Differences between isolates of the English serotype of tomato black ring virus in their transmissibility by an English population of *Longidorus attenuatus* (Nematoda : Dorylaimoidea)

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SUMMARY

In a field population of *Longidorus attenuatus* from England, 57 % of individual nematodes were found to be transmitting tomato black ring virus (TBRV) whereas in a field population from Germany the proportion of nematodes transmitting the virus was only 10 %. In laboratory tests, the population of *L. attenuatus* from England transmitted four English isolates of TBRV much more frequently than three German isolates. The proportion of individual *L. attenuatus* from England that transmitted the English isolates ranged from 26 to 78 % whereas only 1 to 15 % transmitted the German isolates. All the English and German isolates belonged to the English serotype. Although some of the isolates exhibited minor serological differences, these were not fully correlated with transmissibility of the isolates by the English *L. attenuatus* population. Reasons for the discrepencies between immunological reactions observed and the nematode transmissibility of the viruses are discussed.

RÉSUMÉ

Differences entre isolats des sérotypes anglais du virus de la tache annulaire de la tomate dans leur transmissibilité par une population anglaise de Longidorus attenuatus *(Nematoda : Dorylaimoidea)*

Dans une population de *Longidorus attenuatus* d'Angleterre, 57 % des nématodes ont transmis le virus de la tache annulaire de la tomate (TBRV), alors que dans une population provenant d'Allemagne la proportion des nématodes transmettant le même virus a été de 10 %. Au laboratoire, la population de *L. attenuatus* d'Angleterre a transmis quatre souches anglaises du TBRV beaucoup plus fréquemment que trois souches allemandes du même virus. La proportion de *L. attenuatus* d'Angleterre qui a transmis les souches anglaises a été de 26 à 78 % alors qu'elle était de 1 à 15 % pour les souches allemandes. Toutes les souches anglaises et allemandes appartiennent au sérotype anglais. Alors que certaines souches ont montré quelques différences mineures dans leurs réactions sérologiques, ces différences n'étaient pas bien corrélées à leur transmission par la population anglaise de *L. attenuatus.* La discussion porte sur les relations entre les réactions immunologiques observées et la transmissibilité par les nématodes.

Longidorus attenuatus Hooper, 1961 and the nepovirus tomato black ring or TBRV (Smith, 1946; Murant, 1970) which it transmits (Harrison, 1964), are endemic in several European countries including Britain (Brown & Boag, 1977). Isolates of TBRV fall into two distantly serologically related clusters or " serotypes " (Harrison, 1958; Bercks, 1962). One serotype, here called " English ", contains the type, lettuce ringspot (Smith & Short, 1959), celery yellow vein (Hollings, 1965) and potato bouquet (Köhler, 1950) isolates; the other serotype, here called " Scottish ", contains the beet ringspot (Harrison, 1957) and potato pseudo-aucuba isolates (Harrison, 1958; Köhler, 1955). Isolates of the English and Scottish serotypes have different specific vectors, being transmitted in nature respectively by *L. attenuatus* and *L. elongatus* (de Man, 1876) Thorne & Swanger, 1936 (Harrison, Mowat & Taylor, 1961; Harrison, 1964; Forghani, Sänger & Grossman, 1965; Taylor & Murant, 1969; Harrison & Murant, 1977; Rüdel, 1977; Migliori, Marzin & Rana, 1984). The existence of different specific vectors for different serotypes was also shown for another nepovirus, raspberry ringspot (Harrison, 1964; Taylor & Murant, 1969) and may be a general phenomenon in this virus group.

All nepoviruses, including TBRV, have single-stranded RNA genomes in two parts, called RNA-1 and RNA-2, and in many instances pseudo-recombinants can be prepared that contain RNA-1 from one serotype and RNA-2 from another. Thus, with TBRV, Randles *et al.* (1977) prepared a pseudo-recombinant containing RNA-1 from the potato bouquet isolate (English serotype) and RNA-2 from the beet ringspot isolate (Scottish serotype), though the converse pseudo-recombinant was apparently not viable. Studies with such pseudo-recombinants have shown that serological specificity and nematode transmissibility are both specified by determi-

nants carried on RNA-2. These properties, both being a function of the surface structure of the virus particles, are considered different expressions of the coat protein gene (Harrison, 1964; Harrison, Robertson & Taylor, 1974; Harrison *et al.*, 1974; Harrison & Murant, 1977). This is in agreement with much other evidence (Harrison & Murant, 1984) that the protein coat of virus particles plays an important part in their transmission by vectors.

Differences between populations of the same nematode species in their ability to transmit a single nepovirus isolate have also been reported : for *L. elongatus* with strains of RRV and TBRV (Van Hoof, 1966; Brown & Taylor, 1981) and for *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939 with strains of arabis mosaic and strawberry latent ringspot viruses (Brown & Taylor, 1981; Brown & Trudgill, 1983; Brown, 1985, 1986). In this paper we report, for TBRV, a new type of result : differences between closely related isolates of the same serotype in their transmissibility by a single population of *L. attenuatus.*

Materials and methods

A virus-free population of *L. attenuatus* from *Beta vulgaris* L. in Norfolk, England, was maintained in a heated glasshouse on *Lolium perenne* L. Viruliferous populations of *L. attenuatus* were obtained from *Daucus carota* L. in Norfolk, England, and from *Vitis vinifera* L. in Geisenheim, Federal German Republic (FGR). Nematodes from these populations were extracted and used in bait tests immediately upon receipt.

The isolates of TBRV used in this work are listed in Table 1; all were propagated in *Chenopodium quinoa* Willd. or *Nicotiana clevelandii* Gray by manual inoculation. Virus preparations purified by *n*-butanol clarification (Fritsch, Mayo & Murant, 1978) were used as antigens in gel diffusion serological tests in 0.6 % agarose. Rabbit antisera to isolates TBRV-S and TBRV-G used in these tests were from the SCRI antiserum collection.

Nematode-transmission experiments were done in 25 cm^3 plastic pots which were kept at 90 % relative humidity in a temperature-controlled cabinet (Taylor & Brown, 1974) at 18 ± 1° and with supplementary lighting to maintain a minimum daylength of 16 h. Experimental procedures were as described by Brown and Trudgill (1983), Trudgill and Brown (1978) and Trudgill, Brown and McNamara (1983). The plastic pots were filled with a growth medium consisting of an air-dried mixture of two parts sand and one part steam-sterilized loam with a particle and aggregate size between 150 and 1 500 µm, and each pot was planted with a single seedling of *Petunia hybrida* Vilm. The plants were inoculated manually with the virus isolate to be tested and, after one or two days, virus-free nematodes were added to the soil. After a further four weeks, the nematodes were recovered by a decanting and sieving procedure (Brown & Boag, 1988) and transferred in small replicated groups, by hand-picking, on to the sand/loam growth medium in pots containing virus-free *P. hybrida* bait plants. Nematodes recovered from field soils were tested in the same way. Infection of the roots, and of the aerial parts of most of those plants in which virus was recovered from the roots, was detected by return manual inoculation to *C. quinoa.*

Table 1

Isolates of tomato black ring virus used in this work

Isolate/source	*Abbreviation*	*Origin/History*
	ISOLATE FROM SCOTLAND	
Beet ringspot	S	Harrison (1958)
	ISOLATES FROM ENGLAND	
Carrot	ED	Norfolk (Murant, 1982)
Celery yellow vein	EC	Suffolk (Hollings, 1965)
L. attenuatus 1	E	Fritsch *et al.* (1980). Originally recovered in 1971 from a *Petunia hybrida* bait plant grown in soil from a tulip field in Norfolk
L. attenuatus 2	EL	Recovered in 1977 from a *Petunia hybrida* bait plant grown in soil from a sugar beet field in Norfolk
	ISOLATES FROM GERMANY (FGR)	
Potato bouquet	G	Köhler (1950); Harrison (1958); Randles *et al.* (1977)
Grapevine mild	GM	Recovered from *Vitis vinifera* plants sent by Dr M. Rüdel in 1981. In *Chenopodium quinoa* the mild isolate induced faint chlorotic local lesions and very mild systemic mottle, whereas the severe isolate resembled all the other isolates listed in inducing necrotic local lesions and severe systemic necrosis.
Grapevine severe	GS	

Results and discussion

As expected, all the English and German isolates were transmitted by *L. attenuatus* (Tabs 2, 4). However, the proportion of test plants infected by TBRV-EL transmitted by *L. attenuatus* from the field population from

England was substantially larger than the proportion infected with TBRV-GM transmitted by *L. attenuatus* from the field population from Germany (Tab. 2).

Table 2

Transmission of isolates of tomato black ring virus from England and Germany (FGR) by their naturally associated field populations of *Longidorus attenuatus*

Nematode population/ TBRV isolate	*Bait plants*				
	No. nematodes per plant		*No. root galls*	*No. plants infected/ no. tested*	*P**
	used	*recovered*			
English/EL	1	0.8	0.8	17/30	0.567
	5	3.5	3.0	20/20	> 0.451
German/GM	2	1.4	3.1	4/21	0.100
	5	3.0	5.0	0/4	< 0.056

*P, the estimated proportions of nematodes transmitting virus, calculated using the equation of Gibbs and Gower (1960).

Whether there are real differences in the vector efficiency of these populations is not certain because factors such as the host from which the nematodes acquired virus or delays in testing the nematodes sent from Germany could have affected the proportions of the nematodes that were viruliferous. The nematodes from the two populations were anatomically similar (Tab. 3) but the *L. attenuatus* from England had a smaller mean spear length (121 µm) than those from Germany (138 µm).

Table 3

Morphometric mean values of male and female *Longidorus attenuatus* from English and German (FGR) populations

	Unit	*English population*		*German population*	
		male	*female*	*male*	*female*
n		2	25	1	10
L	mm	6.66	7.10	7.22	7.50
a		155	153	154	166
b		15.8	18.0	17.4	18.3
c		133	142	147	143
c′		1.45	1.47	1.23	1.53
V or T	%	47	48	42	49
Odontostyle	µm	81	81	91	94
Odontophore	µm	40	40	41	44
Spear (total)	µm	121	121	132	138
Tail	µm	50	50	49	53
Male supplements		1 + 10	—	1 + 10	—
Spicule	µm	50	—	50	—

No further experiments were possible with *L. attenuatus* from Germany, but two experiments were done on the transmission of English and German isolates of TBRV by *L. attenuatus* from England (Tab. 4). In the first experiment, TBRV-EL was transmitted by about 30-40 % of individual nematodes but TBRV-G was not transmitted at all, despite the fact that galls were produced on the roots of source and bait plants, indicating that the nematodes had fed on them. In the second experiment, TBRV-EL was again transmitted by more than 40 % of individual nematodes, and high frequencies of transmission (26-78 %) were observed with three other English TBRV isolates, TBRV-ED, TBRV-E and TBRV-EC. In contrast, TBRV-G and two other German isolates were transmitted by only 1-15 % of the English *L. attenuatus*.

These differences in efficiency of transmission of the English and German isolates of TBRV by the English population of *L. attenuatus* were unexpected because all the isolates belong to the same serotype : vector specificity of nepovirus isolates has previously been shown to be correlated with their antigenic specificity (Harrison, 1964; Harrison, Robertson & Taylor, 1974; Harrison *et al.*, 1974; Harrison & Murant, 1977) and is therefore considered to be determined by the structure of the particle coat protein (Harrison & Murant, 1984). The serological properties of the isolates were therefore examined in more detail. Purified preparations of each of the English and German isolates and of the type isolate of the Scottish serotype, TBRV-S, were tested against a series of doubling dilutions of antisera to TBRV-S and TBRV-G in gel diffusion tests. The TBRV-S antiserum had a titre of 1/512 to isolate TBRV-S, 1/128 to isolates TBRV-E, TBRV-EL, TBRV-ED, TBRV-GM and TBRV-GS, and 1/64-1/128 to isolates TBRV-EC and TBRV-G. The TBRV-G antiserum had a titre of 1/128 to isolate TBRV-S, 1/256-1/512 to isolate TBRV-EC and 1/512 to all the other English and German isolates. These results justified the classification of all the English and German isolates as, broadly, of the English serotype. Further investigation by spur formation tests with antisera to TBRV-S and TBRV-G (Fig. 1) confirmed that all the English and German isolates were distinct from the type isolate of the Scottish serotype, TBRV-S. The English and German isolates were all indistinguishable from each other when tested against antiserum to TBRV-S. However, when they were tested against antiserum to TBRV-G, three minor serological variants were found. Isolates TBRV-GS, TBRV-GM, TBRV-E, TBRV-EL and TBRV-ED appeared identical to each other, but the remaining two isolates, TBRV-G and TBRV-EC, were distinguishable from these five and also from each other.

These minor serological variations among the English and German isolates were not fully correlated with differences in their transmissibility by the English population of *L. attenuatus;* for example, they do not explain

the poor transmissibility of isolates TBRV-GM and TBRV-GS compared with that the apparently serologically identical isolates TBRV-ED, TBRV-E and TBRV-EL (Tab. 4). These isolates must therefore differ in ways that were not revealed by our serological tests. Differences might perhaps be detected by using other antisera, or monoclonal antibodies, or other kinds of serological test. However, the possibility also remains that the regions in the coat proteins of these viruses that are important for attachment to, and/or release from, the sites of retention in the vector nematode are not involved in the immunological reaction. Yet a further possibility is that the isolates differ in some other property that could affect the results of bait tests, for example, in the proportion of bait plants in which the infection spreads away from the site of inoculation. Such a property, perhaps determined at least in part by RNA-1, was suggested to explain the poorer transmissibility of a pseudo-recombinant isolate of TBRV than of the parental source of its RNA-2 (Harrison & Murant, 1977). No evidence for this was seen in the present studies (indeed all *P. hybrida* bait plants that were positive in back-tests gave rise to many lesions per inoculated leaf of *C. quinoa*) but further work is required to establish the true nature of the differences in transmissibility we have observed.

Table 4

Transmission of English and German (FGR) isolates of tomato black ring virus by *Longidorus attenuatus* from Norfolk, England

TBRV Isolate	*Source plants*		*Bait plants*				
	No. nematodes used per plant	*No. root galls per plant*	*No. nematodes used per plant*	*Mean no. nematodes recovered*	*Mean no. root galls per plant*	*No. plants infected/ no. tested*	*P**
EXPT 1							
EL	30	14	1	0.9	1.6	12/28	0.429
			2	1.2	1.2	39/63	0.383
			5	2.7	2.7	28/33	0.311
G	28	18	2	1.3	1.3	0/34	< 0.015
			5	1.9	3.1	0/18	< 0.011
Control (virus-free)			33	23	25	0/21	< 0.002
EXPT 2							
ED	40	36	2	1.5	1.6	19/20	0.776
			5	3.8	4.7	11/11	> 0.381
E	35	27	2	1.0	1.8	14/20	0.452
EL	38	32	2	1.6	1.6	28/40	0.452
			5	3.0	3.8	15/15	> 0.418
EC	35	25	2	1.0	1.6	22/40	0.329
			5	2.9	3.6	11/14	0.265
GM	40	36	2	0.9	2.0	2/20	0.051
			5	4.5	4.9	5/11	0.114
GS	40	37	2	1.3	1.8	1/20	0.025
			5	4.4	4.1	6/11	0.146
G	38	31	2	1.4	1.7	2/39	0.026
			5	3.6	4.7	0/21	< 0.010
Control (virus-free)			40	33	30	0/17	< 0.002

*P, the estimated proportions of nematodes transmitting virus, calculated using the equation of Gibbs and Gower (1960).

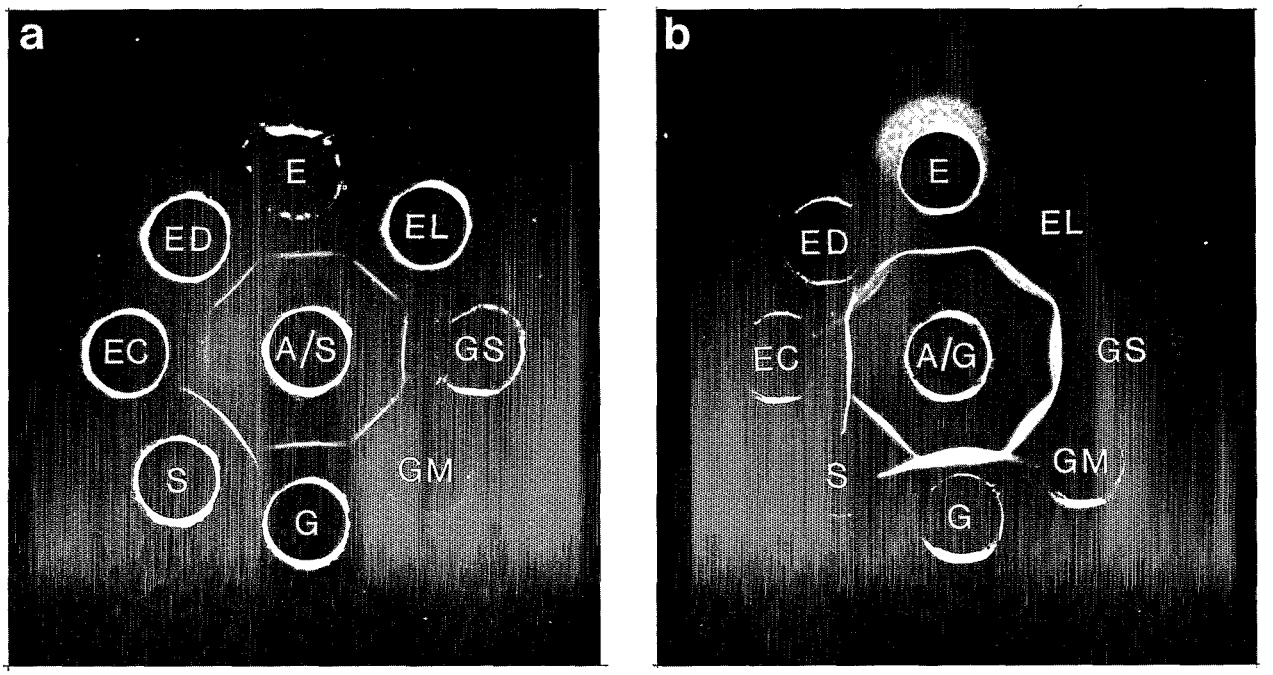

Fig. 1. Gel diffusion serology with TBRV isolates. a : Centre well, 1/20 dilution of antiserum to TBRV-S (A/S); b : Centre well, 1/20 dilution of antiserum to TBRV-G (A/G). Outer wells, purified preparations of TBRV-S (S), TBRV (G), TBRV-GM (GM), TBRV-GS (GS), TBRV-E (E), TBRV-EL (EL), TBRV-ED (ED) and TBRV-EC (EC).

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