

**Aerial Strands of *Erwinia amylovora*: Structure and
Enhanced Production by Pesticide Oil**

H. L. Keil and T. van der Zwet

Research Plant Pathologists, Plant Science Research Division, ARS, USDA, Beltsville, Maryland 20705.

We thank J. M. Wehrung, Sperry Rand Microanalysis Laboratory, Rockville, Maryland, for preparation of the scanning electron microscope pictures.

Accepted for publication 22 October 1971.

ABSTRACT

Sprays of Superior "70" oil enhanced production of aerial bacterial strands from the blighted shoots of pear and apple trees infected with *Erwinia amylovora*. Strand production was greater on pears than on apples, and was increased over that for the water control even at the lowest (0.125%) oil concentration. Proportionately more strands were produced at each higher concentration. Oil increased aerial strand production even when applied 10 days before or after inoculation. Strands were produced at relative humidities as high as 94%, but not at ca. 100%. Apparently, the oil clogs some of the natural openings of the epidermis, and thereby effects in the shoots an increase in their internal pressure that results in extrusion

of rapidly proliferating bacteria in the form of strands. Bacteria in the strands were virulent, and caused more infection in injured than in noninjured plants. Examination of the strands with a scanning electron microscope revealed their variation in morphological structure and the approximate ratio of bacterial cells to matrix. Disintegration of the strands in water did not result in complete separation of the cells. Instead, many individual cells and clumps of cells remained held together by a cobweblike network. The possible importance of the strands in the dissemination of *E. amylovora* is discussed.

Phytopathology 62:355-361.

Additional key words: *Pyrus communis*, *Malus sylvestris*, fire blight.

Numerous studies have been made on the dissemination of *Erwinia amylovora*, the causal agent of fire blight of pear and apple. The blight organism may be spread by wind-driven rains from oozing

cankers or blighted branches (1, 6, 14, 15, 19, 20), or by bees and other pollinating insects from infected to healthy flowers (4, 10, 16). These types of dissemination, however, do not adequately explain

severe outbreaks of fire blight in pear and apple orchards in which the disease has not been observed for 1 to several years (7, 12, 21).

One type of dissemination seldom mentioned in fire blight literature involves aerial strands of *E. amylovora*. Bacterial strands or threads of *E. amylovora* were first described in 1937 by Ivanoff & Keitt (11) in Wisconsin. They reported strands only a few millimeters long. About 31 years later, Bauske (2) in Iowa, and Sprague & Covey (18) in Washington, described similar strands.

Recently, we observed in the greenhouse profuse production of aerial strands on artificially inoculated Bartlett pear trees sprayed with a commercial spray oil (13). We report herein the nature, structure, and production of these aerial strands and their potential role in the dissemination of *E. amylovora*.

MATERIALS AND METHODS.—Pear (*Pyrus communis* L. 'Bartlett') and apple (*Malus sylvestris* Mill. 'Jonathan') whips, 60-90 cm tall, were used. Cultures of *E. amylovora* were grown for 24 hr on slants of nutrient yeast dextrose agar. Inoculations of an aqueous suspension (10^6 cells/ml) were made by hypodermic needle into two sites in the top 12 mm of the whip. The inoculated plants were kept on the greenhouse bench or placed in an incubation chamber (25 C and ca. 100% relative humidity) for 5 days and then moved to the greenhouse bench until observations were made.

We used commercial Superior "70" spray oil (70 second viscosity paraffinic oil). The oil was diluted with water, and the resulting mixture was applied at 70 psi with a paint sprayer until the mixture ran off the plant surfaces. Plants were always allowed to dry 1-2 hr before they were placed in the incubation chamber. In one experiment, we tested mixtures that contained 1, 0.5, 0.25, and 0.125% oil. Each mixture was applied to five Bartlett trees. The tips of the whips were inoculated and immediately sprayed with an oil mixture, and the trees were kept on the greenhouse bench throughout the study. In another experiment, we determined strand production in response to a 1% oil mixture applied as a spray to each of three Bartlett whips 10 days before or 10 days after inoculating them with *E. amylovora*.

Strands were mounted in "70" oil or dimethyl sulfoxide for examination under the light microscope. Plant specimens examined under a scanning electron microscope (SEM) were first mounted on an aluminum pedestal with a silver paste, then coated with gold-palladium in a vacuum evaporator.

Pathogenicity of the bacteria in the strands was tested by dissolving the strands in water and injecting, by means of a hypodermic needle, a concentrated aqueous suspension of the bacteria into succulent Bartlett whips. We tested the effect of certain temperatures on cell viability by placing plants bearing strands in a room maintained at 0 C and holding excised shoots with strands in the laboratory at ca. 23 C.

Infectivity studies were conducted on 20 Bartlett and 20 Jonathan whips by placing drops of sterile water in the leaf axils, 5 cm from the apex, and

adding to the drops a few fragments of 7-day-old strands about 5 mm long. Half these plants were injured with a sterile needle inserted once through the drop into the plant stem; the remaining plants were uninjured. Five plants of each group were kept in the greenhouse, and the remaining five were held in a humidity chamber (25 C and ca. 100% relative humidity) for 5 days.

RESULTS.—*Production of aerial strands.*—Long aerial strands of *E. amylovora* occurred in abundance on inoculated Bartlett pear whips sprayed with 1% oil (Fig. 1-A). Strands were fewer in number, shorter in length, and far less conspicuous on plants not sprayed with oil (Fig. 1-B). Short strands on unsprayed plants are difficult to distinguish from trichomes.

distinguish from trichomes.

In one experiment using Bartlett pear whips, strand production began 3 days after the plants were sprayed, and increased in amount each day thereafter for 4 days, when final observations were made. Many strands were produced at the lowest concentration of oil (0.125%) tested and proportionately more were observed at each higher concentration (0.25%, 0.5%, and 1%). Relatively few strands were observed on the inoculated plants sprayed only with water (Fig. 1-B). Strand production was about the same on plants sprayed with 1% oil whether the oil was applied 10 days before or after inoculation. In other experiments, strand production on Jonathan apple whips was more frequent at oil concentrations of 1% than at lower concentrations.

Many more strands were produced on pear than on apple shoots. The strands were most abundant on petioles and lower midribs of leaves (Fig. 1-C, D, E). They usually were seen first about 1 day before the plant tissues became necrotic, and they became more abundant as the tissues collapsed. The strands usually increased in length for at least 4 days after blight symptoms first appeared. As the affected tissues dried, strand production ceased. However, new strands were produced on the newly blighted parts as blight infection progressed down the shoot.

Inoculated Bartlett plants sprayed with oil and held on the greenhouse bench always produced aerial strands. Similar plants held at 25 C and ca. 100% relative humidity never produced strands. Additional experiments showed repeatedly that plants held for 5 days under the latter conditions began to produce strands within 1 day after they were moved to the greenhouse bench, and continued to do so as long as blight infection remained active. Strands were produced on sprayed blighted Bartlett shoots at relative humidities up to 94%. We did not study their production at 95-99% relative humidity. Strands disappeared within 0.5 hr when exposed to a mist.

Nature and infectivity of strands.—The strands at first glistened and appeared colorless, then became creamy white, and finally, light brown. Most strands were smooth on the outside, of more or less uniform diameter throughout their length, and somewhat thicker at the tip. The diameter of 20 smooth strands selected randomly varied from 11.9 μ to 35.9 μ , and averaged 19.1 μ . These measurements were similar to

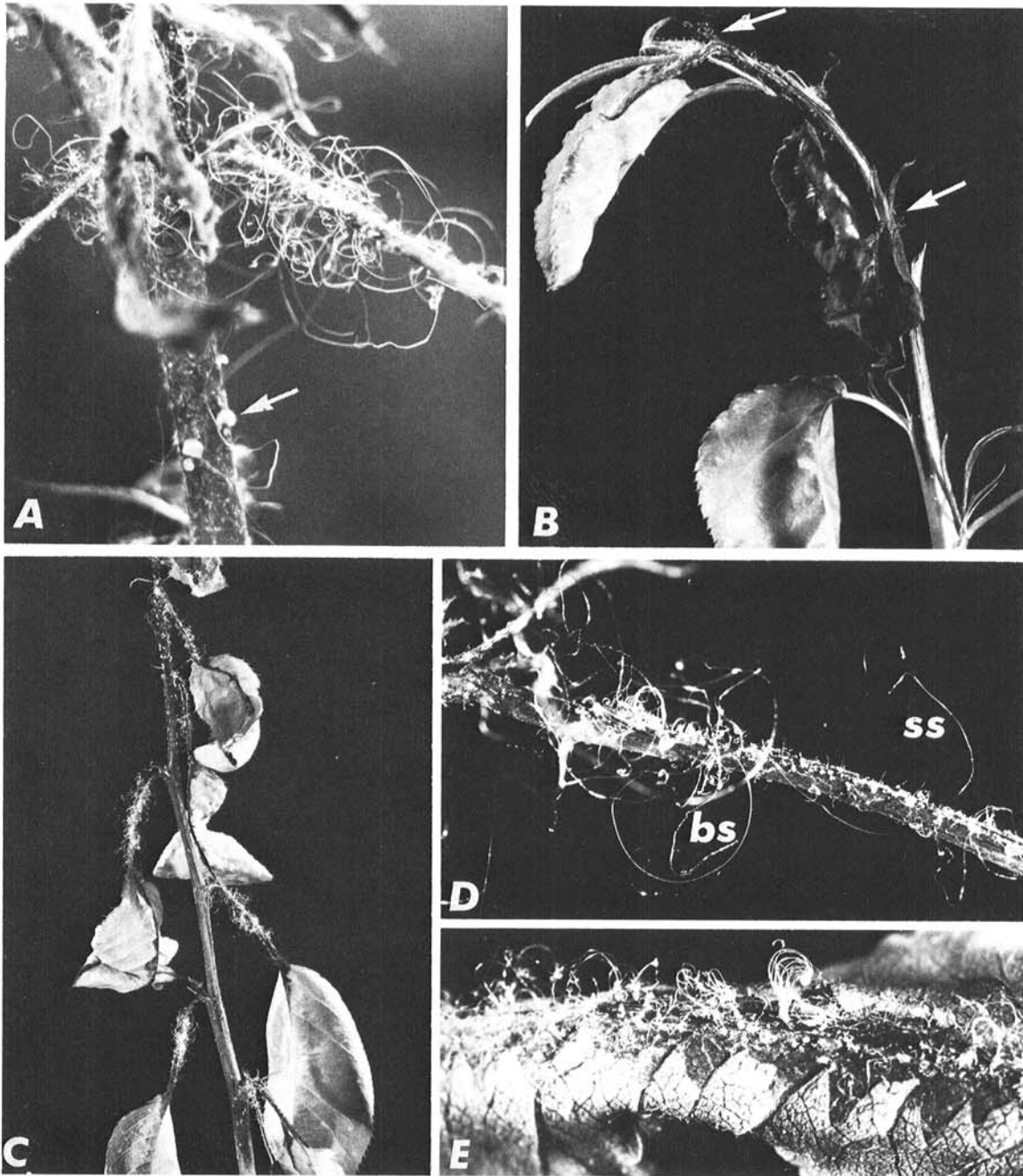


Fig. 1. Aerial strands of *Erwinia amylovora* on Bartlett pear produced 10 days after artificial inoculation. **A)** Profuse strands on plant sprayed with 1% commercial spray oil. Note ooze droplets (arrow) on main stem. **B)** Sparse production of short aerial strands (arrows) on plant not sprayed with oil. **C)** Abundant strand production on petioles sprayed with 1% commercial spray oil. **D)** Portion of petiole from plant sprayed with oil, showing extremely long smooth strands (ss) and beaded strands (bs). **E)** Lower side of leaf midrib, showing intermediate strand production following oil application.

those reported by Bauske (2), but somewhat smaller than those reported by Ivanoff & Keitt (11). Strands from control plants were 1-15 mm in length, whereas those from oil-sprayed plants usually were 30-75 mm long (Fig. 1-D). One unusual strand measured 13 cm in length. Sometimes the strands resembled a string of

beads (Fig. 1-D). Occasionally, strands were twisted or coiled like a spring; some were coiled twice, one coil within another.

Examination of aerial strands with SEM showed both the smooth and beaded types (Fig. 2-B). Smooth strands of about equal diameter throughout

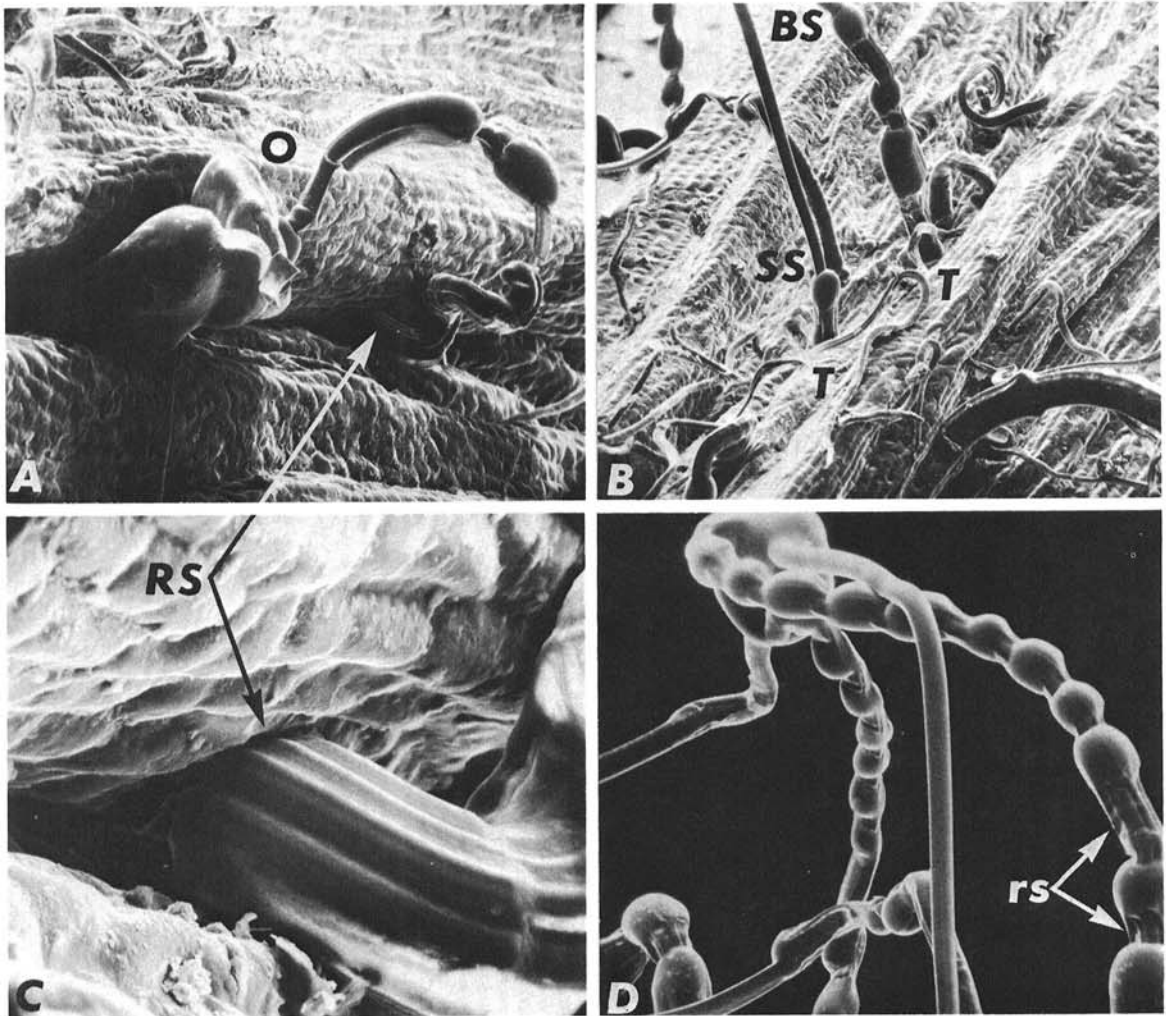


Fig. 2. Scanning electron micrographs of aerial strands of *Erwinia amylovora* on petioles of Bartlett pear. A) Ridged strand (RS) and odd-shaped strand attached to bacterial ooze (O) protruding from lenticles in petiole (X 200). B) Smooth strands (SS) and beaded strand (BS) among trichomes (T) on petiole surface (X 120). C) Ridged strand extruded from lenticle in A (X 1,000). D) Magnified view of B showing smooth and beaded strands, one with ridged segments (rs), all attached to dried ooze droplet in upper left (X 200).

their length seemed to be formed by a uniform quantity of matrix extruded through natural openings of the epidermis. Upon exposure to air, the matrix solidified to produce the strand. As more of the matrix was extruded basipetally, the strand increased in length.

Beaded strands appeared to be formed by extrusion of the matrix in spurts. One strand extruded from a lenticle was ridged (Fig. 2-A, C), as were some segments of other strands (Fig. 2-D). Frequently, we observed masses of ooze with attached strands (Fig. 2-A). Strands associated with ooze may have been produced initially by extrusion through a small aperture which later ruptured, allowing rapid extrusion of the matrix as bacterial ooze.

Strands were very firm and rigid (Fig. 3-E), became brittle with age, and, when crushed, broke or

shattered like glass into many fragments with irregular ends (Fig. 3-F). Broken ends of the strands suggested that they were composed largely of matrix rather than of bacteria (Fig. 3-G, H); preliminary analyses indicated a composition of 80% matrix and 20% bacterial cells. When the strands were dissolved in water which then was evaporated under vacuum, the bacterial cells remained connected by a thin, cobweblike network (Fig. 3-K). When strands were dissolved in water, the suspension was diluted with 95% ethyl alcohol or acetone, and the liquid was then evaporated under vacuum, the network disappeared and the bacteria could be seen as individual cells (Fig. 3-L). Flagella were not observed, and may have been detached during preparation of the samples (Fig. 3-M).

Strands placed in water dissolved in a few seconds. When they were mounted in Superior "70" oil, they

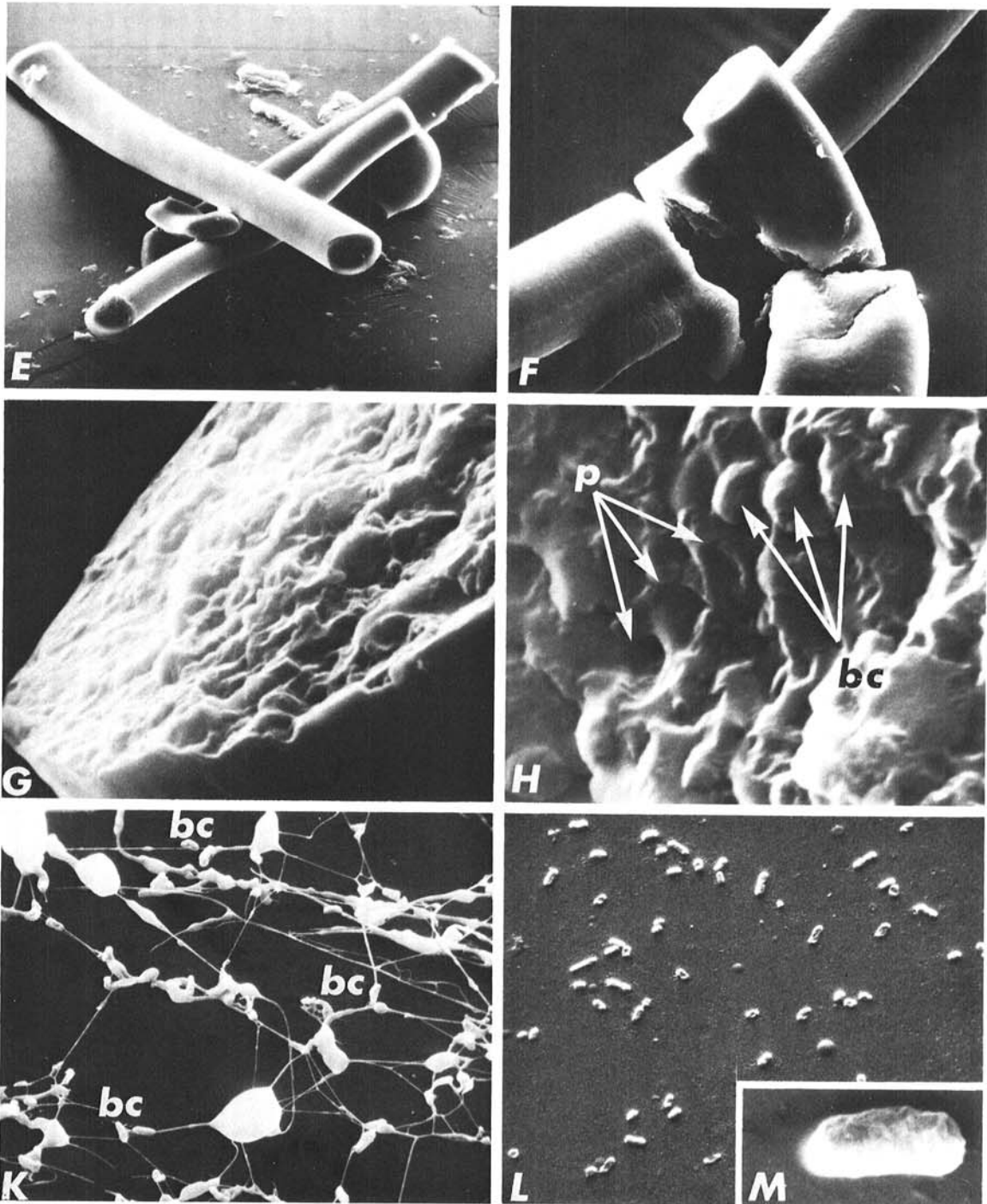


Fig. 3. Scanning electron micrographs of aerial strands of *Erwinia amylovora* on petioles of Bartlett pear. E) Several portions of broken, smooth strands (X 450). F) Pieces of broken, smooth strands showing irregular breaks and cracks (X 1,300). G) View of strand end shown in upper center in F (X 13,000). H) Magnified portion of cross section in G showing bacterial cells (bc) and cavities (p) in the cementing material where cells probably were present (X 26,000). K) Clumps and individual bacterial cells (bc) connected by a cobweblike network resulting from strands dissolved in water and the liquid then evaporated under vacuum (X 2,600). L) Individual bacterial cells resulting from strands dissolved in water and resuspended in 95% ethyl alcohol. Note collapse of cell walls due to prolonged exposure to electron beam (X 2,600). M) Individual cell of *E. amylovora* (X 26,000).

TABLE 1. Infectivity of *Erwinia amylovora* bacteria from aerial strands to Bartlett pear and Jonathan apple trees as influenced by plant injury

Treatment		Trees blighted/trees inoculated	
		Bartlett	Jonathan
Injured			
Incubated:	on greenhouse bench in moist chamber	3/5 4/5	5/5 4/5
Uninjured			
Incubated:	on greenhouse bench in moist chamber	1/5 0/5	0/5 0/5

maintained their shape for at least 6 months without disintegration. When mounted in dimethyl sulfoxide, they slowly disintegrated after 2-3 days, and showed cracking similar to that found when they were crushed.

The bacteria in the strands were virulent when mixed with water and injected into tender, succulent Bartlett shoots. Bacteria remained viable and pathogenic in strands attached to plants held for 4 days at 0 C, and on excised shoots held 3 months in a glass beaker in a laboratory at 22-25 C. Bauske (2) found them viable after 1 year at 5 C.

The bacteria in strands from oil-sprayed whips infected more injured Bartlett and Jonathan plants than noninjured ones (Table 1). Only 5% of the noninjured plants, compared to 80% of the injured plants, blighted. Infectivity of the bacteria was the same whether plants were kept in the chamber or on the greenhouse bench.

DISCUSSION.—Aerial strands of bacterial plant pathogens are infrequently mentioned in the literature (2, 11, 17, 18). Aerial strands of *E. amylovora* appear to be another form of ooze production. This is suggested by preliminary studies which showed that both aerial strands and ooze are composed of about four-fifths matrix and one-fifth bacterial cells. The matrix around the cells might act as a protective coating and a cementing material. Because *E. amylovora* cells remain viable for 15-25 months in dried, natural ooze (9) and up to 12 months in aerial strands (2), both ooze and aerial strands are potential sources of inoculum in the fire blight syndrome.

It has been reported that only one to a few *E. amylovora* cells are required for each new blight infection (8). This is not tenable, however, because most studies indicate that for infection to occur, the inoculum suspension must contain many cells per unit volume. This discrepancy remained unexplained until the SEM studies were conducted. These studies showed that when strands are dissolved in water, the bacteria are connected by a cobweblike network. If the strands get caught up in meteoric water, clumps of bacteria could thus be deposited in an infection court and provide large numbers of cells for infection.

We have shown that strands are produced abundantly under certain conditions and may play an important role in fire blight epidemiology. They may have gone undetected because of their similarity to

trichomes, especially on cultivars with excessive natural pubescence. In Illinois, J. B. Mowry (*personal communication*, Southern Illinois University, Carbondale) observed aerial strands as a haze in ambient air in apple orchards. D. Powell (*personal communication*, University of Illinois, Urbana) observed abundant strands, some as much as 5 mm long, on holdover fire blight cankers in an orchard of Willow Twig apple trees; these occurred most frequently in June on days when the temperature was about 27 C and the relative humidity, 70%. Powell, in the same orchard, obtained pure cultures of *E. amylovora* by exposing petri plates containing potato-dextrose agar.

Bacterial strands are easily detached or broken into fragments by wind or disintegrated by fine mist (2), which may account for the infrequent observation of their presence on blighted trees in the orchard. It is conceivable that they may be wafted high into the sky by air currents, then dissolved in moisture in the clouds. Temperature in the upper air is such that strands could remain viable and return to fruit orchards in raindrops many miles away. This would explain the occurrence of blight epidemics in orchards where the disease has not been seen for long periods.

Timing of oil sprays might be important relative to production of bacterial strands. Our studies show that application of oil close to an infection period is not the only time that abundant strand production is induced, as enhanced production was obtained when oil was applied 10 days before or after the infection period. We have not tested longer periods. The oil apparently plugs some natural epidermal openings, resulting in a buildup of pressure within the plant, and causing the rapidly proliferating bacteria to be extruded in the form of strands.

The profuse production of *E. amylovora* strands on infected plants sprayed with Superior "70" oil suggests some intriguing possibilities. In some areas, it is common practice in summer to spray fruit trees with oil for control of mites and psylla. In other areas, oil is sprayed for control of scales and insect and mite eggs during spring just before budbreak, or as a delayed dormant application after budbreak. It is conceivable that by these practices, bacterial strands are produced which may result in more fire blight infection.

Borden & Thomas (3) reported in 1943 from

California that Bartlett trees sprayed with oil in summer had 17% of the fruit infected with *E. amylovora*, whereas trees not sprayed with oil had only 0.5% of the fruit infected. Moreover, a shipment of apparently healthy pears, originating from orchards sprayed in summer with oil for control of red spider mites, had 30-50% of the fruit infected when it arrived in Hawaii (5). We wonder whether the oil sprays induced strand production and played a role in spread of fire blight in these orchards.

In some orchards, oil or oil derivatives may be used as pesticide solvents in liquid concentrates. Such concentrates are usually used at 1-2 pints/100 gal water as a dilute spray. According to our results, 1 pint of Superior "70" oil is sufficient to induce significant strand production. Thus, even oil used as a pesticide solvent in liquid concentrates may induce production of bacterial strands of *E. amylovora* that play a significant role in the epidemiology of fire blight.

LITERATURE CITED

- BAUSKE, R. J. 1967. Dissemination of waterborne *Erwinia amylovora* by wind in nursery plantings. *Amer. Soc. Hort. Sci.* 91:795-801.
- BAUSKE, R. J. 1968. Bacterial strands: a possible role in fire blight. *Iowa State J. Sci.* 43:119-124.
- BORDEN, A. D., & E. THOMAS. 1943. Fire blight and oil sprays. *Pacific Rural Press* 146 (3):68.
- BROOKS, A. N. 1926. Studies of the epidemiology and control of fire blight of apple. *Phytopathology* 16:665-696.
- CALIFORNIA AGRICULTURAL EXTENSION SERVICE. 1966. Do summer oil sprays favor fire blight development in pear fruit? *Fruit, Nut, Grape Dis.* January. p. 2-3.
- GOSSARD, H. A., & R. C. WALTON. 1917. Fire-blight infection—rain proves an important carrier of disease in open orchards. *Ohio Agr. Exp. Sta. Monthly Bull.* 2:357-364.
- GOURLEY, C. O., C. L. LOCKHART, & R. E. C. LAYNE. 1966. Fire blight of pear and some other hosts in Nova Scotia. *Can. Plant Dis. Survey* 46:139-142.
- HILDEBRAND, E. M. 1937. Infectivity of the fire-blight organism. *Phytopathology* 27:850-852.
- HILDEBRAND, E. M. 1939. Studies on fire blight ooze. *Phytopathology* 29:142-156.
- HILDEBRAND, E. M., & E. F. PHILLIPS. 1936. The honeybee and the beehive in relation to fire blight. *J. Agr. Res.* 52:789-810.
- IVANOFF, S. S., & G. W. KEITT. 1937. The occurrence of aerial bacterial strands on blossoms, fruits, and shoots blighted by *Erwinia amylovora*. *Phytopathology* 27:702-709.
- KEIL, H. L., B. C. SMALE, & R. A. WILSON. 1966. Role of injury and longevity of *Erwinia amylovora* in the epidemiology of fire blight of pear. *Phytopathology* 56:464-465.
- KEIL, H. L., & T. VAN DER ZWET. 1970. Role of oil sprays in production of aerial strands by *Erwinia amylovora*. *Phytopathology* 60:1298 (Abstr.).
- KEITT, G. W., J. A. PINCKARD, & A. J. RIKER. 1934. Rain-borne bacteria important in the spread of fire blight. *Wis. Agr. Exp. Sta. Ann. Rep.* 50:86-88.
- MILLER, P. W. 1929. Studies of fire blight of apple in Wisconsin. *J. Agr. Res.* 39:579-621.
- PARKER, K. G. 1936. Fire blight: overwintering, dissemination, and control of the pathogen. N.Y. (Cornell) *Agr. Exp. Sta. Mem.* 193. 42 p.
- SMITH, E. F. 1920. An introduction to bacterial diseases of plants. W. B. Saunders Co., Philadelphia, Pa. 688 p.
- SPRAGUE, R., & R. P. COVEY. 1969. Fungous and bacterial pear diseases of eastern Washington. *Wash. Agr. Exp. Sta. Circ.* 498:1-14.
- STEVENS, F. L., W. A. RUTH, & C. S. SPOONER. 1918. Pear blight wind borne. *Science* 48:449-450.
- TULLIS, E. C. 1929. Studies on the overwintering and modes of infection of the fire blight organism. *Mich. Agr. Exp. Sta. Tech. Bull.* 97. 32 p.
- VAN DER ZWET, T., H. L. KEIL, & B. C. SMALE. 1969. Fire blight in the Magness pear cultivar in north central Arkansas. *Plant Dis. Repr.* 53:686-689.