

Identification and Spread of *Erwinia amylovora* on Pear in Turkey

M. TIMUR MOMOL, Plant Protection Department, Faculty of Agriculture, University of Akdeniz, Antalya, Turkey, and WOLFGANG ZELLER, Federal Biological Research Centre, Institute for Plant Protection in Fruit Crops, 6915 Dossenheim, Germany

ABSTRACT

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Diagnostic and pathogenicity tests of two isolates of bacteria from blighted pear trees in Turkey were compared with tests of known isolates of *Erwinia amylovora*. Turkish isolates formed orange-yellow colonies with deep orange centers and clear margins on modified Miller-Schroth medium; they did not fluoresce under ultraviolet light when grown on King's medium B. Bacterial ooze was produced on immature pear fruit, and susceptible pear shoots were blighted in 6 days. Based on these tests, the two Turkish isolates were positively identified as *Erwinia amylovora*. The pattern of spread of fire blight in the Middle East, the Balkans, and the Mediterranean area since 1982 suggests that infections in Turkey may be part of an expanding epidemic originating in Egypt.

Fire blight of pear (*Pyrus communis* L.), caused by *Erwinia amylovora* (Burrill) Winslow et al, is one of the most serious diseases of pomaceous fruit trees and some ornamental plants. A severe outbreak of fire blight was observed on pears in the Nile Delta of Egypt in 1982

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(1,9). Since then, the disease has spread rapidly through the eastern Mediterranean and Balkan areas (10). In 1984, fire blight was detected on the island of Cyprus, and severe infection occurred in 1985 (2). Fire blight was first observed in Israel in 1985 (2). Therefore, the appearance of fire blight in Turkey was not unexpected. Symptoms resembling fire blight were first observed in Turkey in 1985 in a pear orchard at Sultandag,

Afyon province, (7) and were observed in 1989 in several pear and quince orchards in Korkuteli, Antalya province (5). In 1989 and 1990, bacteria resembling *E. amylovora* were isolated from diseased trees in Antalya and Burdur; however, the isolates were not confirmed as *E. amylovora* (6).

The objective of this study was to confirm the presence of *E. amylovora* in pear orchards in Turkey. Diagnostic and pathogenicity tests were performed on two isolates of *E. amylovora* from Turkey and were compared with tests of known cultures of *E. amylovora*.

MATERIALS AND METHODS

Identification. Isolations from diseased pear trees were performed using nutrient saccharose agar medium (1). Bacteria in fresh ooze were streaked on the medium and incubated 2–3 days at 26 C. Two resulting isolates (TREA90 and TREA91), collected in the 1990 and 1991 seasons, were maintained on nutrient agar to which 0.2% yeast extract

was added (8). The two Turkish isolates (TREA90 and TREA91), one German isolate (C 6/6), and one Egyptian isolate (E 1/81) (German and Egyptian isolates were obtained from W. Zeller) were plated on modified Miller-Schroth (MS) medium (3). Colonies of each of the four isolates and one isolate of *Pseudomonas syringae* pv. *syringae* van Hall (PSS) were also grown on King's medium B (4) and examined for fluorescence under ultraviolet light.

A slide agglutination test was performed with an antiserum obtained from the plant virology laboratory, Institute für Viruserologie der Biologischen Bundesanstalt, Braunschweig, Germany. A loopful of bacteria of each isolate was mixed with one drop of antiserum on a glass slide. In 3–5 min, the reaction was observed under a light microscope. Reactions were considered positive if bacteria showed an agglutination reaction (1).

Pathogenicity. Pathogenicity of the four isolates was tested by immature pear fruit assay (9). Fruit were surface disinfested with 70% ethanol and air-dried, then cut in slices, six slices per isolate. A loopful of bacteria was spread aseptically on the cut surface. After incubation in moist petri dishes for 3 days at 26 C, the inoculated surfaces were examined for bacterial ooze. The test was repeated twice.

Pathogenicity of the isolates was also tested on succulent pear shoots (cv. Comice). Shoots (four per isolate), about 40-cm long, were placed in an Erlenmeyer flask with tap water, and the tips were inoculated with 0.3 ml of bacterial suspension (10^8 cfu/ml, prepared with tap water) injected with a hypodermic needle. The shoots were maintained in a growth chamber at 23 C and 80% relative humidity. Shoots were observed for symptoms of fire blight (black necrosis and oozing) 6 days after inoculation. The test was repeated twice.

RESULTS AND DISCUSSION

Identification. Isolates E 1/81, C 6/6, TREA90, and TREA91 formed orange-yellow colonies with deep orange centers and clear margins on modified Miller-Schroth medium, typical of *E. amylovora* (8). None of the four isolates fluoresced on King's medium B under ultraviolet light, whereas PSS showed characteristic fluorescence. All four isolates showed a positive reaction in the slide agglutination tests, whereas PSS reacted negatively (Table 1).

Pathogenicity. Bacterial ooze developed on immature pear fruit inoculated with the two Turkish isolates and *E. amylovora* isolates C 6/6 and E 1/81. No substantial differences were observed in the degree of oozing. In contrast, inoculations with PSS did not result in oozing (Table 1). All isolates of *E. amylovora* caused fire blight symptoms on pear

shoots 6 days after inoculation; the blighted portion of the shoots was about one third of their total length. These tests confirmed that the two Turkish isolates were *E. amylovora*; they were similar to isolates C 6/6 and E 1/81.

Distribution of fire blight. The distribution of fire blight between 1982 and 1990 in Europe and the Middle East was mapped (Fig. 1). The spread of *E. amylovora* in the Middle East, the Balkans, and the southeastern Mediterranean area since 1982 has been very rapid, under apparently favorable epidemiological conditions. Based on the reported pattern of occurrence (10) of fire blight (Fig.

1), we hypothesize that the infections in Turkey may be part of an expanding epidemic originating in the Nile Delta in Egypt.

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Table 1. Reactions of two Turkish isolates (TREA90 and TREA91), two known isolates of *Erwinia amylovora* (E 1/81 and C 6/6), and *Pseudomonas syringae* pv. *syringae* (PSS) to various diagnostic tests

Isolate	Modified MS ^a	King's medium B ^b	Immature pear fruit ^c	Succulent pear shoots ^d	Slide agglutination ^e
E 1/81	+	NF	O	N	+
C 6/6	+	NF	O	N	+
TREA90	+	NF	O	N	+
TREA91	+	NF	O	N	+
PSS	... ^f	F	NO	...	-

^a Modified Miller-Schroth medium; orange-yellow colonies (+).

^b Under ultraviolet light, no fluorescence (NF) or fluorescence (F).

^c Oozing (O) or no oozing (NO).

^d Necrosis (N).

^e Positive (+) or negative (-) reaction.

^f No test performed.

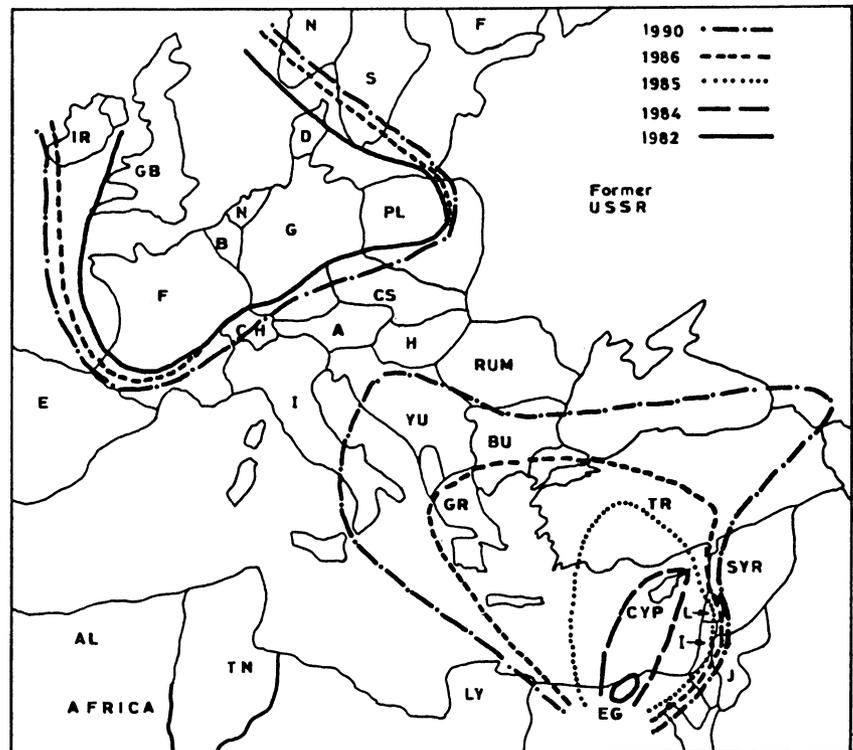


Fig. 1. Hypothesized spread of fire blight in Europe and the Middle East from 1982 to 1990. Country codes are as follows: A = Austria, AL = Algeria, B = Belgium, BU = Bulgaria, CH = Switzerland, CS = Czechoslovakia, CYP = Cyprus, D = Denmark, E = Spain, EG = Egypt, F = Finland and France, G = Germany, GB = Great Britain, GR = Greece, H = Hungary, I = Israel and Italy, IR = Ireland, J = Jordan, L = Lebanon, LY = Lybia, N = Netherlands and Norway, PL = Poland, RUM = Romania, S = Sweden, SYR = Syria, TN = Tunisia, TR = Turkey, YU = Yugoslavia.

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