

## Survival of *Erwinia rubrifaciens* in Soil

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*Erwinia rubrifaciens* Wilson, Zeitoun, and Fredrickson (1967) causes an important disease of Persian walnut (*Juglans regia* L.) in California (4). A copious slime that oozes through cracks in the bark of trunks and branches (Fig. 1-A) contains large numbers of the causal organism. Exudate commonly drips to the soil during late summer, but large amounts remain on the bark until washed away by rains, which usually do not occur until early October.

This paper reports on survival of the bacteria in the soil. An early phase of this work was briefly reported (3).

The selective medium used for isolation of *E. rubrifaciens* had the following composition: 10 ml glycerol, 5 g ammonium sulfate, 2 g dipotassium phosphate, 0.4 g eosin Y, 0.065 g methylene blue, 250 mg cyclohexamide (85-100% active ingredient), 40 mg novobiocin (pure), 40 mg neomycin sulfate (680 µg/mg), 15 g agar, and 1 liter distilled water. The many soil-inhabiting bacteria that require organic nitrogen to grow (2) cannot utilize ammonium sulfate, on which *E. rubrifaciens* grows readily. Eosin-methylene blue helps in the diagnosis of *E. rubrifaciens* (4), and inhibits development of gram-positive bacteria (1). For enhanced selectivity, cycloheximide was added to suppress fungus growth, and novobiocin and neomycin were added to inhibit bacterial growth further. Each ingredient was added prior to autoclaving at 15-lb pressure for 15 min. Table 1 compares the development of bacteria in soil from four walnut orchards on Bacto EMB, the selective medium without antibiotics and the complete selective medium.

Colonies of *E. rubrifaciens* became visible on this

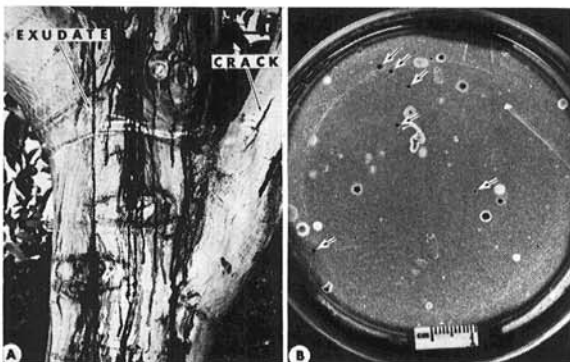


Fig. 1. A) External symptoms (cracking) and signs (exudate) of bacterial phloem canker on walnut limbs. B) Effectiveness of the selective medium in suppressing soil bacteria. Soil sample 10-7 taken on 9 January 1969 (Table 1). Arrows point to colonies of *Erwinia rubrifaciens*.

TABLE 1. Exclusion of soil bacteria by the selective medium

Soil sample	Type of soil	Colonies <sup>a</sup> of soil bacteria/petri plate on:		
		Bacto EMB with cyclohexamide	Selective medium	
			Without antibiotics <sup>b</sup>	With antibiotics
1	Loam	++ <sup>c</sup>	+	1.6
2	Sandy loam	+	119	0.5
3	Sandy loam	++	116	1.0
4	Clay loam	+	35	0.5

<sup>a</sup> Average of six replications: 1-1,000 dilution of the soil samples.

<sup>b</sup> Medium contained cyclohexamide but not neomycin or novobiocin.

<sup>c</sup> ++ = Too many colonies to count. ++ = Plates almost completely covered by bacterial colonies.

medium in 4 days at 27 C, and were 1-2 mm in diam in 6 days. They were circular, with entire margins and a convex surface. The dye had been absorbed to the extent that, by transmitted light, the colony was deep blue with translucent margins. When the colonies were numerous, the surrounding medium developed a greenish metallic sheen.

Fig. 1-B shows the recovery in January of *E. rubrifaciens* from an orchard soil which had been contaminated earlier with exudate from an infected tree. The bacteria from such characteristic colonies were streaked on a medium containing yeast extract, dextrose, finely divided calcium carbonate, and agar (YDC). Only those colonies producing a bright-red pigment in the medium, a characteristic reaction produced by *E. rubrifaciens* on that medium (4), were considered positive. Final proof of identity was obtained by inoculating walnut trees. In all cases, representative colonies from each soil sample produced typical symptoms of phloem canker (4).

Survival of *E. rubrifaciens* in a sandy-loam soil was determined by marking an exudate site beneath five walnut trees on 2 October 1968. Samples obtained from the top 3 inches of soil were taken to the laboratory for immediate assay. Duplicate 1-g lots of soil were added to 9 ml of sterile water, shaken on a vortex mixer, and diluted 1:10. One-tenth-ml lots of the suspension were pipetted onto the surface of three petri plates containing the selective medium, and evenly spread with an L-shaped glass rod while the plate was turning. Samples were taken on 2 October, 3 November, and 5 December 1968, and on 9 January and 4 April 1969. By 3 November, 3 inches of rain had fallen, washing all visible exudate from the surface of the soil. Frequent rains kept the soil moist until mid-February.

On 2 October the number of cells of *E. rubrifaciens*/g of soil was over a billion, and this number had not decreased notably by 3 November. Although the numbers were reduced considerably by 12 December, they were still relatively large even on 9 January. Three months later, however, no viable cells of *E. rubrifaciens* were found at any location (Table 2).

TABLE 2. Survival of *Erwinia rubrifaciens* in a sandy loam orchard soil

Sample location	No. colonies/g of soil				
	2 October 1968	3 November 1968	8 December 1968	9 January 1969	4 April 1969
5-4	$>1 \times 10^8$	$>1 \times 10^8$	$3 \times 10^4$	$4 \times 10^3$	0
10-2	$>1 \times 10^8$	$>1 \times 10^8$	$1 \times 10^7$	$1 \times 10^4$	0
10-3	$>1 \times 10^8$	$>1 \times 10^8$	$4 \times 10^3$		
10-7	$>1 \times 10^8$	$>1 \times 10^8$		$1 \times 10^3$	0
11-9	$>1 \times 10^8$	$>1 \times 10^8$	$4 \times 10^5$	$3 \times 10^4$	0

Schaad & Wilson (3) reported that the *E. rubrifaciens* was not recovered from soil 30 days after it had been contaminated by exudate from an infected tree. These samples were taken, however, in the late summer of 1967 when the soil was dry.

We conclude that *E. rubrifaciens* can survive in soil for at least 90 days under the conditions prevailing during the winter of 1968-69 in California. Just what

constitutes favorable soil moisture and soil temperature conditions remains to be determined.

Theoretically, the trunks of walnut trees could be contaminated by bacteria carried from the soil in spattered rainwater. Whether this means of inoculation plays a significant role in development of the disease also remains to be determined.

#### LITERATURE CITED

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