Fire Blight on Pear, a New Disease in Israel

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ABSTRACT

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In early May 1985, symptoms characteristic of fire blight of pear were observed in an orchard in the Upper Jordan Valley of Israel. An extensive survey of other pear orchards revealed similar symptoms in eight pear orchards throughout the country. Inoculation of immature pear fruits with suspected isolates produced whitish bacterial exudates 76 hr after infection. Pathogenic isolates induced a hypersensitive reaction in tobacco leaves. In agglutination assays and dot-ELISA, the eight Israeli isolates reacted in a manner identical to that of the reference cultures with three sera: two produced against confirmed isolates of *Erwinia amylovora* and one against an Israeli suspect.

Fire blight, caused by *Erwinia amylovora*, is a serious disease of pears and related plants in the family Rosaceae in North America, Europe, and some other areas of the world (8). In the Mediterranean countries, however, this disease has only recently become a problem. In 1982 and 1983, Egyptian plant pathologists reported severe losses (1), and an epiphytotic of this disease also occurred in 1984 (9). That same year, fire blight was reported from Cyprus (4). In Israel, fire blight had not been found, although extensive cultivation of host plants as well as the climate provided conditions favorable for disease development.

Symptoms characteristic of fire blight were observed in early May 1985 in a young pear orchard at Rosh Pinna in the Upper Jordan Valley and during May and June in seven other pear orchards (Table 1). In this report, we present evidence confirming the presence of fire blight in Israel and *E. amylovora* as the causal agent.

MATERIALS AND METHODS

Collection of plant material. An extensive survey of all pear orchards in Israel (about 1,000 ha) was conducted during May and June 1985. Blighted terminal branches and flower buds bearing desiccated foliage were collected from five pear orchards spaced over a distance of 100 km in Galilee in the north and from three adjacent orchards 200 km south of Galilee in the southern coastal plain.

Isolation and identification of causal agent. The pathogen was isolated from the blighted branches by excising pieces of discolored woody tissue from the margins of the lesions and homogenizing these in sterile distilled water. Dilutions of the homogenates were plated on both 5% sucrose agar and the selective high sucrose medium of Crosse and Goodman (3,5,6). Incubation for colony development was at 27 C for 48–72 hr or until levan-forming colonies typical of *E. amylovora* were observed. Transfers from such colonies were made onto King's B medium, and colonies that did not produce green fluorescence were used in subsequent pathogenicity and immunological tests (5).

Pathogenicity tests. Bacterial suspensions, prepared in sterile phosphate-buffered saline (0.1 M, pH 7.2) (PBS), were adjusted to about 5×10^8 cfu/ml by measurement of optical density at 440 nm. Immature pear fruit was sliced in half lengthwise and inoculated by piercing the cut surface with a needle dipped in the bacterial suspension. The fruit was incubated in Pyrex dishes covered with plastic wrap in the dark at 27 C for 3 days. For comparison, inoculations were made with *Pseudomonas syringae* pv. *syringae* van Hall, originally isolated from pear, as well as

Table 1. Comparison of pathogenicity and immunological reactivity of various Israeli bacterial isolates with those of reference strains of *Erwinia amylovora*

		Reactions expressed		
Isolate	Source	Dot-ELISA[a]	Pear fruit[b]	Tobacco
EA 105/9 (IL-1)	Rosh Pinna	(C) +	+	HR
IL-2	Lavi	(E) +	+	···
IL-3	Allonim	(F) +	±	HR
IL-4	Dovrat	(G) +	+	HR
IL-5	Yad Mordekhay	(H) +*	+	HR
IL-6	Gevaram	(I) +	±	HR
IL-7	Yakhini	+*	+	HR
IL-8	Baram	+*	±	HR
EA 595	England (J. C. Crosse)	(A) +	+	HR
Erwinia sp-1(1069)	England	(B) −	−	−
Erwinia sp-2	Yad Mordekhay	−*	−	−
Erwinia sp-3	Allonim	−*	−	−
Erwinia sp-4	Metulla[c]	(J) −	−	−
Pseudomonas syringae	Rosh Pinna	(D) −	−	HR
E-8	United States (R. N. Goodman)	(K) +	−	HR
E-9	United States (R. N. Goodman)	(L) +	+	HR

[a] Letters refer to positions of bacteria in Figure 1. Each bacterial isolate was tested twice with each of three antisera, except those marked with an asterisk, which were tested only once. + = Positive reaction and − = negative reaction.
[b] All fruit infections were duplicated. + = Ooze formation, − = no ooze formed, and ± = opposing results in two tests.
[c] Isolated from pear fruit surface and has characteristic growth of *Erwinia* sp. on media (3,5,6).

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reference isolates of *E. amylovora* obtained from England (J. C. Crosse) and from the United States (R. N. Goodman, Missouri) (Table 1). Control pears were inoculated with sterile buffer only. Ooze formation was interpreted as a positive indicator of pathogenicity (5,6). Pathogenicity assays were conducted twice with most isolates. Induction of the hypersensitive reaction was tested on leaves of young tobacco plants (*Nicotiana tabacum*) by injection with a bacterial suspension (6). The plants were kept for 2–3 days in a greenhouse at 25 C until clear hypersensitive reactions developed.

Biochemical tests. Selected isolates and the confirmed *E. amylovora* isolates were tested for similarities with a number of biochemical assays outlined by Schroth and Hildebrand (6) for differentiation of *Erwinia* species. In addition, the oxidase test detailed by Lelliott (5) was also performed.

Immunological tests. *Preparation of antisera.* Two antisera were prepared against one presumptive isolate from the Rosh Pinna pear orchard (EA 105, IL-1) and the reference culture obtained from J. C. Crosse (NCPPB EA 595). New Zealand rabbits were injected with a bacterial suspension according to the schedule outlined by Lelliott (5). The animals were bled 21 days after the first inoculation.

The activity of the antisera prepared in Israel was compared with that of one prepared against a confirmed isolate of *E. amylovora* (IPO 7043-7072) supplied by H. J. Miller (Plant Protection Service, Wageningen, Netherlands).

Immunoassays. Slide and tube agglutination tests were carried out on the three sera in a preliminary screening for immunological activity. Subsequent assays were performed with the dot-ELISA technique on nitrocellulose membranes (NCM).

Dot-ELISA technique. Two-microliter drops of serial 10-fold dilutions (in PBS) of bacterial suspension were applied to strips of nitrocellulose (Schleicher and Schuell, Dansell, West Germany; 0.45-μm pore size) with a Hamilton microsyringe. After air-drying, the NCM were placed in an oven at 80 C for 30 min to stabilize binding. Unreacted sites were blocked by incubation for 15 min in the soluble fraction of 3% casein (w/w) (Sigma, St. Louis, MO) in tris-buffered saline [50 mM tris (hydroxymethyl)-aminomethane, 150 mM sodium chloride + 0.01% Thiomersal] (TBS). The strips were then incubated for 10 min in dilutions of antisera in TBS containing 1% bovine serum albumin (BSA, Sigma), 0.05% Tween 20 and 0.01% Thiomersal (TBS-BSA-Tween). This was followed by five 1-min rinses in TBS-Tween (0.05% Tween 20) and incubation for 90 min in a 1:1,000 dilution of alkaline phosphatase-conjugated Protein A in TBS-BSA-Tween (Bio Yeda, Rehovot, Israel). Unbound conjugate was rinsed away as previously with TBS-Tween. All incubations and washes were performed in plastic trays with gentle agitation on a platform shaker at room temperature.

Enzyme activity was determined by covering the strips with a solution of substrate composed of naphthol AS-MX phosphate (0.4 mg/ml) and fast red TR salt (6 mg/ml, both from Sigma) in 0.1 M Tris buffer, pH 8.0, containing 1 mM $MgCl_2$. Incubation was carried out until a definitive reaction was observed.

RESULTS

Fire blight symptoms were observed in eight of a large number of orchards surveyed (80) throughout the country. In three of the orchards, 5–10% of the trees showed some symptoms, whereas in the five others, only isolated trees were affected. Fire blight infections were observed in all of the main cultivars grown, Spadona (75% of the acreage), Gentile, and Costia, as well as on the less common Butirra Rosetta. Bacterial colonies isolated from blighted branches on selective medium (3) had morphological features identical to those of the confirmed *E. amylovora* isolate. The colonies were white-domed, mucoid, levan type on 5% sucrose (5,6), were not fluorescent on King's B medium (5), and on high sucrose, they had a cratered appearance (3).

Isolates from blighted tissues reacted positively in slide and tube agglutination tests with dilutions to 1:10,000 of sera produced against a confirmed *E. amylovora* isolate (IPO 7043) as well as sera produced in Israel against a reference culture (EA 595) and an Israeli suspect (EA 105). In dot-ELISA (Fig. 1, columns C and E–I), they gave positive reactions identical to those of the reference isolates (Fig. 1, columns A, K, and L) with the three antisera tested. Reactions were discernible when as few as 1,000 bacterial cells were applied to the membrane. Dilutions as low as 1:16,000 were active but required longer incubation in the antisera for a conclusive reaction. Some isolates that by morphological features were considered to be *Erwinia*-like did not react with any of the sera (Fig. 1, columns B and J). A *P. syringae* isolate obtained from fire blighted twigs also did not react (Fig. 1, column D).

Results of pathogenicity tests demonstrated that most isolates that were positive in dot-ELISA were also pathogenic on pear fruit, although some variability in pathogenicity was obtained with three isolates in the two fruit tests conducted (IL-3, IL-6, and IL-8) (Table 1). Four isolates that were negative in immunoassay were also nonpathogenic on pears (*Erwinia* sp-1 to sp-4) (Table 1).

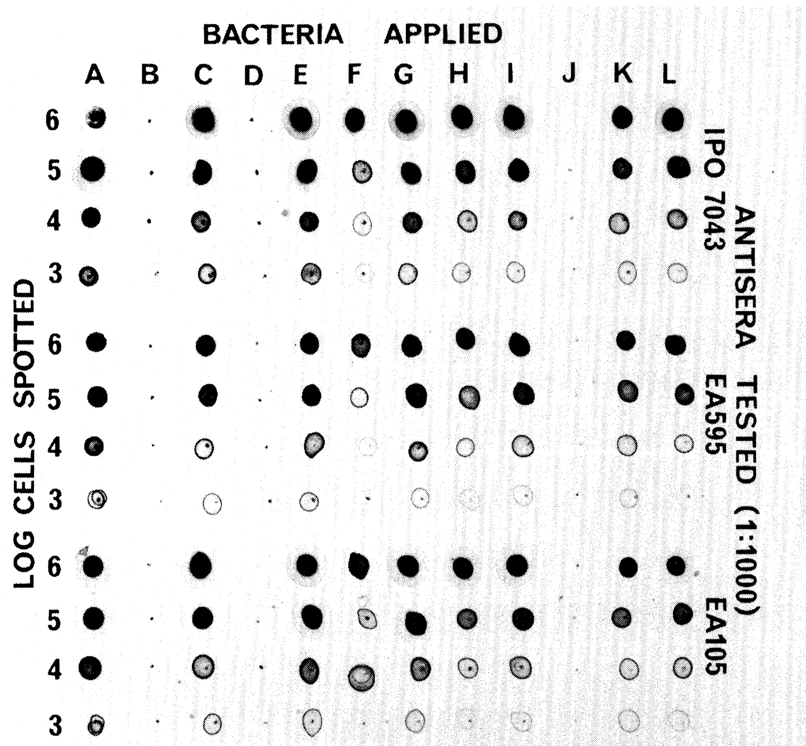

Fig. 1. Dot-ELISA test. Reactions of 12 bacterial isolates (A–L) with three serum preparations (1:1,000 dilutions). Serum IPO 7043 was obtained from Wageningen, sera EA 595 and EA 105 were prepared in Israel (EA 595 against a reference *Erwinia amylovora* [A] culture obtained from England, and EA 105 [C] against an Israeli suspect). Dilutions of bacterial suspensions were applied in 2-μl volumes to nitrocellulose membrane. A, K, and L are reference *E. amylovora* isolates from England (A) and the United States (K and L). B is an *Erwinia* sp. from England. D is a pathogenic *Pseudomonas syringae* isolate from pear. C, E, F, G, H, and I are Israeli *E. amylovora* suspects IL-1 to IL-6 (Table 1). J is an *Erwinia* sp. saprophyte isolate from pear fruit.

Isolate E-8, an avirulent *E. amylovora* isolate obtained from R. N. Goodman, was not pathogenic on pear fruit but reacted positively in the dot-ELISA (Table 1; Fig. 1, column K).

Results of a single test on the ability of the various strains to elicit the hypersensitive reaction on tobacco showed good correlation with ooze formation on pear fruit (Table 1), except in the case of the avirulent U.S. isolate (E-8) of *E. amylovora* (Table 1) and of *P. syringae* (Table 1). The eight Israeli isolates (IL-1–IL-18) reacted similarly to the reference isolate (EA 595) from England in the biochemical tests as outlined by Schroth and Hildebrand (6).

DISCUSSION

Symptoms, colony morphology on selective media, serology, and pathogenicity assays all provided evidence to confirm the existence of fire blight in Israel and the fact that *E. amylovora* is the pathogenic agent. No individual test by itself, however, can be relied on positively to identify pathogenic isolates of *E. amylovora*. Dot-ELISA gave consistently reproducible reactions with the isolates tested when their reactivity with three antisera was compared. Dot-immunobinding assays have now been found extremely useful for characterization and identification of numerous biological compounds (7) including viruses (2). The specificity one is able to attach to the test is of course directly dependent on the properties of the serum used. In our tests, all three antisera gave a positive identification of eight Israeli isolates from infected pear twigs and the reference *E. amylovora* isolates from abroad. *P. syringae* and several *Erwinia*-like isolates were not identified by any of the sera, but the number of such isolates tested is not deemed sufficient to ascribe absolute specificity to the sera. None of the sera differentiated between the avirulent and virulent strains obtained from the United States (Fig. 1, columns K and L; Table 1, E-8 and E-9).

The isolation of *E. amylovora* from eight orchards demonstrates that the pathogen has become established in both the southern and northern regions of Israel. Infection, however, appears to be extensive in only three of the eight orchards with the disease. Although the source of the inoculum has not been identified, present suspicions are centered on natural import of the pathogen by the prevailing winds that pass over Cyprus (T. van der Zwet, *personal communication*), where the disease is known to be present (4).

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