

Erwinia salicis as the cause of dieback in *Salix alba* in the Netherlands and its identity with *Pseudomonas saliciperda*¹

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Abstract

Research on a bacterial disease of willow (*Salix alba*) has been carried out in the Netherlands. Isolations made from diseased branches yielded a peritrichous bacterium which proved to be *Erwinia salicis* (Day) Chester. Evidence of its identity with *Pseudomonas saliciperda* Lindeijer is demonstrated.

Introduction

The first reliable report on the bacterial disease discussed in this paper originated from Britain and was given by Day (1924). He described the occurrence of *Bacterium salicis* Day in cricket-bat willow, *Salix alba* L. 'Calva,' and in *Salix alba* L. as the cause of leaf wilting and dieback of branches, resulting in death of the trees.

In the Netherlands a similar disease of various varieties of *Salix alba* was investigated in detail and its cause attributed to *Pseudomonas saliciperda* Lindeijer (Lindeijer, 1932). Experimental work proved this organism to be pathogenic to willow.

Dowson (1937), continuing Day's work in Britain, reported on the same willow disease named 'watermark disease'. He also proved the pathogenicity of *Bacterium salicis*, an organism afterwards renamed *Erwinia salicis* (Day) Chester (Breed et al., 1957).

Since considerable revival of the disease in *Salix alba* has been observed in the Netherlands, a detailed review of the disease symptoms and its occurrence has been given (Jansen, 1969).

Experiments

Isolations were made from willow branches showing leaf wilting symptoms as well as characteristic water – soaked discolorations of the wood. Various types of bacteria were obtained. After screening, isolates V, 192a, 193b, 195b, 208 and 305 were used for inoculation work in the field during 1967 and 1968. Artificial wounds were made with a special apparatus on one and two year old branches and the wounds inoculated with a bacterial suspension. The results obtained in one year show a low percentage of infection and although this phenomenon cannot be explained so far, this is in agreement with Lindeijer's observations. Isolates V and 208 proved to be pathogenic to *Salix alba* 'Liempde'.

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Identification

For identification purposes, isolates V and 208 were grown on yeast-peptone-glucose agar. After 1, 3, 7, 10, 15 and 24 days of culture, bacteria were stained using Gray's method to investigate number and mode of attachment of flagella.

At first, one "polar" flagellum per rod was noticed, suggesting identity with *Pseudomonas saliciperda* (Lindeijer, 1932). However after modification of staining technics, a peritrichous organism with up to seven flagella was observed (Fig. 1). The "polar" attachment seemed to be due to a coherence of the flagella as a result of imperfect staining methods.

Table 1 compares the reactions of isolates V and 208 with both *Erwinia salicis* and

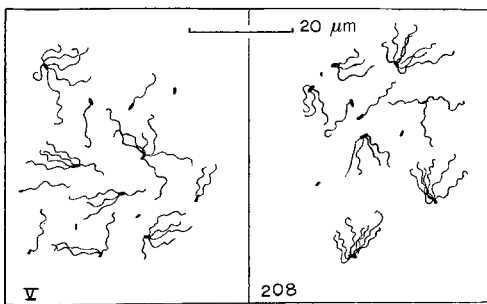


Fig. 1. *Erwinia salicis* (Day) Chester. Isolates V en 208 obtained from diseased branches of *Salix alba* L.

Fig. 1. *Erwinia salicis* (Day) Chester. Isolaten V en 208 verkregen uit zieke takken van *Salix alba* L.

Table 1. Comparison between *E. salicis*, *P. saliciperda* and some Dutch isolates.

Characters	<i>Erwinia salicis</i> ¹	<i>Pseudomonas saliciperda</i> ²	Isolates Nos V and 208
Shape	single rods or in pairs	single rods or in pairs	single rods or in pairs
Flagella	5-7, peritrichous	1, polar	up to 7, peritrichous
Gram's staining	—	—	—
Litmus milk	no change	no change	No V: no change No 208: not investigated
Nitrate reduction	+	+	+
H ₂ S	—	—	No V: — No 208: not investigated
Indole production	—	+	—
Gelatin stab	—	—	—
Voges Proskauer	+	not invest.	+
Glucose	+	+	+
Lactose	—	—	—
Broth	turbidity	turbidity	turbidity
Potato plug	yellow pigment	no pigment	no pigment

¹ After Dowson (1937).

² After Lindeijer (1932).

³ After Bergey's Manual, 7 ed. (1957).

Tabel 1. Vergelijking van *E. salicis*, *P. saliciperda* en enkele Nederlandse isolaten.

Table 2. Inoculation experiments on *Salix alba* L. 'Liempde'.

Isolates	Number of plants inoculated			Results one year after inoculation
	July '67	June '68	July '68	
V	7	—	—	1/7
192a	7	—	—	0/7
193b	6	—	—	0/6
195b	5	—	—	0/5
208	6	—	—	1/6
V	—	10	—	5/10
208	—	10	—	0/10
305	—	10	—	0/10
208	—	—	15	0/15

Tabel 2. Inoculatie proeven op *Salix alba* L. 'Liempde'.

Pseudomonas saliciperda. They differ from *E. salicis* by lack of yellow pigment on potato plug; from *P. saliciperda* in the number and mode of attachment of flagella and production of indole.

Based on these reactions in vitro (Table 1) and pathogenicity tests on *Salix alba* (Table 2) both isolates are considered to be identical with *Erwinia salicis*. In addition, the following data have convinced us that *Pseudomonas saliciperda* is nothing but *Erwinia salicis*:

- 1 Disease symptoms on willow described by Day (1924) and Dowson (1937) and those mentioned by Lindeijer (1932) are the same;
- 2 Inoculation experiments proved that both organisms are pathogenic to willow;
- 3 Bacteriological tests did not show striking differences;
- 4 The differences between *E. salicis* and *P. saliciperda* based on number of flagella and attachment are merely the result of staining technics applied.

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Samenvatting

Erwinia salicis, de oorzaak van afsterven van *Salix alba* en de identiteit met *Pseudomonas saliciperda*

Het onderzoek over de oorzaak van het afsterven van schietwilgen (*Salix alba* L.) in Nederland heeft zich gedurende de afgelopen jaren vooral bezig gehouden met het isoleren van de ziekteverwekker uit aangetaste takken, identificatie van de verkregen organismen en het verrichten van inoculaties aan een- en tweejarige wilgetakken om de pathogeniteit van de isolaten aan te tonen.

Na voorafgaande selectie in de geïsoleerde bacteriën werden de isolaten V, 192a, 193b, 195b, 208 en 305 voor inoculatiewerkzaamheden gebruikt. Hiervan bleken V en 208 in staat typische ziekteverschijnselen bij de cultivar 'Liempde' te veroorzaken (Tabel 2).

Ook werden beide isolaten op bacteriologische wijze getoetst en vergeleken met *Erwinia salicis* en *Pseudomonas saliciperda* (Tabel 1). Aanvankelijk werden na fixatie en kleuring bacteriën met polaire flagellen geobserveerd, welke in vele opzichten overeenkomst vertoonden met de bacterie die door Lindeijer (1932) als *Pseudomonas saliciperda* werd beschreven. Na wijziging van de kleurtechniek kon echter worden aangetoond dat beide isolaten V en 208 in werkelijkheid een peritriche soort vertegenwoordigen, die identiek is met *Erwinia salicis*.

Op grond van de hier volgende vier punten worden deze identiek geacht:

- 1 De ziekteverschijnselen beschreven door Day (1924) en Dowson (1937) voor *Bacterium salicis* (*Erwinia salicis*) en die vermeld door Lindeijer (1932) voor *Pseudomonas saliciperda* verschillen niet;
- 2 De inoculatieproeven met beide organismen uitgevoerd, bewezen dat beide pathogeen voor wilg zijn;
- 3 Het bacteriologisch onderzoek (Tabel 1) leverde geen opvallende verschillen op;
- 4 Het enig overblijvende onderscheid tussen beide organismen, berustende op het verschil in aantal flagellen en de wijze van bevestiging, is slechts toe te schrijven aan de gebruikte kleurtechniek.

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