other tested isolates of *E. carotovora* var. *aroideae* as well as of *E. atroseptica* induce lesions similar to those produced by the avocado isolate on avocado and other hosts. The pathogenicity temperature range of the avocado isolate is wider, however. Infection spreads more rapidly at higher than at lower temperature. The optimal temperature for infection is 30-32°C; very good infection is produced at 36-37°C, and good, though slow infection is induced at 14°C. No infection occurs above 38 or below 12°C.

The close relationship of the avocado organism and other isolates of *E. carotovora* var. *aroideae* with *E. atroseptica,* the causal organism of the black leg disease of potatoes is discussed.

A bacterial soft-rot disease in avocado fruits was found in Israel for the first time in December 1953 on several fruits picked from a single tree of the California Variety H. L. The disease was caused by an organism belonging to the soft-rot group of bacteria originally identified as *Erwinia aroideae* (Townsend) Holland (Bergey et al. 1948) and later by Rudd Jones, Dowson and others as a non-gas-forming strain of *Bacterium carotovorum* (L. R. Jones) Lehman et Neumann (Rudd Jones 1950), or *Erwinia carotovora* (Jones) Holland (Bergey et al. 1948) or *Pectobacterium carotavoruin* (Jones) Waldee (Dowson 1957). This organism was not yet mentioned in the literature in connection with avocado. It was finally identified as *Erwinia carotovora* var. *aroideae* or *Pectobacterium carotavorum* var. *aroideae* (Dowson 1957). A short account of the disease and its cause has been given elsewhere (Volcani 1954).

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In this paper the results of further experiments comprising the pathogenicity of the organism to 4 different winter varieties of avocado fruits (Fuerte, Anaheim, Nabal and Benik) to tomato and pepper fruits and to potato plants are described. The results of infection experiments on avocado fruits of the Nabal variety with 2 other isolates* of *E. carotovora* var. *aroideae* and 2 isolates** of *E. atroseptica* (van Hall) Jennison (Bergey et al. 1948) are also reported. Finally, the close relationship of the avocado organism with isolates of *E. atroseptica.* is discussed.

Healthy unripe detached fruits were inoculated either by pricking through drops of sterile distilled water suspension of 48 hours old slant cultures, or by spraying with the same suspension without pricking. The inoculated fruits and controls were kept in bell-jars over water or without water at temperatures of 12, 14, 20, 25, 30-32, 36-37 and 38°C.

Healthy potato plants grown in pots were inoculated on their stems by the methods described above, and kept in the incubator under bell-jars overnight at 25°C. They were then put outdoors, and the bell-jars were removed. Temperatures ranged from 14-16°C during the night to 20-30°C during the day.

Positive results were obtained only with material which had been pricked through drops of the suspension of the organism. Infection experiments with reisolations from lesions gave similar results. Microscopic examination of Gram-stained affected tissues of inoculated specimens revealed numerous Gram-negative rod-shaped bacteria. The organisms recovered from the lesions of inoculated hosts were identical with the respective original isolations.

All tested organisms required relatively high humidity to produce infection. Progress of infection was more rapid at temperatures of 30-37°C for the avocado and pepper isolate, than at 25 and 20°C, and attained its maximum rate for the former isolate at 30-32°C. No infection was produced at below 20°C with the pepper isolate, but good, though slow infection was produced at 14°C with the avocado isolate. The progress of infection, for the tobacco isolate and the 2 isolates of *E. atroseptica* was greater at 25 than at 20 and 32°C; no infection was produced at 36-37°C.

Infection was produced on all four varieties of the avocado fruits inoculated with the avocado isolate, and there were no significant differences in the sizes of the lesions. The lesions were similar to those observed in the original fruits except with the Benik variety. They appeared as soft dark flat spots around centres of infection (Figure 1). Eight or nine days after inoculation at 32°C, the spots coalesced and covered the entire unripe fruits. In longitudinal section of inoculated fruits, the infected tissue appeared softer than the healthy and light to dark brown in colour. Sunken concave dark-brown pits often appeared under the skin around the centre of inoculation (Figure 2). Putrid smell evolved from all infected fruits.

On unripe Benik fruits the lesions appeared at first as dark spots quite distinct from the green healthy skin. But as soon as this fruit starts to soften, it normally darkens, and it was therefore impossible to distinguish between the healthy and diseased area. When

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* No. 37 isolated from pepper fruits in Israel (Volcani 1953a); No. 66 isolated from tobacco in Uganda, received from Dr. W. J. Dowson, Cambridge, England.

** No. 74 isolated from potato stem in Israel (Volcani 1953b); No. 274 an American potato isolate received from Dr. Dowson.

the fruit was cut, however, the diseased tissue under the skin showed lesions similar to those described above.

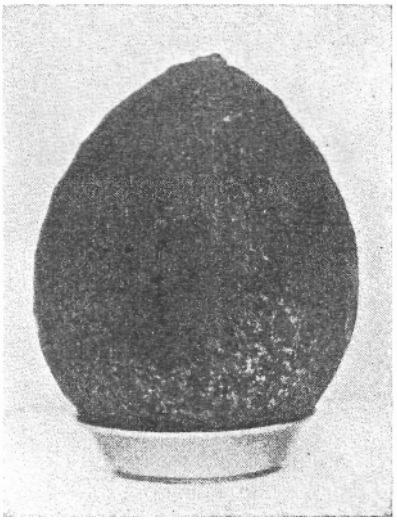

Figure 1. Inoculated unripe Nabal avocado fruit showing soft dark flat spots around the center of infection.

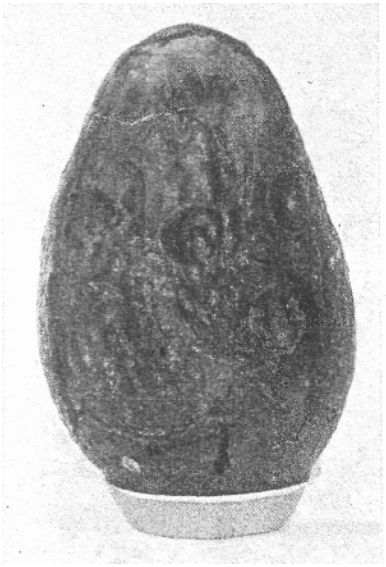

Figure 2. Inoculated Anaheim avocado fruit showing sunken concave dark brown pits under the skin around center of infection.

Lesions similar to those produced by the avocado isolate appeared on the Nabal fruits inoculated with the 2 isolates of *E. carotovara* var. *aroideae,* and the 2 isolates of *E. atroseptica.* The progress of infection was greater, however, with the avocado isolate than with the four other organisms at their respective optimal temperatures.

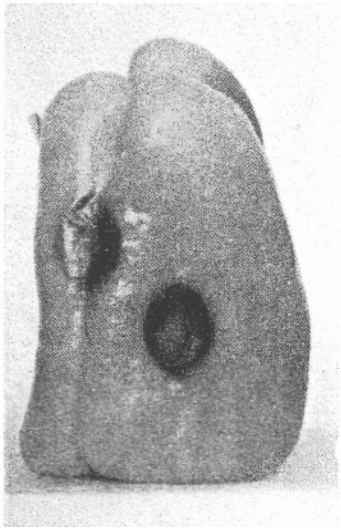

Figure 3. Inoculated pepper fruit showing soft, flat, brown spots.

Lesions on tomato and pepper fruits as well as on potato and carrot slices were similar for all 5 organisms. Inoculated green tomato and pepper fruits showed light to dark-brown, soft, flat spots around centre of inoculation (Figure 3). Following inoculation at 25°C, the spots coalesced and a soft rot of the entire fruit ensued after 3-4 days with, the avocado organism, and after 5-8 days with the others. The skin of the tomato fruits was often raised and shrivelled, and deep cracks developed in the infected area (Figure 4). Ripe tomato fruits were affected with soft-rot to the same extent as the green fruits. Inoculated potato and carrot slices in water developed soft rot of the parenchyma.

Distinct signs of black leg similar to those produced by the *E. atroseptica* isolates appeared within 24 hours on potato plants inoculated with the avocado organism. The inoculation-wounds had developed into elongated dark brown to black

lesions, and brown streaks appeared on the stems (Figure 5). The upper inoculations developed more rapidly than did the basal. The basal lesions appeared within 3 days after inoculation, but wilting of the leaves followed by collapse of the stems and disintegration ensued 3-4 days later.

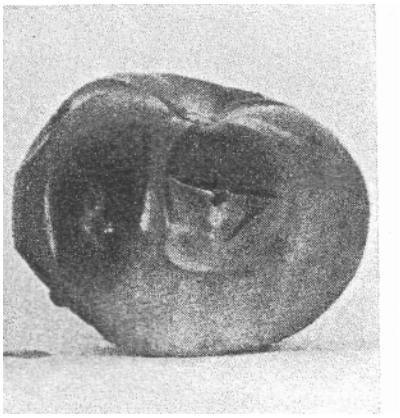

Figure 4. Inoculated tomato fruit showing soft, flat, light brown, cracked spots.

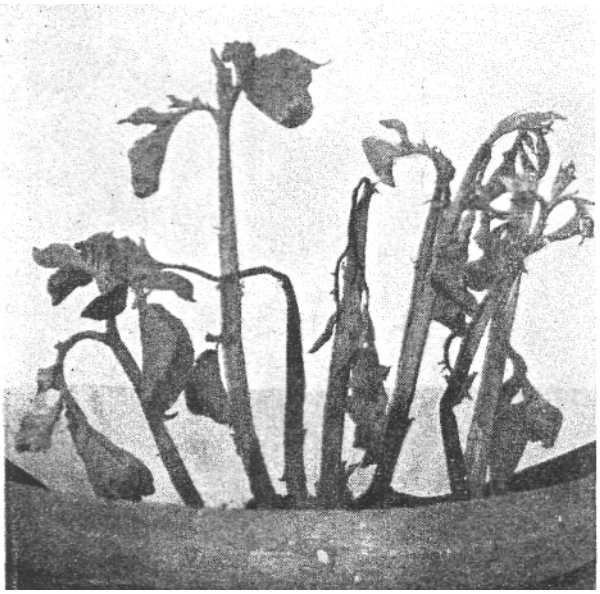

Figure 5. Inoculated potato plants showing typical black leg symptoms 3 days after inoculation. The tips of the stems in the upper inoculations are bent over, wilted and turned black. The basal inoculations show dark brown elongated lesions and long brown streaks developing along the stems upwards from the wound.

The avocado isolate has also been found to be closely related in many of its biochemical activities as well as in its pathological characteristics with the black leg organism *Erwinia atroseptica;* it differs only in its maximum and optimum growth and pathogenicity temperatures which are higher.

These results are in agreement with the author's preliminary findings and with Hellmers's and Dowson's experiments showing that various isolates of potato black leg and soft rot bacteria produce similar effects when inoculated into the vascular bundles of potato plants (Hellmers and Dowson 1953). Furthermore, another strain of *E. carotovora* var. *aroideae* more recently isolated from maize plants (Volcani 1958) was also found to cause typical black leg symptoms when inoculated on stems of potato plants.

These results emphasize once more the conclusion of Hellmers and Dowson contrary to Burkholder and Smith (1949), that the group of organisms identified as *E. atroseptica* are strains or varieties of the group of the soft rot organisms identified in Bergey as *E. carotovora* (Jones) Holland (Bergey et al. 1948), which Hellmers and Dowson preferred to term *Bacterium carotovorum* (L. R. Jones) Lehman et Neumann (Hellmers and Dowson 1953) and lately as *Peclobacterium carotovorum* var. *atrosepticum* (Dowson 1957).

Experiments have shown that the avocado organism is not specific to the H L Californian variety, but is capable of infecting other winter varieties of avocado fruits. Since its maximum pathogenicity temperature is above 37°C, it is likely that the organism can infect summer varieties of fruits under favourable humidity conditions.

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