Infection of Cricket Bat Willow (Salix alba var. caerulea) Sm. by Erwinia salicis (Day) Chester detected in the Field by the Use of a Specific Antiserum

by W. C. WONG and T. F. PREECE

Agricultural Sciences Building, Department of Plant Sciences, The University of Leeds, LS2 9JT.

SUMMARY

The use of a slide agglutination test on squeezed-out sap of cultivated cricket bat willow trees allowed rapid diagnosis of infection by Erwinia salicis in the field. By this means, infection of “classically” diseased willow trees with both red leaf and wood staining was confirmed. Trees with either red leaf or wood stain, but not both symptoms, were also shown to be infected. Symptomless infection of cricket bat willow trees was detected. E. salicis was viable in the stumps of trees felled five years previously. Some of these stumps were in an advanced state of decay and some were producing symptomless leafy shoots. Trees dying with severe die-back symptoms and infected with Armillaria mellea, were shown to be infected with E. salicis. Salix caprea and S. vitellina, two wild species of willow, were also shown to be infected.

INTRODUCTION

Although watermark disease is known and troublesome in England (Day, 1924) and the Netherlands (Lindeijer, 1931), the mode of survival and of transmission of the causative agent has not been elucidated (Anon., 1962). Insects have not been definitely implicated as the principal vectors, in spite of many suggestions (Callen, 1939; Gray, 1940) that this is so. For the last 40 years, control of this disease in Essex and other Eastern counties of England has been by careful field inspection and the felling of visibly affected trees showing the classical symptoms – a dark stain in the wood and reddening of the leaves (Anon., 1962, 1966). We are engaged in a study of the epidemiology and physiology of this disease, especially in Essex. This paper reports some of the findings using a slide agglutination test in the field alongside more usual methods of isolating the organism from willow trees.

PREPARATION OF ANTISERUM AND METHODS OF TESTING

The antiserum was prepared in rabbits in the normal way without adjuvant using a formalised suspension ($1 \times 10^{10}$ cells/ml) of the organism grown on glycerol nutrient agar (G.N.A.). The titre of the antiserum was $>1/640$, and no preservative was used in the storage of the antiserum at $-20^\circ$. The antiseraum was transported frozen solid in 1 ml quantities in a Dewar flask. Drops of sap were collected by cutting a shoot from the tree and squeezing the freshly cut wood with sterilized pliers. A drop of antiseraum was added. Good positive agglutination was visible almost immediately. Control slide agglutination tests using killed suspensions of Erwinia salicis, of E. herbicola, of Erwinia-like organism and of Escherichia coli, as well as using sap from known uninfected trees and serum from uninoculated rabbits, were performed with each batch of antiseraum. Confirmatory slide and tube agglutination tests were later performed in the laboratory. Using G.N.A., attempts were made to isolate the causative organism of the disease from all of the trees tested. During this work it has
become apparent that only suspensions of \textit{E. salicis} will stain the wood of detached cricket bat willow shoots in the laboratory, and all isolates were tested in this way by standing 20 cm lengths of terminal shoots in a suspension of the isolate under test for 10 days in a controlled environment cabinet.

\textbf{FIELD OBSERVATIONS AND RESULTS}

During the summer and autumn of 1972, under the direction of the senior inspector of willows to the Essex County Council, T. H. Nash, some 200,000 cricket bat willow trees were examined in Essex, Cambridgeshire, Suffolk, Hertfordshire and Bedfordshire. Trees tested for infection were not selected at random, but were either visibly affected or growing on either side of an affected tree.

A variety of disease symptoms was seen on willows, such as red leaf but no internal staining of the wood and vice versa. Very severe die-back, sometimes associated with infection by \textit{Armillaria mellea} (Fr.) Quél., but with none of the classical watermark symptoms, was also investigated (Table 1). When trees infected with \textit{E. salicis} are felled, the stumps subsequently either decay and appear dead, or produce leafy shoots. No symptoms were seen on the shoots growing on these stumps. Table 1 shows that infection is present in symptomless trees. Four of the 17 symptomless trees were 10 years old, six were 3 years old and the other seven were between 4 and 15 years old. The finding of symptomless infection in the leafy shoots growing from the stumps of felled trees and in the roots of apparently rotten and dead stumps is also reported in this Table. Of the 11 stumps shown to be infected, four had been felled in 1967 and four in 1969. The others could not be accurately dated.

\begin{table}[h]
\centering
\caption{The results of slide agglutination tests using \textit{Erwinia salicis} antiserum and the sap from trees of cricket bat willow (\textit{Salix alba} var. caerulea) compared with symptom picture, the result of culturing sap on glycerol nutrient agar and a detached shoot staining test on isolates.}
\begin{tabular}{|l|c|c|c|c|}
\hline
Usual symptoms & No. tested & Sap agglutination & Isolation on GNA & Detached shoot tests on isolates \\
& & +ve & -ve & +ve & -ve \\
\hline
None & 17 & 10 & 7 & 10 & 7 & 10 & 0 \\
Red leaf only & 5 & 2 & * & 5 & 0 & 5 & 0 \\
Wood stain only & 16 & 16 & 0 & 15 & 1 & 15 & 0 \\
Red leaf and wood stain & 41 & 41 & 0 & 38 & 3 & 38 & 0 \\
Tree dying, looks like \textit{Armillaria} attack & 9 & 2 & † & 6 & 3 & 6 & 0 \\
Leafy old stumps, with no symptoms on leaves & 4 & 4 & 0 & 4 & 0 & 4 & 0 \\
Decayed stumps & 7 & 4 & 3 & 2 & 5 & 2 & 0 \\
\hline
\end{tabular}
\end{table}

*3 not examined †7 not examined.

As we came across other \textit{Salix} species in the field, we also examined them for infection by \textit{E. salicis}. The results are shown in Table 2. The previous report of infection in \textit{Salix caprea} (Anon., 1962) is confirmed. We found no symptomless infected trees of this species, but on the other hand, every tree found with classical watermark disease symptoms was shown to be infected. To date, we have not detected the disease in trees of \textit{S. fragilis}, \textit{S. viridis} and \textit{S. cinerea}, but infected trees of \textit{S. vitellina} have been found. Of various (as yet unidentified) \textit{Salix} species, only one tree, which was dying with severe die-back symptoms and with wood staining but no red leaf, has been shown to be infected.
Table 2

The results of slide agglutination tests using Erwinia salicis antiserum and the sap from trees of spp. of Salix other than cricket bat willow, compared with the symptom picture, the results of culturing sap on glycerol nutrient agar and a detached shoot staining test on isolates

<table>
<thead>
<tr>
<th>Species of Salix</th>
<th>Symptoms</th>
<th>No. tested</th>
<th>Sap agglutination</th>
<th>Isolation on GNA</th>
<th>Detached shoot test on isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. fragilis</td>
<td>None</td>
<td>13</td>
<td>*</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>S. caprea</td>
<td>None</td>
<td>18</td>
<td>†</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>S. caprea</td>
<td>Red leaf and wood stain</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>S. vitellina</td>
<td>Red leaf and wood stain</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Salix spp. (not identified)</td>
<td>None</td>
<td>11</td>
<td>‡</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Salix spp. (not identified)</td>
<td>Severe die-back and wood stain</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*10 not examined †11 not examined ‡7 not examined

Discussion

Our findings indicate that symptomless cricket bat willow trees infected with Erwinia salicis may be more common than hitherto suspected. Use of agglutination tests may be useful in the selection of healthy propagating material.

Old stumps left behind after the felling of infected trees are still infected five years later and probably longer. The need for chemical destruction of infected stumps, or their removal, seems to be indicated from the results reported here.

Whereas infection of S. caprea has been reported previously, the discovery of heavily infected trees of S. vitellina growing near water in a gravel pit emphasises that the location of infected wild willow trees needs considering more closely in relation to the epidemiology of watermark disease in crops of cricket bat willows.

A detailed report on the distribution of diseased willow trees and sources of infection by E. salicis in Essex will be published elsewhere.

References
