Trichodorid vectors of serologically distinguishable strains of tobacco rattle tobravirus occurring in Germany and the use of antagonistic plants to suppress „spraing“ disease in potato

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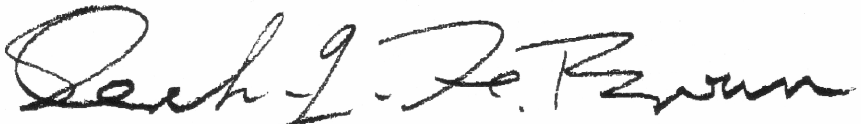

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I INTRODUCTION

I.A The Tobraviruses

I.A.1 Background

Several members of the plant ectoparasitic nematode genera *Trichodorus* and *Paratrichodorus* transmit tobraviruses. Of the three tobraviruses, the type member tobacco rattle virus (TRV) is the most economically important causing 'TRV-spraing' disease of potatoes (*Solanum tuberosum* L.; Taylor & Brown, 1997). The disease results in brown necrotic arcs in the potato tuber flesh that render the crop unmarketable. The nematodes feed on root epidermal and root hair cells. At the commencement of the feeding cycle the nematode uses its onchiostyle to penetrate several individual cells, but then it abandons the cell leaving it intact. Eventually it selects cells upon which he feed, and most of the cell contents are removed during the feeding process causing death of the cell (Wyss, 1971b and 1975; Fritzsche *et al*., 1985). During the feeding process virus particles present in a cell are ingested along with the cell cytoplasm and retained by the nematode in its feeding apparatus. Subsequently, when the nematode begins its feeding cycle on an uninfected cell, or plant, these virus particles are transferred into the new cell resulting in transmission of the virus (Trudgill, 1976).

'Mauche' disease of tobacco growing in Germany was considered by Behrens (1899) to be soil-borne, and Böning (1931) demonstrated that the disease was caused by an agent that passed through bacterial filters. Subsequently, Quanjer (1943) referred to the agent as tobacco rattle (ratel) virus, the name referring to the noise made when wind blew through infected tobacco leaves. Sol *et al*. (1960) were first to report that the natural vector of the virus were trichodorid nematodes.

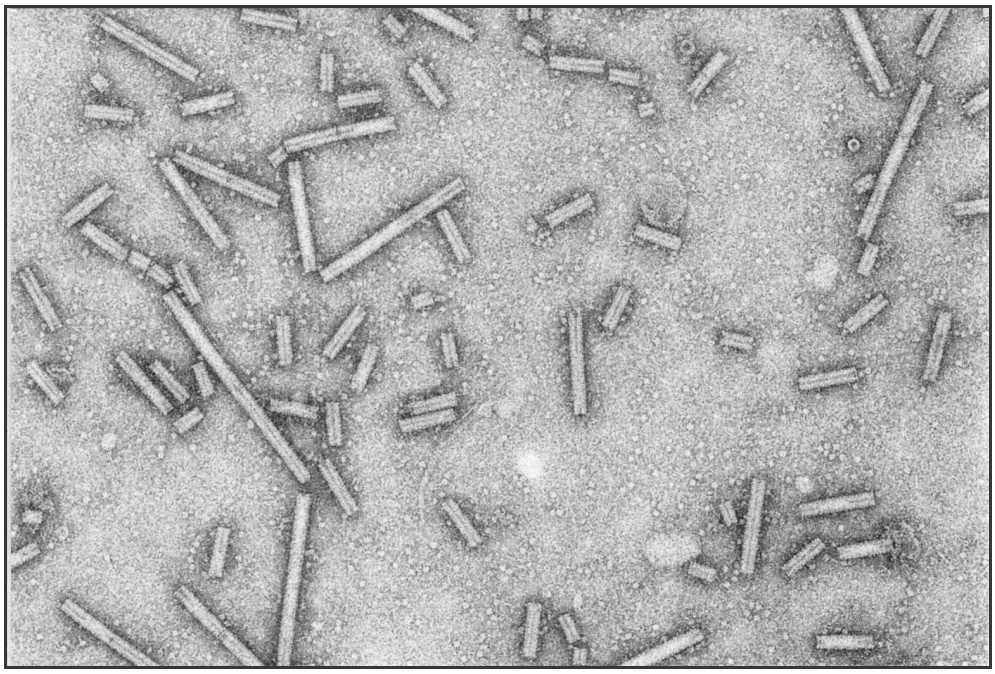
TRV was named as one of the members of the NETUvirus group (NEmatode transmitted TUbular particle viruses), due to their transmissibility by nematodes and their tubular particles (Cadman, 1960). After a taxonomic revision of viruses, the term NETUvirus was replaced with the term Tobraviruses, in recognition of the type member of this virus genus. In a recent classification of viruses *Tobravirus* was established as a genus containing three viruses, each named after one of the hosts that the virus infects: tobacco rattle virus, the type-member of the genus (TRV), pea early-browning virus (PEBV), and pepper ringspot virus (PRV) that was originally referred to as the CAM-strain TRV, and which occurs only in Brazil (Van Regenmortel *et al*., 2000).

I.A.2 Properties and characteristics of the Tobravirus *genus*

The bipartite RNA-viruses of the *Tobravirus* genus are comprised of two rod-shaped, straight tubular particles of different length (Ploeg & Brown, 1997). The longer particle, known as the RNA-1 segment of the viral genome, is required *inter alia* for RNA-synthesis. The shorter particle, the RNA-2, codes for the production of the viral capsid and vector transmissibility (Steck, 1971; Linthorst & Bol, 1986). Both RNAs are single-stranded, with positive polarity.

The average length of the long and short particles, depending on the specific isolate, is 180-215nm and 45-115nm, respectively. The particles have a diameter of 21-25nm. (Bokx, 1972; Bos, 1983; CMI/AAB, 1973; CMI/AAB, 1970; Brunt *et al.*, 1990). The axial canal is obvious, being 4-5nm in diameter (Brunt *et al.*, 1990).

Figure 1: TRV-particles; EM-photograph



The RNA1 is highly conserved, and contains several open reading frames (ORFs) that code for, *i.e.*, RNA-replication, cell-to-cell-movement and partially for seed transmission, whereas the RNA2 is genetically variable and contains ORFs coding for the viral capsid, partially for seed transmission and one or more genes code for non-structural proteins, of which at least one is essential for vector transmission (CMI/AAB, 1970; MacFarlane *et al*., 1996).

A typical serologically distinguishable strain of TRV, strain SYM, has an RNA1 that contains a 134/194K ORF that codes for the replicase gene, a 29K ORF that is involved in cell-to-cell-transport of the virus and induction of some symptoms in hosts (Angenent *et al*., 1989a; Boccara *et al*., 1986; Hamilton *et al*., 1987; MacFarlane *et al*., 1989; Ziegler-Graff *et al*., 1991), and a 16K ORF whose function is unknown, but which is homologous to a 16K ORF in the RNA2, that overlaps with a 13K ORF, whose function also is unknown. It has been speculated that the

16K ORF modulates the host plant nucleic acid metabolism, and therefore is considered to be associated with plant cell nuclei (Liu *et al*., 1991). A similar analysis of the RNA1 of PEBV strain SP-5 revealed that it contains four ORFs similar to those of TRV RNA1, with molecular masses of 201, 141, 30 and 12kDa, and similar biological properties to those in TRV.

The RNA2 contains one to four ORFs, including the coat protein (CP) gene and one to three non-structural protein (NSP) genes (Angenent *et al*., 1986; Hernandez *et al*., 1995; MacFarlane & Brown, 1995). The RNA2 of different TRV strains is variable in length and sequence.

Figure 2: exemplary structure of the two RNAs of TRV; RNA1 from SYM; RNA2 from TCM

The three *Tobraviruses* occur as several serologically distinct strains, and particularly with TRV the RNA2 sequences of the different strains results in substantial genome diversity that arises by spontaneous deletion in the RNA2 (Harrison & Woods, 1966; Hernandez *et al*., 1995). Also,

recombinant isolates of TRV occur naturally that contain PEBV-RNA2 sequences in the RNA2 segment of the genome (Robinson *et al*., 1987; Robinson, 1994).

Tobravirus isolates are classified by means of molecular hybridization techniques based on sequence homology of the highly conserved RNA1. Serological methods are not reliable for classifying *Tobraviruses* as they identify epitopes on the viral capsid and there could be similar epitopes on different viruses and these epitopes are not related to physico-chemical or biological characteristics of the virus. The genetic sequence of the viral capsid gene of *Tobraviruses* is variable (Harrison & Robinson, 1986) and the natural occurrence of PEBV sequences in the RNA2 segment can each result in a misleading identification of a particular virus isolate.

TRV in plants occurs either as an M (multiplying) or an NM (non-multiplying) type of infection (Crosslin & Thomas, 1995). These two types of infection can be distinguished by repeated freezing and thawing *in vitro*, after which only the M-type remains infectious. M infections contain both the RNA1 and the RNA2 segments of the virus genome. Thus, M infections are characterised by the presence of virus particles, they are serologically detectable, and are vector transmissible. In contrast, NM infections contain only the RNA1 segment of the genome, thus virus particles are absent and consequently these isolates are not vector-transmissible.

I.A.3 Genetic determinants of vector transmissibility

Using pseudorecombinants of nematode-transmissible (PpK20) and non-nematode-transmissible (PLB) TRV strains Ploeg *et al*. (1993b) demonstrated that the genetic determinants of vector-nematode-transmissibility of *Tobraviruses* are encoded by the RNA2 segment of the virus genome.

Initially, it was considered that only the virus coat protein (CP) was involved in vector transmission, however two other ORFs on the RNA2, each of which encodes for a non-structural protein (NSP), have been shown to be involved in nematode transmission of *Tobraviruses.* cDNA clone-transcripts of PEBV RNAs enabled the viral determinants of specific tobravirus-trichodorid interactions to be studied (MacFarlane *et al*., 1995). Infectious cDNA clones of TRV and PEBV wild type isolates revealed that, when the coat protein gene from a non-transmissible isolate (PEBV-SP5) was replaced with that of a transmissible isolate (TRV-PpK20), the recombinant virus remained non-vector-transmissible. Therefore, the coat protein alone did not confer vector transmissibility to tobraviruses (MacFarlane *et al*., 1995). Introducing mutations to each of the four RNA-2 genes of the transmissible isolate PEBV TpA56-RNA2 showed that nearly all the proteins (CP and some NSPs) were involved in vector transmission as essential (CP and 2b for example) or as mediators (2c for example) (MacFarlane & Brown, 1995; MacFarlane *et al*., 1995 and 1996), whereas with TRV-PpK20 the CP and only one of the two NSPs were required for vector transmission (Hernandez *et al*., 1997).

The involvement of viral proteins in insect-, fungal- and nematode-vector virus transmission is a common characteristic of plant virus-vector interactions (Gray, 1996). Helper-protein dependent

transmission has been broadly studied in the field on *Poty-* and *Caulimovirus* genera (Pirone & Blanc, 1996) and a specific correlation was reported between the coat and helper proteins in aphid transmission of tobacco vein mottling potyvirus (Blanc *et al*., 1997). It appears probable that the NSPs of TRV and PEBV RNA2 encode for proteins of similar function to those involved in virus transmission by aphids.

I.A.4 Identification of Tobravirus *isolates*

Isolates of TRV and PEBV can exhibit extensive antigenic variation. Also, several TRV isolates have been found to be natural recombinants, whose genome consists of a TRV-RNA1 strand and sequences of PEBV-RNA2 (Harrison & Robinson, 1986).

The most frequently used procedure for detecting the occurrence of TRV is the "bait test" method. A container is filled with field soil, a herbaceous virus free "bait plant" is then grown in the soil for several weeks and the roots of the pant subsequently tested for the presence of virus (Taylor & Brown, 1997). Immunological and molecular detection, or confirmation of the presence, of TRV in potato tubers is extremely difficult and largely unreliable due to the requirement to use a wide range of antisera and the presence of inhibitors in the tuber flesh (Weidemann, 1993a; Xenophontos *et al*., 1998; Crosslin & Thomas, 1995).

Characterisation of an isolate of TRV infecting herbaceous plant leaves or roots can be reliably carried out by using polyclonal antisera, prepared against fully identified isolates of the virus. These immunological tests are done using electron microscope techniques such as immunosorbent electron microscopy (ISEM), or serological methods such as enzyme linked

immunosorbent assay (ELISA). As noted, because of the extensive variability of TRV isolates a large number of different polyclonal antisera are required to unequivocally identify and characterise an M-type isolate of the virus in serological tests (Robinson & Harrison, 1985; Harrison & Robinson, 1986; Ploeg *et al*., 1993a).

More reliable techniques have been introduced for identifying *Tobraviruses* with the development of molecular biology, and particularly the polymerase chain reaction (PCR) technique. This method utilises an *in vitro* molecular hybridization technique, with specific probes to RNA1 conserved sequences (Robinson, 1989). Most recently, the reverse transcriptase-PCR (RT-PCR) technique has been developed for distinguishing isolates of TRV, PEBV and PRV (MacFarlane, 1996). This method provides a rapid diagnostic procedure for determining the existence of TRV and analysis of the naturally occurring strains of the virus in plant material, and directly from the body contents of nematodes (Boutsika *et al.*, 1999). RT-PCR enables a diagnostic test for the detection of TRV to be completed within 3 days of receipt of a sample and offers a potentially rapid and reliable method for detecting TRV in potato tubers.

I.A.5 Host range and plant diseases

TRV has an extensive host range, probably the widest of any plant virus, and has been shown to infect at least 600 species in more than 50 families, dicotyledonous and monocotyledonous, (Schmelzer, 1957; CMI/AAB, 1970; Spaar & Hamann, 1974; Weidemann, 1981; Arias & Bello, 1988). Plant species belonging to the Hydrophylaceae, Solanaceae, Primulaceae and Linaceae families can become systemically infected, while those in the Cucurbitaceae and Leguminosae

are only infected locally. Members of the Rosaceae and Graminae families are not hosts for TRV (Schmelzer, 1957).

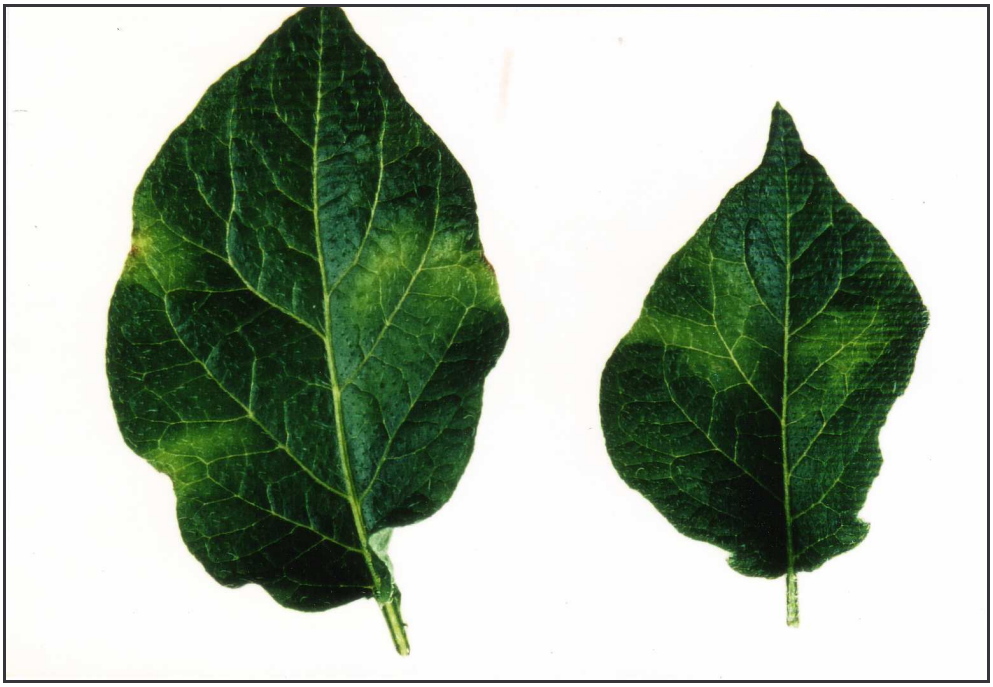
The symptoms and their severity induced in plants by Tobraviruses vary both amongst the three viruses and amongst serlogically distinguishable strains of the viruses. Plant hosts such as tobacco, potato, bulbous ornamentals, peppers, tomatoes, French bean (*Phaseolus vulgaris*), pea, spinach, sugar beet, lucerne, lettuce and onion can show obvious symptoms induced by Tobraviruses. Other hosts such as pine, clover, elm, maize, chestnut, cherry, spruce, redcurrant, *Stellaria media*, *Viola tricolor* and cucumber whilst readily infected by Tobraviruses do not show symptoms of virus infection (Cooper & Thomas, 1970; CMI/AAB, 1973; Cooper, 1971b). Crops most seriously affected by Tobraviruses include potato, tulip, narcissi, hyacinth and tobacco (Maas & Rothuis, 1973; Ploeg & Brown, 1997). In tobacco TRV causes necrotic spots and arcs on the leaves of infected plants. Infection results in the leaves crinkling as they become dry and brittle (Steck, 1971), and the English disease name "rattle" derives from the rustling sound of the dry leaves moving in the wind (Heinicke, 1983). TRV causes notched leaf in gladiolus, ringspot in aster and pepper, yellow blotch in sugar beet, colour-break in tulip, and unnamed diseases in lettuce, hyacinth, and narcissus (CMI/AAB, 1970).

TRV can over-winter in perennial weed species and weed seeds in the absence of nematodes, for example in *Viola tricolor* (Cooper & Harrison, 1973). Approximately 1 to 5% of seeds from an infected weed species carry TRV, and these infected weed seeds pose a particular problem. The infected seeds may lie dormant for several years, and even decades, in soil before germinating and presenting a source from which vector nematodes can acquire the virus (Cooper, 1971a and 1971b).

TRV induces a wide range of symptoms in different potato cultivars, with about 50% of currently used cultivars showing symptoms of infection (Reepmeyer, 1973b; Steck, 1971). Infection and any consequent disease symptomatology are highly dependent on the potato genotype and the strain of TRV. Infection of tubers is also dependant on soil moisture conditions prevailing during tuber initiation, as a high soil moisture is necessary for vector nematode mobility and hence efficient virus transmission.

Potato cultivars can be resistant (not infected), tolerant (infected but without any visible symptoms in the tuber flesh), or susceptible (classical 'TRV-spraing' disease symptoms occur in the tuber flesh in which of brown necrotic arcs are readily observed). Symptoms caused by TRV infection in potato leaves and stems are relatively rare in comparison to tuber symptoms (aid Auswertungs- und Informationsdienst, 1997; Weidemann, 1981), and usually are visible as chlorotic yellow spots and chevrons, leaf-tip necrosis, ("stem mottle"; Fig. 3; Bonituranleitung, 1981).

Figure 3: TRV-symptoms at potato-plants: stem-mottle



TRV infection in potato tubers can cause external symptoms such as necrotic spots (brown or rust-red, faded, with a slightly darker centre; Bonituranleitung, 1981) and sunken rings that can tear open later and continue inside the tuber (Stippigkeit, Pfropfenkrankheit) as spots (Fig. 4) or arcs (Fig. 5) or bung-shapes (Fig. 6) (Spaar & Hamann, 1974). During heavy infection tubers can appear deformed (Fig. 7). Symptoms in tubers develop during growth of the tuber, are evident at harvest and their severity increase little during storage (Dale & Solomon, 1988).

Figure 4: TRV-symptoms at potato-tubers: spots

Figure 5: TRV-symptoms at potato-tubers: arcs

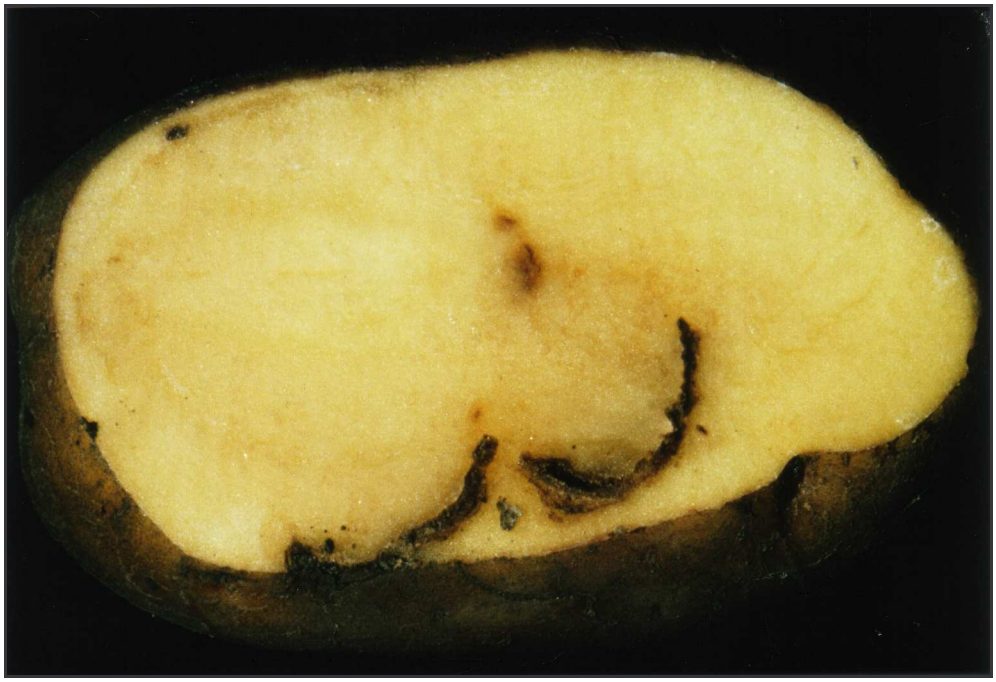


Figure 6: TRV-symptoms at potato-tubers: bungs

Figure 7: TRV-symptoms at potato-tubers: heavily infected deformed tubers

Potato tubers showing TRV-spraing disease symptoms are unacceptable for ware and processing markets *i.e.* whole potatoes, crisps, chips, potato fritters, or french fries (Schütz, 1973). If more than 2% of the tubers in a consignment show TRV-spraing-damage, the entire consignment is rejected (Spaar & Hamann, 1974). With tolerant cultivars used for seed losses of 25% can occur in daughter crops as a result of the virus causing an increased number of smaller unmarketable tubers being produced (Dale *et al*., 1998).

In the Hannover region about 2.5 Mill.t of potato are harvested annually of which approximately 90,000t of 1 Mill.t that show signs of TRV-spraing are rejected (D. Heinicke, personal information). Germination of potato seed tubers showing TRV-spraing symptoms is clearly inhibited (aid Auswertungs- und Informationsdienst, 1997), and these plants often produce fewer tubers (Cadman, 1959).

I.A.6 Virus-spread within plants and between plant generations

Translocation of a soil-borne virus in a plant initially is cell to cell radiating from the initially infected root cell, and eventually the virus enters the phloem which provides a pathway for rapid spread throughout the plant (Bokx, 1972). TRV particles can be found in all parts of a systemically infected host plant; in the cytoplasm, mitochondria and often in the cells arranged around them (Brunt *et al.*, 1990), but the virus is frequently aggregated within the plant. Upon infecting a plant *via* transmission by a vector nematode TRV usually remains in the roots of the plant for a considerable time before spreading throughout the plant (Bokx, 1972). TRV requires at least 4 hours to move from the primary inoculated cell into adjacent cells (Derrick *et al*., 1992), moving symplastically across cell walls (intercellular) *via* plasmodesmata that have a maximum diameter of 0.9nm. Virus nucleic-acid and proteins are too large to move in this way, consequently movement proteins encoded by the virus cause these openings to enlarge and increase plasmodesmatal permeability until the diameter reaches an average of 3.1nm. The enlarged diameter enables the movement of the viral ribonucleoprotein complexes through the altered plasmodesmata.

In many plant species TRV apparently remains localised at the initial site of infection inducing chlorotic and/or necrotic lesions (hypersensitive reaction resulting in death of the infected cell), regardless of whether infection originated from mechanical inoculation of leaves or root inoculation by virus-vector trichodorids. In other plant species TRV spreads systemically eventually infecting the entire plant. TRV-spraing symptoms in potato tuber flesh are regarded as extreme examples of a hypersensitive response in the plant host. However, as stated in Section I.A.5 different potato cultivars react differently to different strains of TRV and can be

classified as resistant (= not infected), susceptible (= tubers show typical TRV-spraing symptoms), and tolerant (= entire plant infected with TRV but TRV-spraing symptoms absent in tubers) (Xenophontos *et al*., 1998).

Vector transmission of TRV to potato tubers is believed to occur in newly forming tubers when the nematode feeds on lenticells when the phelloderm is thin (Cooper, 1971b). Mechanical inoculation of TRV infected tuber flesh onto leaves of virus indicator plants rarely causes infection, and especially not after a period of storage of the tubers as a result of virus inhibitors in the tuber flesh (Cooper, 1971b).

With tolerant potato cultivars, TRV infected tubers do not show any symptoms (Xenophontos *et al*., 1998), and the plants that grow from these tubers are systemically infected and produce symptomless daughter-tubers. Tubers from these tolerant cultivars when infected with TRV provide a highly efficient pathway for dispersal of TRV, and provide an excellent source from which the virus can be acquired by the associated vector nematodes present at sites at which the virus was not previously present (Xenophontos *et al*., 1998).

Potato tubers with TRV-spraing symptoms mostly contain NM-strains, and only a small proportion of plants growing from such infected seed tubers eventually develop stem mottle symptoms. Daughter-tubers of these stem-mottle-plants only very rarely develop “secondary TRV-spraing” symptoms (Xenophontos *et al*., 1998). As a result of viral inhibitors TRV is distributed unevenly in tolerant potato tubers and in tubers showing typical TRV-spraing symptoms the virus is usually found only within the necrotic arc region. Consequently, negative results obtained when testing potato tubers for the presence of TRV are not reliable and do not provide a guarantee of virus-freedom (Xenophontos *et al*., 1998).

I.B The Family Trichodoridae

I.B.1 Systematics and taxonomy

De Man described the species *Dorylaimus primitivus*, in 1880. Cobb (1920) erected *Trichodorus* with a single species, *T. obtusus*, which was later synonymized with *T. primitvus* by Micoletzky (1922). When describing *Dorylaimus primitivus*, de Man (1880) also described a new genus, *Diphtherophora*, with a single species, *D. communis*, and in 1935 Thorne, recognising similarities between *Diphtherophora* and *Trichodorus*, proposed the family Diphtheropteridae with two subfamilies, Diphtherophorinae and Trichodorinae, in the Dorylaimoidea.

In subsequent classifications the two genera were further separated by raising the Trichodorinae to family status by Siddiqi (1961) and Clark (1961), the latter also proposing the superfamily Diphtherophoroidea. Clark (1961) assigned this superfamily to the suborder Alaimina within the Dorylaimida. Coomans and Loof (1970) raised Diphtherophoroidea to suborder rank but retained Trichodoridae as a family. Siddiqi (1974) raised Trichodoridae to superfamily rank under Diphtherophorina which was retained under Dorylaimida. However, he drew attention to some of the unique features of the group, such as the spicule musculature, possession of a single testis, presence of a gubernaculum, prominent excretory pore and absence of a prerectum - these features are not shared by other dorylaims. On the basis of these morphological differences he placed the Diphtherophorina, including the trichodorids, in the order Triplonchida (Siddiqi, 1983).

The family Trichodoridae contained only the single genus *Trichodorus* but Siddiqi (1974) proposed splitting the genus into two genera, *Trichodorus* and *Paratrichodorus* with the latter genus divided into three subgenera, *Paratrichodorus*, Atlantodorus and *Nanidorus*.

Andrassy (1976) subsequently proposed *Monotrichodorus* for the monodelphic *Trichodorus monohystera*, the brief description of which was expanded by Rodriguez-M. and Bell (1978) when they also erected *Allotrichodorus*, another monodelphic genus originating from South America. Siddiqi (1980) later raised his subgenera of *Paratrichodorus* to generic rank, but Decraemer (1980a) rejected the concept of splitting *Paratrichodorus* into either subgenera or genera and recognized Trichodoridae as comprising only four genera, *Trichodorus, Paratrichodorus, Monotrichodorus* and *Allotrichodorus,* an opinion which has been widely accepted and is incorporated in the classification presented in Table 1.

Table 1: Classification of virus-vector trichodorid nematodes (after Coomans, 1996 and Maggenti, 1983 and 1991)

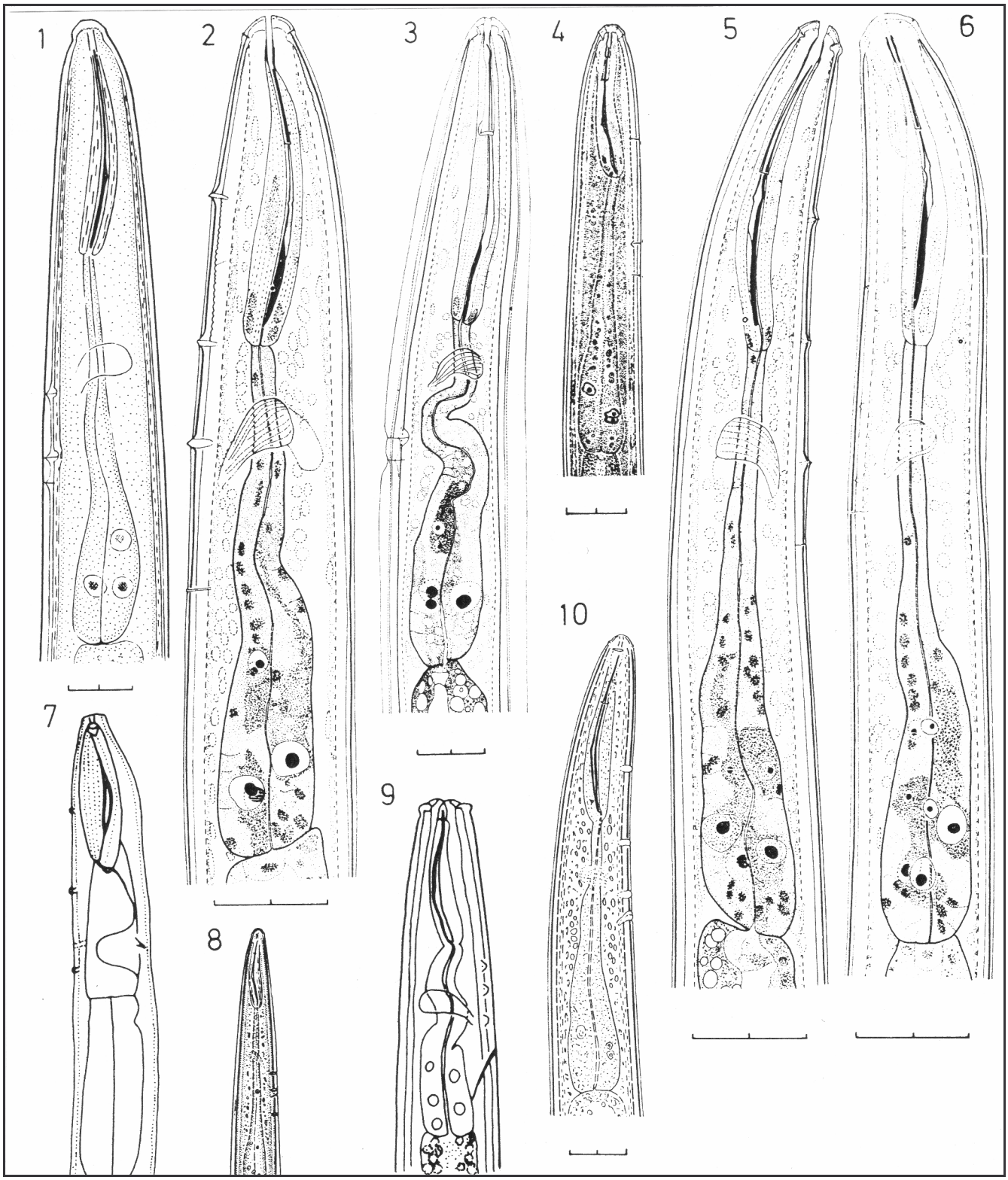
Order: TRIPLONCHIDA	
Suborder: Diphtherophorina	
Superfamily: Trichodoroidea	
Family: Trichodoridae	
Genera:	*Allotrichodorus*
	Monotrichodorus
	Paratrichodorus
	Trichodorus

I.B.2 General morphology

Trichodorid nematodes are soil-inhabiting relatively small cylindrical cigar-shaped root ectoparasites. Their body length varies from 0.35 to 1.8mm. The head is rounded and the tail is usually blunt. An important characteristic of trichodorid species is that they have a characteristically elongated, solid tipped and ventrally curved tooth-like onchiostyle, which distinguishes them from dorylaimid nematodes. When heat-killed and fixed the cuticle of *Paratrichodorus* species usually swells, whereas that of *Trichodorus* species usually does not.

The most easily recognised characters useful for species identification are the shape and structure of the vulval region in females and the spicules region in males (Decraemer, 1995). However, accurate identification of trichodorids to the specific level requires considerable taxonomic skill. The feeding apparatus (Fig. 8) is described here in detail, as TRV particles are specifically retained within this region in vector species. The buccal cavity (terminology based upon Decraemer, 1995) is located between the mouth opening and the pharynx. For trichodorids, this area is referred to as the stoma, or cheilostome, and is formed by an infolding of the outer body wall cuticle. The pharyngeal lumen has a regularly triradiate symmetry, but at the zone of the onchium it appears hexagonal in cross-sections. Its anterior part accommodates the onchiostyle (spear), which consists of two parts: the anterior tooth-like onchium and the posterior onchiphore. The onchium is solid and comprised of cuticular tissue. At the level of fusion of the onchiophore/pharyngeal wall an infolding forms what is referred to as the “guide ring“. Posterior to the onchistyle, the isthmus (mid-portion to the pharynx) is narrower in diameter and composed of muscles. Its triradiate lumen is surrounded by the nerve ring. Posterior to the nerve ring, the pharynx expands to a “pear-shaped“ basal bulb that contains one large dorsal and two pairs of ventrosublateral gland cells that possess ducts that open into the lumen of the alimentary tract.

Figure 8 (after Decraemer, 1980a):
Oesophageal region in *Trichodorus* (scale = 20µm). 1: *T. hooperi* (after Loof, 1973). 2: *T. primitivus*. 3: *T. californicus* (specimen, courtesy E.M. Noffsinger). 4: *T. lusitanicus* (after Siddiqi, 1974). 5: *T. viruliferus* (specimen, courtesy D. De Waele). 6: *T. similis*. 7: *T. castellanensis* (redrawn after Arias-Delgado, Jimenez-Millan & Lopez-Pedregal, 1965). 8: *T. pakistanensis* (after Siddiqi, 1962). 9: *T. kurumeensis* (redrawn after Yokoo, 1966). 10: *T. variopapillatus* (after Hooper, 1972).



The pharyngeal bulb acts as a pump operating by means of thin radial muscles that open the lumen against the turgor pressure of the bulb. The pump functions during the salivation and the ingestion phases. A one-way valve present at the pharyngeal/intestinal junction connects the pharyngeal lumen with the intestine.

I.B.3 Life Cycle

The life cycle of nematodes consists basically of six stages: the egg (= embryo), four developmental juvenile stages (J1, J2, J3 and J4) and the final adult stage. Juveniles moult to become the next stage, and during the moulting process the external cuticle that surrounds the nematode`s body, becomes separated (apolysis) from the underlying tissue (hypodermis). The old cuticle is shed along with the cuticular lining of the stomatal and pharyngeal lumina (ecdysis) (Taylor & Brown, 1997). Thus, during ecdysis any virus particles adsorbed on the internal cuticle become shed along with the cuticle (Taylor & Robertson, 1977).

Trichodorids have relatively short life cycles, and all life stages are present at any one time in the field (Taylor & Brown, 1997). Under natural field conditions in Europe trichodorids probably survive for three or four years. However in laboratory studies with *T. christiei* on tomato at 27ºC, a parthenogenetic species indigenous in the United States and that exhibits an "r" life-strategy, the J1 emerged from the egg on the 4th day after the latter was laid, and immediately moulted to J2. The J3 developed seven days after egg-deposition, the J4 developing on the tenth day and the adults in two weeks (Morton & Perry, 1968).

Coiro & Sasanelli (1994) observed the life-cycle of *Trichodorus sparsus* under laboratory conditions on S. Lucie cherry (*Prunus mahaleb* L.) at 27ºC. This species was found to have a life span of 22 weeks, a reproductive span of 16 weeks, and a total reproductive capacity of 215-225. From egg till adult 40-42 days were usual. *Trichodorus viruliferus* in the UK/Scotland has 2 generations per annum (Cooper & Thomas, 1971).

Under field conditions trichodorids reproduce from spring to autumn (Alphey, 1985) when soil temperatures and soil moisture levels are above a minimum threshold required for reproduction. Large seasonal variation in reproduction is not common, although larger populations have been recorded during autumn in Europe (Brown & Boag, 1987). The latter seasonal variation is probably linked to the annual growth cycle of the respective plant host (Brown & Boag, 1987).

I.B.4 Feeding behaviour

Trichodorids are strictly root ectoparasites, that feed on epidermal cells (Rhoades, 1965; Rohde & Jenkins, 1957; Russel & Perry, 1966; Schilt & Cohn, 1975). However, van Hoof (1964) noted that trichodorids will feed upon leaf cells when leaves are buried in soil, and he used this feeding behaviour to develop a bait-leaf method for determining virus transmission by trichodorids. The nematodes aggregate and feed on cells immediately behind the apical meristem of the root cap, within a tract of 1-3mm (Pitcher, 1967). They have been observed aggregating at the zone of root elongation (Coiro & Sasanelli, 1994) feeding preferentially on root tips (Pitcher & McNamara, 1970; Wyss, 1975, 1977 and 1982) of actively growing roots (Högger, 1973; Zuckerman, 1961).

Trichodorids feed on individual cells using their onchistyle to puncture the cell wall and then use secretions glands in the oesophageal bulb to form a feeding tube through which they withdraw the cell contents (Wyss, 1971a and 1975). During this process TRV particles present in a cell are ingested and a proportion of them become specifically retained in the feeding tract. Transmission occurs subsequently, when the nematode next feeds. Virus particles are introduced into the living cell, then dis-assemble, interact with the cell constituents and begin replication and re-assembly, thus establishing an infection in the cell and eventually in the whole plant. However, trichodorids commonly kill the cell being fed upon, but during the early stages of a feeding cycle the nematode punctures a proportion of the cells, forms a feeding tube, but does not remove the cell contents. It is during the formation of the feeding tube that secretions from the glands in the oesophageal bulb release and move virus particles retained in the feeding tract into the cell. In those that cells remain intact the virus can establish an infection in the plant.

A feeding cycle includes a series of consecutive feeds and an intermediate inactive period. The feeding process on a single cell rarely exceeds six minutes and consists of five distinct phases, *i.e.* exploration, perforation, salivation, ingestion, and withdrawal (Wyss, 1971a). After completing feeding on one cell, the trichodorid moves to a neighbouring cell. While feeding on a cell, the nematode forms a hollow feeding tube at the perforation site, through which they can ingest cell sap. Because this feeding tube is sufficiently wide, they are the only nematodes that can ingest cell organelles (Wyss, 1975). They are also the only nematodes that thrust their onchiostyle continuously during feeding (Chen & Mai, 1965; Wyss, 1977) and they have the ability to distinguish between dead and living cells, adapting their feeding behaviour accordingly.

I.B.4.a Root cell and tissue responses

Trichodorids follow the root growth through the soil and finally disperse when the root is critically damaged (Pitcher, 1967). Also, they have occasionally been observed feeding on secondary, fine, lateral, and on “feeder” roots when thicker, extending roots were absent (Pitcher, 1967). The symptoms induced by trichodorid feeding vary amongst different plant hosts and with different trichodorid species. Generally, there is a decline of root growth followed by cessation of growth if the apical meristem is attacked. The nematodes move to lateral root tips that eventually become stubby and turn brown or black, probably as a result of secondary infection or necrosis. Symptoms of trichodorid feeding are: yellowing on the root-surface, root tip swelling, and typically deformed stele of cherry (Coiro & Sasanelli, 1994); darkening, abnormal growth, shortening and proliferation of roots, and stunting of tomato (Rohde & Jenkins, 1957); decrease of growth and substantial reduction of root weight, chlorotic condition of the plant, and root-lesions of St. Augustine grass (Rhoades, 1965); cracks in the epidermis and enlargement of tissues underlying fed upon cells of apple, loss of meristematic activity, browning of epidermal and hypodermal cells and in some cases shrinkage of these brown cells in apple (Pitcher, 1967); and inhibition of root hair formation in *Brassica rapa* var. silvestris, *Fragaria vesca* var. semperflorens, and *Nicotiana tabacum* (Wyss, 1975).

The reactions observed within the cells that have been fed upon by trichodorids are probably a result of chemical or enzymatic reaction to the nematode secretions, however they may derive from mechanical injury (Wyss, 1975). It is most likely that a combination of these is responsible for the overall cell responses.

I.B.5 Host range

Trichodorid nematodes are polyphagus ectoparasites that can be found in a wide range of habitats (arable land, grassland, woodland) and are associated with numerous species, including weeds, bulbous ornamental, annual and perennial crops, and some woody species.

Rohde and Jenkins (1957) grouped plants into four categories as hosts of *P. minor* (only examples given):

Excellent hosts include those plants upon which the number of nematodes increase at least ten times: Graminae: *Avena sativa* (oat); Crucifera: *Brassica rapa* (turnip); Leguminosae: *Trifolium incarnatum* (crimson clover); Solanaceae: *Lycopersicon esculentum* (tomato); Compositae: *Helianthus annuus* (sunflower).

Good hosts are those plants upon which the number of nematodes increase up to nine times: Taxaceae: *Taxus baccata* (English yew); Graminae: *Lolium perenne* (rye grass); Liliaceae: *Allium cepa* (onion); Polygonaceae: *Fagopyrum exculentum* (japanese buckwheat); Chenopodiaceae: *Beta vulgaris* (beet); Leguminosae: *Phaseolus vulgaris* (bean); Ericaceae: *Rhododendron* sp. (azalea); Cucurbitaceae: *Cucumis melo* (muskmelon).

Poor hosts are those plants upon which the number of nematodes decrease: Graminae: *Secale cereale* (rye); Chenopodiaceae: *Spinacia oleracea* (spinach); Cruciferae: *Brassica napus* (rape); Rosaceae: *Fragaria chiloensis* var. ananassa (strawberry); Leguminosae: *Pisum sativum* (pea);

Solanaceae: *Nicotiana tabacum* (tobacco); Cucurbitaceae: *Cucumis sativus* (cucumber); Buxaceae: *Buxus sempervirens* var. suffruticosa (English boxwood); Compositae: *Chrysanthemum morifolium* (chrysanthemum).

Non-hosts comprise only four out of the 45 plant species tested, from which trichodorids were not recovered: Liliaceae: *Asparagus officinalis* var. altilis (asparagus); Leguminosae: *Crotalaria spectabilis* (showy crotalaria); Euphorbiaceae: *Euphorbia pulcherima* (poinsetia); Solanacae: *Datura stramonium* (jimsonweed).

I.B.6 Ecology and distribution

Trichodorids have been reported from all the main continents, and are particularly widespread and prevalent in Europe and North America. The geographical distribution of trichodorids within Europe was comprehensively presented in the European Atlas of Longidoridae and Trichodoridae (Alphey & Taylor, 1986). Subsequently, several new species have been described since this publication (Decraemer, 1995).

In Germany 8 *Trichodorus* and 5 *Paratrichodorus* species have been found (Sturhan, 1994). Trichodorids are present in Lower Saxony in *ca.* 90% of the potato-soils (light sand), 65% of these contain TRV (Meyer & Schönbeck, 1972). In the former DDR, Horneburg (1989) reported that TRV occurs, causing various amounts of damage in the years 1981 to 1988, in 11% to 32% of the potato cultivation areas.

During a comprehensive survey of virus-vector nematode species in the UK trichodorid nematodes were recovered from 22% of all sites sampled (Alphey & Boag, 1976). At least 10-15%, and 15-20% of the arable hectarage in Scotland was found to be infested with TRV and with trichodorids, respectively (Cooper, 1971b). In other areas of Europe TRV was present in more than 50% of irrigated land in Switzerland (Gugerli, 1977a); 12% of the potato area is affected in Great Britain (Cooper, 1971c) and in Belgium 15% of potato land was found infected (De Pelsmacker & Coomans 1987).

Trichodorids prefer sandy soil, especially arable land (Cooper & Harrison, 1973; Cooper, 1971c). Their frequency of occurrence in forests and pastures is much lower (Alphey & Boag, 1976). Under natural conditions about 90% of trichodorids occur in the upper 50cm soil depth, but they have also been recovered from soil depths of 1.2m (Weidemann, 1981). The depth is dependant on the particular nematode species and plant host (Alphey, 1985). Under barley 90-95% of the trichodorids are situated in the upper 30cm (Banck, 1988). Potato-tubers grow mainly in < 20cm depth, and thus when soil moisture levels are high trichodorids exploit this soil depth in search of the available food source (Cooper & Harrison, 1973).

Trichodorids disperse horizontally slowly, usually at a maximum of 1m per year (aid Auswertungs- und Informationsdienst, 1997), and wind-dispersal is suspected to occur under particular conditions (Cooper & Harrison, 1973).

Under natural conditions trichodorids have an aggregated distribution in fields (Boag *et al.*, 1986), probably as a result of their movement for mate-finding for reproduction, feeding on host-roots and in response to soil-structure and fluctuating soil-humidity (Alphey, 1985). Suitability

of a habitat for trichodorids is probably most dependent on temperature and water, with prolonged periods of drought resulting in inactivity or even death of the nematode. Also, Winfield and Cooke (1975) reported that trichodorid nematodes principally inhabit free-draining, coarser textured sandy or slightly loamy soils, thus these nematodes are particularly likely to encounter very dry soil conditions. Therefore, population numbers in the soil are often correlated with rainfall (Winfield & Cooke, 1975).

I.C The Tobravirus-Trichodorid Association

Sol *et al*. (1960) and Walkinshaw *et al*. (1961) reported *P. pachydermus* and *P. minor*, respectively, as natural vectors of TRV. Subsequently, further reports of *Trichodorus* and *Paratrichodorus* nematodes transmitting TRV, pea early-browning (PEBV), and pepper ringspot viruses (PRV) were published (van Hoof, 1962; Salomao, 1973; Taylor & Brown, 1997) and these nematodes and their associated tobraviruses have been shown to be of significant economic importance.

Currently, there are 47 valid *Trichodorus* species and 31 *Paratrichodorus* species (Decraemer, 1995), of which 4 and 9 species, respectively, are virus vectors (Taylor & Brown, 1997). Trichodorids frequently occur as species-mixtures, and each species is a potential vector of a different virus strain (Taylor & Brown, 1997). *Paratrichodorus pachydermus* and *T. primitivus* are the most prevalent, and thus most economically important, European vectors of TRV (Alphey & Boag, 1976).

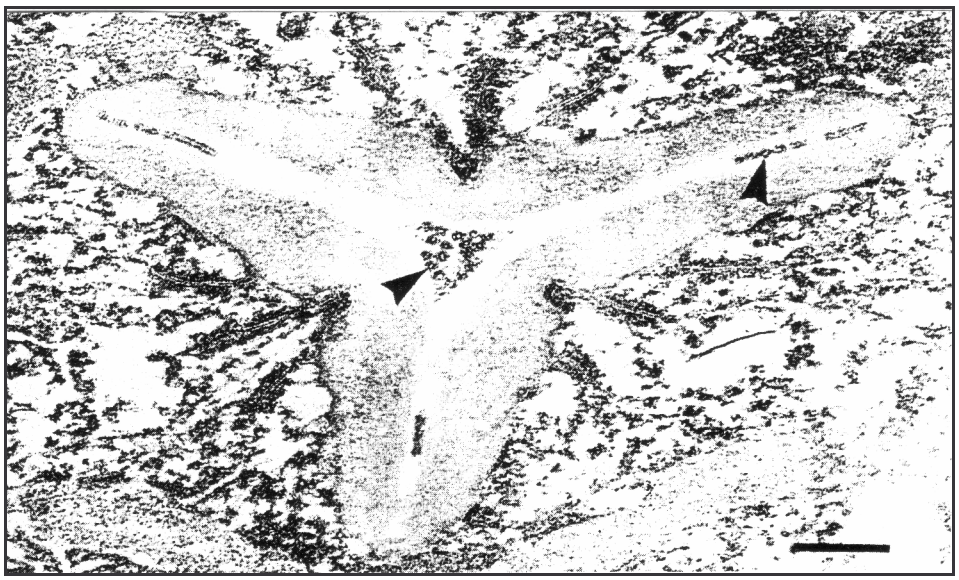
TRV remains viable and can be transmitted by trichodorids after the nematodes have been starved for 20 weeks (Ayala & Allen, 1968), and trichodorids stored in soil in plastic-bags were able to transmit TRV after 3 years (van Hoof 1970).

Trichodorids transmit better in moist soil (min. 15%, optimum 30%; Cooper & Harrison, 1973) and transmission is strongly temperature dependant. European vector trichodorids transmit TRV at the highest rates at soil temperatures of 20°C, less frequently at 24°C and at 29°C transmission does not occur (Cooper & Harrison, 1973). Above 25°C trichodorids became inactive, thus accounting for the absence of transmission at the higher temperature (PSA Ahlem, 1974).

I.C.1 General remarks

A proportion of TRV particles ingested by a vector trichodorid when feeding on a virus infected plant are specifically adsorbed to the lining of the pharyngeal tract of the nematode (Wyss, 1975). Subsequently, when the nematode next feeds these particles are available for release and thus establish an infection in a plant. Virus particles retained at the sites of retention within the vector do not multiply (Weidemann, 1981), and can remain invective for extended periods of time, up to several years, within the vector nematode (Trudgill, 1976).

Figure 9: crossways cut by the oesophagus-area of a *Trichodorus similis*; virus-particles (arrows) in the inner space and the runner of the oesophagus (from: Brown *et al.*, 1996)



Sol *et al*. (1960) were first to report a trichodorid nematode, *P. pachydermus*, as a vector of tobacco rattle tobravirus (TRV). Subsequently, several tobravirus strains have been shown to be transmitted and disseminated by numerous trichodorid species (Brown & Weischer, 1998), with male, female and juvenile stages each capable of transmitting virus (Ayala & Allen, 1966; Gibbs & Harrison, 1964; van Hoof, 1964; Sol, 1963).

I.C.2 Transmission specificity

Of the approximately 4000 plant parasitic nematodes thus far identified only members of the families Longidoridae and Trichodoridae are known to act as vectors of viruses. The longidorid and trichodorid virus-vector species transmit viruses belonging to only two genera of plant viruses, nepoviruses and tobraviruses, respectively. Only a few species in each nematode genus

are able to transmit virus: 8 *Longidorus* spp., 1 *Paralongidorus* sp., 9 *Xiphinema* spp., 9 *Paratrichodorus* spp and 4 *Trichodorus* spp. Transmission specificity is defined as the specific relationship between a plant virus and its vector nematode, *viz*. a recognition event between the virus and the site of retention in the vector (Brown & Weischer, 1998; Brown *et al*., 1995; Cadman, 1963; Harrison *et al*., 1974).

Trudgill *et al*. (1983) proposed a set of criteria that should be fulfilled before regarding a longidorid nematode as a vector of a nepovirus. Subsequently, Brown *et al*. (1989) modified these criteria for application to tobraviruses and trichodorid nematodes. The criteria are:

- infection of the bait (healthy) plants must be demonstrated
- transmission experiments should be done with hand-picked nematodes
- it must be shown unequivocally that the given nematode is the vector of the virus and that no other contamination factor or alternative vector was present
- the nematode must be fully identified
- the virus must be fully characterised

A similar specificity in the association of trichodorids with tobraviruses was suspected and Ploeg *et al.* (1992b) confirmed this by demonstrating transmission of tobraviruses by using individual specimens of various trichodorid species and populations. It is now widely accepted that specific associations occur between trichodorid virus-vector species and their associated tobraviruses and serologically distinguishable strains of the viruses (Ploeg & Brown, 1997; aid Auswertungs- und Informationsdienst, 1997).

Ayala and Allen (1966) reported that only two of three trichodorid-species transmit a strain of TRV present in California. Van Hoof (1968) reported that *P. pachydermus* transmitted TRV

only when the virus isolate and the nematode population came from the same field site (van Hoof, 1968). Subsequently Ploeg *et al.* (1992a) demonstrated that the frequency of TRV transmission by *P. pachydermus* varied with respect to the nematode population, and that most populations deriving from several European countries could effectively transmit the virus.

I.C.3 Exclusivity and complementarity

Exclusivity and complementarity are important characteristics of virus-vector specificity (Vassilakos *et al*., 1997; Brown & Weischer, 1998). Exclusivity is defined as "the case where a nematode species transmits only one virus or one serologically distinct virus strain and the virus or virus strain has only a single vector". Complementarity is defined as "the case where a nematode species transmits two or more viruses or serologically distinct virus strains, and where two or more viruses or virus strains share the same vector species" (Brown & Weischer, 1998). Complementarity is the main feature of tobravirus transmission by trichodorids (Vassilakos *et al*., 1997), whereas exclusivity is relatively uncommon with only *P. hispanus* and *P. tunisiensis* having been found to exclusively transmit a Portuguese and an Italian TRV serotype, respectively.

I.C.4 Transmission efficiency

Differences in the efficiency of transmission frequently occur in virus-vector nematode interactions. As the process involves three main components *viz*., virus, vector nematode and

host plant species, any variability found associated with the process may result from each of these components individually, but more commonly is a consequence of a combination, and interactions, amongst all three components.

After access to infected plants, acquisition of the virus particles can take place within 1 hour (Brown *et al*., 1995). Even a single brief feed on a virus-infected plant is probably sufficient for virus acquisition to occur. Conversely, a nematode vector does not necessarily become viruliferous just by feeding on a host infected with a virus that it normally transmits (Taylor & Robertson, 1970). For example, Taylor and Robertson (1977) reported that only particles free in the cytoplasm of plant cells could become adsorbed at the site of retention and subsequently be transmitted. Harrison *et al*. (1974) suggested that lack of acquisition or retention or dissociation can lead to transmission failure.

TRV has been transmitted by individuals of all nematode developmental stages with equal efficiencies. However, during the moulting process of the juveniles, retained particles are shed along with the cuticle lining the feeding apparatus (Harrison *et al*., 1974). Also, virus particles do not pass through the nematode egg (Ayala & Allen, 1968; Taylor & Robertson, 1977).

Only one nematode is needed to infect a plant (Ayala & Allen, 1966), and tobraviruses can be transmitted serially by an individual nematode to more than one plant (van Hoof, 1964). Thus, it may be assumed that once an adult nematode acquires virus, it remains viruliferous for extended periods of time, and possibly for the remainder of its life (van Hoof, 1964). However, the nematode may release only a proportion of the retained virus particles when feeding, and the nematode may re-acquires new virus particles from the plant that it originally infected. The ability of the vector nematode to retain virus for extended periods without diminution of the

virus infectivity provides a relatively efficient pathway for virus maintenance, especially over-winter (Taylor & Brown, 1997).

Environmental factors such as temperature and soil moisture and experimental procedures as bait plant for example also can affect vector efficiency, as has been shown in laboratory studies, *e.g*. (Brown *et al*., 1995).

I.C.5 Retention and dissociation of tobravirus particles in the vector

Factors determining the specific retention of virus particles at the sites of retention within the vectors are unknown. It has been suggested that particle adsorption at the site of retention may be induced by surface charge density, and that dissociation occurs due to pH changes generated by salivation (Harrison *et al*., 1974). Consequently, different strains of the same virus, that require different vectors would be expected to have different surface charge densities, whereas two distinct viruses transmitted by the same vector would have similar charges (Taylor & Robertson, 1977).

Virus retention probably depends on both interacting surfaces *i.e.* the cuticular lining of the pharyngeal tract in the vector nematode, and the outer coat of virus particles. The application of carbohydrate staining revealed the presence of a thin layer between acquired particles and the cuticle lining of the pharyngeal lumen in *P. pachydermus* (Robertson & Henry, 1986). This suggests that there could be a complementary reaction between carbohydrates within the food canal and lectin-like molecules on the virus coat protein. More recently, it has been shown that the viral capsid and the 2b non-structural protein each encoded by the RNA-2 of TRV are essential for successful transmission of TRV. Probably the 2b protein acts as a helper factor in

facilitating the attachment of the virus particle at the site of retention within the vector (Brown *et al*., 1995; MacFarlane *et al*., 1996; Taylor & Brown, 1997).

The specific site of retention is located all along the pharyngeal lumen in close association with the cuticle lining the lumen of the pharyngeal tract. Virus-particles are usually found attached as a monolayer in the apices of the pharyngeal lumen, the open part of the lumen, in the region of the onchiostyle with a central hollow core (Brown *et al*., 1996) and in the pharyngeal bulb, probably associated with the presence of a mucus layer along this region (Robertson & Henry, 1986). The mucus layer is suspected to protect retained particles from the flow of plant sap during ingestion (Taylor & Robertson, 1970). The sites of virus retention are similar in *Trichodorus* and *Paratrichodorus* species (Brown *et al*., 1996).

I.D Control Measures

As with most plant viruses the diseases caused by TRV are not easy to control. Soil-disinfection products provide efficient control only for a limited number of growing seasons, sometimes only for the season after application. Other chemicals that inhibit nematode movement thus preventing the nematode from transmitting virus are effective for only a few weeks after application and thus have to be applied every growing season. Few non-chemical strategies for controlling TRV have been proposed, and most do not provide a high degree of success or efficiency. As the disease problem caused to crops by TRV have steadily increased, and the procedures for direct control of the diseases are limited, new methods must be developed to protect vulnerable crops such as potato growing on land with vector-nematodes and TRV.

Trichodorids and TRV each have an extensive number of weed species as hosts, thus trichodorids can readily acquire TRV from infected weeds (Kegler *et al.*, 1989) and subsequently transmit it many months later (Heinicke, 1983). Therefore, efficient weed control is a pre-requisite in any cultural control method being proposed.

The most efficient and reliable method to control TRV in commercial crops is to breed virus-resistant cultivars. However, use of resistant potato cultivars is problematic as TRV-strains differentially affect potato cultivars (Xenophontes *et al.*, 1998) and the most appropriate cultivar for suppressing disease symptoms may not be the preferred marketable cultivar (Maas, 1975). Breeding is based on selection for resistance or a hypersensitive response. Recently, seed potato tubers of cultivars that do not exhibit visible symptoms of infection with TRV have been shown to present a potentially efficient pathway for spread of the virus to new sites at which the associated vector trichodorid species is present (Robinson *et al.*, 1995).

I.D.1 Chemical

Nematicides and nematastats can be used to reduce the number of trichodorids, or to temporarily immobilise them, and consequently reduce the impact of TRV at field sites (Cooper & Thomas, 1971). However, such control is not fully effective as some nematodes always survive (Alphey, 1985). There are only a limited number of authorised products, but several of these are effective particularly when TRV infection is low (Reepmeyer, 1973a). In growing seasons with cool, damp weather 100% control of the virus infection in a susceptible crop may not be achieved

(Heinicke, 1983). Also, in many potato-growing areas the oxime-carbamate group of chemicals is forbidden. These chemicals must be used each time a susceptible crop is grown, and the chemicals are extremely toxic, with the recommended rate of application of these chemicals per square metre sufficient to kill 10 adult humans. Because the limit of tolerance of affected tubers is very low, the use of expensive chemicals such as the oxime-carbamates (>1000DM per hectare) can be problematic, especially if the selling price of the potato crop is low.

Agro-chemicals are now widely regarded appreciated as being a cause of environmental problems (aid Auswertungs- und Informationsdienst, 1997). In the flower-bulb producing areas in the Netherlands chemical disinfection of soils started in the 1960s. Because of environmental pollution the use of nematicides and nematastats have been curtailed since the 1980s (Asjes & Blom-Barnhoorn, 1998). Consequently, in the Netherlands frequency-reduction of soil-fumigation is directly associated with increasing problems with TRV/trichodorids (Hartsema & Molendijk, 1998).

As trichodorids can acquire TRV from weeds, weed-control can have an important effect on suppressing the effects of TRV infection in susceptible crops. However, the use of herbicides (Cooper & Harrison, 1973) in the first year may have little effect as the trichodorids present at a site already have TRV. Also, in the second year there may be sufficient trichodorids with TRV to sustain the TRV problem. An effect from efficient weed control may only be seen during the third or subsequent years, and this is dependent on the susceptibility of the crop being grown. Weed control has to be maintained as TRV infected weed seeds may lie dormant in the soil for many years (>80 years) before germinating and thus presenting a pathway for the nematodes to re-acquire the virus.

Interestingly, efficient weed control in a potato crop may significantly increases TRV infection as the nematodes feed preferentially on the weed species and in the absence of their preferred hosts the nematodes are forced to feed on the potato plants (Cooper & Harrison, 1973). However, weed control is essential in potato crops to facilitate efficient harvesting.

I.D.2 Cultural

Growing of selected inter-crops that are known to reduce the level of virus and/or its vectors in the soil may offer an alternative strategy to reduce or suppress the effects of TRV on the following crop. Few experiments have been carried out to investigate this approach and the results from these studies are often contradictory or inconclusive (Lütke-Entrup, 1973; Seemüller, 1986; Maas, 1974). For example Maas (1974 and 1975) reported less damages at potato after summer-barley as after sugar-beet. In contradiction Kegler (Kegler *et al.*, 1984) published results that after summer-barley an increased infestation could be seen.

Consequently, a comprehensive review of this approach is necessary.

I.E Research Objectives

As a consequence of the increasing problem of TRV infection in the potato growing areas in Germany a research project was established with the following objectives:

I.E.1 Assess the occurrence of TRV and virus-vector trichodorids in potato areas in Germany

Soil samples were collected from selected potato growing areas in Germany and bait-tested for the presence of TRV. The recovered viruses were characterised immunologically and serologically, and virus-vector trichodorids extracted and the species identified. This to provide benchmark data on the occurrence, distribution and potential relationships between virus-vector trichodorid species and their associated serologically distinguishable strains of TRV present in potato growing areas in Germany.

I.E.2 Determine the specificity of association between isolates of TRV and their associated virus-vector trichodorid species.

This was achieved by doing virus transmission experiments in which individual nematodes were used to transmit TRV, thus enabling the virus strain and the nematode species each to be accurately determined. This overcame the frequently occurring problem of several trichodorid species occurring together at a site thus rendering it impossible to determine which species were acting as vectors. Also, several species may be transmitting virus and only one strain may be recovered from the bait plants thus masking the occurrence of other strains of the virus.

I.E.3 Assess TRV strain and potato cultivar interactions

A questionnaire sent to growers requesting information about the occurrence of TRV induced symptoms in potato cultivars provided insight of possible TRV-potato cultivar interactions.

These data identified field sites where particular potato cultivars did and did not exhibit TRV symptoms in tubers. A catalogue of potato cultivars was produced that revealed those cultivars that never, or only infrequently, showed symptoms of TRV infection in tubers.

I.E.4 Develop a diagnostic test for TRV isolates occurring in potato fields in Germany

Three diagnostics were developed based on the use of TRV strain-specific antibodies involving the use of an electron microscope (ISEM, immunosorbent electron microscopy) and ELISA (enzyme-linked immunosorbent assay). The third method involved RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) that provides a rapid procedure for identifying the existence of TRV and the strain of TRV present. The RT-PCR method allows identification of TRV present in plant and in nematode extracts.

I.E.5 Assess the use of selected antagonistic plant species for suppressing TRV and virus-vector trichodorids in potato fields

Selected antagonistic plant species were grown at a field site known to be heavily infected with TRV and trichodorids. Nematodes were extracted at intervals from soil samples collected from the experimental and also soil was bait tested for the presence of TRV at intervals to assess the decrease of nematodes and TRV, respectively, under the different plant species used in the experiment. The results from this experiment identified several crops that may be used to suppress the presence of trichodorids and TRV and offers a potential practical non-chemical approach for the control of TRV in the potato growing areas in Germany, and possibly elsewhere.

II Material and methods

II.A Plants

Host plants for TRV were selected for the following experimental requirements:

Bait plants - seedlings of herbaceous plants that become infected when planted in soil containing viruliferous trichodorid vectors, and thus can be used to assay the presence of infectivity. The bait plants were exposed to the nematodes in soil for 3-6 weeks and then sap from the roots and/or tops was mechanically inoculated to suitable indicator plants.

Indicator plants - when inoculated with TRV, usually in infected plant sap, rapidly develop symptoms in the treated leaves

Local lesion host - used to distinguish strains of TRV

Propagation hosts - plants in which the virus multiplies as the M isolate.

TRV, and its trichodorid vectors, have a very wide host range among cultivated and wild plants. The following species were selected because most of them have been used extensively for various experiments with TRV:

- *Nicotiana tabacum* cv. Samsun: as bait plants
- *Nicotiana tabacum* cv. Xianthi-nc: as propagation hosts for TRV and for PCR and immunological tests
- *Nicotiana clevelandii*: as propagation hosts for TRV for EM preparations
- *Nicotiana tabacum* cv. Nevrocop, *Nicotiana rustica* and *Nicotiana banthamiana*: for their usefulness as virus-propagators
- *Chenopodium quinoa* cv. Willd.: as indicator plants
- *Chenopodium amaranticolor* cv. Coste *et* Reyn: as indicator plants

II.B Soil sampling

Soil samples were collected from fields in northern Germany that had a history of TRV infection *i.e.* spraing symptoms in the tubers. At each site soil samples were taken at random using a 1.5cm diameter auger. Each sample was taken to a depth of approximately 40cm and sufficient samples were taken at each site to provide a composite sample of approximately 2kg soil. The soil cores comprising the composite sample were carefully placed in polythene bags, which were then labelled, and handled with care until they reached the laboratory. Soil samples that could not be dealt with immediately were stored in a refrigerator at 4°C. Each composite sample provided two 200g sub-samples for nematode extraction and two 500g sub-samples for bait testing for the presence of TRV.

II.C Potato tubers

To ascertain the extent of TRV infection in a particular crop of potatoes, tubers were examined for external evidence of TRV infection (misshape, nodules) and then cut in slices for evidence of spraing symptoms (necrotic spots and arcs).

For a more precise assessment of infection all the tubers from the crop were sliced mechanically with each slice assessed according to the following classification (Bonituranleitung, 1981):

symptomless = no visible symptoms

light = symptoms covering <40% of the section

medium = symptoms covering 41-60% of the section

high = symptoms covering >61% of the section

II.D Nematodes

II.D.1 Extraction

Nematodes were extracted from 200g aliquots of the bulk soil sample using Flegg´s (1967) modification of Cobb´s (1918) decanting and sieving method and final separation in a Baermann funnel (1917). This procedure is described in detail by Brown & Boag (1987). The trichodorid specimens obtained were identified and counted (see below), and fresh specimens used for the virus transmission tests.

II.D.2 Vector-determination

Individual specimens of adult trichodorids obtained by extraction from soil samples were placed in a drop of water on a microscope slide and killed by application of gentle heat (*ca.* 65°C). *Paratrichodorus* species were readily distinguished by their swollen cuticle, which did not occur with *Trichodorus* species. Identification of species was made with reference to the shape of the vaginal sclerotizations in the females, especially for *Trichodorus* species, and the shape and structure of the spicules in the males. Tentative identification was confirmed by reference to Decraemer´s (1995) tabular key.

II.D.3 Single nematode transmission

Precise studies of virus transmission can only be made using individual nematodes. This is a method that overcomes the problem of mixed species populations that frequently occurs with

trichodorid vectors. Plastic Beem capsules (size 0.5cm^3) were one-third filled with sieved air-dried sand with a particle size of 500-1500μm. Water was added to half fill the capsule with the surface 2mm above the surface of the sand.

A single specimen from the trichodorid population under investigation was then floated on to the surface of the water in the capsule, a bait plant seedling (*N. tabacum* cv. White Burley or cv. Samsun or *N. clevelandii*) was added and further sand added to fill the capsule.

The filled capsules were partly plunged into a container of wet sand to minimise the loss of water by evaporation and maintained in a controlled environment at about 20°C, with supplementary lighting, for 7 to 14 days.

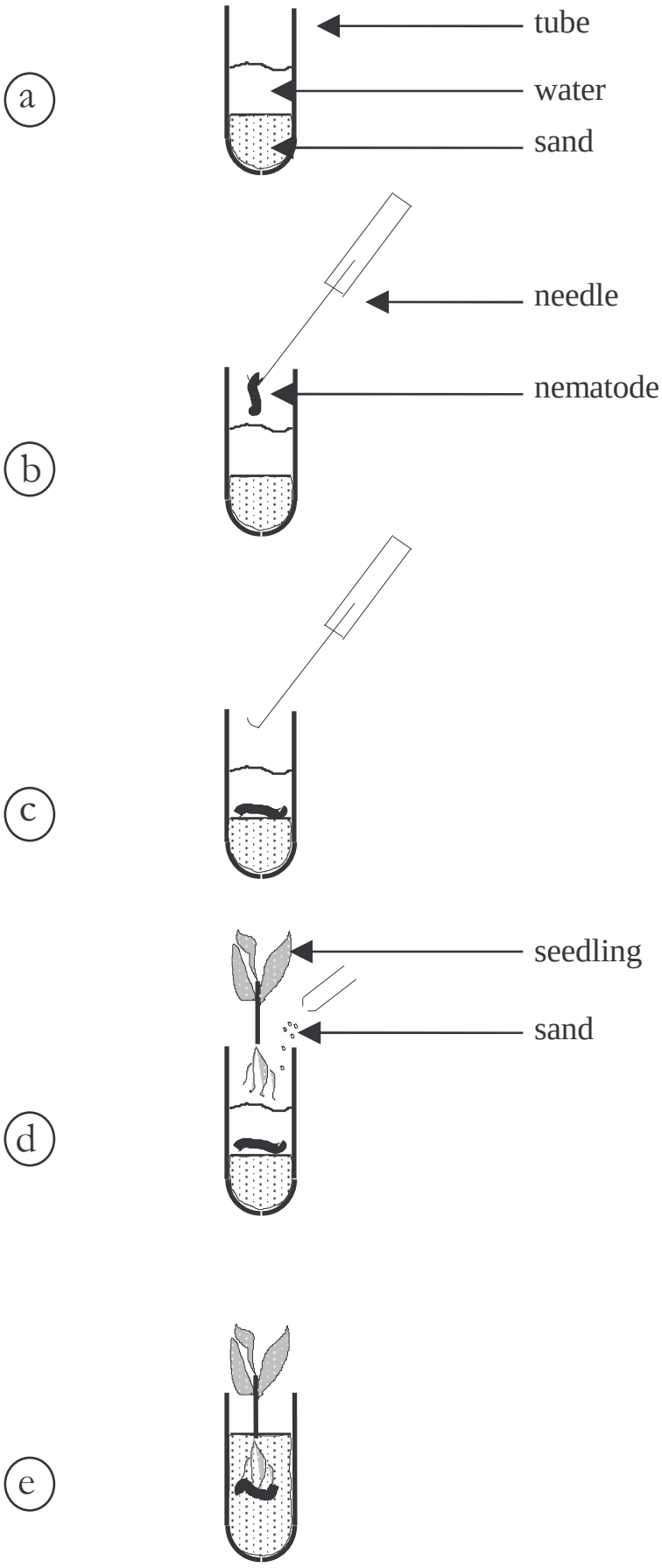
After this period the contents of each capsule were washed into a glass beaker, using a Pasteur pipette and about 5cm^3 water. The roots of the bait plant were thoroughly agitated in the water in the beaker to dislodge the nematode, the contents were gently mixed, and the supernatant poured into a counting dish.

The nematode was located with the aid of a binocular microscope and immediately transferred to a drop of water on a microscope slide and heat killed for identification.

Each of the bait seedlings from the capsules from which a trichodorid nematode was recovered were transferred to small pots containing sterilised potting compost and grown for three to eight weeks to allow for any transmitted virus to multiply. The roots of the seedlings were then washed free from adhering compost and comminuted in a mortar and pestle and the suspension rubbed by finger onto the leaves of *Chenopodium quinoa* or *C. amaranticolor*, test plant.

Test-plants showing typical symptoms, usually after about three days, provided evidence that the single nematode had transmitted TRV to the seedling.

Figure 10: single-nematode-transmission-test; virus-transmission



II.D.4 Cultivation

Trichodorids are difficult to culture and populations multiply slowly. Populations of the trichodorid species used in the various experiments were therefore obtained from field locations in northern Germany with a history of TRV infection in potato crops.

II.E Virus

Irrespective of whether recovery of the virus was direct from the nematode or indirectly by inoculated tobacco bait plants, the symptoms of infection on the leaves of the indicator plants are identical. The various strains of TRV were subsequently identified by electron microscopy and serological methods.

Tobraviruses were easily recognised in the electron microscope by their rod-shaped form and the size of particles (Roberts, 1986a; see IIE7). An exact determination of the virus strain was made with specific antibodies using the technique of immunosorbent electron microscopy (see IIE8; Roberts, 1986a and b) or the decoration test (see IIE9).
Enzyme-linked immunosorbent assay (ELISA; see IIE5), a serological technique, also was used to demonstrate the presence of a specific strain of a virus.

The determination of virus strains is important because the observation of resistance or tolerance of potato cultivars with recommendations to cultivar choice and assessment and mapping of infection risks is based on this determination.

II.E.1 Proof with bait-test

In the tobacco bait test (= bio-test) tobacco seedlings were planted in bulk soil samples and grown for about 5 weeks. This was sufficient time for any vector nematodes to feed on the roots and for the virus to multiply (Taylor & Brown, 1997).

The roots of the bait plant were comminuted in a pestle and mortar (see IIE2) and the suspension rubbed on the leaves of *Chenopodium amaranticolor* and *C. quinoa* indicator plants. After 3-7 days characteristic symptoms of TRV infection appeared on the indicator plants.

II.E.2 Inoculation

Plant-viruses can be mechanically transferred from an infected host plant to another plant in which symptoms characteristic for certain viruses or strains are produced. The potentially virus-containing material (usually fresh or freeze-dried *Chenopodium*- or tobacco-leaves or tobacco-roots) was crushed with as little water as possible in a mortar.

Virus free (Bos, 1983) indicator plants were lightly dusted with 600 carborundum powder (= silicon carbide), which during mechanical inoculation causes small wounds in the epidermal cells and increases the chances of transmission. The cells do not die, so that the virus was transported into living cells where it can replicate (Bokx, 1972).

The suspension of material was rubbed on the indicator plants (usually a single plant of *C. quinoa*, *C. amaranticolor*, *N. tabacum* cv. Xianthi) using a clean finger. If only single leaves were inoculated they were marked with a small hole to distinguish between primary infected leaves and secondary systemic infection (aid Auswertungs- und Informationsdienst, 1997).

Afterwards, the leaves of the plants were washed briefly in a gentle stream of water and placed in a greenhouse, taking care that the plants did not come into contact with one another to avoid transmission through contamination.

The optimum temperature for inoculation depends on the virus and the plant (Bokx, 1972), therefore with TRV a temperature above 28°C was avoided.

II.E.3 Virus-recovery

For the production of a suspension of leaf material for ISEM, decoration, ELISA and RT-PCR a piece of leaf of approximately 1x1cm with symptoms was taken with clean fingers off the plant and pushed into a 0.5ml Eppendorf tube with a new unsharpened toothpick. A small quantity of 600 Carborundum and 3 drops of citrate-buffer were added. The toothpick was put into a mini-drill and the leaf piece crushed thoroughly with the unsharpened end. Citrate-buffer was added and the solution was again crushed thoroughly.

The closed Eppendorf tube was centrifuged for 10min. at 4000rpm to disperse the virus particles into the supernatant whilst forcing plant debris to the bottom of the tube.

II.E.4 Virus strain determination with ELISA

In the ELISA procedure antibodies against different virus strains were attached to a synthetic material in wells. A leaf-suspension was put into each well and a colour reaction occurred when virus was present.

The colour reaction was caused by the enzyme (usually alkaline phosphatase) reacting with the substrate (p-nitrophenyl phosphate) with the substrate turning yellow. The colour change was

measured in a spectrometer (405nm, absorbance). The results of this test indicated the virus strain.

During this work the indirect triple-sandwich-ELISA (Hampton *et al.*, 1990) was used, as it had been shown to be most useful for TRV.

II.E.5 Electron microscopy basic methods

With the electron microscope viruses can be immediately visualised and identified; and this method is more sensitive than serology (Bokx, 1972).

For all of the tests described here a Zeiss EM-10 transmission electron microscope was used.

All work with liquid drops was done on wet paper in a Petri-dish to reduce evaporation. Siliconised slides were used as drop-pads and the treated surface of the slide was used to create sphere-shaped drops that makes it easier to handle the grids.

Grid-production

For ISEM and decoration tests filmed (0.5% pioloform in chloroform) and carbon-coated copper-grids of 300mesh were used.

Negative contrasting with PTA = phosphorus tungsten acid sodium salt

Three to 4 drops of the stain were slowly dripped over the carbon-coated side of the grid, which was held vertically with tweezers. The grid was thoroughly dried with pieces of filter paper that were held against the edge of the grid.

II.E.6 Virus strain determination with drop-tests

One 10µl drop of the suspension to be examined was placed on a siliconised slide and a carbon-coated grid was then floated on the surface of the drop for 5min to 4hours. Each grid was then stained with PTA and examined in the electron microscope for the presence of TRV particles. The number of particles per unit area were counted.

II.E.7 Virus strain-determination with ISEM

If the number of virus-particles in the test material was very low, as occurs frequently with TRV, virus-particles may not be found despite examination of many grid-meshes. This false-negative result because of low virus concentration was overcome by using ISEM (immunosorbent electron microscopy; Bos, 1983).

In this technique support films on EM-grids were precoated with specific antibodies. To these, homologous or closely related virus particles were trapped and concentrated from plant or nematode material. The virus-particles were absorbed selectively by the antibodies serologically related to them. The grids then are observed by EM.

ISEM indicated the serological relatedness of virus-particles in samples and the virus-antibodies that were used. If the increase of the relative number of virus-particles by ISEM per standard area was 3 times or more, this was considered proof of the relationship of the virus and antibody. The greater the enrichment (*e.g.* an enrichment factor >100) the closer the virus was related to the antibody.

By using serologically different antibodies a classification of relationships could be established between the different viruses/ isolates.

Also, by using antibody mixtures it was possible to increase the probability of finding antibodies that recognised the virus being investigated, and thus reduced the time required to identify the virus.

ISEM-method

A 1:1000 solution of antisera was produced in a 0.5ml Eppendorf tube in 0.06M phosphate-buffer PH6.5. 10µl drops of this solution were placed on a siliconised slide in a glass-Petri-dish. A filmed and carbon-coated copper grid was incubated on each of the drops for 60 minutes at 30°C. The antibodies were absorbed tightly to the grid surface. The grids were then tipped at the edge briefly on a filter paper and immediately placed in a vessel (diameter 18mm) filled with 0.06M phosphate-buffer PH6.5 with positive meniscus for a 10 minutes washing period. Grids floating on the surface were moved by rotation, using a plastic-pipette, three times to ensure thorough cleaning. After this they were again tipped briefly on filter paper and floated on 10µl drops of the prepared leaf extract. These were placed in similarly prepared Petri-dishes and were incubated for 4 hours at 4°C.

After 2-4 hours the grids were removed from the drops and stored under vacuum. The grids were examined in the EM within 2 days. The number of visible virus-particles were counted at a magnification of x10,000 in several fields of view, and the average per field of view was calculated and converted to a defined standard area. This enabled comparisons to be made between different grids. The relative virus-particle-numbers with and without ISEM were compared and an enrichment of at least x3 was considered evidence of the identity of the virus.

Figure 11: production of antibody-coated grids (ACG) and ISEM-method

example with 3 samples and the antibodies Rostock and Onion
incubation-time 2 and 4 hours

ACG-production

1h., 30°C
o = antibody-solution with a grid on it

ROS-A/S

ON-A/S

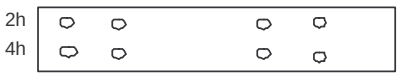
tip on filter-paper
↓
10Min. washing on
phosphate-buffer
↓
tip on filter-paper

ISEM

4°C
o = leaf-sap-drop with ACG on it

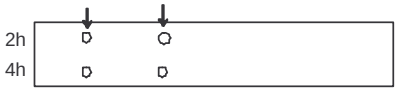
ROS-A/S **control**

1. sample 2. sample 1.sample 2.sample



ON-A/S

1.sample 2. sample



↓
after ISEM contrast with 4 drops PTA
and dry very good

A mesh contains approximately 100 fields of view and if only a few virus particles were present a maximum of 100 fields of view were examined. If no virus particles were found in this number of fields, the next grid was examined. If the number of virus particles was large, only a few fields were examined with 3 fields of view being the minimum examined. For calculation of the ISEM-result the number of virus particles on all 3 grids were added and the number of particles then divided by the sum of the examined fields of view.

Example: 60 virus particles in 30 fields of view, 63 virus particles in 30 fields of view, 61 virus particles in 30 fields of view > (60+63+61) : (30+30+30) = 184 : 90 = 2.04 (the result is rounded to two decimal places). This is the Av. P/FV = Average Particle by Field of View.

This result is multiplied by the factor 118, which was calculated from the visual qualities of the TEM used in this work and its magnification. This is the P/SA = Particles by Standard-Area, which means the number of virus-particles on an area of 1000µm^2.

A control (grid without antibody-layer) from the same leaf-sap was always prepared and counted. The P/SA of the control and the P/SA of the ISEM-Grid were compared and this provided the means for calculating the enrichment-factor.

Examples:

	P/SA ISEM	:	P/SA control	=	enrichment-factor
a	4.51		4.51		1
b	9.00		4.50		2
c	5.50		1.45		3.79
d	44.50		1.55		28.71
e	1.50		2.60		0.58

a: the number has remained same; the antibodies have not caused enrichment, thus the result is negative *i.e.* the antibodies have not recognised the virus.

b: the number has doubled; the antibodies have caused an enrichment factor of 2, but this result is considered negative as the enrichment factor must be at least 3 before being considered as evidence of recognition between the antibodies and the virus.

c, d: the number has increased by a factor of at least 3; thus the antibodies have caused an enrichment and this is considered evidence of positive recognition between the antibodies and the virus.

e: the number has decreased; consequently the antibodies have not caused any enrichment and this result is negative.

The decline-phenomenon (e) can be caused by enzymes (proteases) that are contained in the leaf-extract, and which break the antigen-antibody-bond resulting in a decrease in the particle-number.

II.E.8 Virus strain-determination with decoration, titer-decoration

To investigate quantitatively the degree of relatedness between a virus and antibody a decoration test was carried out. Antibodies were used in excess and if they adhered to virus-particles this was because of an antibody-virus recognition event. As a large amount of antibodies were used, a visible “coat“ could be observed in the electron microscope which indicated a positive result. If the virus-particles were not decorated with such a “coat“ at high antibody concentration, this was considered evidence that the virus and antibodies were not related.

To determine the degree of relationship, a series of antibody-dilutions was produced and tested. The dilution at which a clear “coat“ could still be recognised was referred to as the “decoration end point“.

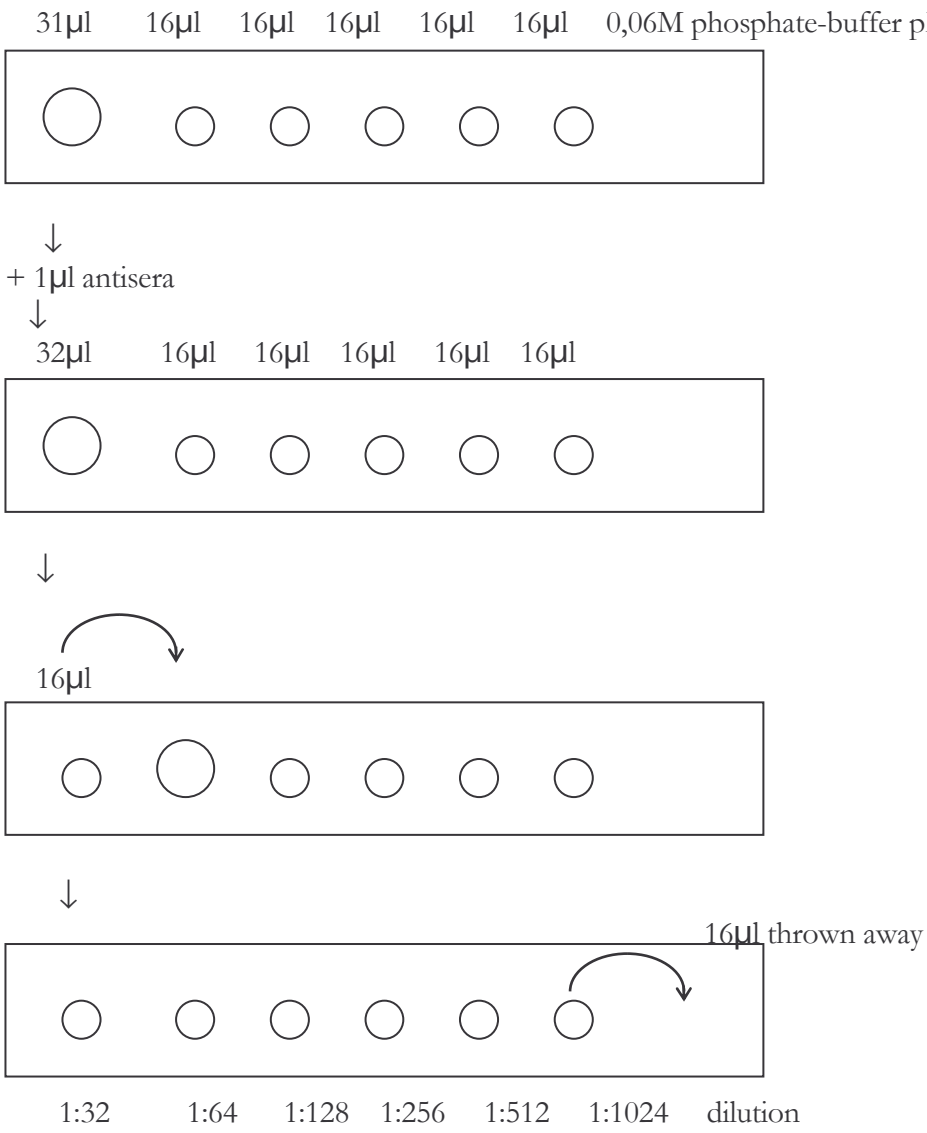
A further advantage of decoration is the recognition of different mixtures of virus-serotypes. If mixtures of serologically different virus particles occur, they become decorated differently depending on their degree of relatedness with the antibodies used and can be distinguished. The thickness of the hem and/or the decoration end points differ, therefore mixtures of viruses are readily recognised.

The standard decoration tests (Roberts, 1984) were carried out using AVMs (antibody-virus-mixtures). This method of production of an antibody dilution-series contained the following parts:

6 drops of phosphate-buffer were placed on a silicon treated slide. The first contained 31µl, the others 16µl. 1µl of the antisera was added to the first drop with a capillary-pipette and was mixed by agitation using a 16µl Eppendorf-pipette. Similarly, 16µl was removed at the end of the mixing period and added to the second drop and mixed with in the same way. This was repeated until the last drop and the surplus of 16µl was discarded. Consequently, a row of 6 drops with 16µl antisera solution of the dilution-degrees of 1:32 1:64 1:128 1:256 1:512 1:1024 was produced.

4.5µl of the leaf-sap were added to each of the drops and mixed with a clean Eppendorf-pipette. The virus-particles in the leaf-sap came into direct contact with the antibodies through this method and stuck to them. This mixture was incubated at 4°C. After 4 hours a grid was

Figure 12: decoration-test



placed on the mixture, and then immediately removed and stained with PTA. This procedure was carried out with each drop.

The virus-concentration was increased by this method by factors of 2 to 20 times in contrast to the usual drop preparations.

Decoration assessment

Examination of the preparations was started with the grids that had been exposed to the most concentrated antibody solution (mostly 1:32). The TRV isolates were examined and their appearance compared with that of other viruses, bacteria-flagelli or dirt-particles in their surroundings. If the coat of the viruses appeared more thick in comparison to the “coat" of the other particles, the decoration was considered positive.

Grids with less concentrated antibody solutions were examined next. The antibody coat always became thinner with lower antibody dilution. The final dilution, at which the coat was still obviously thicker around the TRV particle, was determined to be the end of the decoration and was regarded as a positive result.

II.E.9 Particle-measurement

The size of TRV-particles can be used to estimate strain-differences. To estimate particle-size, at least 100 particles were measured from photographic negatives analysed under a binocular microscope. The measurements were classified into groups of 10 or 25nm creating a Histogramm to calculate the normal length of the particles.

III Biological assessment

III.A Introduction

The extent to which TRV is a problem in Germany was investigated by undertaking a survey. Soil samples were obtained from locations where TRV was believed to be present and the presence of the virus was subsequently confirmed in laboratory tests (see Chapter II). Questionnaires (see IIIB2) were then sent to selected farmers and agricultural advisory officers in those areas where TRV infections had been identified.

III.B Material and methods

III.B.1 Biological assessment questionnaire

The questionnaire contains 6 different headings:

1. location:

With these data the sites were localised and assigned to different areas. This provided insight of any accumulation of infected sites in specific areas.

2. soil characteristics:

The influence of soil conditions on the expression of symptoms, and the occurrence of TRV damage, is not known. Some observations have suggested that high soil moisture increases the mobility of nematodes and therefore the spread of the virus. Collection of relevant data as supplied in the questionnaire could be helpful in confirming this.

3. occurrence:

Information about the history of occurrence of TRV symptoms can reveal how farmers deal with TRV problems, particularly if they have tried different potato cultivars, or if they ceased growing potatoes because of a single incidence of TRV damage.

4. rotation of crops:

The influence of different crops on the occurrence of trichodorids and/or TRV has been speculated. Also, in this connection the crop rotation of the preceding years needs to be examined to identify any changes in TRV damage after specific rotation patterns.

5. symptoms:

This information identifies the main symptoms in the areas examined and the characteristic symptoms that are associated with, and influenced by, different cultivars.

6. potato cultivar:

Information supplied under this heading potentially could identify TRV resistant potato cultivars. As there is different and often contradictory information about the resistance characteristics of potato cultivars, an examination of the actual situation occurring particularly with German potato cultivars and strains of TRV occurring in Germany, is essential.

To obtain an overview of the occurrence of trichodorid vector species the soil samples from the various locations were processed and the identification of the specimens determined (see IID1 and IID2).

Figure 13: questionnaire about TRV-infected sites

Essay: „on the transmission of tobacco-rattle-virus (TRV) by nematodes in potato-cultivation“

processing-number: _____
(please do not fill out)

QUESTIONNAIRE
for soil-samples in the case of suspected tobacco-rattle-virus infestation (multiple answers are possible)

Please describe the precise location.

land registry number: _____
post code: _____
area: _____

What damage has occurred?

Spots ☐
arcs ☐
bungs ☐
star-formed fissures ☐
brown areas in the vascular-bundle ☐
others _____

How would you estimate the damage?

light ☐
middle ☐
strong ☐

When did the damage last occur?

_____

Which potato cultivars have been affected?

_____

Have you noticed this damage on potatoes at this site before?

yes ☐
no ☐

Which potato cultivars have been affected?

_____

_____

_____

Have other potato cultivars been planted on the same field, that do not show any symptoms? If yes, which cultivars and when?

_____

_____

What type of soil applies to the site?

Sand _____

sandy clay _____

clay _____

Has the field been watered?

yes ☐
no ☐

Which soil humidity applies to the site?

very dry ☐
dry ☐
normal ☐
humid ☐
very humid ☐
temporary flooded ☐

Please describe the rotation of crops as far as possible.

19__ : main crop _____

inter-crop _____

19__ : main crop _____

inter-crop _____

19__ : main crop _____

inter-crop _____

III.B.2 Examination of soil samples

The soil samples were tested for TRV by bait-testing (see IIE1) followed by ISEM (see IIE8). The nematodes were extracted (see IID1) and trichodorids present were determined to species level (see IID2). Affected tubers sent together with the soil sample were visually inspected by slicing them (see IIC).

III.C Results

III.C.1 Diversity of sites

Fifty-six samples were investigated in the study, and 36 of them (64%) proved to be TRV-positive.

III.C.1.a Location

Some of the farmers were reluctant to provide details of the exact location of their infected fields due to commercial confidentiality. However, most were willing to provide full details of the area in which their fields are located (Tab. 2) and the extent of any crop infection with TRV.

Because of confidentiality, location data (name, address, contact) are not published here, but may be obtained from the author after consultation and agreement from the farmer concerned.

S54 and S55:

Tubers from these sites were inspected, and showed unequivocal symptoms of TRV infection. Soil-samples from these sites gave negative results which may have been due to an inappropriate method of taking the samples (wrong depth, wrong time of the year, *etc.*); method of storing the samples (wrong temperature, too long a period until processing); or samples taken from only a few places at the site (no nematodes in the sample because of the aggregated distribution of the nematodes).

Because of the unequivocal symptoms in the potato tubers, these sites are included in this work.

Tab. 2: location-data of the TRV-positive sites

site		state	post code	area	district	administration district
S1	=	NiS	30900	Mellendorf	Burgdorf	Hanover
S2	=	NiS	21397	Vastorf	Lüneburg	Lüneburg
S5	=	NiS	21442	Toppenstedt	Harburg	Lüneburg
S7	=	NiS	29556	Suderburg/ Hasenwinkel	Uelzen	Lüneburg
S10	=	NiS	29227	Osterloh	Celle	Lüneburg
S14	=	NiS	29485	Bockleben	Lüchow-Dannenberg	Lüneburg
S15	=	NiS	29485	Bockleben	Lüchow-Dannenberg	Lüneburg
S16	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S17	=	NiS	27327	Martfeld	Diepholz	Hanover
S19	=	NiS	38518	Neubokel	Gifhorn	Braunschweig
S21	=	BW	79258	Hartheim-Feldkirch	Breisgau /Hochschwarzwald	Freiburg
S22	=	BW	79206	Breisach-Gündlingen	Breisgau/Hochschwarzwald	Freiburg
S29	=	NiS	29499	Zernien	Lüchow-Dannenberg	Lüneburg
S30	=	NiS	29499	Zernien	Lüchow-Dannenberg	Lüneburg
S31	=	NiS	29485	Bockleben	Lüchow-Dannenberg	Lüneburg
S32	=	B	97359	Hörblach	Kitzingen	Unterfranken
S34	=	NiS	27374	Visselhövede	Rotenburg, Wümme	Lüneburg
S35	=	NiS	26160	Wehnen/ Westerstede	Ammerland	Weser-Ems
S36	=	NiS	49356	Schwering-hausen	Diepholz	Hanover
S37	=	NiS	49356	Schmalvörden	Diepholz	Hanover
S40	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg

S41	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S42	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S43	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S44	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S45	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S46	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S48	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S49	=	NiS	29562	Suhlendorf	Uelzen	Lüneburg
S50	=	S	01683	Nossen	Meißen	Dresden
S51	=	BW		Breisgau	Hochschwarzwald	Freiburg
S52	=	B	86666	Straß	Erding	Oberbayern
S53	=	NiS	26160	Wehnen/Bad Zwischenahn	Ammerland	Weser-Ems
S54	=	B	85622	Ingolstadt/ Feldkirchen	München	Oberbayern
S55	=	B	85452	Moosinning	Erding	Oberbayern
S56	=	NiS	49835	Wietmarschen	Grafschaft Bentheim	Weser-Ems
state:		NiS = Niedersachsen / Lower Saxony BW = Baden-Württemberg B = Bayern / Bavaria S = Sachsen / Saxony				

III.C.1.b Mapping

III.C.1.b.1 Lower Saxony

Twenty-seven of the 35 sites are located in Lower Saxony (Fig. 14).

From these 27 sites:

- 20 are in the administration district of Lüneburg (Fig. 15 and 16):
    - 11 in the district of Uelzen,
    - five in Lüchow-Dannenberg,
    - one each in Celle, Harburg, Lüneburg and Rotenburg/Wümme.
- four sites are part of the administration district of Hanover (Fig. 15 and 16):
    - three in the district of Diepholz,
    - one in the district of Burgdorf.
- two sites are situated in the administration district of Weser-Ems (district Ammerland) (Fig. 15 and 16) and
- one site in the administration district of Braunschweig (district Gifhorn) (Fig. 15 and 16).

The large number of sites in this State is a consequence of the exceptional co-operation received from the Plant-Protection Department in Hanover.

III.C.1.b.2 Bavaria

Four sites are in Bavaria (Fig. 14).

- Three in the administration district of Oberbayern:
    - two in the district of Erding,
    - one in the district of München
- One in the administration district of Unterfranken (district Kitzingen).

Figure 14: location of the TRV-sites in the different states of Germany

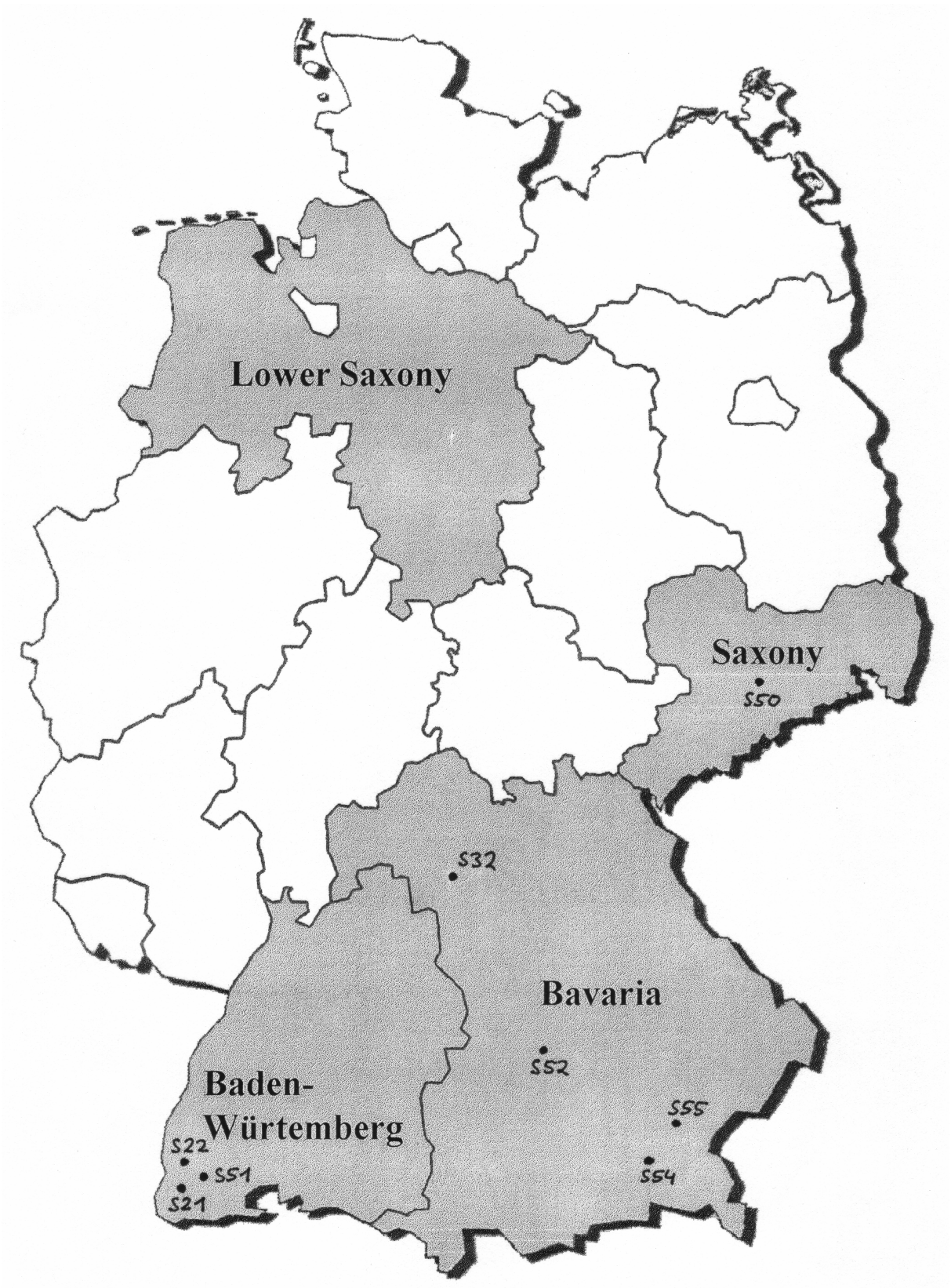


Figure 15: location of the TRV-sites in the different administration districts of Lower Saxony

Figure 16: location of the TRV-sites in the different districts of Lower Saxony

III.C.1.b.3 Baden-Württemberg

Three sites are in the state Baden-Württemberg (Fig. 14)

- administration district of Freiburg
    - two in the district of Breisgau-Hochschwarzwald,
    - one in the district of Hochschwarzwald.

III.C.1.b.4 Saxony

One site is located in Saxony (administration district Dresden, district Meißen) (Fig. 14).

III.C.2 Diversity of soil

Farm land used for potato cultivation was sandy and dry, which is based on the requirements of potato cultivation and available farm land. Of the TRV-positive sites in this study 62% were sandy and 38% sandy clay. The humidity ranged from very dry (27%), dry (40%) to normal (27%). Only two sites (6%) were characterised as wet, and no site was very wet or temporarily flooded.

Irrigation of fields is necessary to obtain a satisfactory crop, but this has financial implications for the farmer. Irrigation was used at 62% of the sites. A high irrigation intensity produces a high yield, for example in Lower Saxony the average yield is 414.2dt/ha, as compared to an overall average for Germany of 376.2dt/ha.

Table 3: characterisation of the soil of TRV-positive sites

site	sand	sandy clay	clay	watered	not watered	very dry	dry	normal	humid	very humid	temporary flooded
S1	x				x		x				
S2											
S5											
S7	25-28			x				x			
S10	18-24			x			x				
S14		38-40			x			x	x		
S15	25-30			x			x				
S16	24			x			x				
S17	25			x			x				
S19	22			x			x				
S21		40		x			x				
S22		30		x			x				
S29		35		x				x			
S30		30		x				x			
S31		30-35			x		x	x			
S32	35			x		details	not	given			
S34	28				x		x				
S35	28-30				x			x			
S36		x			x		x				
S37		x			x		x				
S40	25			x		x					
S41	25			x		x					
S42	25			x		x					
S43	25			x		x					
S44	25			x		x					
S45	25			x		x					
S46	25			x		x					
S48	25			x		x					
S49	25			x		x					
S50		x			x			x			
S51		40		x			x				
S52		35			x			x			
S53	27				x			x	x		
S54		70			x			x			
S55		x			x			x			
S56	25-33				x			x			

III.C.3 Diversity of disease occurrence

The data on occurrence ranged between 1993 and 1999.
Mostly the damage has occurred before (81%). That means that most of the farmers try to solve their TRV-problem by using other cultivars, crop rotation-patterns or cultivation-techniques. They do not give up potato cultivation because of one year of damage.

Table 4: chronological occurrence of damages of the TRV-positive sites

site	last occurrence of damages	first occurrence	damages also occur before	no damages
S1	1993		x	
S2	1994			
S5	1994			
S7	1994		x	
S10	1993		x	
S14	1998		x	
S15	1994		x	
S16	1994		x	
S17	1997		x	
S19	1994	x		
S21	1994		x	
S22	1994		x	
S29	1995	x		
S30	1995	x		
S31	1998		x	
S32	1995	details not given		
S34	1995	x		
S35	1995		x	
S36	1996			x
S37	1996			x
S40	1996		x	
S41	1996		x	
S42	1996		x	
S43	1996		x	
S44	1996		x	
S45	1996		x	
S46	1996		x	
S48	1996		x	
S49	1996		x	
S50	1997		x	
S51	1998	x		
S52	1998		x	
S53	1997		x	
S54	1999		x	
S55	1998		x	
S56	1998	x		

III.C.4 Diversity of crop rotation patterns

The most important rotation crops before potatoes are grain crops (barley, wheat, rye). Twenty-nine sites (85% of the 34 potato sites for which information was available) had this rotation.

The main group (21 sites) of these had combinations of grain crops with other crops:

- sugar-beet (5 sites),
- maize (5 sites),
- others (turnips, tobacco/rape, 11 sites)

Rotations with only grain crops (7 sites) or grain crops and grass (1 site) are relatively rare.

In only a few instances (4 sites, 12%) grain crops were not included in the crop rotation:

- maize at two sites
- maize/sugar-beet at one site
- sugar-beet/onion at one site

Inter-crops are not generally used. Only at 15 sites (44%) had the farmer planted an inter-crop between the main crops. The principal inter-crop was oil-radish (14 sites), and at individual sites lupins, winter-rape, or *Brassica rapa* had been planted. At 9 sites a mixture of legumes had been planted (see Chapter VII.)

Figure 17: grain crop-rich crop-rotation-patterns of the TRV-positive sites

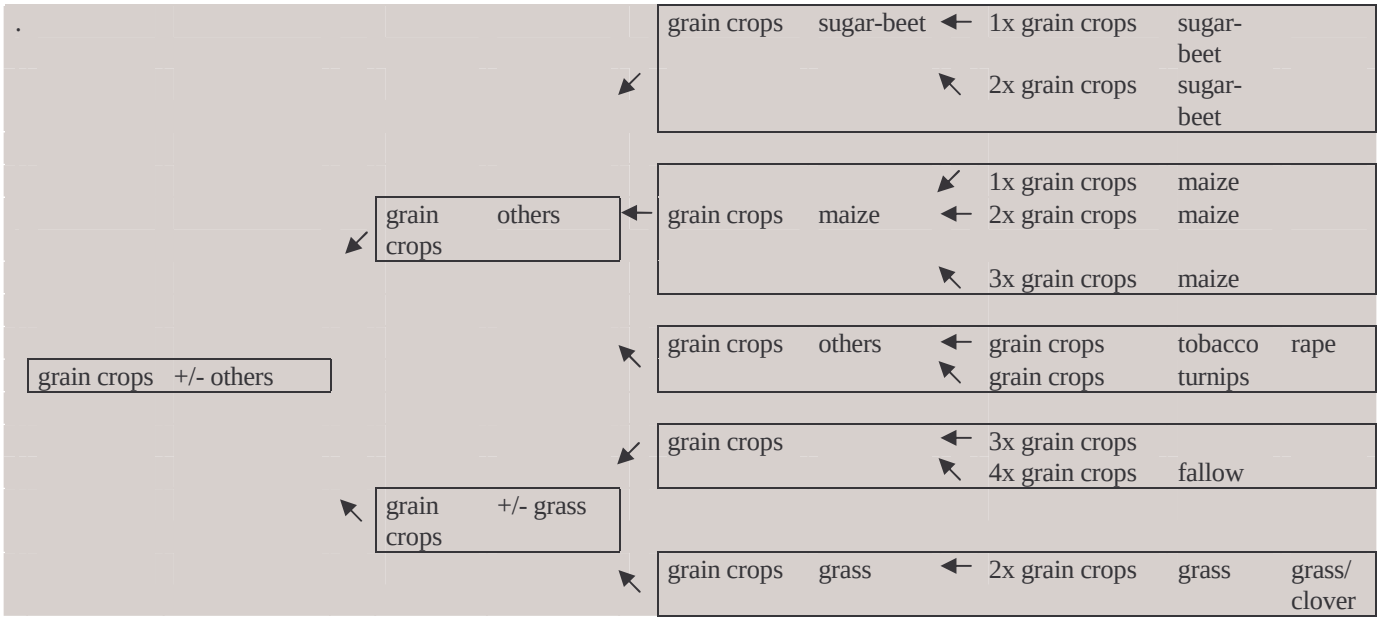


Figure 18: grain crop-free crop-rotation-patterns of the TRV-positive sites

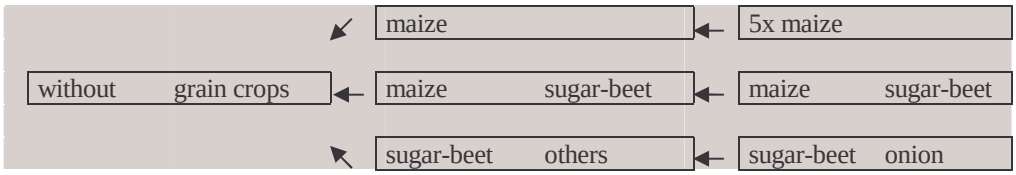


Table 5: rotation of crops of the TRV-positive sites

	year of damages		year before		year before		year before		year before		year before	
site	main crop	inter-crop	main crop	inter-crop	main crop	inter-crop	main crop	inter-crop	main crop	inter-crop	main crop	inter-crop
S1	potatoes		grain crops									
S2												
S5												
S7	potatoes		winter-barley	oil-radish	sugar-beet	lupins	summer-barley		potatoes			
S10	potatoes		summer-barley		summer-barley		sugar-beet		potatoes		summer-barley	
S14	potatoes		winter-barley		winter-barley		Triticale		potatoes		maize	
S15	potatoes		maize		Triticale		winter-barley		potatoes			
S16	potatoes		sugar-beet	oil-radish	summer-barley		potatoes		summer-barley		sugar-beet	oil-radish
S17	potatoes		winter-barley		winter-rye and potatoes							
S19	potatoes	oil-radish	winter-rye		summer-barley		potatoes	winter-rape	summer-barley			
S21	potatoes		sugar-beet-seed		maize							
S22	potatoes		sugar-beet		onions							
S29	potatoes		sugar-beet		Triticale		winter-barley		potatoes		parsley	
S30	potatoes		summer-barley		sugar-beet		winter-barley		potatoes			
S31	potatoes		maize		Triticale		winter-barley		potatoes			
S32	potatoes and carrots											
S34	potatoes		maize									
S35	potatoes		Triticale	oil-radish	summer-barley		maize		summer-barley			
S36	tobacco		wheat		rape		tobacco		wheat		rape	
S37	tobacco		wheat		rape		tobacco		wheat		rape	
S40	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S41	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S42	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S43	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S44	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S45	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S46	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S48	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S49	potatoes	mixture of legumes	grain crops		turnips	oil-radish	potatoes					
S50	potatoes		grass		winter-wheat		winter-barley		clover-grass			

S51	potatoes		maize	*Brassica rapa*	maize		maize		maize		maize	
S52	potatoes		winter-wheat		maize							
S53	potatoes		summer-barley	oil-radish	winter-barley	oil-radish	fallow land		winter-barley		Triticale	
S54	potatoes		winter-wheat		winter-barley							
S55	potatoes		grain crops									
S56	potatoes		winter-rye		winter-rye							

III.C.5 Genetic diversity of potato cultivars

III.C.5.a Symptomatology

The appearance of TRV symptoms (see IA5) is a characteristic dependent on the potato cultivar, the virus strain, and the local conditions (see soil characteristics and weather influence). Affected potato tubers from the examined sites mostly showed necrotic spots (44%), arcs (25%) and cones (66%). Brown necrotic areas in the vascular-bundle occurred only in 16% of the cases and star-formed fissures only twice (6%). In most of the cases the damage was assessed as light (62%) or medium (31%). Extensive symptoms only occured in 21% of crops.

Table 6: shaping of the potato-symptoms (symptomatology) of the TRV-positive sites

site	spots	arcs	cones	star-formed fissures	brown areas in the vascular-bundle	others	light	middle	strong	no damages
S1	x	x	x	x	x	x	x	x	x	
S2										
S5										
S7	x	x	x				x			
S10	x								x	
S14	x							x	x	
S15		x							x	
S16		x	x						x	
S17	x		x				x			
S19			x					x		
S21	x				x		x			
S22					x					
S29	x			x	x				x	
S30			x					x		
S31	x				x					
S32	x		x							
S34		x	x					x		
S35	x	x	x					x		
S36										x (tobacco)
S37										x (tobacco)
S40			x				x			
S41			x				x			
S42			x				x			
S43			x				x			
S44			x				x			
S45			x				x			
S46			x				x			
S48			x				x			
S49			x				x			
S50	x		x				x			
S51		x						x		
S52	x						x	x		
S53	x	x						x		
S54			x				x			
S55			x				x			
S56	x						x			

An examination of symptoms in relation to potato cultivar revealed that most cultivars show different symptoms. From the six sites where the described symptoms can be assigned to a single potato cultivar, only the cultivars Bintje and Saturna showed one symptom (spots or cones) exclusively.

Describing symptoms is not easy, and the method of examination of tubers can vary, which makes the assessment of symptoms problematic. Also, symptoms can be influenced by the virus strain, or local conditions.

Table 7: affected potato cultivars of the TRV-positive sites that can be assigned to the described symptoms

Site	affected cultivars	spots	arcs	cones	star-formed fissures	brown areas in the vascular-bundle
S17	Hansa	x		x		
S19	Saturna			x		
S29	Thomana	x			x	x
S30	Solara			x		
S34	Elkana		x	x		
S56	Bintje	x				

III.C.5.b Potato cultivars

Affected cultivars are defined here as showing TRV-symptoms after harvesting from a particular site.

Resistant cultivars are those that were planted at the same site as previously, but did not show symptoms.

It is necessary to differentiate between those instances where simultaneous cultivation of the affected and “resistant“ cultivars has occurred at a single site, and where theses cultivars have been grown at the same site but in different years.

In the first instance the “resistance“ may have resulted from the vector and virus occurring in patches, *i. e.* one cultivar may have grown in an infected area the others in non-infected areas.

In the second instance resistance also is uncertain, because of the influence of weather-conditions. One year the conditions for the vector can be so poor that infection is limited to a very small area. In a subsequent year conditions can be totally different.

Table 8: affected and non-affected potato cultivars of the TRV-positive sites

site	affected cultivars	potential resistant potato cultivars	no damages
S1	see Chapter IV	see Chapter IV	
S2	Hansa		
S5	Secura		
S7	Hansa Hela Roxy	Cilena	
S10	Amigo Cilena Granola Grata	Linda Solara	
S14	Gesa Granola Hela Linda	Cilena	
S15	Gesa Granola Hela Linda	Cilena	
S16	Hansa Saturna Solara		
S17	Hansa		
S19	Saturna		
S21	Atica Clivia Granola Karat Karatop Nicola Secura Sieglinde	Aiko Berber Cilena Ilona Karla Karlena Likaria Liu Ponto Quarta Rita Ute	
S22	Atica Christa	Berber Gloria Karatop Karla Rita	
S29	Thomana	Hansa Quarta	

S30	Solara	Cilena	
S31	Gesa Granola Hela Linda	Cilena	
S32	unknown	unknown	
S34	Elkana		
S35	Aurora Granola Hansa Indira Producent		
S36			nothing but tobacco-cultivation
S37			nothing but tobacco-cultivation
S40	Elkana Granola		
S41	Elkana Granola		
S42	Elkana Granola		
S43	Elkana Granola		
S44	Elkana Granola		
S45	Elkana Granola		
S46	Elkana Granola		
S48	Elkana Granola		
S49	Elkana Granola		
S50	test-cultivars	test-cultivars	
S51	Aula Exempla Nicola	Cilena Granola Quarta Satina Selma	
S52	test-cultivars	test-cultivars	
S53	Florijn Rikea	70 test-cultivars	
S54	Saturna test-cultivars	20 test-cultivars	
S55	3 test-cultivars	85 test-cultivars	
S56	Bintje		

III.C.6 Species-diversity of vector-nematodes

Most of the soil collected from the various sites was used for bait-tests. Consequently, only a little soil was available for examination of trichodorids. Because of this and the occurrence of trichodorids in very small numbers and in distinct patches, nematodes were recovered from only 14 samples (39%).

The investigation of TRV-positive soil-samples for trichodorids resulted in 7 different trichodorid species being identified (*Trichodorus primitivus*, *T. similis*, *T. viruliferus*, *Paratrichodorus pachydermus*, *P. teres*, *P. nanus*, *P. anemones*; Tab. 10). At most of the sites (83%) two to six species were present (see Tab. 9). *P. teres* was the most commonly occurring species (8 sites, 67%) followed by *T. similis*, *T. viruliferus* and *P. pachydermus* (each at 6 sites, 50%). *T. primitivus* was present at 5 sites (42%), *P. anemones* at 4 (33%), and *P. nanus* at one (8%).

The distribution of the different species (Fig. 20 and 21) show no obvious geographical pattern.

Table 9: distribution of trichodorid-species at the TRV-positive sites in the German samples of this work

site	trichodorid-species
S1	*P. pachydermus**
	*P. teres**
	*P. nanus**
S2	*P. sp.*
S7	*T. primitivus**
	T. similis°
	T. viruliferus°
	P. pachydermus°
	P. teres°
	P. anemones°
S10	*T. primitivus°*
	T. similis°
	T. viruliferus°
	P. pachydermus°
	P. teres°
S14	*T. primitivus**
	T. similis°
	T. viruliferus°
S15	*T. primitivus°*
	T. similis°
	T. viruliferus°
	P. pachydermus°
	P. teres°
	P. anemones°
S19	*T. similis°*
	P. teres°
S29	*P. teres°*
S31	*T. primitivus°*
S40	*T. sp.*
S42	*T. sp.*
	P. teres°
S48	*T. viruliferus°*
	P. anemones°
S49	*T. sp.*
	P. pachydermus°
	P. teres°
S53	*T. viruliferus°*
	T. similis°
	P. anemones°
	P. pachydermus°

* = virus-vectors

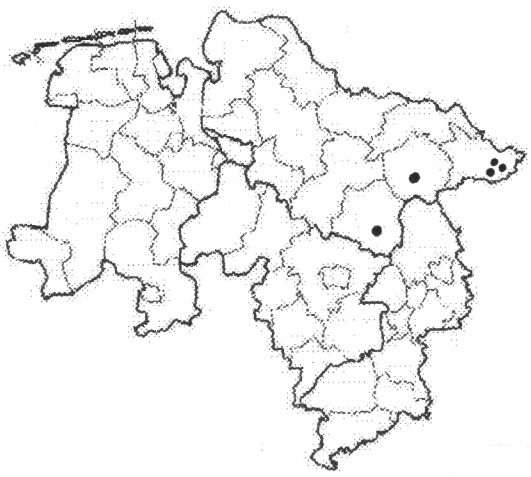
° = according to literature potential vectors, but till now at this location not proved as vectors

sp. = only juveniles found

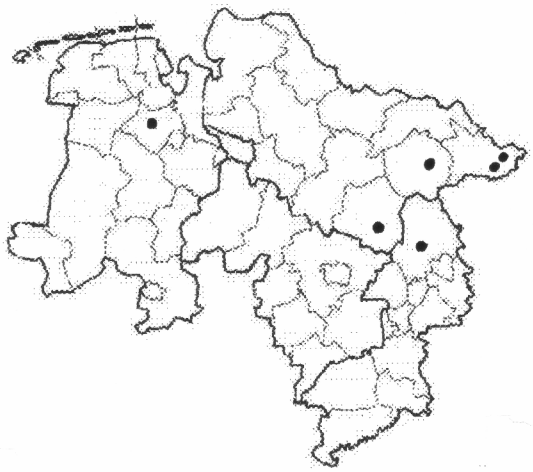
Table 10: distribution of trichodorid-species at the TRV-positive sites in the German samples of this work

trichodorid-species	site
Trichodorus primitivus	**S7** **S10** **S14** **S15** **S31**
Trichodorus similis	**S7** **S10** **S14** **S15** **S19** **S53**
Trichodorus viruliferus	**S7** **S10** **S14** **S15** **S48** **S53**
Paratrichodorus pachydermus	**S1** **S7** **S10** **S15** **S49** **S53**
Paratrichodorus teres	**S1** **S7** **S10** **S15** **S19** **S29** **S42** **S49**
Paratrichodorus nanus	**S1**
Paratrichodorus anemones	**S7** **S15** **S48** **S53**

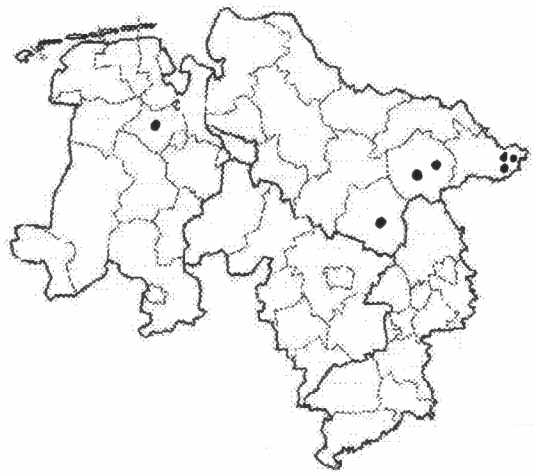
Figure 19: distribution of *Trichodorus*-species at the TRV-positive sites in the German samples of this work



Trichodorus primitivus

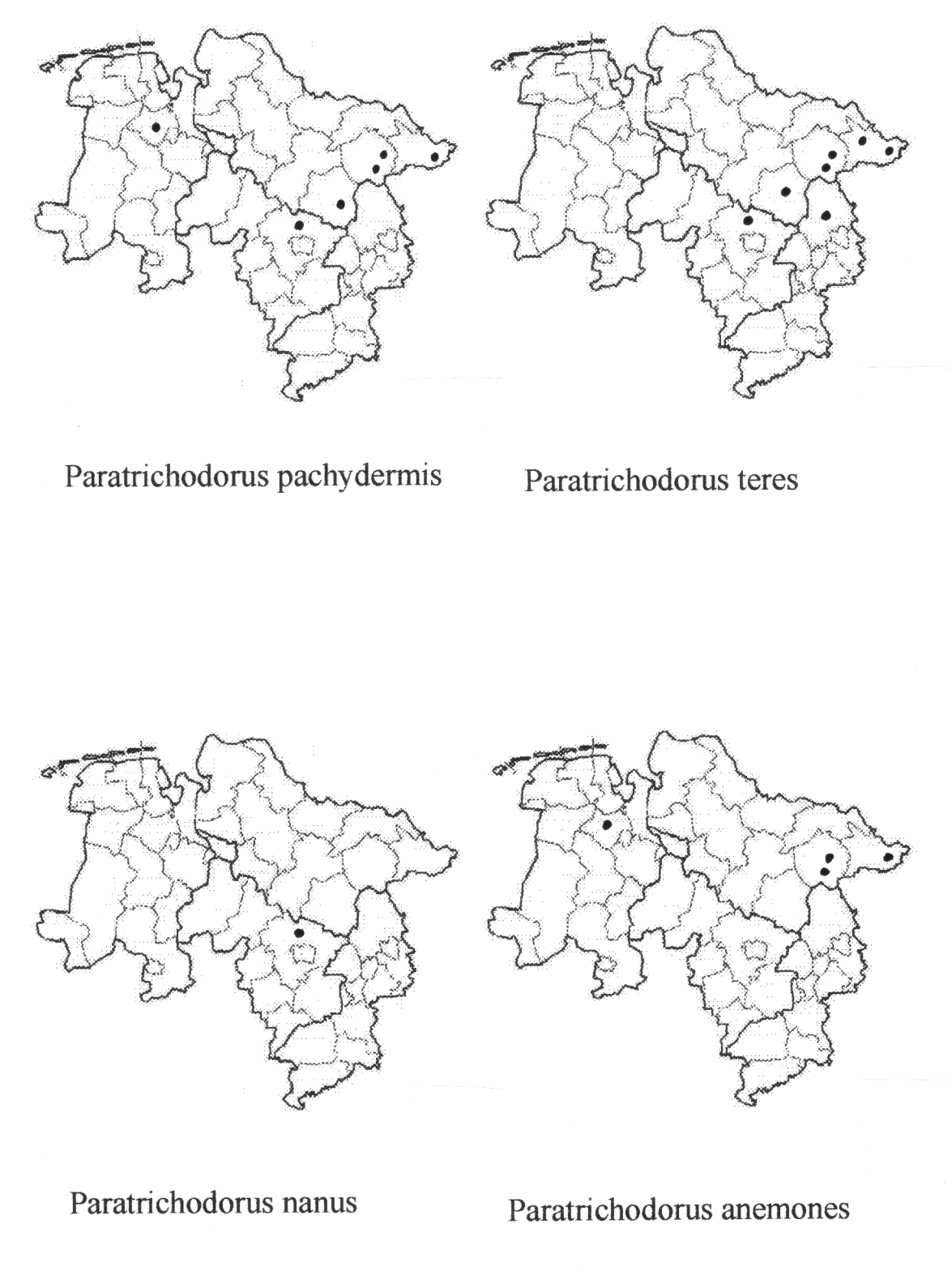


Trichodorus similis



Trichodorus viruliferus

Figure 20: distribution of *Paratrichodorus*-species at the TRV-positive sites in the German samples of this work



III.D Discussion

TRV was found at all the areas in Germany that were examined and appears to occur throughout the entire country. The amount of damage appeared to depend on the extent of potato-cultivation. The more extensively the crop is planted, the more samples that were available for investigation as a result of the increased interest of the farmers in disease control. If there is a direct relationship between potato cultivation and the incidence of infected sites, this can only be assessed with a more extensive survey involving all sites with this crop. In the present study only sites where the disease was suspected to occur were investigated; a more thorough assessment should be the subject of a more comprehensive and unbiased survey.

The influence of soil-conditions on symptom production and occurrence of TRV-damage is not known. Some observations (Cooper & Harrison, 1972) revealed that high humidity *viz.* rainfall, irrigation, increases the mobility of nematodes and therefore the spread of virus. It is suspected, but not proven that irrigation of potato fields in Germany has resulted in an increase of TRV damage, but this needs to be investigated separately.

Information about the history of occurrence of TRV-symptoms provided some insight of how farmers deal with TRV-problems. Most farmers apparently have tried different potato cultivars to decrease their losses. However, this approach is not under-pinned by scientific investigation, and this requires to be addressed as a matter of urgency.

Any influence of particular crop species on the occurrence of trichodorids and/or TRV has been little studied. Similarly, the influence of crop rotations used in years preceding the potato crop have been little studied.

At the sites examined in the present study the dominant rotation-pattern included grain crops (barley, wheat, rye). In literature (Spaull,1980; Weidemann, 1981) the high multiplication rate of trichodorid nematodes under grain crops has been reported, and a large population density increases the possibility for virus-transmission to occur. However, it is important to appreciate that transmission may be independent of total numbers or trichodorids. For example in a mixed species population only a small percentage of the total nematodes may be the vector species.

Information on TRV symptoms in potato cultivars grown in the different areas in Germany was requested from farmers and was assessed directly from tubers examined during the study.
TRV diseased tubers mostly exhibited spots, arcs and cones of necrotic tissue.
In most instances the damage was classified as light to moderate, however a 1-2% infection rate can result in the entire crop being rejected for human consumption.

A principal objective of the study was to identify potato cultivars potentially resistant to infection, *i. e.* not exhibiting TRV symptoms. At the TRV-infected sites investigated in the study 43 different potato cultivars and up to 85 test cultivars were examined. A comparison of symptoms and potato cultivars revealed considerable diversity of symptoms, and in the occurrence of symptoms all of which appeared to be compounded by a site/strain of virus effect (see Chapter IV).

To obtain an overview of the occurrence of vector trichodorid nematode species the TRV-positive soil-samples were investigated and the species present were identified. Seven different

trichodorid species were identified and most samples contained mixtures of two to six species. The distribution of the individual species was independent of geographical constraints.

In conclusion, TRV was found to occur in all of the areas examined in the study and caused damage to specific cultivars. Therefore, research into the problem is required to identify methods to reduce crop losses caused by the virus.

IV Resistance/tolerance/susceptibility of potato cultivars

IV.A Introduction

The biological assessment (see Chapter III) revealed that TRV is widespread in Germany. This presents a problem for potato production, particularly with regard to potatoes that are grown in many areas for direct food consumption, or for processing *e. g.* French-fries or chips. In many countries ´mashed´ potato is now selling more than chips or French-fries - it turns ´grey´ if TRV infected tubers are used! Potato cultivars that are resistant (= no spread and no multiplication of the virus in the plant and therefore no symptoms) or tolerant (= spread and multiplication of the virus in the plant without developing symptoms) to TRV provide a possible economic solution to the problem in contrast to susceptible cultivars (= little spread and multiplication of the virus in the plant but with symptoms in tubers). However, the selection of an appropriate potato cultivar is complicated because the virus exists as several different strains and there is a strain/cultivar interaction. A specific prediction procedure is required to select the resistant or tolerant cultivars that would be suitable for each locality. Currently, cultivars are tested in the field at only one location in Germany (Mellendorf), but this is not reliable because of the uneven distribution of the virus and the influence of the weather and crop rotation patterns. A nematode can transmit TRV to up to 3 plants within 4 days (Ploeg & Brown, 1997). Spread of infection is therefore possible even with very low population densities of trichodorids if the nematodes are vigorous and there is sufficient moisture in the soil to encourage their mobility.

Direct inoculation of the potato-tuber until now is not acceptable because of the low infection rate (Xenophontos *et al.*, 1998). Also, the virus is not transmitted from seed-tubers of susceptible cultivars to the new daughter tubers (= seed tubers) (Weidemann, 1994).

Growing cultivars at several different carefully selected sites to expose them to different strains of TRV is effective but expensive. Therefore testing potato cultivars directly against various virus strains would be the most effective and reliable means for identifying resistance.

TRV damage can be expressed as susceptibility (% infected tubers) and sensitivity (severity of symptoms) (Cooper, 1971b). Affected tubers are unacceptable as food potatoes or for industrial purposes, particularly those that are processed for crisps and chips (Schütz, 1973). With more than 2% of tubers affected the complete crop is rejected for human consumption (Spaar & Hamann, 1974) and can only be used for starch production and animal feed (Schütz, 1973). In a year of over-production of potatoes a lesser percentage of rattle-symptoms (in Scotland 1-2%) may lead to rejection of the crop for human consumption.

Damage to tubers may also make them more susceptible to fungi and bacteria and hence their storage may be adversely affected or their sprouting ability reduced (Heinicke, 1983).

A reduction in yield has not been observed (Heinicke, 1983), but recent investigations have shown that TRV infection can cause a reduction in the size of tubers to the extent that they are not marketable (Dale *et al.*, 1998). Another problem is that tubers may contain TRV without showing symptoms of infection (Xenophontos *et al.*, 1998) These tolerant cultivars represent a high risk for the spread of TRV at other virus-free sites.

In Germany about 132 potato cultivars are cultivated commercially (aid VerbraucherDienst, 1990). According to information acquired from the literature, 50% of these can be classified as susceptible to TRV infection (Reepmeyer, 1973b).

The "descriptive list of potato cultivars" of the cultivar office of the Federal Government shows a susceptibility-level for TRV for 106 food potato cultivars.

Table 11: susceptibility of potato cultivars to TRV; from "descriptive list of potato cultivars" 1995

susceptibility	**rating**	**number of food cultivars**
low	3	47
low-medium	4	28
medium	5	21
medium-high	6	9
high	7	1

From these data 47 cultivars with a susceptibility rating of "low" and 28 with "low-medium“ are available for low-input farming. Unfortunately, because of strain-specific reaction differences, the ratings given in the "descriptive list of potato cultivars" are not reliable. Consequently, the cultivars with low susceptibility such as Roxy, Saturna, Secura and Solara during the growing seasons of 1977 to 1995 on average had 17.23% of the crops showing TRV symptoms with 1.33% of the crops being rejected. Conversely, Berber and Agria, cultivars with medium susceptibility, showed only 2.81% disease incidence, which led to the rejection of only 0.48% of the crops. The various virus strains and environmental conditions are possible reasons for these discrepancies.

A reliable and strain-specific susceptibility diagnosis is required to identify cultivars with low or nil susceptibility to enable them to be used at TRV-infected sites. The integrated protection of plants could be introduced to affected areas taking the points mentioned above into account and thus providing a method for growing potatoes at TRV-infected sites without any requirement for the use of chemicals to control the trichodorids.

IV.B Material and methods

The results obtained during ten years from a cultivar screening test site at Mellendorf, Lower Saxony, provided by Dr. Dieter Heinicke, plant-protection-office Hannover, have been evaluated. Results from the sites examined in this work (see III) have been incorporated into this data base and the full results are provided here.

IV.C Results

IV.C.1 Mellendorf, country-cultivar-test-area

At Mellendorf the weather dependent minimum-/maximum-infestation has been officially recorded. In the calculation of this infestation-index:

(1x number of tubers with light symptoms + 2x number of tubers with medium symptoms + 3x number of tubers with heavy symptoms) / total number of tubers

the degree and extent of the symptoms are included.

During the present study soil-samples from this site were examined. The presence of TRV was confirmed and one of the strains was identified with ISEM (see IIE8) as reacting with the PRN antiserum. It is not impossible that other TRV strains also occur at this site, but none were recorded during the present study.

Five vector-species have been determined at this site: *Paratrichodorus pachydermus, P. nanus, P. teres, T. cylindricus* and *T. primitivus*. All of these species, except *T. primitivus,* have been proven as vectors of TRV at this site. Consequently, it is probable that four different strains of

TRV may be present at this site. Consequently, the evaluation of cultivar/TRV strain interaction cannot be accurately determined at this site.

With the evaluation of the cultivar-symptom-lists of 1984-1993 one can identify the cultivars that are susceptible, or not susceptible, against one or all of the strains that occur at this site (Tab. 12). These may not be resistant or susceptible at other locations at which other strains may occur as the symptoms are probably the result of strain-specific cultivar interactions.

Table 12: testing-field Mellendorf; evaluation of the TRV-symptoms 1984-1993

	infestation in %			**infestation-index**		
cultivar	**minimum**	**maximum**	**average**	**minimum**	**maximum**	**average**
Aiko	0	0	0,0	0	0	0,0
Atica	0,5	60	19,3	0,5	89	25,9
Aula	0	77	18,2	0	161	33,8
Berber	0	0	0,0	0	0	0,0
Berolina	0	1	0,2	0	1	0,2
Christa	0	0	0,0	0	0	0,0
Cilena	0	0	0,0	0	0	0,0
Clivia	0,4	76	21,8	0,4	154	40,0
Cosima	6	66	41,5	7	139	70,2
Datura	19	19	19,0	24	24	24,0
Desiree	0	77	18,1	0	131	26,1
Gloria	0,4	0,4	0,4	0,7	0,7	0,7
Granola	0	79	21,7	0	164	36,2
Grata	44	44	44,0	78	78	78,0
Hansa	0	6	2,0	0	8	2,5
Ilona	0	0	0,0	0	0	0,0
Indira	1,5	1,5	1,5	1,8	1,8	1,8
Isola	2	2	2,0	2	2	2,0
Karat	0	0	0,0	0	0	0,0
Karatop	0	0	0,0	0	0	0,0
Karla	0	0	0,0	0	0	0,0
Karlena	0,3	0,3	0,3	0,3	0,3	0,3
Likaria	0	0	0,0	0	0	0,0

Liu	0,3	0,3	0,3	0,9	0,9	0,9
Maritta	0	5	2,0	0	7	2,7
Maxilla	0	0	0,0	0	0	0,0
Nicola	0	8	2,1	0	18	4,6
Ponto	0	0	0,0	0	0	0,0
Prima	44	44	44,0	66	66	66,0
Producent	0,2	0,2	0,2	0,2	0,2	0,2
Quarta	0	0	0,0	0	0	0,0
Rebecca	0,2	0,2	0,2	1,5	0,5	0,5
Saskia	45	45	45,0	74	74	74,0
Secura	0	0	0,0	0	0	0,0
Sieglinde	0	76	21,4	0	169	38,1
Ukama	0	56	14,5	0	98	23,1
Ute	0	0	0,0	0	0	0,0

IV.C.2 Other sites

At the sites examined in this study the cultivars that were grown are listed in Table 13, together with their origin, type and susceptibility-level, gathered from the "descriptive list of potato cultivars" of the cultivar office of the Federal Government.

Table 13: Cultivars that have been planted on the tested fields; hatched = should be susceptible (susceptibility-level 5-9)

Cultivar	type	susceptibility
Aiko	middle-early, economic sort	4
Amigo	middle-late, economic sort	7
Atica	very early, food sort	5
Aula	middle-early, food sort	5
Aurora	-	
Berber	very early, food sort	5
Berolina		
Bintje	-	
Bonanza	middle-late, economic sort	
Christa	very early, food sort	3
Cilena	early, food sort	3

Clivia	middle-early, food sort	
Cosima		
Datura		
Desiree	middle-early, food sort	5
Elkana	-	
Florijn	middle-late, economic sort	7
Gesa	middle-early, food sort	
Gloria	very early, food sort	5
Granola	middle-early, food sort	6
Grata	middle-early, food sort	5
Hansa	middle-early, food sort	4
Hela	very early, food sort	4
Ilona	early, food sort	3
Indira	middle-late, economic sort	6
Isola		
Karat	early, food sort	3
Karatop	very early, food sort	3
Karla	very early, food sort	3
Karlena	early, economic sort	3
Likaria	middle-early, food sort	4
Linda	middle-early, food sort	4
Liu	middle-early, food sort	3
Maritta		
Maxilla	middle-late, economic sort	
Nicola	middle-early, food sort	6
Pamir	middle-early, food sort	
Ponto	middle-early, economic sort	7
Prima		
Producent	middle-late, economic sort	5
Quarta	middle-early, food sort	3
Rebecca	middle-late, economic sort	
Rikea	early, food sort	6
Rita	very early, food sort	3
Rosella	middle-early, food sort	
Roxy	middle-early, food sort	3
Saskia		
Satina	middle-early, food sort	3
Saturna	middle-late, food sort	3
Secura	middle-early, food sort	3
Selma	middle-early, food sort	5
Sieglinde	early, food sort	6
Solara	middle-early, food sort	3
Thomana	middle-early, economic sort	6
Ukama	very early, food sort	4
Ute	early, economic sort	3

IV.C.3 Comparison

The data in Table 14 show the reactions of the cultivars at the different sites involved in the biological assessment (column S2-S56) of this study (see III). Column S1 represents the observations at the test site at Mellendorf (see IVC1).

legend:
S = has shown symptoms
R = has not shown symptoms, potential resistant
hatched = should be susceptible (5 till 9)
not hatched = should be not susceptible (1 till 4)
sus. = susceptibility = for spraing-disease:

1 = very low	4 = low till medium	7 = strong
2 = very low till low	5 = medium	8 = strong till very strong
3 = low	6 = medium till strong	9 = very strong

Table 14: view of the reaction of the involved potato-cultivars

cultivar	sus.	S1	S2	S5	S7	S10	S14	S15	S16	S17	S19	S21	S22	S29	S30	S31	S34	S35	S40	S41	S42	S43	S44	S45	S46	S48	S49	S51	S53	S54	S56
Aiko	4	R										R																			
Amigo	7					S																									
Atica	5	S										S	S																		
Aula	5	S																										S			
Aurora	-																	S													
Berber	5	R										R	R																		
Berolina		S																													
Bintje	-																														S
Christa	3	R											S																		
Cilena	3	R			R	S	R	R				R			R	R												R			
Clivia		S										S																			
Cosima		S																													
Datura		S																													
Desiree	5	S																													
Elkana	-																S		S	S	S	S	S	S	S	S	S				
Exempla	4																											S			
Florijn	7																												S		
Gesa							S	S								S															
Gloria	5	S											R																		
Granola	6	S				S	S	S				S				S		S	S	S	S	S	S	S	S	S	S	R			
Grata	5	S				S																									
Hansa	4	S	S		S				S	S				R				S													
Hela	4				S		S	S								S															
Ilona	3	R										R																			
Indira	6	S																S													
Isola		S																													
Karat	3	R										S																			
Karatop	3	R										S	R																		
Karla	3	R										R	R																		
Karlena	3	S										R																			
Likaria	4	R										R																			
Linda	4					R	S	S								S															
Liu	3	S										R																			
Maritta		S																													
Maxilla		R																													
Nicola	6	S										S																S			
Ponto	7	R										R																			
Prima		S																													
Producent	5	S																S													
Quarta	3	R										R		R														R			
Rebecca		S																													
Rikea	6																												S		
Rita	3											R	R																		
Roxy	3				S																										
Saskia		S																													
Satina	3																											R			
Saturna	3								S		S																			S	
Secura	3	R		S								S																			
Selma	5																											R			
Sieglinde	6	S										S																			
Solara	3					R			S						S																
Thomana	6													S																	
Ukama	4	S																													
Ute	3	R										R																			

Through the comparison of suspected and observed susceptibility three groups of cultivars were determined:

1. Cultivars that have shown only sensitive reactions (Tab. 15)
2. Cultivars that have shown only potential resistant reactions (Tab. 16)
3. Cultivars that have shown sensitive and potential resistant reactions according to the site (Tab. 17).

Table 15: potato cultivars that only have shown sensitive reactions; legend see Table 14

cultivar	**sus.**	**S1**	**S2**	**S5**	**S7**	**S10**	**S14**	**S15**	**S16**	**S17**	**S19**	**S21**	**S22**	**S29**	**S31**	**S34**	**S35**	**S40**	**S41**	**S42**	**S43**	**S44**	**S45**	**S46**	**S48**	**S49**	**S51**	**S53**	**S54**	**S56**
Amigo	**7**					**S**																								
Atica	**5**	**S**										**S**	**S**																	
Aula	**5**	**S**																									**S**			
Aurora	**-**																**S**													
Berolina		**S**																												
Bintje	**-**																													**S**
Clivia		**S**										**S**																		
Cosima		**S**																												
Datura		**S**																												
Desiree	**5**	**S**																												
Elkana	**-**															**S**		**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**				
Exempla	**4**																										**S**			
Florija	**7**																											**S**		
Gesa							**S**	**S**							**S**															
Grata	**5**	**S**				**S**																								
Hela	**4**				**S**		**S**	**S**							**S**															
Indira	**6**	**S**															**S**													
Isola		**S**																												
Maritta		**S**																												
Nicola	**6**	**S**										**S**															**S**			
Prima		**S**																												
Producent	**5**	**S**															**S**													
Rebecca		**S**																												
Rikea	**6**																											**S**		
Roxy	**3**				**S**																									
Saskia		**S**																												
Saturna	**3**								**S**		**S**																		**S**	
Sieglinde	**6**	**S**										**S**																		
Thomana	**6**													**S**																
Ukama		**S**																												

Table 16: potato cultivars that only have shown potential resistant reactions; legend see Tab. 14

cultivar	sus.	S1	S2	S5	S7	S10	S14	S15	S16	S17	S19	S21	S22	S29	S31	S34	S35	S40	S41	S42	S43	S44	S45	S46	S48	S49	S51	S53	S54	S56
Aiko	**4**	**R**										**R**																		
Berber	**5**	**R**										**R**	**R**																	
Ilona	**3**	**R**										**R**																		
Karla	**3**	**R**										**R**	**R**																	
Likaria	**4**	**R**										**R**																		
Maxilla		**R**																												
Ponto	**7**	**R**										**R**																		
Quarta	**3**	**R**										**R**		**R**													**R**			
Rita	**3**											**R**	**R**																	
Satina	**3**																										**R**			
Selma	**5**																										**R**			
Ute	**3**	**R**										**R**																		

Table 17: potato cultivars that have shown sensitive and potential resistant reactions according to the site; legend see Table 14

cultivar	sus.	S1	S2	S5	S7	S10	S14	S15	S16	S17	S19	S21	S22	S29	S30	S31	S34	S35	S40	S41	S42	S43	S44	S45	S46	S48	S49	S51	S53	S54
Christa	**3**	**R**											**S**																	
Cilena	**3**	**R**			**R**	**S**	**R**	**R**				**R**			**R**	**R**												**R**		
Gloria	**5**	**S**											**R**																	
Granola	**6**	**S**				**S**	**S**	**S**				**S**				**S**		**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**		
Hansa	**4**	**S**	**S**		**S**				**S**	**S**				**R**				**S**												
Karlena	**3**	**S**										**R**																		
Karat	**3**	**R**										**S**																		
Karatop	**3**	**R**										**S**	**R**																	
Linda	**4**					**R**	**S**	**S**								**S**														
Liu	**3**	**S**										**R**																		
Secura	**3**	**R**		**S**								**S**																		
Solara	**3**					**R**			**S**						**S**															

The first group (sensitive reactions) shows an agreement of suspected and observed susceptibility in 75% of the cases (susceptibility level 5-9), and a difference in 25% of the cases (susceptibility level 1-4).

The second group (potential resistant reactions) shows an agreement in 73% of the cases (susceptibility level 1-4), and a difference in 27% of the cases (susceptibility level 5-9).

In the third group (sensitive and potential resistant reactions) 17% of the cultivars were found to be sensitive (susceptibility level 5-9) and 83% to be resistant (susceptibility level 1-4).

In conclusion, it can be stated that the reliability of the susceptibility characteristic of a cultivar described in the "descriptive list of potato cultivars" of the cultivar office of the Federal Government is on average only 75%. Consequently, this procedure is considered inappropriate and recommendations need to be developed based upon strain-specific interactions.

IV.D Discussion

The recommendations in the "descriptive list of potato cultivars" of the cultivar office of the Federal Government have been obtained from data collected only at one site, and these statements can only be related to the TRV-strains present at this testing site. However, the cultivar-reaction is probably strain-specific (Weidemann, 1993b) and therefore a cultivar may react quite differently when exposed to other strains at other sites. The examination of this work results in a reliability of the official recommendations of only 75%. This is not acceptable for the farmer, who risks a total crop-loss because of the wrong choice of cultivars.

V Interaction of virus strain, vector species and potato cultivar

V.A Introduction

The use of resistant cultivars is an economically attractive solution to the problem of TRV infection of potatoes. In Germany, an official potato breeding programme which has been operating since 1977 includes TRV-resistance as one of its parameters. However, the trials have been conducted in only one location and therefore the breeding lines have been exposed to only one strain of the virus and possibly only to one potential vector species.

Different potato cultivars vary in their reaction to TRV infection and different strains of the virus may induce varying reactions in the same cultivar; this is virus strain-specific resistance (Weidemann, 1993b). The symptoms of infection depend on the potato genotype, the environmental conditions at the location, and particularly the virus strain that is present.

Recent investigations have indicated that each strain of TRV can only be transmitted by a specific trichodorid species (Ploeg & Brown, 1997). In Germany different strains have been identified together with some that have no known antiserum. For the benefit of the potato breeding programme and to provide information prior to planting commercial crops it is necessary to determine the occurrence and identity of TRV strains in Germany, together with their trichodorid vector species.

V.B Material and methods

See IIC (tuber symptom survey), IIE1 (bait-test), IIE8 (ISEM), IIE9 (decoration) and VIB (RT-PCR)

The antisera used are characterised as follows:

Table 18: characterisation of antisera

	abbreviation	name	specification
1	ON	Onion	Onion-Furo-Virus, 817 v. 15.10.96
2	ROS	Rostock	908 TRV Rostock-Kraut v. 3.9.97, 3. Abn., from Stefan Winter
3	WEY	Weyhausen	Tobacco Rattle V. Weyhausen, Kan. 455 v. 8.11.82
4	MEL	Mellendorf	Tobacco Rattle V. Mellendorf, Kan. 397 v. 18.5.82
5	PRN	PRN	PRN γ-glob., from Dr. I. Roberts, SCRI, Dundee, Scotland
6	RQ	RQ	RQ γ-glob., from Dr. I. Roberts, SCRI, Dundee, Scotland
7	Greek	Greek	Greek γ-glob., from Dr. I. Roberts, SCRI, Dundee, Scotland
8	Turin	Turin	Rohserum: from Dr. Heinze
9	USA	USA	from Phil Berger/Patrick Shiel, University of Idaho
10	Dutch PEBV	Dutch PEBV	from Wageningen, Serum E116, Titer 256, from Dr. Heinze

1: Onion

- Has been produced by Prof. Dr. Koenig, BBA Braunschweig, using plant material from infected onions (Koenig, personal information).
- It should be a member of the TRV-group because of nearly 100% homology of the RNA1. TRV-Onion also shows homology with Hypochoeris-mosaic-virus.
- Its RNA2 includes 3 ORFs: for the coat protein (83% of its aminoacids are homologous to TRV-TCM), for a 27kDa- and for a 9kDa-protein.

2: Rostock comes from Germany

3: Weyhausen comes from Germany

4: Mellendorf comes from Germany

5: PRN

- PRN (Potato Root Necrosis) was prepared against an isolate of TRV from potato (cv. Kerr´s Pink) from Scotland.
- It is transmitted by *Paratrichodorus nanus* and *P. pachydermus*.

6: RQ comes from Scotland.

7: Greek

- Greek (Brown *et al.*, 1996) did not react with 10 different antisera in serological tests but with the RNA1 of TRV-SYM in spot hybridization tests. It therefore seems to be a new serotype.
- It is transmitted by *Trichodorus similis*.
- Its long particles have a size of 290S (RNA1 = $2.2x10^6$D), its short 184S (RNA2 = $1.2 x10^6$D).

8: Turin comes from Italy

9: USA has been produced by Phil Berger/Patrick Shiel, University of Idaho.

10: Dutch PEBV (Edwardson & Christie, 1990) is transmitted by *P. anemones*, *P. teres*, *P. pachydermus*, *T. primitivus* and *T. viruliferus*. Its RNA1 consists of 4 ORFs.

V.C Results

V.C.1 Determination of TRV with bait-tests

Of the 56 sites that were sampled, bait tests and symptoms in the tubers that were sampled were positive in 36. From these, 8 were selected for detailed examination (Fig. 21) with ISEM and decoration.

V.C.2 Strain-determination with ISEM and decoration

Samples from 8 sites (see IIIC1) S10, S19, S29, S30, S31, S45, S46 and S49 were examined using ISEM and decoration. Controls of healthy plant material, plant material with known virus-content and pure buffer-solution were included in all tests.

V.C.2.a Comparison

Figure 21: comparison

Site \ Antiserum	ROS	ON	WEY	MEL	PRN	RQ	GREEK	TURIN	USA	Dutch PEBV
S 29	+									
S 10	+									+
S 46	+									+
S 45	+	+								+
S 49	+	*					+			+
S 31	+	+								+
S 19	+			+	+			+		
S 30	+			+	+			+	*	

(confirmed and assessed by Dr. Ian Roberts, SCRI)

(* = results indifferent)

Twenty-two of the 23 positive ISEM-results results were confirmed by positive decoration-tests. The ISEM-result of S49 with Onion-A/S failed in this test with a dilution 1:32, and this test should have been continued with higher A/S-concentrations. Consequently, this result remains questionable but seems to be positive.

One (S30 with USA-A/S) of the ISEM-result was indeterminate although the decoration result was positive. Because the USA-A/S never reacted with other isolates in ISEM- or decoration-tests this A/S might not be useful for serological tests. Therefore the result is considered as negative.

In overview from the serological tests four groups can be distinguished:

group 1 (S29):

reacted only with Ros-A/S

group 2 (S10, S46):

reacted with Ros- and Dutch PEBV-A/S

group 3 (S45, S49, S31):

reacted with Ros-, Dutch PEBV- and Onion-A/S and one isolate (S49) also with Greek-A/S

group 4 (S19, S30):

reacted with Ros-, Mel-, PRN- and Turin-A/S (perhaps S30 also with USA s.a.)

Group 2 and 3 seem to show a closer relationship between Ros and Dutch PEBV although the members of group 3 also react with other antisera.

Group 4 appears to be a mixture of different strains because of the mixed positive results.

The above results should have been specified by intensive characterisations of the antisera and their cross-reaction-features. But they show that serologically different strains of TRV occur in German soil and therefore different reactions of potato cultivars at different sites can be expected.

V.C.2.b S10

The isolate from site S10 reacted in ISEM with the Rostock- and the Dutch PEBV-antisera. The results also have been confirmed by positive decoration-tests.

The degree of relatedness was higher with Rostock (decorated till 1:512) than with Dutch PEBV (1:256).

Table 19: S10

site	result	ISEM with A/S	for	P/SA before ISEM	P/SA after ISEM	increase-factor	decorated till dillution
S 10	pos.	Ros	2h	385	2848	7,4	1:512
			4h	264	4589	17,4	
	neg.	On	2h	385	244	0,6	
			4h	264	614	2,3	
	neg.	Weyhausen	2h	385	116	0,3	
			4h	264	71	0,3	
	neg.	Mellendorf	2h	385	116	0,3	
			4h	264	71	0,3	
	neg.	PRN	2h	385	506	1,3	
			4h	264	684	2,6	
	neg.	RQ	2h	385	506	1,3	
			4h	264	684	2,6	
	neg.	Greek	2h	385	163	0,4	
			4h	264	307	1,2	
	neg.	Turin	2h	385	163	0,4	
			4h	264	307	1,2	
	pos.	Dutch PEBV	2h	385	1927	5	1:256
			4h	264	2701	10,2	
	neg.	USA	2h	207	236	1,1	
			4h	207	79	0,4	

V.C.2.c S19

The isolate from site S19 reacted in ISEM with the Rostock-, Mellendorf-, PRN- and Turin-antisera. The results also have been confirmed by positive decoration-tests.

The degree of relatedness was higher with Mellendorf (decorated till 1:64) than with Rostock, PRN and Turin (1:32).

Table 20: S19

site	result	ISEM with A/S	for	P/SA before ISEM	P/SA after ISEM	increase-factor	decorated till dillution
S19	pos.	Ros	2h	5	43	9,2	1:32
			4h	18	47	2,7	
	neg.	On	1h	38	50	1,3	
			2h	53	56	1,04	
	neg.	Wey	1h	38	112	2,96	
			2h	53	64	1,19	
	pos.	Mel	2h	12	1046	88,7	1:64
			4h	9	2250	260	
	pos.	PRN	2h	12	152	12,9	1:32
			4h	9	72	10,3	
	neg.	RQ	2h	12	1	0,1	
			4h	9	22	2,6	
	neg.	Greek	2h	12	6	0,5	
			4h	9	14	1,7	
	pos.	Turin	1h	4	22	5,7	1:32
			2h	10	34	3,5	
	neg.	Dutch PEBV	2h	5	9	1,9	
			4h	18	2	0,1	
	neg.	USA	2h	5	3	0,7	
			4h	18	24	1,3	

V.C.2.d S29

The isolate from site S29 reacted in ISEM with the Rostock-antiserum. The result also has been confirmed by positive decoration-tests.

The degree of relatedness was 1:32.

Table 21: S29

site	result	ISEM with A/S	for	P/SA before ISEM	P/SA after ISEM	increase-factor	decorated till dillution
S 29	pos.	Ros	2h	36	56	1,5	1:32
			3,5h	21	87	4,1	
	neg.	Onion	2h	36	30	0,8	
			3,5h	21	59	2,8	
	neg.	Weyhausen	2h	36	18	0,5	
			3,5h	21	2	0,1	
	neg.	Mellendorf	2h	36	18	0,5	
			3,5h	21	2	0,1	
	neg.	PRN	2h	36	27	0,7	
			3,5h	21	5	0,2	
	neg.	RQ	2h	36	27	0,7	
			3,5h	21	5	0,2	
	neg.	Greek	2h	36	18	0,5	
			3,5h	21	15	0,7	
	neg.	Turin	2h	36	18	0,5	
			3,5h	21	15	0,7	
	neg.	Dutch PEBV	2h	36	4	0,1	
			3,5h	21	21	1	
	neg.	USA	2h	3	2	0,6	
			4,5h	3	1	0,4	

V.C.2.e S30

The isolate from site S30 reacted in ISEM with the Rostock-, Mellendorf-, PRN- and Turin-antisera. The results also have been confirmed by positive decoration-tests. The results with the USA-antiserum were inconclusive.

The degree of relatedness was higher with Mellendorf (decorated till 1:1024) than with Turin (1:256) and Rostock and PRN (1:128).

Table 22: S30

site	result	ISEM with A/S	for	P/SA before ISEM	P/SA after ISEM	increase-factor	decorated till dillution
S 30	pos.	Ros	2h	55	115	2,1	1:128
			3,5h	25	45	1,8	
	neg.	On	2h	55	52	1	
			3,5h	25	53	2,1	
	neg.	Wey	2h	44	21	0,5	
			4,5h	9	24	2,8	
	pos.	Mel	2h	44	586	13,2	1:1024
			4,5h	9	1377	159,1	
	pos.	PRN	2h	44	268	6	1:128
			4,5h	9	905	104,5	
	neg.	RQ	2h	44	48	1,1	
			4,5h	9	13	1,4	
	neg.	Greek	2h	44	8	0,2	
			4,5h	9	6	0,7	
	pos.	Turin	2h	44	336	7,6	1:256
			4,5h	9	88	10,2	
	neg.	Dutch PEBV	2h	55	30	0,5	
			3,5h	25	22	0,9	
	?	USA	2h	44	80	1,8	1:128
			4,5h	9	30	3,5	
			1h	9	3	0,3	
			2h	2	6	3,3	

V.C.2.f S31

The isolate from site S31 reacted in ISEM with the Rostock-, Onion- and Dutch PEBV-antisera. The results also have been confirmed by positive decoration-tests.

The degree of relatedness was higher with Rostock (decorated till 1:256) than with Onion and Dutch PEBV (1:32).

Table 23: S31

site	result	ISEM with A/S	for	P/SA before ISEM	P/SA after ISEM	increase-factor	decorated till dillution
S31	pos.	Ros	2h	10	118	11,8	1:256
			4h	6	156	24,8	
	pos.	On	2h	10	13	1,2	1:32
			4h	6	30	4,8	
	neg.	Weyhausen	2h	10	2	0,2	
			4h	6	1	0,1	
	neg.	Mellendorf	2h	10	2	0,2	
			4h	6	1	0,1	
	neg.	PRN	2h	10	5	0,5	
			4h	24	9	0,4	
	neg.	RQ	1h	7	9	1,3	
			2h	10	11	1,08	
	neg.	Greek	2h	10	19	1,8	
			4h	6	7	1,1	
	neg.	Turin	2h	10	19	1,8	
			4h	6	7	1,1	
	pos.	Dutch PEBV	2h	10	41	4	1:32
			4h	6	92	14,7	
	neg.	USA	2h	10	1	0,1	
			4h	6	15	2,4	

V.C.2.g S45

The isolate from site S45 reacted in ISEM with the Rostock-, Onion- and Dutch PEBV-antisera. The results also have been confirmed by positive decoration-tests.

The degree of relatedness was higher with Rostock and Onion (decorated till 1:64) than with Dutch PEBV (1:32).

Table 24: S45

site	result	ISEM with A/S	for	P/SA before ISEM	P/SA after ISEM	increase-factor	decorated till dillution
S45	pos.	Ros	2h	3	126	40	1:64
			4h	1	385	490	
	pos.	On	2h	3	9	3	1:64
			4h	1	34	43,5	
	neg.	Weyhausen	2h	20	7	0,4	
			4h	2	3	1,9	
	neg.	Mellendorf	2h	20	7	0,4	
			4h	2	4	2,5	
	neg.	PRN	2h	20	7	0,4	
			4h	2	9	6	
	neg.	RQ	2h	20	11	0,6	
			4h	2	6	3,5	
	neg.	Greek	2h	3	2	0,8	
			4h	1	2	2	
	neg.	Turin	2h	3	2	0,8	
			4h	1	2	2	
	pos.	Dutch PEBV	2h	3	9	2,8	1:32
			4h	1	102	130	
	neg.	USA	2h	3	5	1,5	
			4h	1	0	0	

V.C.2.h S46

The isolate from site S46 reacted in ISEM with the Rostock- and Dutch PEBV-antisera. The results also have been confirmed by positive decoration-tests.

The degree of relatedness was higher with Rostock (decorated till 1:256-1:512) than with Dutch PEBV (1:64).

Table 25: S 46

site	result	ISEM with A/S	for	P/SA before ISEM	P/SA after ISEM	increase-factor	decorated till dillution
S 46	pos.	Ros	2h	218	2635	12,1	1:256 - 1:512
			4h	244	1982	8,1	
	neg.	On	2h	218	275	1,3	
			4h	244	291	1,2	
	neg.	Weyhausen	2h	218	126	0,6	
			4h	244	118	0,5	
	neg.	Mellendorf	2h	218	126	0,6	
			4h	244	118	0,5	
	neg.	PRN	2h	218	194	0,9	
			4h	244	358	1,5	
	neg.	RQ	2h	218	194	0,9	
			4h	244	358	1,5	
	neg.	Greek	2h	218	139	0,6	
			4h	244	173	0,7	
	neg.	Turin	2h	218	139	0,6	
			4h	244	173	0,7	
	pos.	Dutch PEBV	2h	218	751	3,5	1:64
			4h	244	1408	5,8	
	neg.	USA	2h	92	147	1,6	
			4h	228	254	1,1	

V.C.2.i S49

The isolate from site S49 reacted in ISEM with the Rostock-, Onion-, Greek- and Dutch PEBV-antisera. The results also have been confirmed by positive decoration-tests except the Onion-result where no reaction was visible at 1:32.

The degree of relatedness was higher with Rostock (decorated till 1:64) than with Greek and Dutch PEBV (1:32).

Table 26: S 49

site	result	ISEM with A/S	for	P/SA before ISEM	P/SA after ISEM	increase-factor	decorated till dillution
S49	pos.	Ros	2h	8	114	14,5	1:64
			4h	14	207	14,6	
	pos.	On	2h	8	59	7,5	no coat at 1:32
			4h	14	45	3,1	
	neg.	Weyhausen	2h	8	2	0,3	
			4h	14	22	1,6	
	neg.	Mellendorf	2h	8	2	0,3	
			4h	14	22	1,6	
	neg.	PRN	1h	11	25	2,2	
			2h	12	15	1,3	
	neg.	RQ	1h	11	0	0	
			2h	12	1	0,05	
	pos.	Greek	2h	33	43	1,3	1:32
			4h	9	39	4,2	
	neg.	Turin	2h	33	15	0,5	
			4h	9	26	2,7	
	pos.	Dutch PEBV	2h	8	35	4,5	1:32
			4h	14	119	8,4	
	neg.	USA	2h	8	8	1	
			4h	14	1	0,1	

V.C.3 TRV-proof with different methods

The following synopsis presents an overview of the results of all TRV-detection-methods used in this study.

All ISEM- and decoration-tests were confirmed and assessed by Dr. Ian Roberts and the RT-PCR-results by Dr. David Robinson. Both are authorities in their field at the SCRI, Dundee, Scotland and have long-term experience with TRV. Most tests were performed under supervision from these experts during visits to the SCRI.

Table 27: TRV-proof with different methods

Site	TRV-proof with bait-test/tuber-survey	TRV-proof with RT-PCR (RNA1)	TRV-proof with ISEM/decoration	correspondence
S1	positive	positive	positive	both methods function
S2	positive	positive	negative	only RT-PCR functions
S3	negative			
S4	negative			
S5	positive	positive	positive	both methods function
S6	negative			
S7	positive	positive	negative	only RT-PCR functions
S8	negative			
S9	negative			
S10	positive	positive	positive	both methods function
S11	negative			
S12	negative			
S13	negative			
S14	positive	positive	negative	only RT-PCR functions
S15	positive	negative	positive	only ISEM/decoration functions
S16	positive	negative	negative	no methods functions
S17	positive	negative	negative	no methods functions
S18	negative			
S19	positive	negative	positive	only ISEM/decoration functions
S20	negative			
S21	positive	positive	positive	both methods function
S22	positive	positive	negative	only RT-PCR functions
S23	negative			
S24	negative			
S25	negative			
S26	negative			
S27	negative			
S28	negative			

S29	positive	positive	positive	both methods function
S30	positive	positive	positive	both methods function
S31	positive	positive	positive	both methods function
S32	positive	negative	negative	no methods functions
S33	negative			
S34	positive	negative	negative	no methods functions
S35	positive	positive	positive	both methods function
S36	positive	negative	negative	no methods functions
S37	positive	negative	positive	only ISEM/decoration functions
S38	negative			
S39	negative			
S40	positive	positive	negative	only RT-PCR functions
S41	positive	negative	positive	only ISEM/decoration functions
S42	positive	negative	negative	no methods functions
S43	positive	positive	negative	only RT-PCR functions
S44	positive	positive	negative	only RT-PCR functions
S45	positive	positive	positive	both methods function
S46	positive	positive	positive	both methods function
S47	negative			
S48	positive	negative	positive	only ISEM/decoration functions
S49	positive	positive	positive	both methods function
S50	positive	not tested yet	not tested yet	not tested yet
S51	positive	not tested yet	not tested yet	not tested yet
S52	positive	not tested yet	not tested yet	not tested yet
S53	positive	not tested yet	not tested yet	not tested yet
S54	positive	not tested yet	not tested yet	not tested yet
S55	positive	not tested yet	not tested yet	not tested yet
S56	positive	not tested yet	not tested yet	not tested yet

In 11 of the positive cases (38%) both RT-PCR and ISEM/decoration worked with the isolates.

In 7 of the cases (24%) only RT-PCR functioned and it was discovered that in each case NM-strains, that only consist of RNA1, were present. ISEM/decoration needs a coat-protein, that is encoded on the RNA2, to show positive results, thus only RT-PCR could react with NM-strains.

In 5 of the cases (17%) only ISEM/decoration reacted, and possibly primer-problems existed so that the primers used could not detect the isolate-specific sequences.

In 6 of the cases (21%) no method gave a positive result, and it is assumed that the presence of NM strains and primers not fitting the isolate caused theses failures in detection.

The results show that there is no single method that is usable with all TRV-strains. Related to the problems with NM-strains and primer-problems the method that leads to detection of TRV depends on the isolate-features. If one method does not give a positive result, other methods should be used before the sample is designated as being negative

V.D Discussion

Different strains of TRV have been shown to occur in Germany. Also it has been shown (see IVC3) that potato cultivars vary in their reaction at different sites, which can be regarded as proof that potato cultivars react differently depending on the virus strain present.

In order to recommend a particular potato cultivar the reaction of a cultivar to a virus strain must be studied. A prerequisite for this is that the virus strain must be accurately determined. For this, different methods (ISEM, decoration, ELISA, RT-PCR) are available.

It can be concluded from the discrepancies shown in this chapter that ISEM-, decoration- and ELISA-results are not totally comparable. Each method has its pros and cons. A totally dependable and unequivocal method has not yet been found. Perhaps nucleic acid-based methods such as RT-PCR could provide a solution in the future.

Serological methods (ISEM, decoration, ELISA) are well established and relatively cheap, but they need high-technology-equipment (EM, ELISA-reader) that is not available at all plant-protection offices.

Further, the operator has to have experience (especially with ISEM, decoration) with the virus. Also these methods do not work with NM-strains.

Comparing ISEM- and ELISA, ELISA is much easier and quicker. ISEM/decoration delivers no false positive or false negative results.

RT-PCR-technology has the advantage of reacting with NM-isolates, and therefore should be the method of choice in the future. However, reactions with NM-isolates, as they involve only the highly conserved RNA1 segment of the TRV genome, will not provide identity of the strain of virus. Consequently, this method/reaction will provide evidence of the occurrence of TRV, but not specific strain identity.

The results of all these methods are not totally comparable and any comparison should therefore be viewed with caution.

VI Preliminary development of a non-expert diagnostic method for TRV (RT-PCR)

VI.A Introduction

TRV presents a variety of problems namely:

- NM-strains often occur but are not detectable with serological methods;
- Different serological methods often are not comparable because of differences in sensitivity (see V);
- TRV mostly occurs in very low concentrations (Crosslin & Thomas, 1995) and in potato-tubers the virus-content may be so low that serological or biological proof may not be obtainable (Weidemann, 1993a). Also, TRV is distributed very unevenly in tubers of susceptible cultivars (Crosslin & Thomas, 1995), and transmission of TRV from tubers to indicator-plants (*Chenopodium quinoa*) is not reliable (Crosslin & Thomas, 1995).

It is therefore necessary to develop a fast diagnostic procedure for identification of tobacco rattle virus and the differentiation of virus strains in an area. A starting point for this is RT-PCR (reverse transcriptase polymerase chain reaction)-technology that is based on nucleic-acid because of the large detection-sensitivity that this technology has shown. The aim is to develop a method that is simple, practical and with low staff and material expenditure. The results of a soil test should be available within a few days by this procedure.

The objective of this procedure would be a pre-plant test and secondarily the cause-clarification of already damaged potatoes.

The RT-PCR method does not identify specific strains of TRV which is the basis for the resistance-testing. Therefore strain determination has been carried out on a serological basis (ISEM, decoration, ELISA). However, with PVY a differentiation between strains with RT-PCR works (Weidemann & Maiß, 1993) and therefore a practical simplification and expansion of the RT-PCR method for the specific strains of TRV should be a future objective.

RT-PCR-tests can be undertaken with several materials:

1. leaves of indicator-plants from bait-tests: to provide an early warning test for a site
2. vector-nematodes: to speed up this early warning test by avoidance of indicator-plants
3. potato tubers that show symptoms of TRV infection

Specific TRV-strains are transmitted by specific vector species. Consequently, identification of the vector species enables a prediction to be made of the associated virus strain, and *vice versa*. To simplify the species-determination, which traditionally requires much experience, a RT-PCR method should be developed. Different scientists are currently working on this goal and preliminary results indicate that a reliable technique has been developed both for conventional RT-PCR and also for the quantitative “real-time“ multiplex TaqMan® RT-PCR system (D. Brown, pers. comm.).

The RT-PCR-test-results described in this chapter have been undertaken to ascertain if this technology works with TRV isolates from Germany and how the results differ with the use of different materials (tubers and indicator-plant-leaves).

VI.B Material and methods

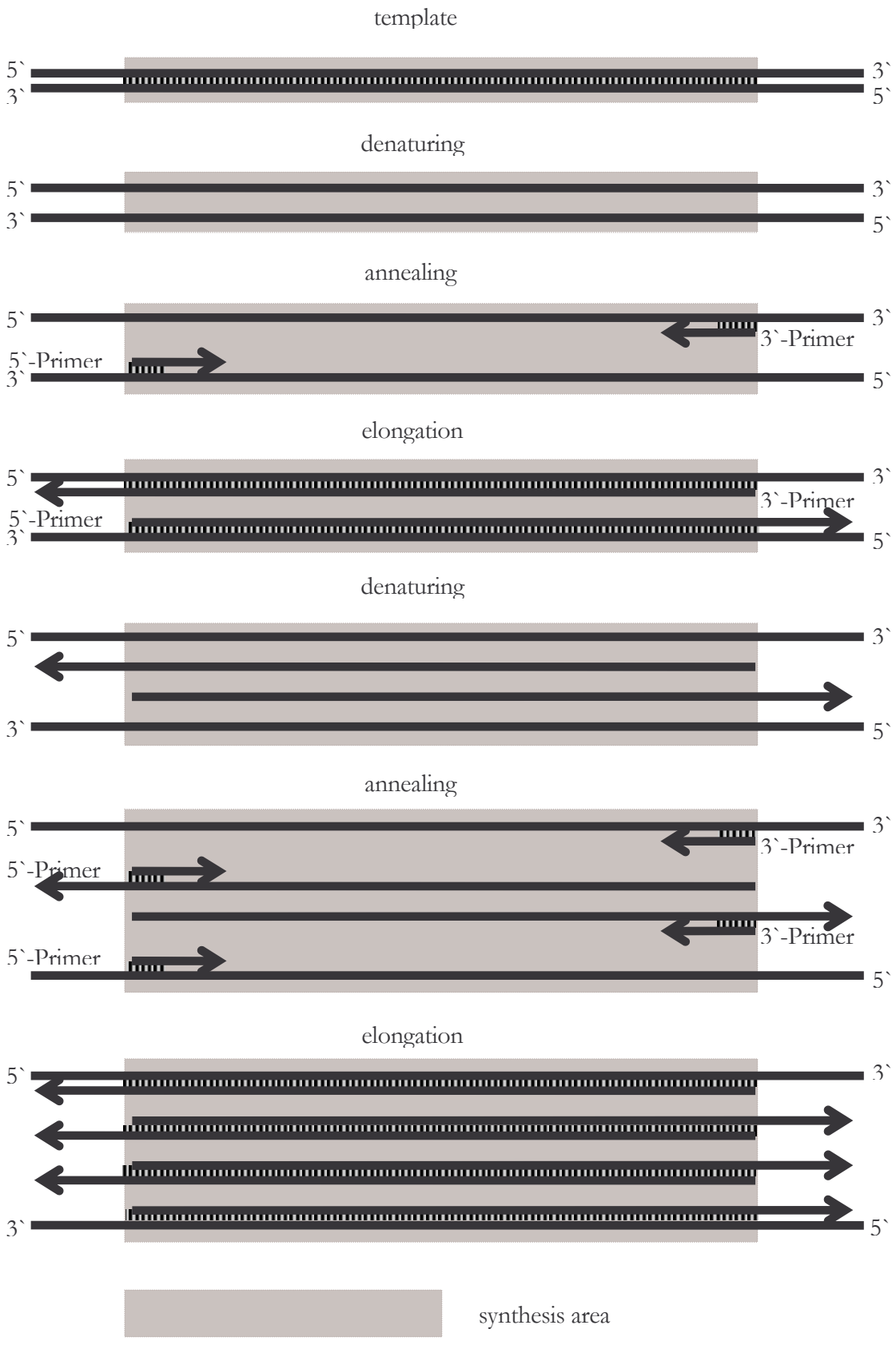
In summary the RT-PCR-method amplifies a region of interest on a nucleic-acid, that is only available in a very low concentration. To detect this region its sequence must be amplified in a high amount to reach detection-level. For this, primers must be designed that bind specifically at the edges of this region. These primers get recognised by a polymerase that performs the amplification of this sequence in contrast to all the other sequences in the sample. As the template, RNA (with a reverse transcriptase step before) as well as DNA can be used. The amplification takes place by alternating denaturing (separation of strands if the template is double-stranded), annealing (= primer binding) and elongation of the copy-strand by polymerase (see Fig. 22).

There are several published reports about the difficulties with TRV-detection and -transmission in tubers (Crosslin & Thomas, 1995). RT-PCR-proof of TRV in tubers works only with the detection of TRV-RNA1 (Weidemann, 1993a). Here primers are made (Weidemann) that are situated in the area of the 16K-ORF that fits several strains (SYM, PRN, Oregon mild). The technique also works with infected but symptom-less tubers.

Also, others have reported successful detection of TRV by RT-PCR in tubers (RNA1) and also in *Nicotiana clevelandii* and *Narcissus* leaves (Crosslin & Thomas, 1995). The proof of origin of TRV by hybridisation of the RT-PCR-products also has been made with a non radio-actively-labelled complementary DNA probe (prepared from purified TRV-RNA) in Southern blot (Crosslin & Thomas, 1995).

A strain-specific detection is not yet possible.

Figure 22: PCR-principle



The results following have been obtained by (Robinson 1992; Hamilton *et al.*, 1987) Phenol-Chloroform-extraction (tubers), RT-PCR and ethidiumbromid-gel-electrophoresis with the following primers (that are situated in the 16K-ORF of the RNA1; constructed with the sequence of TRV-SYM):

Primer A (405): 5´-CAGTCTATACACAGAAACAGA-3´
Primer B (406): 5´-GACGTGTGTACTCAAGGGTT-3´

VI.C Results

Table 28: RT-PCR results

site	1. RT-PCR	2. RT-PCR
S1	positive	positive
S2	positive	positive
S5	positive	positive
S7	positive	positive
S10	positive	positive
S14	negative	negative
S15	negative	negative
S16	negative	negative
S17	negative	negative
S19	negative	negative
S21	positive	positive
S22	positive	positive
S29	positive	positive
S30	positive	positive
S31	positive	positive
S32	negative	negative
S34	negative	negative
S35	positive	positive
S36	negative	negative
S37	negative	negative
S40	positive	positive
S41	negative	negative
S42	negative	negative
S43	positive	positive
S44	positive	positive
S45	positive	positive
S46	positive	positive
S48	negative	negative
S49	positive	positive

Infected and symptom-bearing tubers from the Mellendorf site (S1, see III) were tested in RT-PCR using areas of the tubers that included symptoms and from adjacent areas.

From 13 unequivocally infected tubers from a site proven to contain TRV, only one showed a positive result in RT-PCR. This is probably because of inhibitors in the tuber.

In another test-series leaves of indicator-plants were used. The TRV-isolates were obtained from TRV-positive sites (except the sites S50-S56 from which TRV was subsequently obtained; see III).

The RT-PCR-tests were repeated twice with all samples:

VI.D Discussion

The RT-PCR method used here requires further development to provide reliable results with TRV. Until now it has not been reliable, especially with tubers, probably because of inhibitor-systems. And it is not yet strain-specific. Because the potato-reaction is also strain-specific (see IV) a strain-determination is a prerequisite for reliable cultivar recommendation at sites.

The RT-PCR method is expensive but provides a potentially reliable analysis-method for TRV-detection, and in the future could also be developed to be strain-specific.

For testing of resistance against various strains serology should be used until the RT-PCR method is reliably developed.

VII Control of TRV and vector-trichodorids with antagonistic plants

VII.A Introduction

There are many plant species that are not hosts for TRV or its vectors. The use of these antagonistic plant species could therefore represent a potential control measure for TRV-damage if used as inter-crops.

Two types of antagonistic plants can be differentiated:

One that is directly toxic to the nematode in the foliage, roots or root exudates (Caswell-Chen & Sharma, 1996), and the second being non-hosts for the nematodes or virus. Thus direct and indirect effects can be distinguished.

Direct effects are the production of toxins; that are chemical suppressants that inhibit, for example, the nematode life cycle or biology *e.g.* egg hatch, movement, root penetration, development, fecundity and mate finding.

Indirect effects are the induction of suppressive rhizospheres.

Plants that are antagonist to nematodes act through modification of functional groups, for example, more starch/gelantine-hydrolyzation, production of siderophores or hydrogen cyanide, phenol-oxidation, and/or alter the community structure of rhizosphere bacteria (Kloepper *et al.*, 1996).

The effectiveness of antagonistic plants against nematodes is dependant on the nematode-species. For example, *Chloris gayana*, *Digitaria decumbens*, *Raphanus sativus*, *Tagetes patula*

and *T. erecta* have been reported to control different nematode species (Caswell-Chen & Sharma, 1996).

Marigolds (*Tagetes* sp.) reduced cyst nematodes (*Heterodera schachtii*) and root-knot nematodes (*Meloidogyne hapla*) when planted at infected sites or if their root-exudates were added to soil. They do not work with lesion nematodes (*Pratylenchus penetrans*) (Riga & Potter, 1998). *Tagetes* sp. are effective only with endo-parasites and not with ecto-parasites such as Trichodorids.

Some companies (Schlathölter & Petersen, 1997) breed nematode-resistant oil-radish- and mustard-cultivars and with such plants a 90% reduction in infection was recorded with *Heterodera schachtii*.

If a good host for trichodorids and not for TRV is present, the trichodorids feed on the plant and thus release virus particles, however the virus does not establish an infection in the plant (Maas, 1975) and eventually the nematodes become virus-free.

Cereals and grasses are frequently used as inter-crops with potatoes.

Less TRV-damage has been reported after summer-barley than after sugar-beet, maize and potatoes (Maas, 1974 and 1975).

Barley (summer- and winter-cultivars; Weidemann, 1981) and perennial ryegrass (Cooper & Harrison, 1973) are non-hosts for TRV, but are excellent hosts for trichodorids (Weidemann, 1981). The number of *Paratrichodorus anemones* under wheat was twice as high as in unplanted soil, and under barley was three times as high (Spaull, 1980). Also Italian ryegrass (*Lolium multiflorum*) is a good host for trichodorids but is a non-host for TRV (Aartrijk, 1996).

Many weed species are hosts both for the nematodes and TRV, therefore good weed-control is very important.

The frequently used *Raphanus sativus/* oil-radish is a non-host for TRV and trichodorids (Aartrijk, 1996), and therefore is suitable as an inter-crop.

crop-rotation-patterns:

In the Netherlands different crop rotations with potato, winter-wheat, fodder radish, sugar-beet, onion, tulip and green manure crops, were examined, and the best result was obtained when fodder radish was planted before potato (Hartsema & Molendijk, 1998).

In Germany several green-manure-plants have been tested in infected pots in which trichodorids and TRV had been established (PSA Ahlem, 1971 and 1974). A very strong infestation (= TRV damage) occurred with yellow mustard; strong infestation with *Phacelia*, winter-rape and *Brassica rapa* (var. *rapa* winterform/turnip rape), weak infestation with *Brassica rapa* (turnip), *Lolium sp.* and *Brassica rapa* (var. *rapa* winterform/turnip rape Perko) and very weak infestation with oil-radish and lupins.

In field-tests at three different sites the effect on TRV infection in potato was investigated following the growing of green-manure-plants (PSA Ahlem, 1975). Three potato cultivars were examined and the damage had decreased after lupins, oil-radish and *Brassica rapa* (var. *rapa* winterform/ turnip rape Perko), with winter-rape, *Brassica rapa* (var. *rapa* winterform/ turnip rape) and yellow mustard the infection rate had not significantly decreased.

Also, a decrease in TRV infection was recorded in pot- and field-tests after oil-radish, *Brassica rapa* var. *rapa* winterform/turnip rape (Perko) and lupins had been planted before potatoes (Kegler *et al.*, 1984).

In preliminary examinations TRV-infection with trichodorids was higher under beet and potatoes and lower under barley (especially summer-barley) (Meyer & Schönbeck, 1972; Meyer & Schönbeck, 1976). However, these results need to be confirmed in a field-experiment where potatoes have been planted after the different crop-rotations. Rotations of summer-barley > yellow mustard, summer-barley > winter-barley and *Beta vulgaris* var. *crassa* resulted in a higher rate of TRV infection in potatoes. Less TRV-damage was present after summer-barley > oil-radish and summer-barley > winter-barley, as a result of a significant decrease in nematode numbers.

Results from pot-tests with naturally infected soil (*Trichodorus pachydermus*, *T. viruliferus* and TRV) identified *Stellaria media* and tobacco as good hosts for TRV and trichodorids. Many *Graminea* (summer-, winter-barley), *Brassica rapa* (var. *rapa* winterform/turnip rape), yellow mustard and *Brassica rapa* (turnip) are hosts only for trichodorids, and potato, Phacelia, winter-rape and yellow mustard are hosts only for TRV (Symalla, 1972). Asparagus is a non-host for both trichodorids and TRV (Rohde and Jenkins, 1957).

Table 29: summary

plant species/group	TRV-host	trichodorid-host	TRV-damage	literature
Graminea	no			(Meyer & Schönbeck, 1976)
		yes		(Meyer & Schönbeck, 1976)
barley	no			(Cooper & Harrison, 1973) (Weidemann, 1981) (Kegler et al., 1984) (Meyer & Schönbeck, 1976)
		yes		(Spaull, 1980) (Weidemann, 1981) (Kegler et al., 1984) (Meyer & Schönbeck, 1976)
			increase	(Kegler et al., 1984)
			decrease	(Maas, 1974) (Maas, 1975)
wheat		yes		(Spaull, 1980)
winter-rye	yes			(Kegler et al., 1984)
			decrease	(Kegler et al., 1984)
perennial ryegrass	no			(Cooper & Harrison, 1973)
Italian ryegrass		yes		(Aartrijk, 1996)
			increase	(Aartrijk, 1996) (Asjes et al., 1999)
Lolium sp.			decrease	(PSA Ahlem, 1974)
lupins	no			(Meyer & Schönbeck, 1976)
			decrease	(PSA Ahlem, 1974) (PSA Ahlem, 1975) (Kegler et al., 1984)
Raphanus sativus = oil-radish	no			(Aartrijk, 1996) (Meyer & Schönbeck, 1976)
		no		(Aartrijk, 1996) (Meyer & Schönbeck, 1976)
			decrease	(PSA Ahlem, 1974) (PSA Ahlem, 1975)
fodder radish			decrease	(Hartsema & Molendijk, 1998) (Asjes et al., 1999)
yellow mustard	yes			(Meyer & Schönbeck, 1976)
		yes		(Meyer & Schönbeck, 1976)
			increase	(PSA Ahlem, 1974) (Meyer & Schönbeck, 1976)
phacelia	yes			(Meyer & Schönbeck, 1976)
			increase	(PSA Ahlem, 1974)
Brassica rapa var. *rapa* winterform/ turnip rape		yes		(Meyer & Schönbeck, 1976)
			increase	(PSA Ahlem, 1974)

Brassica rapa/ turnip		yes		(Meyer & Schönbeck, 1976)
			decrease	(PSA Ahlem, 1974)
Beta vulgaris var. *crassa*		no		(Meyer & Schönbeck, 1976)
winter-rape	yes			(Meyer & Schönbeck, 1976)
			increase	(PSA Ahlem, 1974) (Kegler et al., 1984)
Brassica rapa var. *rapa* winterform/ turnip rape (Perko)			decrease	(PSA Ahlem, 1974) (PSA Ahlem, 1975)
Lucerne			decrease	(Kegler et al., 1984)
red clover	no			(Meyer & Schönbeck, 1976)
asparagus	no			(Meyer & Schönbeck, 1976)
		no		(Meyer & Schönbeck, 1976)

Table 30: assessment of the plants referred to in the literature

scientific name	used abbreviation	TRV-host	trichodorid-host	TRV-damage
Trifolium incarnatum L.	clover			
Lolium westerwoldicum L.	grass			decrease
Lolium perenne L.	grass +	no		
Trifolium repens L.	white clover			
Fagopyrum tataricum (L.) Gaertn.	Buckwheat			
Raphanus sativus L.	oil-radish	no	no	decrease
Sinapis sp. L.	yellow mustard	yes	yes	increase
Secale cereale L.	rye	yes		decrease
Lupinus sp. L.	lupins	no		decrease

VII.B Material and Methods

In an extensive field-experiment at Osterloh (S10) in Lower Saxony near Celle the influence of selected potential antagonistic plants on vectors, TRV and potato-symptoms were investigated. Trichodorids and TRV had previously been detected at this site, and field soil was also used in concurrent pot tests.

The inter-crops tested were:

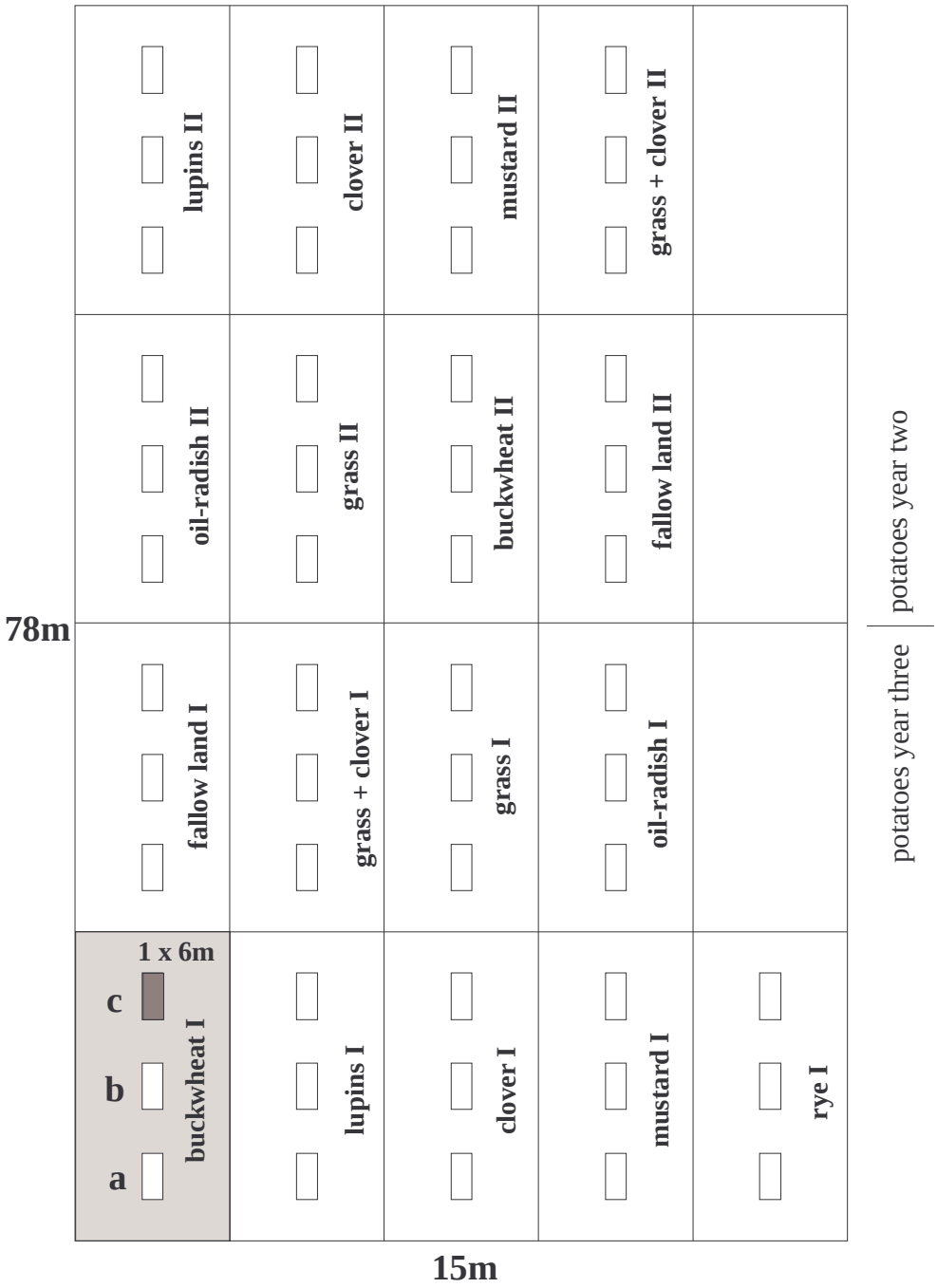
Table 31: inter-corps

used abbreviation	**English name**	**German name**	***scientific name***	***cultivar (DSV)***
clover	crimson clover	Inkarnatklee	*Trifolium incarnatum* L.	Opoolska
grass	annual ryegrass	einjähriges Weidelgras	*Lolium westerwoldicum* L.	Lifloria
grass +	perennial ryegrass	deutsches Weidelgras	*Lolium perenne* L.	Limes (90% of mixture M2)
white clover	white clover	Weißklee	*Trifolium repens* L.	Lirepa (10% of mixture M2)
buckwheat	buckwheat	Buchweizen	*Fagopyrum tataricum* (L.) Gaertn.	Lifago
oil-radish	oil-radish	Ölrettich	*Raphanus sativus* L.	Rufus
yellow mustard	yellow mustard	Gelbsenf	*Sinapis sp.* L.	Hohenheimer
rye	rye	Roggen	*Secale cereale* L.	
lupins	lupins	Lupinen	*Lupinus sp.* L.	

Each crop was planted in two plots (I and II) that were situated randomly to compensate for soil differences. Each plot was 3x19,5m and contained 3 subplots of 1x6m (a, b, c).

The field test had the following layout:

Figure 23: layout of the test-field



Year one:

Eight different inter-crops (buckwheat, lupins, clover, mustard, rye, grass, grass + white clover, oil-radish) were planted in each of two plots, also two fallow plots were included. Rye only was planted in one plot. Because of a very dry period the inter-crops had to be replanted early in the growing season.

Year two:

Half of the plots were planted with the same inter-crops as year one, whereas in the other part of this area a TRV susceptible potato cultivar (Amigo, starch cultivar) was planted. The potatoes were grown in 16 rows on 12m.

Year three:

The area on which the inter-crops had been planted in year one and two were planted with the potato cultivar Amigo. The other area was not examined, because the cultivation of potatoes for two years in succession is not common.

During spring and autumn, soil-samples were taken from each subplot from the surface to 30cm depth (see IIB). The soil was used for the following examinations:

The vector species were extracted, determined and counted (see IID1 and IID2).

The virus content of the soil was determined by bait-testing (see IIE1) followed by ISEM (see IIE8). The inter-crop-species and the weeds present were tested for TRV (see IIE1 + IIE8).

The percentage of virus-containing vectors in the total trichodorid-population was determined by transmission tests with single nematodes (see IID3).

The potato tubers of years two and three were examined for TRV-spraing disease symptoms. This was done by cutting the potato-tubers in 5mm slices and examining each slice immediately for disease symptoms.

VII.C Results

VII.C.1 Potato-tuber-survey

The severity of the symptoms was assessed following the index of Richardson (1970).

The occurrence-severity-index includes the number of tubers showing symptoms and the influence of the severity of symptoms.

The severity-index also expresses the percentage of symptomless tubers in the crop.

Because of its greater importance only the occurrence-severity-index is dealt with in the interpretation of the results.

occurrence-severity-index

$$\frac{\left(\begin{array}{c}\textbf{number of tubers with light symptoms +}\\ \textbf{2x number of tubers with moderate symptoms +}\\ \textbf{4x number of tubers with strong symptoms}\end{array}\right)}{\textbf{total number of tubers (with and without symptoms)}}$$

severity-index

$$\frac{\left(\begin{array}{c}\textbf{number of tubers with light symptoms +}\\ \textbf{2x number of tubers with moderate symptoms +}\\ \textbf{4x number of tubers with strong symptoms}\end{array}\right)}{\textbf{total number of tubers with symptoms}}$$

VII.C.1.a Comparison

Table 32: comparison

	year two					year three			
crop	OSI	STS	SD	conclusion		OSI	STS	SD	conclusion
fallow land	100%	100%	35-44%	control		100%	100%	17%	control
rye	-	-	-	-		58%	53%	111-119%	insignificant
buckwheat	644%	450%	53-54%	increase		100%	78%	75-80%	constant
lupins	56%	49%	18-20%	decrease		58%	51%	141-143%	insignificant
clover	244%	190%	63-64%	increase		69%	73%	120-124%	insignificant
mustard	1322%	878%	24-31%	increase		125%	107%	116-127%	insignificant
grass + white clover	333%	240%	59-60%	increase		3%	4%	0-35%	decrease
grass	444%	359%	48%	increase		250%	209%	19-26%	increase
oil-radish	222%	166%	53-60%	increase		6%	7%	50-74%	decrease

OSI = occurrence-severity-index
STS = share of tubers with symptoms
SD = standard-deviation

The potatoes harvested at the end of the second year, planted after one year of inter-crops, showed significant differences in the degree of TRV infection:

Lupins caused a lower number of infested tubers as compared to the fallow control.

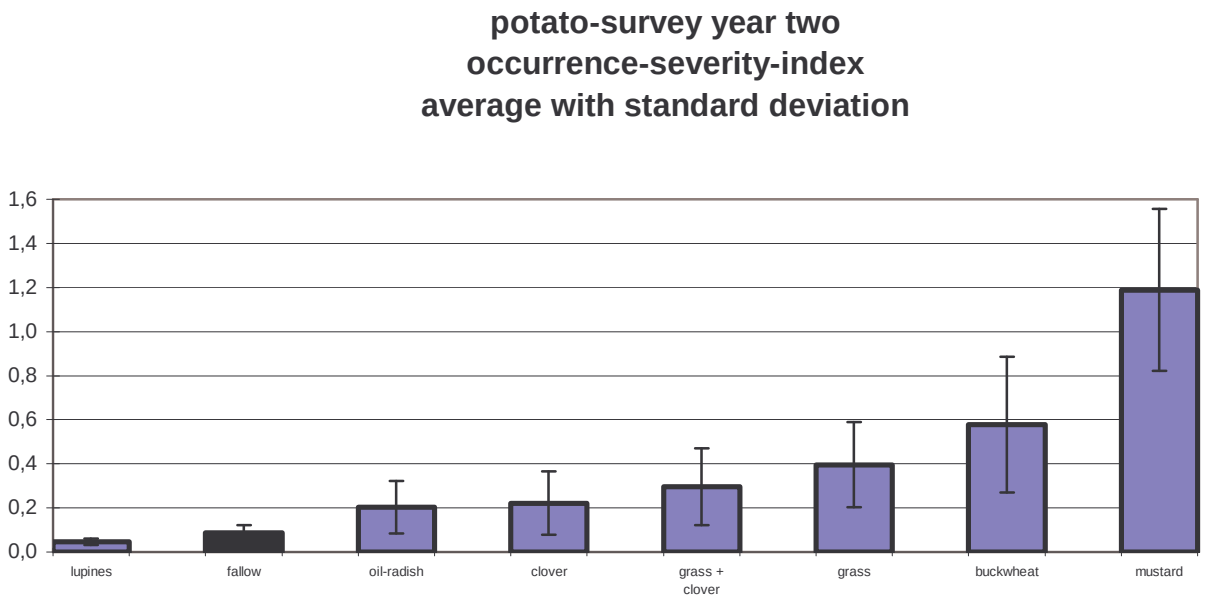
Oil-radish, grass/white clover, clover and grass produced an intermediate level of infection.

Buckwheat and yellow mustard produced an increase in infection.

Also, the infection level was lower in the lupin-plots than in the buckwheat and yellow mustard plots.

The potato-symptoms of the third year, planted after two years of inter-crops, did not show statistically significant differences from the fallow control plots in four of the eight crops (insignificant = results with a standard-deviation of more than 80%). It is probable that the many weeds in the plots, and the extremely dry period in the third year, are responsible for this result (see VIIA1 + VIID).

Figure 24: potato-survey year two; occurrence-severity-index and proportion of tubers with symptoms



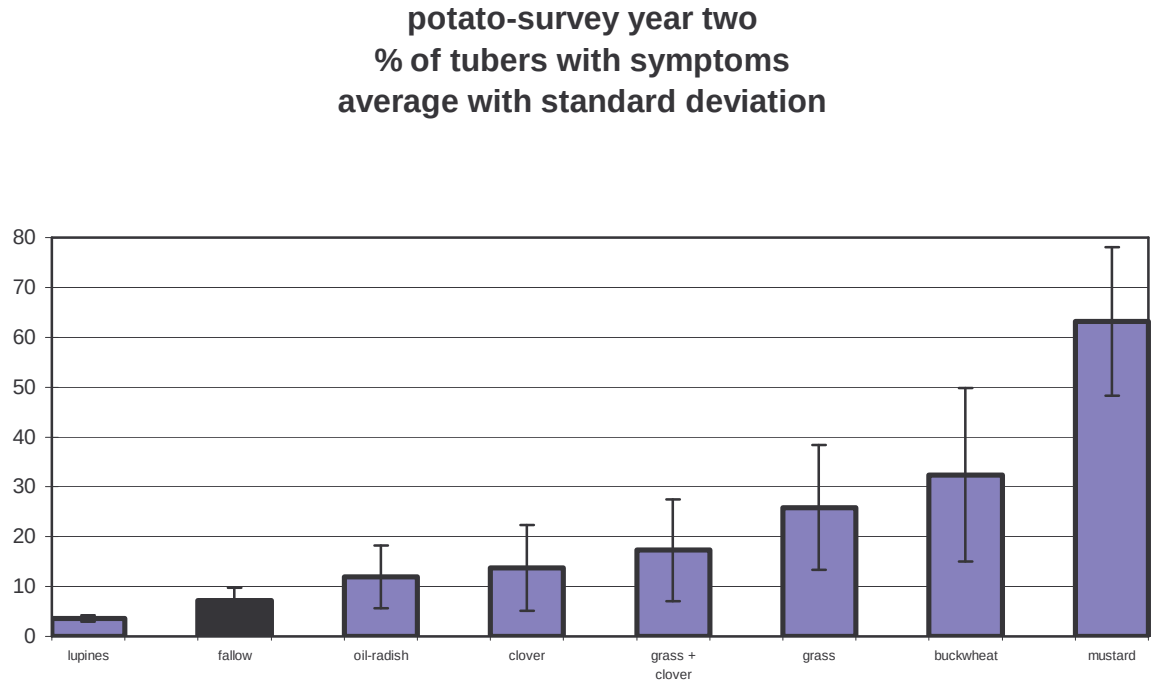
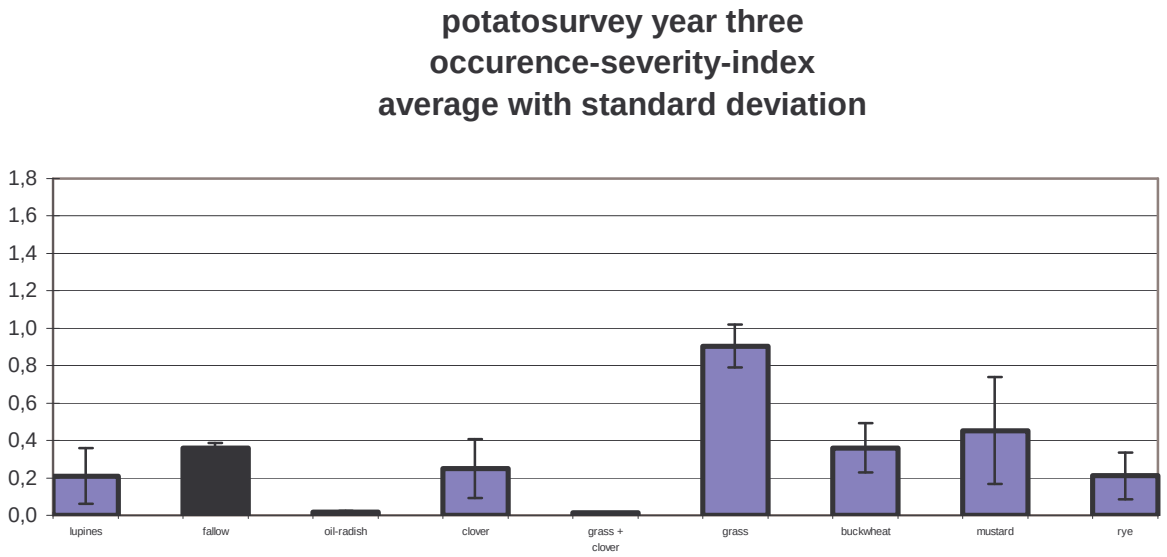
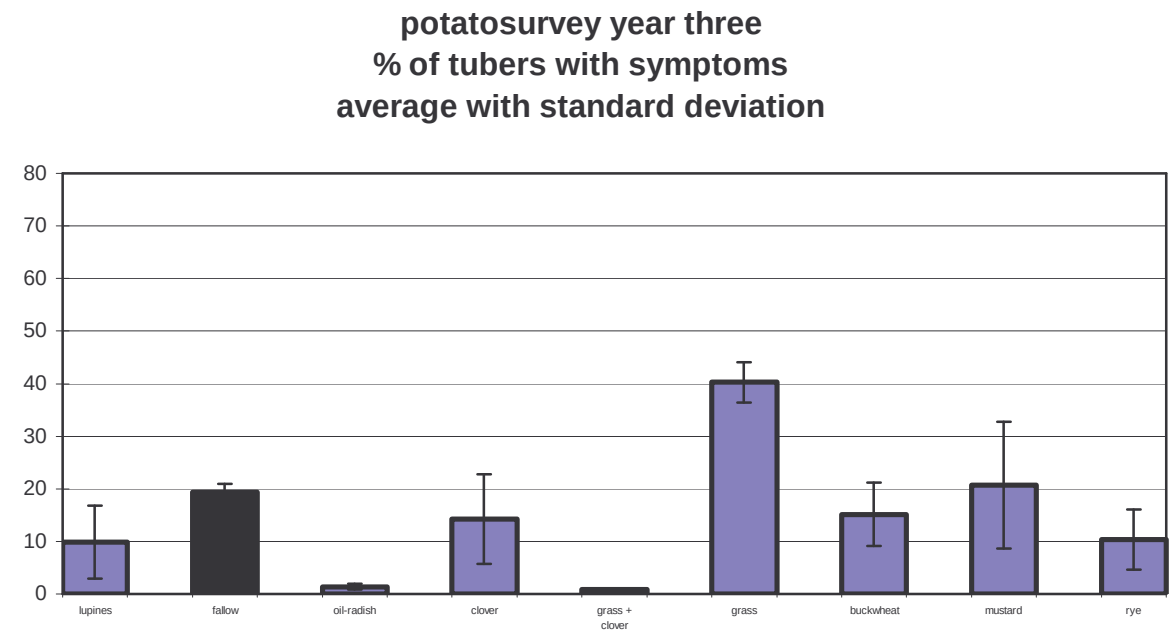


Figure 25: potato-survey year three; occurrence-severity-index and proportion of tubers with symptoms





The fallow plot results were used as a control and as a comparison for the inter-crop-results. The three subplots showed relatively even results (standard-deviation between 3 and 35%)

Rye was planted in the year before potato-symptoms occurred first at this site, therefore it has been included, although rye is not an inter-crop in the common meaning of the term. The rye plot was included only in the second potato-planting year (harvest year three).

The results from the second year revealed a reduction of symptom-occurrence as compared with the fallow-control in the occurrence-severity-index (= OSI; 58%) and in the proportion of tubers with symptoms (= STS; 53%). But the standard-deviation (= SD) was too large (111-119%) to derive any meaningful conclusion from these results.

The buckwheat-plots showed an increase in symptoms in year two to 644% (OSI) resp. 450% (STS) compared with the fallow-control (SD 53-54%).

The OSI in year three was nearly the same as the fallow-control (100%) and the STS had decreased only slightly (78%) (SD 75-80%).

The lupins-plots showed a decrease of symptoms in year two to 56% (OSI) resp. 49% (STS) compared with the fallow-control (SD 18-20%).

The OSI in year three was lower than the fallow-control (58%) and the STS had decreased (51%) but the SD of 141-143% was too large for any meaningful interpretations of these results.

The clover-plots showed an increase of symptoms in year two to 244% (OSI) resp. 190% (STS) compared with the fallow-control (SD 63-64%).

The OSI in year three was lower than the fallow-control (69%) and the STS had decreased (73%) but with a SD of 120-124% no conclusions could be made.

The mustard-plots showed an increase of symptoms in year two to 1322% (OSI) resp. 878% (STS) compared with the fallow-control (SD 24-31%).
The OSI was slightly higher in year three than the fallow-control (125%) and the STS was almost the same as in year two (107%). The SDs were 116-127%, therefore meaningful interpretation was not possible.

The grass + white clover-plots showed an increase of symptoms in year two to 333% (OSI) resp. 240% (STS) compared with the fallow-control (SD 59-60%).
The OSI in year three was lower than the fallow-control (3%) and the STS had decreased (4%) (SD 0-35%).

The grass-plots showed an increase of symptoms in year two to 444% (OSI) resp. 359% (STS) compared with the fallow-control (SD 48%).
The OSI in year three was higher than the fallow-control (250%) and the STS had increased (209%) (SD 19-26%).

The oil-radish-plots showed an increase of symptoms in year two to 222% (OSI) resp. 166% (STS) compared with the fallow-control (SD 53-60%).
The OSI in year three was lower than the fallow-control (6%) and the STS had decreased (7%) (SD 50-74%).

Table 33: results in detail

	year two				year three			
	STS	OSI	SI	n	STS	OSI	SI	n
fallow	**7.2% ± 2.5%**	**0.09 ± 0.04**	**1.17 ± 0.12**	**209**	**19.31% ± 3.33%**	**0.36 ± 0.06**	**1.87 ± 0.05**	**151**
rye	-	-	-	-	**10.32% ± 11.45%**	**0.21 ± 0.25**	**1.57 ± 0.47**	**180**
buckwheat	**32.41% ± 17.35%**	**0.58 ±0.31**	**1.78 ± 0.03**	**221**	**15.13% ± 12.05%**	**0.36 ± 0.27**	**1.66 ± 1.20**	**128**
lupins	**3.54% ± 0.64%**	**0.05 ± 0.01**	**1.29 ± 0.21**	**222**	**9.84% ± 13.92%**	**0.21 ± 0.30**	**0.71 ± 1.01**	**191**
clover	**13.70% ± 8.62%**	**0.22 ± 0.14**	**1.62 ± 0.07**	**200**	**14.19% ± 17.04%**	**0.25 ± 0.31**	**1.41 ± 0.33**	**174**
mustard	**63.18% ± 14.94%**	**1.19 ± 0.37**	**1.86 ± 0.27**	**159**	**20.69% ± 24.08%**	**0.45 ± 0.57**	**1.56 ± 0.55**	**165**
grass + white clover	**17.29% ± 10.19%**	**0.30 ± 0.18**	**1.67 ± 0.17**	**165**	**0.81% ± 0.28%**	**0.01 ± 0.00**	**1.67 ± 0.47**	**165**
grass	**25.87% ± 12.54%**	**0.40 ± 0.19**	**1.53 ± 0.04**	**191**	**40.27% ± 7.69%**	**0.90 ± 0.23**	**2.22 ± 0.23**	**194**
oil-radish	**11.97% ± 6.30%**	**0.20 ± 0.12**	**1.60 ± 0.18**	**221**	**1.39% ± 1.03%**	**0.02 ± 0.01**	**0.75 ± 0.54**	**168**

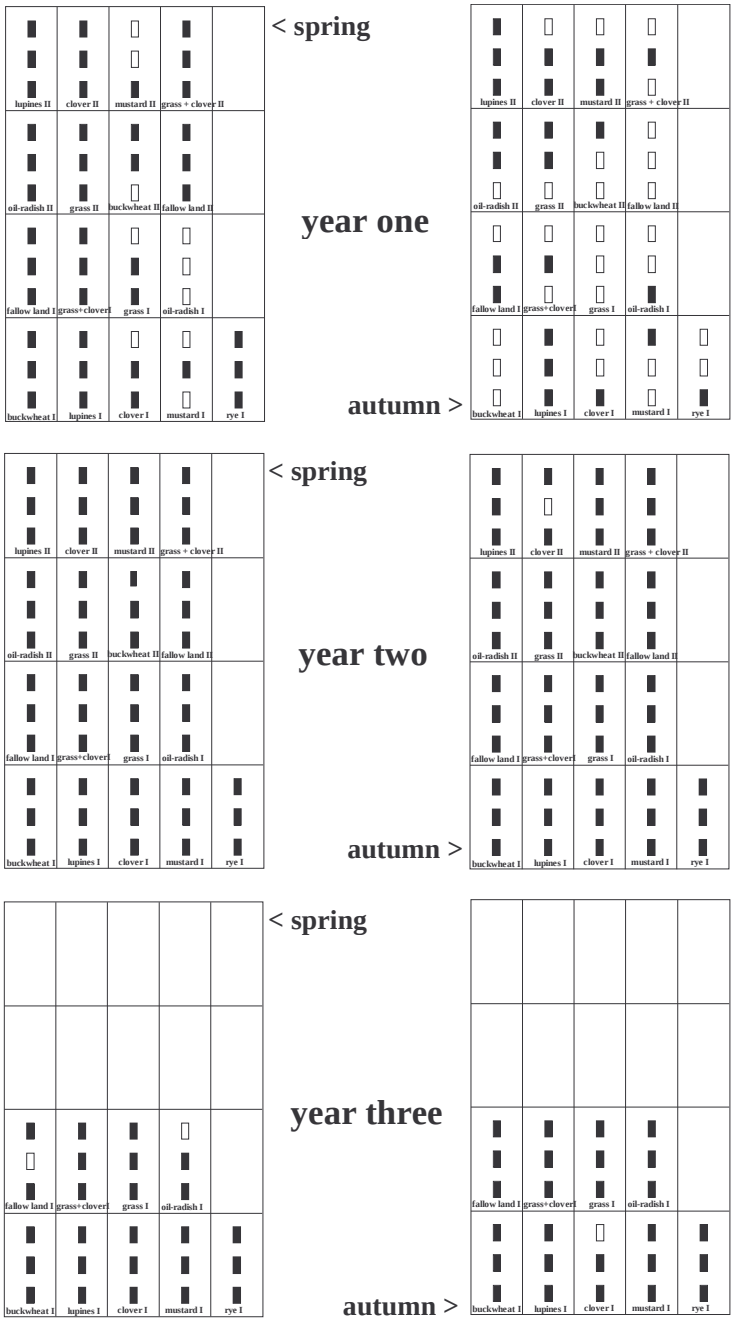
n = total number of tubers
STS = share of tubers with symptoms
OSI = occurrence-severity-index
SI = severity-index

VII.C.2 Virus-test in the soil

A bait test for the detection of TRV was carried out (see IIE1). Except in autumn in year one about the entire area was found contaminated with TRV (88.2 - 100% of the subplots with positive result). The divergent results of autumn in year one (45.1%) was probably the result of a very dry period that had preceded the sampling that had resulted in the trichodorids being present at lower soil depths.

The black fields of the Figure 26 show a positive result in the bait-test, white fields are negative results.

Figure 26: virus-test-results at the test-area



VII.C.3 Nematode-counting and -determination

Trichodorids were extracted, determined and counted from the soil-samples. The first examination yielded five species (determination confirmed by Prof. Dr. Derek J. F. Brown, SCRI):

Trichodorus similis	39%
Paratrichodorus pachydermus	26%
Paratrichodorus teres	22%
Trichodorus viruliferus	9%
Trichodorus primitivus	4%

The number of trichodorids was between 6 and 80 per 100g soil (average 35).

Dependant on plant-species the number of nematodes of 3 to 7 per 100g soil is not unusual (Alphey, 1985; Cooper & Thomas, 1970), thus the data of this examination was higher than expected.

The share of trichodorids in the total number of soil nematodes was 0.3 to 12%.

Trichodorids are usually present in soils extremely unevenly distributed (= aggregated distribution pattern; aid Auswertungs- und Informationsdienst, 1997). Therefore, the results obtained here failed to provide any significant evidence of the affect of inter-crops on nematode numbers.

Potato tubers are infected with TRV at a very young stage, at tuber initiation. A single feed by a vector is enough to transfer virus. Therefore, only very few vectors are needed to cause a total infection of all the tubers and a diminution of trichodorid-numbers therefore could be meaningless if not result in a total extermination of all trichodorids. Also, the number of trichodorids does not indicate the proportion containing virus. The proportion of virus-

containing trichodorids of all occurring trichodorids has been determined, in single-nematode-transmission-tests (see IID3). One *Trichodorus similis* and one *Paratrichodorus teres* of 36 tested specimens transmitted TRV, that is 5,6%, however this test underestimated the number of transmitting nematodes.

Five trichodorid species were present and the different species could possibly be influenced differently or at different degrees by the antagonistic plants.

The fallow land plots served as the negative control. Because no plants had been grown here, no root-material was available for trichodorids to feed on. Therefore, it was suspected that the number of trichodorids should have been less in these plots than in plots with plants.
The total number of trichodorids was between 0 (spring year one, IIc) and 89 (autumn year one, IIb), and the relative share of trichodorids to all nematodes was between 1% (autumn year two, Ia) and 12% (spring year two, IIc).

Rye: The total number of trichodorids ranged from 3 (autumn year two, Ia) to 50 (autumn year one, Ic), and the relative share of trichodorids to all nematodes was between 0.4% (autumn year two, Ia) and 2.3% (spring year one, Ia).

Buckwheat: The total number of trichodorids ranged from 1 (spring year one, Ic) to 135 (autumn year one, IIb), and the relative share of trichodorids to all nematodes was between 0.3% (spring year one, Ic and IIa) and 7% (autumn year one, IIc).

Lupins: The total number of trichodorids ranged from 0 (spring and autumn year one, Ia; autumn year two, Ia and spring year one, Ib) to 59 (autumn year one, IIa), and the relative share of trichodorids to all nematodes was between 0% (spring and autumn year one, Ia; autumn year two, Ia and spring year one, Ib) and 3% (autumn year one, IIa).

Clover: The total number of trichodorids ranged from 0 (autumn year one, Ia; spring year two, Ia and spring year one, Ib) to 159 (autumn year one, IIa), and the relative share of trichodorids to all nematodes was between 0% (autumn year one, Ia; spring year two, Ia and spring year one, Ib) and 7% (spring year two, IIb).

Mustard: The total number of trichodorids ranged from 0 (spring year one, Ib) to 268 (spring year two, IIb), and the relative share of trichodorids to all nematodes was between 0% (spring year one, Ib) and 19% (spring year two, IIb).

Grass + clover: The total number of trichodorids ranged from 6 (spring year one, IIc) to 174 (spring year two, IIa), and the relative share of trichodorids to all nematodes was between 0.7% (autumn year two, Ic) and 12% (spring year two, IIa).

Grass: The total number of trichodorids ranged from 1 (spring year one, IIa) to 132 (autumn year one, IIb), and the relative share of trichodorids to all nematodes was between 0.5% (spring year one, Ia) and 7% (autumn year one, Ic).

Oil-radish: The total number of trichodorids ranged from 1 (spring year one, Ib) to 148 (autumn year one, Ic), and the relative share of trichodorids to all nematodes was between 0.5% (autumn year two, Ia) and 6% (spring year one, Ic).

VII.C.4 Virus-test in inter-crops

The antagonistic plants were examined in bait-tests to determine their susceptibility to TRV infection, but virus was not detected in these tests! Therefore, weed species growing in the plots, and not the antagonistic plants, served as virus reservoirs from which the nematodes required virus.

VII.C.5 Virus-test in weeds

Weeds growing at the test sites were tested for the presence of TRV the year following the completion of the experiment. At this time the field was planted with maize with a very strict weed-control. Therefore very few weeds could be found, and then only at the edge of the field.

The weed-species tested were:

Table 34: weed-species

scientific name	German name	mentioned as a TRV-source* or trichodorid-nutrient# in literature
Galinsoga parviflora Cav.	Kleinblütiges Knopfkraut	* (Edwardson & Christie, 1990)
Geranium pusillum Burm.	Kleiner Storchschnabel	
Achillea millefolium L.	Gemeine Schafgarbe	* (Edwardson & Christie, 1990)
Matricaria maritima L.	Geruchlose Kamille	* (Cooper & Harrison, 1973)
Capsella bursa-pastoris (L.) Med.	Echtes Hirtentäschel	*# (Weidemann, 1981)
Convolvulus arvensis L.	Acker-Winde	
Galium aparine L.	Kletten-Labkraut	
Chenopodium album L.	Weißer Gänsefuß	* (Cooper, 1971b)
Chaerophyllum temulum L.	Taumel-Kälberkropf	
Viola arvensis Murr.	Acker-Stiefmütterchen	* # (Weidemann, 1981; Cooper & Harrison, 1973)

No infected weeds were found probably because only a few weed species in low numbers could be tested.

Soil was collected from the field and weed seeds present allowed to germinate. These weeds were tested for the presence of TRV.

The weed-species tested were:

Table 35: weed-species

scientific name	German name	mentioned as a TRV-source* in literature
Convolvulus arvensis L.	Acker-Winde	
Solanum nigrum L.	schwarzer Nachtschatten	
Chenopodium album L.	Weißer Gänsefuß	* (Cooper, 1971b)
Echinochloa crus-galli (L.) P. B.	Hühnerhirse	

Only a few weed-species, with only a few specimens of each were obtained. Therefore it was not possible to get a positive result above the detection level from these tests.

There are numerous publications about the virus content of weeds (s.a.), thus it is obvious that the negative results obtained here are not representative, particularly from a site known to be heavily infested with TRV.

VII.D Discussion

The field test revealed that antagonistic plants have a significant influence on TRV infection in potato. This influence is independent of the total number of trichodorids and the occurrence of virus.

In both years the virus content (see VIIC3) of the area was between 93 and 100%.

Trichodorids were found to extremely unevenly distributed at the site (aid Auswertungs- und Informationsdienst, 1997). Therefore it is not unusual that the counting of vectors has given no statistically significant results, as to whether or not the inter-crops has performed an influence on the vector number.

Because the potato tuber becomes infected at a very young stage, when it is just formed, only a single feed by a vector is enough to transfer virus. Only very few vectors are therefore needed to cause an effective infection at a site. This could be the reason that the influence of antagonistic plants doesn't relate to the diminution of the number of vectors.

Also, the number of all trichodorids present does not indicate which proportion contains virus and can transmit it.

After the antagonistic plants had examined for virus-content in bait-tests it was clear that the virus did not infect any of the plant species. This result shows that the antagonistic plants do not propagate the virus or serve as a virus-source for the vector. Most weeds are known as very effective virus-sources and these undoubtedly serve as the major source of infection for the vectors (see VIIC5).

The different vector-species could possibly be influenced differently by the antagonistic plants, and the slightly different soil-conditions in the different plots could have a ´micro-influence´ on the results.

The most probable explanation is that the degree of ground cover provided by the plants plays an essential role through weed suppression. The result in the second year was probably caused by the extremely dry weather. During periods of dry weather green-manure growth is usually low and therefore many weeds are able to grow. This causes a very high rate of infestation and therefore falsifies the results (PSA Ahlem, 1975).)

Lupins are effective soil covering plants (Fig. 27), and thus there were almost no weeds that could serve as virus-sources in this crop. If the number of weeds is small, the vectors have little opportunity to acquire virus.

Another possible factor could be the nitrogen-fixing bacteria of lupins, as they may have an influence on trichodorids acquiring/ transmitting TRV.

Figure 27: plot lupins

Figure 28: plot buckwheat

Figure 29: plot yellow mustard

Weed control wasn't carried out during the experiment. The number of weeds occurring was very high in several cultures such as buckwheat and yellow mustard because of their low ground covering ability (Fig. 28 and 29). Here virus-sources were omnipresent and the vectors had substantial opportunity to acquire virus. This could be the reason for the increase of TRV symptoms in the potatoes.

The results of the experiment reveal that the use of antagonistic plants as inter-crops has potential to control TRV-damage, but not for total control of TRV-damage. The choice of a suitable inter-crop can reduce but not eliminate TRV damage and may represent a practical method for the farmer.

VIII General discussion

Information about TRV infected sites was obtained by collecting samples from locations where a TRV-infection was suspected. If this supposition was confirmed by investigation of the soil sample a questionnaire (see IIIB2) was sent to farmers and consultants at plant-protection-offices. The questionnaire contained 6 different parts (see IIIB1): location, soil-characteristics, occurrence, rotation of crops, symptoms and potato-cultivar

To get an overview of the occurrence of vector nematode species the samples were examined for trichodorids and the specimens found determined to species level (see IID1 and IID2).

TRV was found present in all examined areas of Germany and appears to be spread throughout the country.

The level of damage depends on the extent of potato-cultivation, the more this crop is planted, the more samples were investigated because of the increasing interest of growers in disease-control. The connection between potato-cultivation and extent of infected sites can be assessed with a stock-taking of all sites with this crop. In the present study only sites with suspected TRV infection were investigated, therefore a more comprehensive estimation is required to provide an accurate assessment of the TRV disease problem.

The influence of soil-conditions on symptom-expression and occurrence of TRV-damage are not precisely known. Some authors (Cooper & Harrison, 1972) report that high soil humidity increases the mobility of nematodes and therefore the spread of virus. To determine if this hypothesis is correct, information about soil-characteristics must be collected.

The infected sites mostly are sandy and dry - typical for potato-land.

Disease-occurrence appears to be correlated with soil irrigation.

These conditions are optimal for the vector-nematodes: The sandy soil has large pore-spaces for nematode movement. The high water-content provides a fluid film in which they are highly mobile. Both are prerequisites for vector-movement towards the potato plant and therefore for infection with TRV.

Information about the history of occurrence of TRV-symptoms provided insight as to how farmers deal with TRV-problems. For example farmers try different potato-cultivars or stop potato-cultivation because of a single TRV infection.

Most farmers try different potato cultivars to decrease their losses. Because of the high investment this crop requires (storage-buildings with ventilation-systems and temperature-regulation, expensive machines, participation of starch-factories and marketing co-operatives) farmers have considerable pressure to find solutions to the TRV problem.

The influence of different crops on the occurrence of trichodorids and/or TRV was investigated. In this connection also crop-rotation of the preceding years must be examined to explain accumulations of damage after specific rotation-patterns.

At the sites concerned the dominant rotation-pattern included grain crops (barley, wheat, rye). The high multiplication rate of trichodorid vector nematodes under such crops has been well documented (Spaull,1980; Weidemann, 1981). A large population density increases the possibility of virus-transmission.

Because of the rare use of inter-crops comments on their role for TRV control cannot be made. (For further information see chapter VII.)

Symptom-information was used to investigate the characteristic symptoms in the examined areas that could have been influenced by different cultivars.

Affected potato-tubers from the examined sites mostly showed spots, arcs and cones.

In most instances the damage was assessed as light or intermediate.

A principal objective of the investigation was identification of potential resistant potato-cultivars. Because there have been many different, and often contradictory, reports about resistance-characteristics of potato-cultivars, it is imperative to take stock of the actual situation in Germany.

At the TRV-infected sites, 43 different potato cultivars and up to 85 test cultivars were planted. (For further information see chapter IV.)

The comparison of TRV symptoms and potato-cultivar shows that most cultivars exhibit different symptoms.

To get an overview of the occurrence of vector nematode species the TRV-positive soil-samples were investigated for trichodorids and the specimens found determined to species level.

The investigation resulted in identification of 7 different trichodorid species. Mostly the nematodes were present as species mixtures of two to six species.

Examination of the distribution of the different species did not reveal any geographical preferences.

TRV occurred in all examined areas, causing damage to specific cultivars. Therefore, attention to this problem is required to develop methods to reduce crop losses and to avoid further damage.

A biological assessment (see III) revealed that there is a problem with TRV in Germany and apparently the virus is associated with the reaction of different potato cultivars.

The specific use of resistant or tolerant potato cultivars could provide an inexpensive and reliable procedure for eliminating or reducing damage in potato cultivation. To select the right ones from the range of cultivars is very difficult because of the strain-specific reaction of potato genotypes to TRV. A specific prediction procedure is required.

A tolerance/ resistance study was carried out at a single location in Germany (Mellendorf). To test cultivars in the field is not reliable because of the uneven distribution of virus and the influence of weather. Virus-transmission by a single nematode can infest up to 3 plants within 4 days (Ploeg & Brown, 1997). A rapid spread of infection is therefore possible, also when low numbers of trichodorids are present, if the nematodes are vigorous and mobile in soil, through sufficient soil humidity.

Tests in pots are more reliable than field tests and have less variability (Dale & Solomon, 1988).

The most direct test-method, a direct inoculation of the potato-tuber, till now isn't possible because the infection rate is much too low for practical purpose (Xenophontos *et al.*, 1998).

Cultivation of many cultivars at a site at which the virus strain has been determined, is expensive. Therefore testing of potato cultivars directly against various virus strains is the preferred method of choice to enable reliable resistance statements to be made.

Tubers damaged by TRV are unsuitable as food potatoes or for processed products, such as crisps and chips, and inferior as economy potato (Schütz, 1973). Two per cent affected tubers can result in the complete lot being rejected for human consumption (Spaar & Hamann, 1974). These lots can be used only for starch and fodder production (Schütz, 1973). Additional costs arise for the manufacturer and severe losses of income for the farmer through this disease infection. In a year of over-production of potatoes lots with an even smaller percentage of TRV-symptoms (in UK 1-2%) are rejected.

Recently investigations have led to the conclusion that the size of tubers is much reduced, and they are no longer marketable (Dale *et al.*, 1999). Therefore the financial loss can be substantial or even total.

Another risk is that symptomless tubers can contain TRV without showing a reaction (Xenophontos *et al.*, 1998) These tolerant (Adam, 1997; Habekuß *et al.*, 1997) reacting cultivars represent a high risk for the spread of TRV at other further virus free sites.

In Germany about 132 potato cultivars are cultivated (aid VerbraucherDienst, 1990), and 50% of these can be classified as susceptible according to bibliographical references (Reepmeyer, 1973). The "list of potato cultivars" of the cultivar office of the Federal Government shows a susceptibility-level for TRV in 106 food potato cultivars.

Forty-seven cultivars with the susceptibility "low" and 28 with "low-medium“, are available for low-input farming. Unfortunately, because of strain-specific reaction-differences, these details in the "cultivar list" aren't reliable. The cultivars with low susceptibility such as Roxy, Saturna, Secura and Solara on average had 17% of disease incidence and 1.3% of these lots were rejected. The Berber and Agria cultivars, with middle susceptibility, showed only 2.8% disease incidence which led to the rejection of only 0.5% of the lots. The various virus strains and/or the vectors and location conditions are possible reasons for these discrepancies.

On the light soils of northern Germany production of quality potatoes is complicated by TRV infection. A reliable and strain-specific susceptibility-diagnosis is needed, to identify available cultivars with low susceptibility that can be successfully grown. This will improve the quality of potatoes and thus also the income of farmers in these regions.

For a precise cultivar-recommendation the reaction of a cultivar to a virus strain must be studied. A prerequisite for this is that the virus strain must be precisely determined. For this, different methods (ISEM, decoration, ELISA, PCR) are available and each method has its pros and cons.

Serological methods (ISEM, decoration, ELISA) are well established and relatively inexpensive. At the practical level they need high-technology-equipment (EM, ELISA-reader) that is not available at all plant-protection-offices.

Also the worker needs to have experience (especially with ISEM, decoration) with the virus. These methods do not work with the frequently occurring NM-strains.

The pros and cons of EM- and ELISA-methods (see IIE8) in comparison are:

ELISA is much easier and quicker.

ISEM/decoration delivers no false positive or negative results.

PCR-technology has the advantage to react also with NM-strains, and undoubtedly this will be the method of choice in the future.

The results of all these methods are not totally comparable and any comparison should therefore be done with caution. Therefore the development of a universally valid method is urgently required.

The official resistance recommendations, having been only performed at a single TRV infected site, are not reliable (see IV) because of strain-specific reactions and the result that the methods actually used to estimate the interaction of virus strain, vector-species and potato cultivar are not optimal for all isolates. It is therefore necessary for the future to develop a fast diagnostic procedure for the existence of TRV and the identification of the virus strain. PCR(polymerase chain reaction)-technology based on nucleic-acid provides enhanced detection-sensitivity. This method currently has not been fully developed and thus the strain-specific testing with TRV, which is basis for the resistance-testing, needs to be developed.

PCR-tests could be realised with several materials:

1. leaves of indicator-plants form bait-tests: to deliver an early warning test for a site
2. vector-nematodes: to speed up this early warning test by avoidance of indicator-plants
3. tubers: to be used for the cause-clarification of infected potatoes

4. other parts of potato-plants: to deliver another material if tuber-tests show problems because of inhibitor-systems

Another area of research is the determination of vector-species. Specific TRV-strains are only transmitted by specific vector-species therefore identification of the vector nematodes would enable the virus strain to be predicted, or *vice versa*.

Plant species differ in their host function for TRV, and also for the vector nematodes. The use of these plant species could therefore present a method that could be developed for damage control. Two types of antagonistic plants that are not hosts for TRV and/or trichodorids can be distinguished.

The first group act against nematodes or virus, through having a direct influence. Antagonistic properties of this group (Caswell-Chen & Sharma, 1996) reside in foliage, roots or root exudates. In this context direct and indirect effects can be distinguished. Direct effects include the production of toxins; that are chemical suppressants that inhibit for example the nematode life cycle and biology including egg hatch, root penetration, development, fecundity and mate finding. Indirect effects include the induction of suppressive rhizospheres.

The second group of antagonistic plants suppress the nematodes or virus by not being a host.

If a good host for trichodorids, and not for TRV, is present, the trichodorids feed on the plant and transmit the virus which will not be multiplied in the plant (Maas, 1975). Consequently, the nematode can not re-acquire virus and thus become virus-free.

If a non-host for trichodorids is present, the trichodorids do not feed. Therefore the virus stays in them, till they feed on a suitable host-plant.

In an extensive 3-year-field-experiment the influence of selected potential antagonistic plants on vectors, TRV and potato-symptoms was investigated.

Results from this field experiment revealed that antagonistic plants have a significant influence on TRV symptoms in potato.
During two years of the experiment the virus-content (see VIIC3) of the area was between 93 and 100%.

Trichodorids were found with extremely uneven distributions (aid Auswertungs- und Informationsdienst, 1997). Therefore the counting of vectors does not provide statistically significant results i.e. as to whether or not the inter-crops had influenced the vector numbers.

Only a very few vectors are needed to cause an effective infection. This could be the reason that the influence of antagonistic plants doesn't indicate if the diminution of the number of vectors results in the decrease of TRV infection recorded in the experiment. Also, the number of trichodorids does not indicate the number of specimens carrying TRV.

TRV was not detected in the antagonistic plants thus they are probably non-hosts for the virus. This result revealed that it isn't necessary for the antagonistic plants to propagate virus to serve as a virus-source for the nematodes. Most weeds are known as very effective virus-sources (see VIIC5), and an additional source isn't necessary for the nematodes to acquire virus.

The different vector-species could possibly be influenced differently by the antagonistic plants, also, the slightly different soil-conditions of the different plots could have a ´micro-influence´ on the results.

The most probable cause for the reduction in TRV damage was that the degree of canopy of the plants plays an important role in suppressing weed growth, and hence reduces the availability of sources from which trichodorids can acquire virus. Lupins are effective canopy plants, and there were almost no weeds, that could serve as virus-sources, in this crop. If the number of weeds is small, the vectors have little opportunity to acquire virus.
Another possible factor could be the nitrogen-fixing bacteria of the lupine, as they may have an influence on trichodorids and TRV.

Weed control wasn't carried out in the experiment. The number of weeds occurring was very high in cultures such as buckwheat and yellow mustard because of the low ground cover. Here, virus-sources were omnipresent and the vectors had good opportunity to acquire virus.

The use of antagonistic plants as inter-crops is a potentially effective method for controlling TRV-damage, but does not provide total control. The choice of a suitable inter-crop can reduce but not eliminate TRV damage and may provide the only practicable method for the farmer.

IX Publications

talk

- 1/1999
  Petz, C., Heinicke, D., Zunke, U. & Brown, D.J.F.
  „Antagonistische Pflanzen gegen die Pfropfenkrankheit/Stippigkeit der Kartoffel“
  Diplomanden-Doktoranden-Kolloquium, Institut für Angewandte Botanik, Hamburg

- 1998
  Petz, C., Zunke, U., Heinicke, D., Brown, D.J.F., Heinze, C., Bargen, S. von, Willingmann, P. & Adam, G.
  „Entwicklung eines Praxistests zur Unterscheidung der verschiedenen Stämme des Tabak-Rattle-Virus (TRV) und ihrer Vektorbeziehung“
  Jahresvortrag vor dem Projektträger GFP (Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V.)

- 1998
  Petz, C., Zunke, U., Heinicke, D. & Brown, D.J.F. „The use of antagonistic plants to suppress „spraing“ disease in potato caused by tobacco rattle tobravirus, transmitted by trichodorid nematodes“
  24th European society of nematologists´ international symposium

poster

- 7.-12.7.96
  Nieser, C., Zunke, U., Heinicke, D. & Brown, D.J.F.
  „Distribution of strains of tobacco rattle tobravirus and their vector nematodes (Trichodoridae) in Germany“
  Gouadelope, Nematropica, 4, 2.

- 23.-26.9.96
  Nieser, C., Zunke, U., Heinicke, D. & Brown, D.J.F.
  „Verbreitung der verschieden Stämme des Tabak-Rattle-Virus (TRV) und seiner Vektoren, Nematoden der Familie Trichodoridae, in Deutschland“
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  „Trichodorid vectors of serologically distinguishable strains of tobacco rattle tobravirus occuring in Germany“
  Moskau, Russian journal of nematology, 6, 2.

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  „Trichodorid vectors of serologically distinguishable strains of tobacco rattle tobravirus occuring in Germany“
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- 1/99
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  Sachbericht des Verwendungsnachweises 1998 an die Drittmittelgeber GFP und BML, 8, 1-2.

- 1997
  Petz, C. & Zunke, U.
  „Vorkommen und Bedeutung von Nematoden der Gattungen Trichodorus und Paratrichodorus im Kartoffelanbau und ihre Bekämpfung als Überträger des Tabak-Rattle-Virus (TRV)“
  Jahresbericht 1995-1996 des Instituts für Angewandte Botanik, Universität Hamburg, 9, 1-2.

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  Zwischenbericht und Sachbericht des Verwendungsnachweises (Zeitraum 1.5.97-1.5.98) an die Drittmittelgeber GFP und BML, 10, 1-12.

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