Evidence that Virus Translocation and Virus Infection of Non-wounded Cells Are Associated with Transmissibility by Leaf-feeding Beetles

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SUMMARY

Two experimental systems were used to study the translocation of virus particles in plants and translocation followed by infection of non-wounded plant cells. Translocation of virus particles in cut plant stems occurred with the beetle-transmissible viruses southern bean mosaic, bean pod mottle and cowpea severe mosaic, but not with sunn hemp mosaic and tobacco ringspot viruses, which are not transmitted by beetles. When purified viruses were injected below sections of steam-killed stems, only the beetle-transmissible viruses were translocated and infected non-wounded tissue above the steam-killed area. However, sunn hemp mosaic, tobacco ringspot and bean yellow mosaic viruses, which are not transmitted by beetles, did not infect plants above killed portions of the stem. These results suggest that both translocation of virus particles in the xylem of plants and establishment of infection in non-wounded tissue are key properties of viruses that are transmitted by beetles.

Earlier investigations in our laboratory have shown that when plant viruses are inoculated using a technique which mimics beetle feeding (gross wounding inoculation), beetle-transmissible viruses are infective in the presence of beetle regurgitant, whereas those not transmitted by beetles are not (Gergerich et al., 1983; Monis et al., 1986). Evidence was presented that RNase in beetle regurgitant was responsible for the selective inhibition of viruses not transmitted by beetles (Gergerich et al., 1986; Gergerich & Scott, 1988). However, it has also been shown that viruses are not inactivated by beetle regurgitant (Monis et al., 1986). This led us to believe that it is not the interaction of viruses with regurgitant that determines transmission but rather virus behaviour in the plant following delivery by the beetle. In this paper we describe results of comparative studies of translocation and subsequent infection by beetle-transmissible viruses and viruses not transmitted by beetles using two experimental systems.

Four beetle-transmissible viruses [southern bean mosaic (SBMV), the cowpea strain of SBMV (CP-SBMV), cowpea severe mosaic (CPSMV) and cowpea pod mottle (BPMV)] and three viruses not transmitted by beetles [sunn hemp mosaic (SHMV), tobacco ringspot (TRSV) and Scott's bean yellow mosaic (BYMV) (Reddick & Barnett, 1983)] were used in this study. Propagation and purification of SBMV, BPMV, SHMV and TRSV were as described by Gergerich et al. (1983) and of CPSMV was as described by Monis et al. (1986). CP-SBMV was purified from infected Crimson cowpea, Vigna unguiculata (L.) Walp. subsp. unguiculata by chloroform–butanol extraction followed by two or three cycles of differential centrifugation and resuspension in 0.01 M-phosphate buffer pH 7.2. BYMV was partially purified from infected Phaseolus vulgaris L. cv. Cherokee Wax using a modification of the method described by Monis et al. (1986) for zucchini yellow mosaic virus. After precipitation with 8% polyethylene glycol the virus was resuspended in 0.05 M-potassium phosphate, 0.01 M-EDTA, pH 7.5 and centrifuged at 150,000 g for 2 h through a 30% sucrose pad. The pellet was resuspended in 0.05 M-phosphate, 0.1 M-EDTA buffer, pH 7.5.
Table 1. Translocation of purified viruses in cut plants

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Test host</th>
<th>Growing point</th>
<th>Primary leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beetle-transmissible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBMV</td>
<td>Bean</td>
<td>15/28</td>
<td>27/28</td>
</tr>
<tr>
<td>BPMV</td>
<td>Bean</td>
<td>3/25</td>
<td>8/25</td>
</tr>
<tr>
<td>CPSMV</td>
<td>Cowpea</td>
<td>7/20</td>
<td>13/20</td>
</tr>
<tr>
<td>Not transmitted by beetles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHMV</td>
<td>Bean</td>
<td>0/24</td>
<td>1/24</td>
</tr>
<tr>
<td>TRSV</td>
<td>Bean</td>
<td>0/20</td>
<td>0/20</td>
</tr>
</tbody>
</table>

* All viruses were suspended in 0.01 M-phosphate buffer pH 7.2 (except BPMV which was in 0.1 M-phosphate buffer pH 7.2) to a final virus concentration of 5 mg/ml.

To examine the movement of purified virus particles in the xylem of cut stems, Black Valentine bean and Monarch cowpea plants, whose first trifoliolate leaves were just beginning to expand, were severed at the base and then re-cut under water. The severed plants were placed in a concentrated suspension of purified virus (5 mg/ml) for 2 h under a bank of fluorescent lights. When cut plants of bean and cowpea were put into solutions of bromophenol blue under these experimental conditions, the dye moved up the stem and was visible in the leaves within 1 h. The primary leaves (approx. 1 g) and the trifoliolates and growing point (approx. 0.2 g) were assayed separately for virus by grinding the tissue in 0.4 ml and 0.2 ml, respectively, of 0.01 M-phosphate buffer pH 7.2. Assays were made by rub-inoculation to Carborundum-dusted leaves of young Black Valentine bean, a systemic host of all the viruses tested. These plants were assayed for virus 2 weeks after inoculation by Ouchterlony gel-diffusion tests.

Translocation of purified virus particles in the water-conducting elements of young plants occurred much more frequently for beetle-transmissible viruses than for viruses not transmitted by beetles (Table 1). SBMV, which is characteristically transmitted at high levels by beetles was translocated more readily than the other two beetle-transmissible viruses tested.

Although translocation experiments such as those described above indicate that beetle-transmissible viruses move in the xylem of plants more readily than other viruses do, this type of experiment provides no information about the ability of translocated virus particles to establish an infection in non-wounded cells after translocation. Movement of whole virus particles in the xylem and subsequent initiation of primary infection in non-wounded cells were tested using a modification of the steamed stem techniques described by Caldwell (1930) and Schneider & Worley (1959). Systemic hosts of the viruses, Black Valentine bean, Cherokee Wax bean or Monarch cowpea, were grown until the second internode was at least 7 cm long. Individual plants were supported by fastening them to a stake and a portion of the second internode of each plant was killed by a jet of steam as described by Schneider & Worley (1959). One day after the steam treatment, the plants were positioned horizontally and a drop of purified virus was placed on the stem of the first internode. A 27-gauge needle was passed through the drop, forced about half-way through the stem and quickly withdrawn. After 2 min (to allow for virus entry into the stem) the plants were set upright and positioned so that there was no contact between them. Controls were non-steamed plants treated in the same manner. All plants were sprayed with Dithane M-22 (Rohm & Haas, Philadelphia, Pa., U.S.A.) at the second internode to prevent the growth of saprophytic fungi on the dead portion of the stem. Plant tissue above the second internode was assayed for virus 10 to 14 days after inoculation using the Ouchterlony gel-diffusion test.

Plants with steam-killed stem sections survived and grew surprisingly well when given adequate support to prevent bending or breakage at the steam-killed section and if the steamed sections were sprayed with the fungicide. Symptoms of infection in the leaf were observed below the steam-killed stem area in most plants regardless of the virus used. Beetle-transmissible viruses were translocated through the steam-killed stem sections and initiated infection above
Short communication

Table 2. Infection by plant viruses after transport through steam-killed stems

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Concentration (mg/ml)</th>
<th>Host</th>
<th>Proportion of plants infected above the second internode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Steamed</td>
</tr>
<tr>
<td>Beetle-transmissible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBMV</td>
<td>30</td>
<td>Bean</td>
<td>22/24</td>
</tr>
<tr>
<td>CP-SBMV</td>
<td>32</td>
<td>Cowpea</td>
<td>15/30</td>
</tr>
<tr>
<td>BPMV</td>
<td>25</td>
<td>Bean</td>
<td>16/29</td>
</tr>
<tr>
<td>CPSMV</td>
<td>26</td>
<td>Cowpea</td>
<td>9/35</td>
</tr>
<tr>
<td>Not transmitted by beetles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHMV</td>
<td>35</td>
<td>Bean</td>
<td>0/58</td>
</tr>
<tr>
<td>TRSV</td>
<td>22</td>
<td>Bean</td>
<td>1/47</td>
</tr>
<tr>
<td>BYMV</td>
<td>22</td>
<td>Bean</td>
<td>0/35</td>
</tr>
</tbody>
</table>

* All viruses were suspended in 0.01 M-phosphate buffer pH 7.2 (except BPMV which was in 0.1 M-phosphate pH 7.2 and BYMV in 0.05 M-phosphate, 0.01 M-EDTA, pH 7.5).

the steam-killed area of the plant (Table 2). On the other hand, viruses not transmitted by beetles either did not move through the steam-killed sections or, if they did, were not able to infect non-wounded cells above the steam-killed sections.

Several investigators have studied the translocation of virus particles in xylem followed by infection of non-wounded plant cells (Caldwell, 1930; Schneider & Worley, 1959; Roberts & Price, 1967; Roberts, 1970). Caldwell (1930) demonstrated that tobacco mosaic virus did not pass upward through steamed portions of tomato stems and infect the plant above the steam-killed area. Schneider & Worley (1959) and Roberts (1970), however, showed that SBMV moved through steamed portions of bean plants and initiated infection above the steamed section in Pinto bean, a local lesion host. Roberts & Price (1967) demonstrated that tobacco necrosis virus, which is not known to be transmitted by beetles, infected bean plants above steam-killed areas, and Roberts (1970) reported that alfalfa mosaic, tobacco mosaic, cucumber mosaic, BYMV and TRSV (all viruses not transmitted by beetles) did not infect systemic hosts when inoculated in a similar manner.

We have confirmed the work of Schneider & Worley (1959) and Roberts (1970) with SBMV in a systemic host and have shown that three other beetle-transmissible viruses can infect non-wounded cells after movement through the xylem of steam-killed stems of bean or cowpea; the viruses not transmitted by beetles that were tested did not infect plants above the steam-killed areas. From the observations reported here we speculate that two general characteristics of beetle-transmissible viruses are translocation in the xylem of plants and infection of non-wounded plant cells. Tobacco necrosis virus seems to be an intermediate case in that it is translocated and infects non-wounded cells but has not been reported to be transmitted by beetles.

We assume that these two groups of viruses, those transmitted by beetles and those which are not, differ in their mode of translocation and in the manner in which they establish primary infection. This difference is apparent when the viruses are inoculated by beetle feeding, by gross wounding in the presence of beetle regurgitant or RNase, or by injection of virus into stems below steam-killed stem areas. Certainly in the last case the viruses must translocate and initiate infection in non-wounded cells. In the case of beetle feeding or gross wounding inoculation we believe that the presence of regurgitant or RNase at the feeding site or gross wound prevents infection at the point of virus introduction, and that only those viruses which can translocate and infect non-wounded cells are successful under these inoculation conditions.

The lack of translocation of virus particles within a plant is most probably due to some surface property of the virus. The simplest explanation would be specific binding between some plant component, such as the cell wall, and the virus particle resulting in immobilization of the virus. If this is the case, then beetle-transmissible viruses must differ from other viruses in some key surface property which prevents or at least limits binding to plant cell components.
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REFERENCES

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