Nematode transmission of plant viruses - a 30 year perspective

D.J.F. Brown & D.L. Trudgill

Outbreaks of a lethal 'leaf curl' disease in plantations of Baumforth's Seedling raspberry were first observed in eastern Scotland in 1922. The disease did not affect cv. Lloyd George, but further increased 20 years later when cv. Norfolk Giant began to replace Lloyd George. Healthy plants, used to replace diseased plants, subsequently became infected and plants on the periphery of patches of diseased plants became infected. Thus, the size of the diseased patches extended annually.

During the 1950s, research at the Scottish Crop (Horticultural) Research Institute, demonstrated that sap-transmissible viruses caused this and similar diseases. Techniques were developed to recover and serologically type these viruses and to transfer them between herbaceous plants. At the end of the decade, it was discovered in North America that plant ectoparasitic nematodes could transmit some of these viruses.

Following this discovery, research at SCRI identified that two viruses (raspberry ringspot (RRSV) and tomato black ring (TBRV) nepoviruses) damaging raspberry and strawberry in Scotland and other areas of the UK, were transmitted by the nematode *Longidorus elongatus* and that two other viruses (arabis mosaic (ArMV) and strawberry latent ringspot nepoviruses (SLRSV)), more important in England, were transmitted by the nematode *Xiphinema diversicaudatum*. Research elsewhere showed that tobacco rattle tobravirus (TRV), a cause of 'spraing' disease in potato throughout Europe (brown necrotic arcs in the tuber flesh), was transmitted by trichodorid nematodes. This virus and several trichodorid species were identified as being widespread in Scotland.

Control measures developed at SCRI included the use of soil sterilant chemicals, e.g., D-D (dichloropropane-dichloropropene) and the fungicide, Quintozene (pentachloronitrobenzene), both of which control *L. elongatus* and *X. diversicaudatum*. However, early recognition of the problems associated with these highly toxic compounds led to alternative disease control strategies based on epidemiological and ecological studies of the disease.

Raspberry was shown not to be a host for *L. elongatus*, whereas many weed species were identified as hosts for the vector and as over-winter reservoirs for the viruses. The importance of good weed control, to prevent virus spread, was emphasised by the discovery that the virus particles are retained within the nematode's feeding apparatus, similar to the association of non-cir-

culative viruses in aphids. Acquisition of the virus from weeds was shown to be important as the nematodes lost the virus during each of the four moults and only retained the virus for a maximum of a few weeks.

Infected planting material was shown to be another important source for disseminating these viruses. The introduction of a certification scheme for disease-free soft fruit planting material, pre-planting soil tests for nematodes and viruses, and strict weed control in raspberry plantations, all but eliminated nematode-transmitted virus disease problems in the Scottish raspberry industry. Subsequently, these control measures were adopted world-wide but relaxation of weed control measures in soft fruit plantations in the UK during recent years has resulted in a resurgence of nematode-transmitted virus disease problems.

During the 1960s, many further reports were published world-wide of the apparent ability of different nematode species to transmit a variety of plant viruses. However, many of these reports were based on laboratory experiments that frequently did not include appropriate safeguards to exclude the possibility of transmission by an alternative vector or experimental contamination. Techniques and criteria developed at SCRI during the 1970's, including accurate identification of the virus and the nematode and the use of small numbers of hand-picked nematodes, led to two thirds of the then reported virus and nematode associations being rejected. Those which fulfilled the criteria, revealed the existence of a high degree of specificity between the vector species and its associated virus. As a consequence of these highly specialised techniques, with their requirement for multi-disciplinary collaboration between nematologists and virologists, the SCRI became established as the principal centre for research into nematode transmission of plant viruses.

To determine if virus-vector nematodes were a localised or a widespread problem, funding was obtained to undertake a systematic sampling throughout the UK. This survey revealed the ubiquitous distribution of virus-vector nematodes and showed that, in the UK, between 5 and 25% of vector populations were naturally associated with virus. Funding for a pan-European virus-vector nematode survey followed, providing the first systematic approach to identifying distribution patterns of plant pathogens at a continental level. Results from these surveys revealed that trichodorid and *Xiphinema* virus-vector species were widespread in Europe, whereas the *Longidorus* vector species have more localised distributions. Subsequently, the European distribution maps of virus-vector species were used for the preparation of Phytosanitary Regulations.

Research at SCRI showed the ability of nematode species to act as virus vectors is inherited. Populations of vector *Xiphinema* spp. were shown to be highly efficient vectors. Up to 100% of nematodes in a *Xiphinema* population could transmit virus, whereas with most vector *Longidorus* species, generally less than 10% of the population transmitted virus. This discovery led to accurate procedures for risk-assessment when sampling prospective soft fruit planting sites. Also, intra-specific variability in the ability of nematodes to transmit isolates of viruses was demonstrated, leading to the recognition that each population of a virus-vector species requires to be tested for its ability to transmit virus.

During the 1980s, 'spraing' disease in Scottish potato crops increased, partly because movement by and transmission of TRV by the vector trichodorid nematodes was facilitated by increases in crop irrigation. Studies of the transmission of TRV by trichodorid nematodes had made little progress since the 1960s, mainly because of the lack of techniques for working

Nematode	Virus	Serotype
P. anemones	PEBV	TpA56 (English)
	TRV	PaY4 (English)
P. hispanus	TRV	Portuguese
P. minor	TRV	American
P. pachydermus	PEBV	Dutch
	TRV	PpK20 (Scottish)
		PaY4 (English)
P. teres	PEBV	Dutch
	TRV	Oregon (Dutch)
P. tunisiensis	TRV	Italian
T. cylindricus	PEBV	English
	TRV	RQ (Scottish)
		TcB2-8 (Scottish)
T. primitivus	PEBV	TpA56 (English)
	TRV	TpO1 (English)
T. similis	TRV	TsB (Belgian)
		TsD (Dutch)
		TsG (Greek)
T. viruliferus	PEBV	English
	TRV	RQ (Scottish)

Table 1 Specific associations between *Paratrichodorus* and *Trichodorus* species and serologically distinguishable strains of tobacco rattle (TRV) and pea early-browning (PEBV) tobraviruses.

Nematode	Virus	Major crops affected
L. apulus	artichoke Italian latent	artichoke, chicory, grapevine
L. arthensis	cherry rosette	cherry
L. attenuatus	tomato black ring	beet, celery, leek, lettuce
L. elongatus	raspberry ringspot	raspberry, strawberry
	tomato black ring	potato, raspberry, strawberry
L. fasciatus	artichoke Italian latent	artichoke
L. macrosoma	raspberry ringspot	cherry, grapevine, raspberry, strawberry,
L. martini	mulberry ringspot	mulberry
P. maximus	raspberry ringspot	grapevine
X. americanum	cherry rasp leaf	apple, cherry, peach, raspberry
	peach rosette mosaic	blueberry, grapevine, peach
	tobacco ringspot	cherry, grapevine, soybean
	tomato ringspot	apple, grapevine, *Prunus* spp., *Ribes* spp., *Rubus* spp.
X. bricolense	tomato ringspot	see above
X. californicum	cherry rasp leaf	see above
	tobacco ringspot	see above
	tomato ringspot	see above
X. diversicaudatum	arabis mosaic	apricot, blackcurrant, hop, grapevine, peach, raspberry, strawberry,
	strawberry latent ringspot	blackcurrant, grapevine, peach, raspberry, strawberry
X. index	grapevine fanleaf	grapevine
X. intermedium	tobacco ringspot	see above
	tomato ringspot	see above
X. rivesi	cherry rasp leaf	see above
	tobacco ringspot	see above
	tomato ringspot	see above
X. tarjanense	tobacco ringspot	see above
	tomato ringspot	see above

Table 2 *Longidorus, Paralongidorus* and *Xiphinema* virus-vector species, their associated viruses and major crops affected.

with such small animals (<1mm long). Micro-techniques developed at SCRI, which used individual trichodorids in virus transmission experiments, led to specific associations being identified between different trichodorid species and serologically distinguishable strains of TRV and pea early-browning (PEBV) tobraviruses (Table 1). The new understanding derived from this research explained anomalies such as the non-correlation in the field between trichodorid population density and levels of TRV infection. Often, large population densities were found to be comprised of several species, only one of which, frequently present in small numbers, was the vector species. Conversely, small field population densities were of a single, efficient vector species. Also, in multi-species populations, two or more of the species could be associated each with their particular strain of TRV. The association of species with different strains of TRV also explained the observation that potato cultivars grown at different field sites showed differences in their reaction to TRV, i.e., at some sites a cultivar might show few symptoms of infection whereas at other sites it would be highly infected. Most recently, it has been shown that some potato cultivars e.g. Home Guard, King Edward and Wilja, may be infected symptomlessly but act as a source of the virus if planted at sites, formerly free of TRV, where the appropriate vector trichodorid species is present. Current research is examining the impact of trichodorids and TRV infection on potato growth.

The techniques developed at SCRI formed the basis for collaborative studies to investigate the transmission of viruses by nematodes in North America. Transmission of four nepoviruses by several species within the *X. americanum* group of nematodes (which is comprised of 45 parthenogenetically-reproducing, morphologically-similar, putative species) has been reported. Using the micro-techniques developed at SCRI, it was demonstrated that, whereas European longidorid vector species specifically transmit one or at most two serologically distinguishable strains of virus, several American species transmit most, if not all four, of their associated nepoviruses (Table 2).

Associated studies revealed that virus-vector populations of *X. americanum* species from North America had only three juvenile stages whereas species from other areas of the world, which are not associated with viruses, have four juvenile stages. This discovery is being investigated for its utility as a biological marker to identify potential virus-vector species and populations in this group of nematodes. These nematodes and their associated viruses are regarded as important Phytosanitary quarantine organisms, and work under an international EU-funded project on detection and identification of *X.americanum*-group virus-vector nematodes has just started.

The development of molecular virological techniques during the 1990's has resulted in new, fundamental approaches to understanding the unique recognition phenomena between nematodes and their associated viruses. Investigations of the genetic determinants for specificity of transmission of tobravirus are supported by three post-graduate research students from Greece funded by the EU and the Greek State Foreign Scholarship Fund. Non-structural genes on the RNA-2 of tobraviruses have been identified as helper components in vector transmission. Vector-transmitted isolates of tobacco rattle and pea early-browning tobraviruses, each incorporating a green fluorescent protein, are being used in *in vitro* investigations to determine the vector trichodorid feeding mechanism(s) involved in virus transmission and subsequent virus spread from the infection site (= epidermal root cell) (Fig. 1).

Nematode transmission of viruses is an evolving area of study and, with increasing international interest, new virus and vector associations are being recognized world-wide (Fig. 2). Much of this work involves SCRI staff collaborating with international colleagues, i.e., a Royal Society and an internationally-funded research collaboration to investigate nematode-transmitted viruses in China and Brazil, respectively. Also, control of nematode-transmitted virus diseases, with few exceptions, has changed little since the 1960's. However, with societal emphasis on reducing agro-pesticide inputs, more benign nematode and associated virus-induced crop disease control methods are urgently required. The movement to lighter soil types for potato production and a consequent requirement for crop irrigation, has resulted in an increased probability of ‘spraing’ disease caused by tobacco rattle tobravirus. Current research at SCRI is focused on reducing the dependence on chemicals for controlling this disease by seeking to establish cultural methods to prevent virus transmission by the vector nematode, developing natural resistance in potato to tobacco rattle tobravirus and, in collaboration with colleagues from Leiden University, the Netherlands, investigating transgenic resistance. Also, an EU-funded project for improved detection and molecular-based identification of vector trichodorids has just begun. This productive interdisciplinary research relies on the continued, unique collaboration between nematologists and virologists at SCRI and their international colleagues.

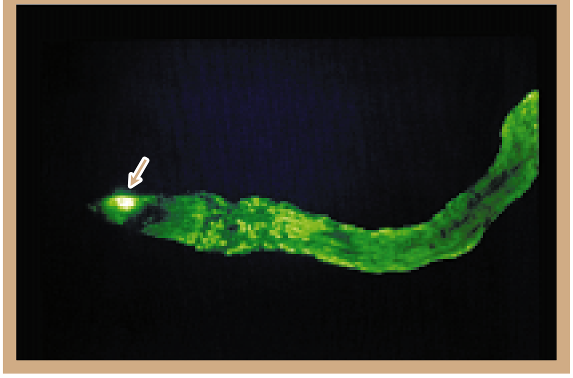

Figure 1 Initial infection site (arrowed) and subsequent systemic cellular spread in root of *Nicotiana clevelandii* of green fluorescent protein-tagged tobacco rattle tobravirus transmitted by *Paratrichodorus pachydermus.*

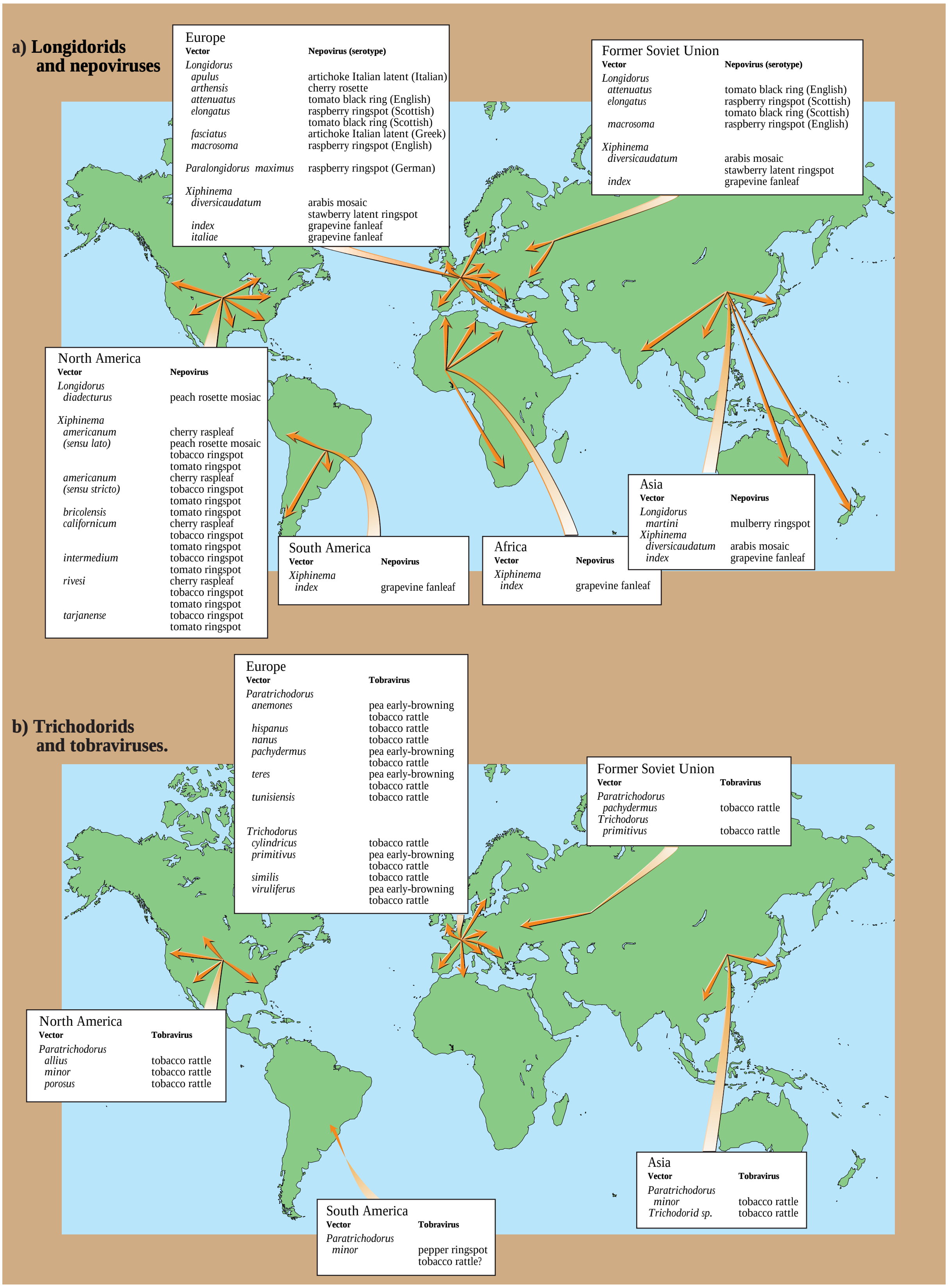

Figure 2 Main world-wide occurrence of virus-vector species in association with viruses: a) longidorids and nepoviruses; b) trichodorids and tobraviruses.