Evidence that Beet Necrotic Yellow Vein Virus RNA-4 Is Essential for Efficient Transmission by the Fungus \textit{Polymyxa betae}

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SUMMARY

The efficiency of transmission by \textit{Polymyxa betae} of beet necrotic yellow vein virus (BNYVV) isolates containing different RNA components was compared using sugar-beet seedlings as test plants. Isolate S-4, containing RNA-1 + 2 + 4, was transmitted by \textit{P. betae} about 100 times more efficiently than isolate S-3 (RNA-1 + 2 + 3) and about 1000 times more efficiently than isolate S-0 (RNA-1 + 2). Isolate S-34 (RNA-1 + 2 + 3 + 4) was transmitted even more efficiently than isolate S-4. Each isolate retained its characteristic RNA composition after transmission by \textit{P. betae}. The virus content, measured by ELISA, of infected rootlets was S-34 > S-3 > S-4 > S-0. In inoculated leaves of \textit{Tetragonia expansa} and \textit{Beta macrocarpa}, isolates S-3 and S-34 multiplied more extensively than did S-4 and S-0. The inefficient transmission of isolate S-3 by \textit{P. betae}, as compared with S-4, cannot be attributed to a poorer ability to spread in root tissue, but the difference in transmissibility of S-3 and S-0 may be explained in this way. These results show that RNA-4 of BNYVV is essential for efficient transmission by \textit{P. betae}, and suggest that RNA-3 may influence the ability of the virus to spread in root tissue. RNA-4 and RNA-3 therefore seem to play important, but different, roles in virus survival and spread in nature.

INTRODUCTION

Beet necrotic yellow vein virus (BNYVV), a causal agent of rhizomania disease of sugar-beet, is transmitted by the soil-inhabiting fungus, \textit{Polymyxa betae} (Tamada, 1975). In sugar-beet plants, BNYVV is usually confined to the roots of the plants, and causes an abnormal proliferation of rootlets and severe stunting of roots and shoots (Tamada, 1975). The rod-shaped virus particles contain four or five RNA components, RNA-1 [6746 nucleotides (nt) excluding the poly(A) tail], RNA-2 (4612 nt), RNA-3 (1774 nt) and RNA-4 (1467 nt) (Putz, 1977; Richards \textit{et al.}, 1985; Bouzoubaa \textit{et al.}, 1985, 1986, 1987) and, in some isolates, RNA-5 (1.4 kb) (Tamada \textit{et al.}, 1989). The two larger RNA species are needed for virus infection, whereas the smaller ones behave like satellite RNA in mechanically inoculated leaves of host plants (Bouzoubaa \textit{et al.}, 1985; Koenig \textit{et al.}, 1986; Tamada \textit{et al.}, 1989). In addition, several isolates with the deleted forms of RNA-3, RNA-4 or RNA-5 have been reported (Bouzoubaa \textit{et al.}, 1985; Koenig \textit{et al.}, 1986; Kuszala \textit{et al.}, 1986; Tamada \textit{et al.}, 1989).

The fact that RNA-3 and RNA-4 (with or without RNA-5) are invariably found in field isolates of BNYVV (Koenig \textit{et al.}, 1986; Tamada \textit{et al.}, 1989) suggests that these smaller RNA species may confer some property that is necessary for virus survival in the field. Indeed, Lemaire \textit{et al.} (1988) reported that full-length RNA-3 and RNA-4 seem to play a role in transmission by the fungus vector. It has also been shown that RNA-3 greatly affects symptom expression in host plants (Kuszala \textit{et al.}, 1986; Tamada \textit{et al.}, 1989). However, it is not known whether either RNA-3 or RNA-4, or both, are needed for fungus transmission. In this paper, we describe the results of transmission tests using virus isolates containing different, or different combinations of, small RNA species, and present evidence that RNA-4 plays an important role in transmission by \textit{P. betae}. 

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**METHODS**

**Virus isolates.** The following isolates of BNYVV containing different combinations of RNA species were used: isolate S-34 (RNA-1 + 2 + 3 + 4), S-3 (RNA-1 + 2 + 3), S-4 (RNA-1 + 2 + 4) and S-0 (RNA-1 + 2) (Tamada et al., 1989). These isolates were obtained by single-lesion transfers in *Tetragonia expansa* leaves. Isolates resembling S-34 are the kind usually found in the field. The virus isolates were propagated in inoculated leaves of *T. expansa* in a glasshouse. Leaf or root extracts for use as virus inoculum were made in 0.1 M-phosphate buffer pH 7-4, containing 0.5% 2-mercaptoethanol.

**Fungus transmission.** The isolate (P-1) of virus-free *Polymyxa betae* used in this study was originally obtained from a root of sugar-beet grown in a non-rhizomania-affected field in Hokkaido (Abe & Tamada, 1986). This isolate was subcultured several times in rootlets of sugar-beet seedlings using an inoculum consisting of small pieces of rootlets containing resting-spore clusters (Abe & Ui, 1986). The freedom of this fungus isolate from BNYVV was confirmed by ELISA. The virus-free isolate was maintained in roots of sugar-beet seedlings and kept as resting spores in dried rootlets.

Sugar-beet seedlings (*Beta vulgaris* cv. Monohill) were used as test plants. They were grown in special test tubes (24 mm x 120 mm) as described by Tamada et al. (1989). The seedlings (one plant per test tube) were supplied with one-fifth strength Hoagland and Arnon solution, pH 7.0, every day and grown in a growth cabinet at 25 °C in light of about 10000 lux for 16 h per day. To prepare inocula of *P. betae*, fresh (0.2 to 0.5 g) or dried rootlets containing numerous resting-spore clusters were ground in 10 ml distilled water with a pestle and mortar. Inoculation was done by pouring the crude homogenate into test tubes (1 ml/tube), into which healthy seedlings were then transplanted or seeds then sown. The concentration of resting-spore clusters in the homogenate was determined from haemocytometer counts for a range of dilutions. Each experiment also included control treatments, which consisted of non-inoculated sugar-beet seedlings and seedlings inoculated with virus-free *P. betae* grown in test tubes at the same time. Infection with *P. betae* was checked by observing the resting-spore clusters and zoosporangia in at least 10 pieces of rootlets (about 10 mm long) from each sugar-beet seedling under the light microscope.

**Detection of BNYVV.** BNYVV was detected by ELISA and, when necessary, by inoculation to *T. expansa* leaves. The ELISA procedure was conducted as described by Tamada & Harrison (1980). The root extracts for ELISA were obtained using a pestle and mortar. After being washed, fresh rootlets (0.05 g) were triturated in 2.5 ml 0.02 M-phosphate buffer pH 7.4, containing 0.15 M-NaCl, 0.05% Tween-20 and 2% polyvinylpyrrolidone (1:50). Further 10-fold dilutions of each extract were also prepared in this extraction buffer. Antiserum to BNYVV prepared by Tamada & Hagita (1982) was used. The microtitre plates (Nunc Immuno Plate) were coated with
Fungus transmission of BNYVV isolates

γ-globulin at 1 μg/ml, and enzyme-conjugated globulin was used at 1/500 or 1/1000. The conjugate was added for 4 h at 37 °C, freshly prepared substrate was added for 30 or 40 min at room temperature, and the A405 was recorded by an NJ-2000 Immuno Reader. BNYVV concentrations in tissue extracts were calculated by interpolating their absorbance values on standard curves produced by plotting the absorbances given by known concentrations of purified virus, when necessary.

Detection of BNYVV RNA. For BNYVV RNA detection, sap from roots of sugar-beet seedlings obtained during the fungus-transmission tests was inoculated to T. expansa leaves. Sap extracted from the lesions produced in inoculated leaves was used as inoculum for further T. expansa leaves. RNA extracted from the virus particles purified from these inoculated leaves was analysed by agarose gel electrophoresis as described by Tamada et al. (1989).

Virus inoculation and infectivity assay. Fully expanded leaves of T. expansa or B. macrocarpa were used. The leaves were dusted with 500-mesh Carborundum and inoculated with sap from virus-infected leaf tissue at different dilutions. The inoculated leaves were washed with tap water immediately after inoculation, and the plants were kept in the glasshouse between 20 °C and 25 °C. Leaf discs (10 mm in diameter) were cut from the inoculated leaves with the aid of a cork borer and were floated on distilled water in Petri dishes at different temperatures for 3 days. In other tests, leaf discs were cut at various times from detached leaves which were incubated at 25 °C. All leaf discs were then stored frozen at −80 °C until sampling had finished. Virus content was determined by ELISA. For ELISA, each leaf disc (about 40 mg) was ground in 4 ml of extraction buffer and the extract was used to make further 10- or 100-fold dilutions. Known concentrations of purified BNYVV were included as standards.

Infectivity was assayed by inoculating half-leaves of T. expansa. After inoculation, the plants were kept for 4 days in the glasshouse, and detached leaves were incubated at 25 °C in darkness. The lesions were counted 2 or 3 days later. These conditions favour the production of readily countable lesions of all isolates.

RESULTS

Transmission of BNYVV isolates by Polymyxa betae

To let virus-free P. betae acquire the virus isolates containing different RNA species, roots of healthy sugar-beet seedlings (three- or four-leaf stage) were inoculated by carefully rubbing them with virus-containing extracts from inoculated leaves of T. expansa. They were then transplanted into special test tubes, and a week later, virus-free P. betae (P-1) was introduced.

After growth at 25 °C for 50 days after Polymyxa inoculation, the virus content of the rootlets was tested by ELISA. BNYVV was detected in roots of several of the 30 seedlings inoculated with each of the virus isolates. Resting-spore clusters of P. betae were observed in rootlets of all sugar-beet plants inoculated. The rootlets in which the virus content was relatively high were selected as sources of inocula for further fungus-transmission tests.

The first set of experiments was made on three isolates, S-3, S-4 and S-0, to confirm that the virus could be acquired and inoculated by the fungus. Crude homogenates from rootlets that contained both the virus and the fungus were inoculated to roots of healthy sugar-beet seedlings (two- or three-leaf stage). Forty-eight days later, almost all inoculated plants were infected with isolates S-4 and S-3, whereas only half were infected with isolate S-0 (Table 1). In addition, the virus concentration, estimated by ELISA in rootlets to which S-0 was transmitted, was much lower than that in rootlets to which S-3 or S-4 had been transmitted (Table 1).

In a second set of experiments, inoculation was done by using as inocula the fresh rootlets which were obtained in the first experiments. With isolate S-4, all seedlings inoculated were infected, whereas with isolates S-3 and S-0, some or many plants, respectively, were not infected, although the virus content of the inocula was relatively high (Table 1).

Because in these two experiments fresh rootlets containing numerous resting spores and zoosporangia were used as inocula, a third set of experiments was made using dried rootlets as inocula, to determine whether the virus is transmitted by zoospores produced by resting spores. Isolate S-34 containing RNA-1 + 2 + 3 + 4 was also used. Inoculation was done by sowing the sugar-beet seeds in test tubes, to which the crude homogenate prepared from dried rootlets had been added. In this experiment, numerous resting-spore clusters were observed 1 month after inoculation in most root epidermal cells of all seedlings. The results showed almost the same trend as those of the first and second experiments (Table 1).

These results indicate that virus-free P. betae was able to acquire and transmit the
Table 1. Transmission of BNYVV isolates containing different RNA species by Polymyxa betae

<table>
<thead>
<tr>
<th>Expt. no. (sequential transmission)*</th>
<th>Virus isolate (RNA species)</th>
<th>Virus content of inoculum†</th>
<th>Transmission to sugar-beet seedlings‡</th>
<th>Mean ELISA values of infected roots (range)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1st transmission)</td>
<td>S-3 (1 + 2 + 3)</td>
<td>&gt;2-00</td>
<td>9/10</td>
<td>&gt;2-00 (0.07-2-00)</td>
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<tr>
<td></td>
<td>S-4 (1 + 2 + 4)</td>
<td>1-45</td>
<td>20/20</td>
<td>&gt;2-00 (1.00-2.00)</td>
</tr>
<tr>
<td></td>
<td>S-0 (1 + 2)</td>
<td>1-07</td>
<td>8/20</td>
<td>0-58 (0-14-1-49)</td>
</tr>
<tr>
<td>2 (2nd transmission)</td>
<td>S-3 (1 + 2 + 3)</td>
<td>&gt;2-00</td>
<td>8/12</td>
<td>&gt;2-00 (0-22-2-00)</td>
</tr>
<tr>
<td></td>
<td>S-4 (1 + 2 + 4)</td>
<td>1-65</td>
<td>12/12</td>
<td>&gt;2-00 (1-40-2-00)</td>
</tr>
<tr>
<td></td>
<td>S-0 (1 + 2)</td>
<td>1-02</td>
<td>1/12</td>
<td>0-48</td>
</tr>
<tr>
<td>3 (2nd transmission)</td>
<td>S-34 (1 + 2 + 3 + 4)</td>
<td>&gt;2-00</td>
<td>24/24</td>
<td>1-67 (1-18-1-98)</td>
</tr>
<tr>
<td></td>
<td>S-3 (1 + 2 + 3)</td>
<td>&gt;2-00</td>
<td>22/23</td>
<td>1-48 (0-95-1-97)</td>
</tr>
<tr>
<td></td>
<td>S-4 (1 + 2 + 4)</td>
<td>1-57</td>
<td>49/49</td>
<td>1-24 (0-54-1-81)</td>
</tr>
<tr>
<td></td>
<td>S-0 (1 + 2)</td>
<td>0-85</td>
<td>20/45</td>
<td>0-35 (0-02-1-16)</td>
</tr>
</tbody>
</table>

* Inocula used in exp. 1 (1st transmission) were prepared from rootlets inoculated both with *P. betae* and, mechanically, with BNYVV. Inocula in exps. 2 and 3 (2nd transmission) were from virus-infected rootlets obtained in exp. 1. Freshly harvested (expt. 1 and 2) or dried (expt. 3) rootlets of sugar-beet seedlings that contained numerous resting-spore clusters were ground with distilled water (20 ml/g fresh root tissue). The crude homogenate was poured into test tubes (1 ml/tube), to which healthy seedlings were then transplanted (expt. 1 and 2) or in which seeds were sown (expt. 3).

† ELISA value for 1 g root tissue per 50 ml extraction fluid.

‡ Virus infection was checked by ELISA at 48 days (expt. 1), 38 days (expt. 2) or 40 days (expt. 3) after inoculation. Numerator is number of sugar-beet seedlings in which the virus was detected, denominator is number inoculated.

§ About 0.05 g of rootlets from each seedling was ground in 2.5 ml extraction buffer (1/50), and the undiluted fluid (expt. 1 and 2) and further 1/10-diluted fluid (expt. 3) was assayed by ELISA.

Mechanically inoculated virus isolates with or without different small RNA species. Isolate S-0 (RNA-1 + 2) was less frequently transmitted by the fungus than isolate S-4 (RNA-1 + 2 + 4) or S-3 (RNA-1 + 2 + 3). The virus transmission was much greater when seeds were sown in test tubes to which virus-carrying *P. betae* had been added, than when the seedlings were transplanted to them.

In further experiments, transmissibilities of different isolates of BNYVV were compared in more detail using dilutions of inocula containing different concentrations of virus. Preliminary ELISA tests showed that when extracts from BNYVV-infected leaves and roots were serially diluted, the absorbance values paralleled those given by purified virus. Therefore, BNYVV concentrations in root extracts were estimated by ELISA, using known concentrations of purified virus as standards. The results shown in Table 2 are summarized as follows. (1) Isolate S-4 was transmitted frequently by *P. betae*, even when the inoculum was greatly diluted. In contrast, isolates S-3 and S-0 were transmitted only when a high inoculum concentration was used. (2) Isolate S-34 was much more efficiently transmitted by *P. betae* than S-4, and also the virus content of rootlets was much higher. (3) When inocula were concentrated and all inoculated plants were infected (100% virus transmission), there was little difference between S-3 and S-4 in the virus contents of infected rootlets. However, when more dilute inocula were used (and 100% virus transmission did not occur) and when there were presumably few infection sites, the virus contents of rootlets infected with S-3 seemed to be higher than those of rootlets infected with S-4 or S-0. (4) For isolates S-34 and S-4, virus infection occurred in some rootlets in which *P. betae* was not detected, following inoculation with highly diluted inoculum.

If the transmission efficiency of BNYVV isolates is estimated from these results, isolate S-4 was about 100 times more efficiently transmitted by *P. betae* than isolate S-3 and about 1000 times more than isolate S-0. Thus RNA-4 plays an important role in BNYVV transmission by *P. betae*, but when RNA-3 is also present, virus transmission is even more frequent and the virus content of the inoculated roots is even greater.

Detection of BNYVV RNA species in roots inoculated by Polymyxa betae

To ascertain whether the RNA species of BNYVV isolates were maintained after inoculation by the fungus, experiments were made to detect RNA in root extracts from seedlings inoculated...
Table 2. Effect of inoculum dilutions on transmission of BNYVV isolates by Polymyxa betae

<table>
<thead>
<tr>
<th>Virus isolate (RNA species)</th>
<th>Virus content of inoculum</th>
<th>Efficiencies of transmission at inoculum concentrations* (number of P. betae resting-spore clusters/ml) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$1 \times 10^6$</td>
</tr>
<tr>
<td>Expt. 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-34 (1 + 2 + 3 + 4)</td>
<td>375†</td>
<td>6/6 (6) 188‡</td>
</tr>
<tr>
<td>S-3 (1 + 2 + 3)</td>
<td>255</td>
<td>3/3 (3) 117</td>
</tr>
<tr>
<td>S-4 (1 + 2 + 4)</td>
<td>108</td>
<td>10/10 (10) 94</td>
</tr>
<tr>
<td>S-0 (1 + 2)</td>
<td>10</td>
<td>7/10 (10) 5</td>
</tr>
<tr>
<td>Expt. 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-3 (1 + 2 + 3)</td>
<td>460</td>
<td>9/9 (9) 78</td>
</tr>
<tr>
<td>S-4 (1 + 2 + 4)</td>
<td>51</td>
<td>9/9 (9) 62</td>
</tr>
<tr>
<td>S-0 (1 + 2)</td>
<td>33</td>
<td>5/9 (9) 6</td>
</tr>
<tr>
<td>Expt. 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-34 (1 + 2 + 3 + 4)</td>
<td>190</td>
<td>–</td>
</tr>
<tr>
<td>S-3 (1 + 2 + 3)</td>
<td>87</td>
<td>–</td>
</tr>
<tr>
<td>S-4 (1 + 2 + 4)</td>
<td>25</td>
<td>–</td>
</tr>
<tr>
<td>S-0 (1 + 2)</td>
<td>27</td>
<td>–</td>
</tr>
</tbody>
</table>

* All inocula (3rd sequential transmission) were prepared from freshly harvested (expt. 1 and 2) and dried (expt. 3) rootlets of sugar-beet seedlings obtained in expt. 3 shown in Table 1. Inoculation was done by sowing the seeds on quartz sand in special test tubes, to each of which 1 ml of a series of 10-fold dilutions of inoculum was added.

† Figure is expressed as ng virus/ml of extraction fluids containing $1 \times 10^4$ resting-spore clusters per ml, estimated by ELISA using known concentrations of purified virus as standards.

‡ Virus infection was checked by ELISA at 35 to 40 days after inoculation. Numerator is number of sugar-beet seedlings in which the virus was detected, denominator is number inoculated. Figure in parenthesis is number of the seedlings in which P. betae was detected. Final figure is the mean virus content for virus-infected rootlets, expressed as μg virus/1 g fresh root tissue.
Fig. 2. Multiplication of different BNYVV isolates in inoculated T. expansa leaves. Intact leaves of T. expansa plants were uniformly inoculated with sap (1:10^2) from virus-infected tissue, and kept at 20 °C in the glasshouse for 1 day. The leaves were then detached, incubated at 25 °C and leaf discs (10 mm diameter) were cut at various times for virus assay. Virus content is the mean value for eight discs per sample, expressed as μg/1 g fresh leaf tissue, and was estimated by ELISA using known concentrations of purified virus as standards. ●, S-34; ■, S-3; ▲, S-4; ○, S-0.

by P. betae carrying each distinct isolate of BNYVV. In another paper (Tamada et al., 1989), RNA components obtained after propagation of the virus in inoculated leaves of T. expansa were shown to be representative of those in sugar-beet roots, to which the virus had been transmitted by P. betae. At least three plants therefore were selected for RNA detection from the virus-infected sugar-beet seedlings in each experiment shown in Tables 1 and 2. Root extract from each plant was inoculated to four or five leaves of T. expansa. After inoculation with isolate S-34 or S-3, leaves always developed yellow spots whereas with isolate S-4, chlorotic lesions appeared. Agarose gel electrophoresis (Fig. 1) showed the same RNA patterns as were given by the original isolates used, indicating that each RNA species present in each isolate was not changed after inoculation by the fungus. However, because the virus content of rootlets infected with isolate S-0 was low, no lesions were produced in the inoculated T. expansa leaves, and the RNA species in this isolate were therefore not confirmed after transmission by P. betae.

Multiplication of different BNYVV isolates in inoculated leaves

Sap from virus-infected leaf tissue (T. expansa or B. macrocarpa) inoculated with each of the BNYVV isolates, S-34, S-3, S-4 and S-0, was diluted 1:10^2 to 1:10^7, and inoculated to half-leaves of T. expansa. The virus content of the same samples was also measured by ELISA. All isolates were infective at dilutions of 1:10^4 to 1:10^5, but not 1:10^6. The virus was detected by ELISA at a dilution of 1:10^6. There were no apparent differences among the three isolates S-3, S-4 and S-0 in either their infectivity or the virus content of infected leaves of T. expansa and B. macrocarpa. Isolate S-34 showed a slightly higher infectivity than the others only at a dilution of 1:10^2.

Further experiments were made to determine whether BNYVV isolates differ in their abilities to multiply in inoculated leaves. Sap from virus-infected tissue (1:10 or 1:10^2) was inoculated to leaves of T. expansa plants, which were kept in the glasshouse at 20 °C to 25 °C for 1 day. In some tests, discs were cut from the inoculated leaves and then incubated at 20 °C, 25 °C or 30 °C for 3 days. In other tests, 1 day after inoculation, the inoculated leaves were detached and then
incubated at 25 °C for 7 days. Leaf discs were cut every day, and their virus contents were
determined by ELISA. Four days after inoculation, no apparent differences were found between
S-3, S-4 and S-0 in the virus content of leaf discs (eight discs in each treatment) at any
temperature. The virus content at 30 °C was much lower than that at 20 °C and 25 °C, but was
similar at 20 °C and 25 °C. In other tests, however, 5 to 7 days after inoculation, the virus
contents of leaves inoculated with S-3 or S-3 were similar but about 1.5- to 2.5-fold greater than
those of leaves inoculated with S-4 or S-0, which were also similar (Fig. 2). These results suggest
that RNA-3 assists the spread of BNYVV in mechanically inoculated leaves, but RNA-4 does not.

**DISCUSSION**

In this paper, we have provided evidence that RNA-4 plays an important role in the
transmission of BNYVV by *P. betae*. Lemaire et al. (1988) showed that two isolates containing
no detectable full-length RNA-3 and RNA-4 were poorly transmitted and that transmissions
were associated with the re-appearance of full-length RNA-3 and RNA-4. They suggested that
full-length RNA-3 and RNA-4 are strongly associated with transmission of BNYVV by the
fungus vector. However, our results indicate that the RNA components of BNYVV isolates do
not change after transmission by *P. betae*, and therefore provide more accurate information on
the role of each of the smaller RNA species in fungus transmission. Isolate S-4, containing
RNA-1 + 2 + 4, was about 100 to 1000 times more efficiently transmitted by *P. betae* than
isolate S-3 (RNA-1 + 2 + 3) or S-0 (RNA-1 + 2) (Table 2). The question may arise as to whether
the virus concentrations in the source roots affect the frequency of transmission by the fungus.
For example, the virus concentration in source rootlets inoculated with isolate S-0 by *P. betae*
was usually much lower than that in rootlets inoculated with isolate S-3 or S-4. Mechanical
inoculation tests using leaf discs, however, showed that the multiplication of isolate S-4 in
inoculated leaves was similar to that of isolate S-0, and thus it is unlikely that RNA-4 alters cell-
to-cell spread of BNYVV in inoculated tissue. Therefore, inefficiency of transmission of isolate
S-0 by *P. betae* is unlikely to be due to an impaired ability to spread in host plants.

It has been shown, on the other hand, that isolate S-3, containing RNA-1 + 2 + 3, was also
poorly transmitted by *P. betae*, although a large amount of virus and many resting spores of the
fungus were present in the inoculum source. Isolate S-3, unlike S-0, can induce severe symptoms
and systemic infection in appropriate host plants (Tamada et al., 1989). Thus, the differences
between S-3 and S-0 in transmissibility may merely reflect differences in ability to spread in root
tissue. For example, virus-carrying *P. betae* usually transmitted isolate S-3 about 10 times more
frequently than isolate S-0, and the concentration of S-3 in inocula was about 10 times greater
than that of S-0 (Table 2). Moreover, when diluted inocula were used, the roots would be initially
infected at only a few sites and virus multiplication in roots would be limited. When comparing
BNYVV isolates in these conditions, the virus content of rootlets infected with S-3 seemed to be
higher than that of rootlets infected with either S-4 or S-0 (Table 2). These results suggest that
RNA-3 may have no effect on transmissibility, and may influence only the ability of BNYVV to
spread in root systems.

Similar results have been obtained with isolates containing RNA-5, which is smaller (1-4 kb)
than RNA-4, and distinct in sequence from either RNA-3 or RNA-4 (Tamada et al., 1989).
RNA-5 is commonly present together with RNA-3 and RNA-4 in field isolates of BNYVV
from Hokkaido, but isolates containing RNA-5 but not RNA-4 were transmitted much less
efficiently by *P. betae* than normal isolates (T. Tamada, unpublished results). This suggests that
RNA-5 resembles RNA-3 in its ability to aid fungus transmission.

Taken together these results indicate that RNA-4 of BNYVV plays a key role in transmission
by the fungus vector. The nucleotide sequences of BNYVV RNA species (Richards et al., 1985;
Bouzoubaa et al., 1985; Ziegler et al., 1985; Kusala et al., 1986) indicate that RNA-3 encodes an
M₅, 25000 polypeptide (P25) and RNA-4 encodes an M₅, 31000 polypeptide (P31). This raises the
question of whether P31 is involved in transmission by the fungus vector and, if so, how it acts.
However, the fact that isolates without RNA-4 are transmitted by *P. betae*, although less
frequently, suggests the possibility that the genomic RNA, RNA-1 and RNA-2, may contain
other genetic information affecting the fungus transmission. Further work is needed to clarify this point.

It is of particular interest that the smaller RNA species of BNYVV control virus spread and survival in nature. Our results show that RNA-4 is strongly associated with the fungus transmissibility, and if RNA-3 is present, virus transmission by the fungus is much more frequent and the virus content of the infected roots is much greater. It has been also shown that RNA-3 confers an ability to intensify symptoms (Kuszala et al., 1986; Tamada et al., 1989) and seems to assist the spread of the virus in infected tissue (Table 2 and Fig. 2). Thus, isolates containing a combination of RNA-3 and RNA-4 are very stable (Koenig et al., 1986; Tamada et al., 1989), suggesting that RNA-3 and RNA-4 play different and important roles in virus survival and spread. We are conducting further experiments on how these RNA species affect the symptom development (rhizomania) in roots of sugar-beet.

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REFERENCES


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