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Molecular Biology of the *Beet necrotic yellow vein virus*

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ABSTRACT

Beet necrotic yellow vein virus is the etiological agent of one of the most devastating sugar beet viral diseases: rhizomania. This review describes the molecular biology of the rhizomania disease, the functions of the BNYVV encoded proteins, the consequences of their expression as well as the biology of the BNYVV vector, *Polymyxa betae*. Root proliferation is an important part of the well-known characteristics of the viral infection that leads to yield and sugar losses. The extensive use of sugar beet cultivars displaying partial resistance or tolerance against virus infection allows containment of sugar yields. However, such extensive uses also permit viruliferous vector to be amplified and therefore the appearance of resistance breaking isolates. We review as well the defence strategies that may be used against rhizomania.

Keywords: *Benyvirus*, rhizomania, *Polymyxa betae*, pathogenicity, ICTVdB Virus Code: 00.088.0.01.001.
Abbreviations: BNYVV, *Beet necrotic yellow vein virus*; **CP**, coat protein; **ORF**, open reading frame; **RB**, resistance breaking; **TGB**, Triple Gene Block

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INTRODUCTION

Sugar beet rhizomania possesses the potential to lower the sugar contents in the roots of susceptible sugar beet cultivars up to 20%. However, sugar companies report yield losses of 50 to 70% during sugar purification (Richard-Molard 1985). Rhizomania was first reported during the fifties in Italy (Canova 1959). Nowadays the disease is present in almost every sugar beet growing area in the world. The etiological agent was discovered by Tamada and Baba in 1973 who demonstrated that rhizomania was induced by the phytovirus named *Beet necrotic yellow vein virus*, BNYVV (Tamada and Baba 1973). Such pathogen classified among the alphavirus-like supergroup is assigned to the genus *Benyvirus* (ICTVdB, http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/). BNYVV is transmitted by the soil-borne obligate parasite *Polymyxa betae* Keskin (Keskin 1964), a protist initially classified in the fungus kingdom. *P. betae* is able to form spores that are extremely resistant to biodegradation, drought and pesticides treatment, that allow viruliferous spores to rest for more than two decades in the infested soils. Breeding efforts were carried out in order to

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Invited Review

maintain the sugar production yields, resulting in the development of sugar beet hybrids partially resistant to BNYVV systemic infection. Such crops are commonly used for commercial production. Their intensive uses among the infested soils applied a positive selection pressure that raised RB isolates. Interestingly, emerging viral species possess sequence variation particularly within RNA-3; the viral component that encodes the pathogenicity determinant p25, involved in root proliferation (Tamada *et al.* 1999).

Actual molecular studies of the rhizomania syndrome tend to reveal the resistance mechanism mediated by the *Rz* resistance gene products as well as the virus induced processes, leading to the root proliferation, particularly by the study of the RNA-3-encoded p25 protein. However, the identification of other *P. betae*-transmitted viruses associated with the rhizomania syndrome rise new questions about the disease etiology. As an example, *Beet black scorch virus* has been shown to induce rhizomania-like symptoms (Weiland *et al.* 2007).

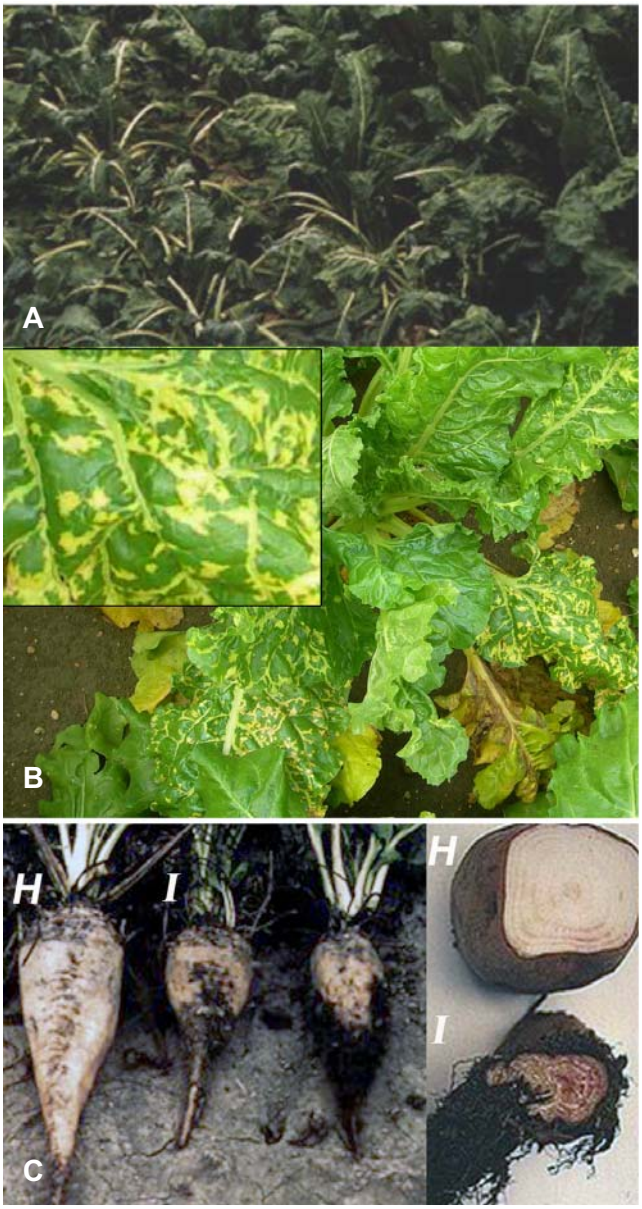

Fig. 1 Symptoms of sugar beet rhizomania. (**A**) During warm period, water deficiencies induced by rhizomania disease provokes plant wilt. (**B**) Foliar symptoms are rather rare and correspond to plant systemic infection. Vein yellowing and yellow chlorotic spots are induced by the expression of the RNA-3-encoded p25 protein. (**C**) Typical root symptoms: infected taproot (I) are shorter, wine glass shaped and display characteristic necrosis and root proliferation compared to healthy plant (H). (Photos **A** and **C**: INRA-Colmar, **B**: Institut Technique de la Betterave, Paris).

RHIZOMANIA DISEASE

Symptoms

The Rhizomania syndrome refers to root madness (Rhizo: root; Mania: madness). Infected sugar beets display more or less a dwarfism that reduces the taproot size, which harbours necrosis (**Fig. 1C**). Infection shapes a wine-glass-like taproot and induces rootlet proliferations that become necrotic, abundant and fragile. These root symptoms reduced water uptake that provoke leaf fading (**Fig. 1A**). Sometimes, when the infection becomes systemic, vein yellowing, necrosis and foliar local lesions appear (**Fig. 1B**). Beet necrotic yellow vein virus was named according to these systemic symptoms.

Histological and biochemical properties of rhizomania diseased plants

Histological sections performed on infected taproot suggested that the infection and/or the necrosis of lateral roots induce the reprogramming of the pericycle cells to meristematic cells, which might cause the synthesis of new rootlets (Pollini and Giunchedi 1989). Moreover, viral sequences could also induce a modulation of the cellular messenger and protein expression profiles leading to root morphogenesis. Thus, root proliferation favors the viral replication and transmission. Indeed, rootlet cells are the targets of BNYVV vector, the parasite *P. betae*.

The plant hormone auxine plays a major role in plant development and particularly on root morphogenesis. Indol-3-acetic acid assays performed onto susceptible and tolerant sugar beet varieties revealed auxin level three times higher in infected plants (Pollini *et al.* 1990). Interestingly, tolerant crops possess lower auxin contents than susceptible plants. One of the questions that still need an answer is to determine if auxin variations are the initiator or the consequence of the cellular disorders induced by BNYVV infection. Comparisons of the root transcriptome of healthy beets with rhizomania infected beets lead to the identification of auxin, cell cycle, defence signalling and ubiquitin-related regulated genes (Schmidlin *et al*. 2008).

BEET NECROTIC YELLOW VEIN VIRUS

Taxonomy

BNYVV possesses a multipartite linear positive-sense single-stranded RNA genome that consists of four to five RNAs possessing 5′ cap and polyadenylated 3′ ends. BNYVV belongs to the supergroup of alphavirus-like and is the type species of the *Benyvirus* genus, which contains as well the *Beet soil-borne mosaic virus* (BSBMV, ICTVdB: 00.088.0.01.002) (Lee *et al.* 2001). BNYVV and BSBMV are rod-shaped, share a similar genomic organization (Lee *et al.* 2001), host range, and are transmitted by the protist vector *P. betae*. However, BSBMV has only been detected within North America. BSBMV and BNYVV are closely related but are distinct viruses since *Rz* resistant genes have no effect on BSBMV accumulation (Wisler *et al.* 2003) and no cross reaction with coat protein antisera, nor cross protection have been described. Recent studies demonstrated that BNYVV RNA-1 and -2 were able to amplify BSBMV RNA-3 and -4, but only in the absence of BNYVV small RNAs (Claudio Ratti and David Gilmer, unpublished). Both viruses could compete for similar host factors. Such hypothesis will be tested as soon as full-length infectious clones of BSBMV RNA-1 and -2 will be available.

Tentative members within *Benyviruses* are *Burdock mottle virus* (BdMV, ICTVdB: 00.088.0.01.004) (Rush 2003) and *Rice stripe necrosis virus* (RSNV, ICTVdB: 00.088.0.01.003) (Morales *et al*. 1999; Van Regenmortel *et al*. 2000; Fauquet *et al*. 2005).

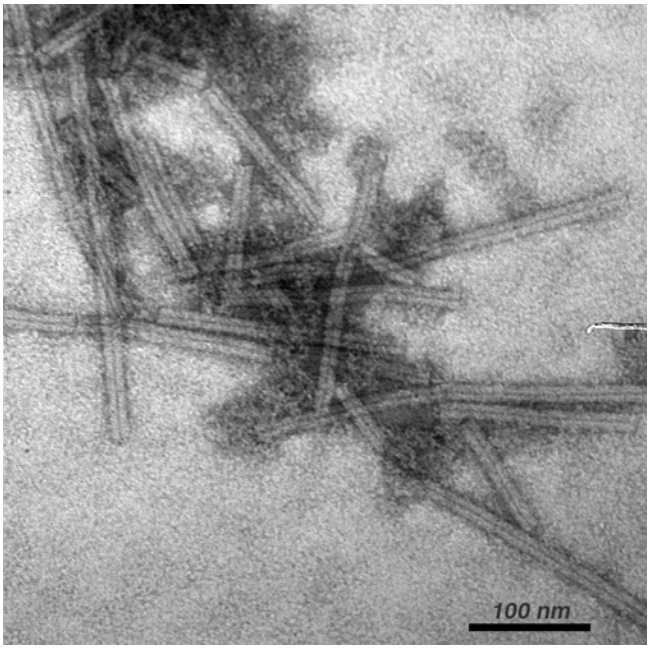

Fig. 2 Immunocaptured BNYVV particles observed by electron microscopy. (Photo M. Erhardt, IBMP).

Host range

BNYVV infects plants within *Amaranthaceae*, *Chenopodiacea* and *Tetragoniaceae* families. Systemic infection occurs naturally onto *Beta vulgaris* (sugar beet) and *Beta macrocarpa*. *Nicotiana benthamiana* and *Spinacia oleracea* are systemically infected even in the absence of RNA-3 and -4. On experimental hosts, BNYVV is propagated onto *Chenopodium quinoa* or *Tetragonia expansa* that respond to the infection by chlorotic or necrotic local lesions, depending on the inoculum composition (Tamada *et al*. 1989; Koenig *et al*. 1991; Jupin *et al*. 1992).

Structure

Viral particles display a right-handed helical symmetry (**Fig. 2**) with a 2.6 nm pitch, 49 capsid protein subunits per repetition of 4 turns, with each subunit interacting with 4 nucleotides (Steven *et al.* 1981). The particles are not enveloped and have a diameter of 20 nm with lengths proportional to the sizes of the encapsidated RNAs, i.e, 390, 265, 105, 89 and 80 nm (Putz 1977; Tamada *et al.* 1989). Coat proteins constitute about 95% of the particle weight. The minor coat protein, a CP amber readthrough gene product (CP-RT) is present at one extremity of particles and participates both to the morphogenesis (Schmitt *et al.* 1992; Haeberle *et al.* 1994) and to the transmission of the virions (Tamada *et al.* 1996).

Genomic organization

BNYVV has a segmented RNA genome composed of 4 to 5 genomic components (Tamada 1999). All components possess a 5′ Cap (m^7GpppG) and a 3′ polyA tail. Sequencing together with the full-length infectious clones (Quillet *et al.* 1989; Link *et al.* 2005) permitted to decipher the viral genomic organization and the main functions of virally encoded proteins (**Fig. 3**).

RNA-1 is involved in the replication of viral RNAs (Gilmer *et al*. 1992a) and RNA-2 is necessary for encapsidation, cell-to-cell movement and RNA silencing suppression (RNA-2), as proven by protoplast and mechanical infection of leaves. RNA-1 and -2 are necessary and sufficient for the infection following leaf mechanical inoculations where small components are dispensable and, if they are present, can undergo deletion or disappear (Bouzoubaa *et al.* 1991). In natural infection, however, these small components are required. Indeed, RNA-3 allows the viral amplification in sugar beet roots and its expression influences symptoms (Tamada *et al.* 1989; Jupin *et al.* 1992), whereas RNA-4 is involved in viral transmission (Tamada and Abe 1989). Moreover, RNA-4-encoded p31 is described as a root specific silencing suppressor (Rahim *et al*. 2007). Therefore, BNYVV is a unique virus as it behaves as a bipartite virus when rub inoculated or as a tetra or pentapartite virus in natural infection. Such a property has been utilized to obtain viral expression vectors, named replicons, and derived from RNA-3 and RNA-5 (Schmidlin *et al.* 2005). Such replicons allow the expression of various sequences within infected tissues (Schmidlin *et al.* 2005).

Genomic functions

RNA-1 is 6746 nts long (without polyA) and harbours only one open reading frame (ORF). It encodes a 237 kDa polypeptide (Bouzoubaa *et al.* 1987) that contains consensus methyl-tranferase (MTR), helicase (HEL), papain-like protease (PRO) and polymerase (POL). Autoproteolysis of p237 between the domains PRO and POL leads to two proteins: p150 and p60, which contain respectively the MTR, HEL, PRO and the POL motifs (Hehn *et al.* 1997). This

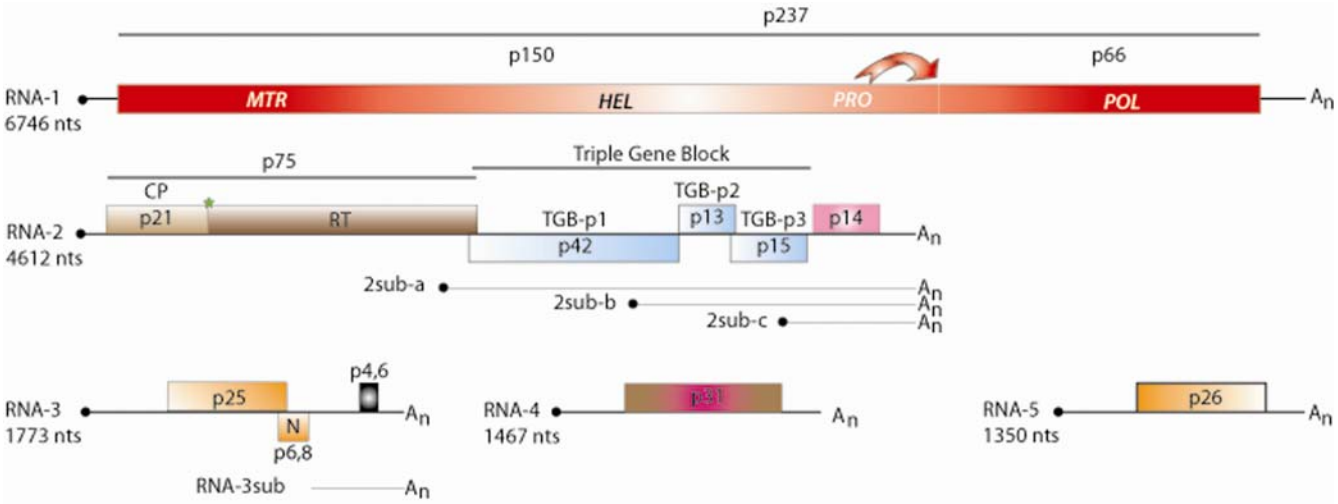

Fig. 3 BNYVV genomic organization. The sizes of the five genomic RNAs are presented. Black dot represents Cap structure and A_n, the polyadenylated sequence. Squares correspond to ORF. Arrow designates the *cis* proteolysis of the RNA-1-encoded p237 polyprotein leading to the p150 and the p66 proteins. MTR, Methyltransferase domain; HEL, Helicase domain; PRO, Protease domain; POL, Polymerase domain. Star denotes the CP amber readthrough mechanism leading to the expression of minor CP-RT protein.

proteolytic clivage of the replicase protein is the significant feature of the *Benyviruses*. However, the stoechiometry of replication complex is not known.

RNA-2 is 4612 nts long and encodes for six proteins.

The first cistron drives the synthesis the 21 kDa major capsid protein, CP. In about 10% of the translation events, CP amber stop codon is suppressed and the resulting read-through leads to the synthesis of the 75 kDa minor coat protein, CP-RT (Niesbach-Klosgen *et al.* 1990; Haeberle *et al.* 1994). CP-RT is needed for the viral transmission (Tamada *et al.* 1996) and for the transient anchoring of the viral particles around mitochondria (Erhardt *et al.* 2001; Valentin *et al.* 2005). These latter studies suggest that the viral genome packaging occurs around mitochondria.

Following the structural protein encoding ORFs, a cluster of three genes, partially overlapping, known as "triple gene block" (TGB) ensures the viral cell-to-cell movement. TGB clusters are also found on other plant viral species, including *Pomo-*, *Peclu-*, *Potex-*, *Carla-* and *Hordeivirus* (Morozov and Solovyev 2003). For BNYVV, these three proteins are encoded by subgenomic RNA-2sub-a for TGB-p1 (42 kDa) and RNA-2sub-b for TGB-p2 (13 kDa) and TGB-p3 (15 kDa) (Gilmer *et al.* 1992a). TGB-p1 protein contains a helicase domain and is able to bind nucleic acids *in vitro* (Bleykasten *et al.* 1996).

Complementation studies of TGB-p1 protein with a virally expressed GFP::TGB-p1 fusion protein permitted to localize TGB-p1 in plasmodesmata only in the presence of the TGB-p2 and -p3 proteins (Erhardt *et al.* 2000). Moreover, TGB-p2 and -p3 are detected within plasmodesmata only when all the TGB proteins are expressed in the same cell (Erhardt *et al.* 2005).

ORF VI product encodes for a cystein-rich protein of 14 kDa. P14 is expressed from RNA-2sub-c. This protein is involved in the regulation of the virus replication (Gilmer *et al.* 1992a), enhances the expression of CP protein (Hehn *et al.* 1995) and acts as a weak silencing suppressor (Dunoyer *et al.* 2002).

RNA-3 is 1773 nts long and is involved in viral pathogenicity. Its presence exacerbates root and foliar symptoms (Tamada *et al.* 1989; Jupin *et al.* 1992). This RNA is also necessary for the long distance movement of the virus (Lauber *et al.* 1998) and is responsible for the rhizomania phenotype on *Beta vulgaris* (Tamada *et al.* 1989; Koenig *et al.* 1991).

p25

The p25 protein is expressed from RNA-3 and modulates foliar (Jupin *et al.* 1992) and root symptoms (Tamada *et al.* 1999). No sequence homology is found with known proteins, except with BNYVV RNA-5-encoded p26, which only shares the $Fx_3FRGPGNx_2L$ motif with p25 (**Fig. 4**).

Nucleo-cytoplasmic localization of p25 has been shown (Haeberle and Stussi-Garaud 1995). Its nuclear addressing involves the N-terminal $_{57}$**KR**IRF**R**$_{62}$ NLS (Vetter *et al.* 2004). Moreover, a nuclear export sequence (NES), $_{169}$**V**YM**V**CL**V**NT**V**$_{178}$, has been found in the C-terminal part of the protein. Hence, such two sequences allow p25 to shuttle between the two compartments by the way of importin alpha and exportin 1, independantly of other viral factors (Vetter *et al.* 2004). Foliar symptom variations observed with p25 mutants suggest a partial correlation between p25 function and its subcellular localization (Vetter *et al.* 2004). If all of the p25 functions are not yet unraveled, the protein displays some common properties with transcription activators (nuclear localization, Zinc-finger-domain, acidic domain). Indeed, in yeast, its fusion to GAL4BD or LexA allows a weak transcription activation of reporter genes (Klein *et al.* 2007), as it has been reported previously for NAC transcription factor (Xie *et al.* 2000). The domain responsible for such transcription activation has been mapped between amino acid residues 103 and 160 (Klein *et al.* 2007). P25 is able to form multimers as well, only if full-length proteins are maintained (Klein *et al.* 2007). Post-translational modifications of p25 have been evidenced by its electrophoretic mobility (Niesbach-Klosgen *et al.* 1990; Klein *et al.* 2007) and its immunodetection after two dimensional gel eletrophoresis reveals different isoforms that mainly differ by their isoelectric point corresponding to phosphate group addition (unpublished). Aspartic acid and alanine scanning mutagenesis of predicted phosphorylation sites revealed the importance of such phosphorylation for the multimerization, nuclear import as well as the transcription activation. Only mass spectrometry analyses will permit the exact identification of such phosphorylation events on the p25 sequence.

RNA-3 potentially encodes two other proteins

BNYVV RNA-3 as well as BSBMV RNA-3 encode potentially for two other proteins. The N-gene could allow the

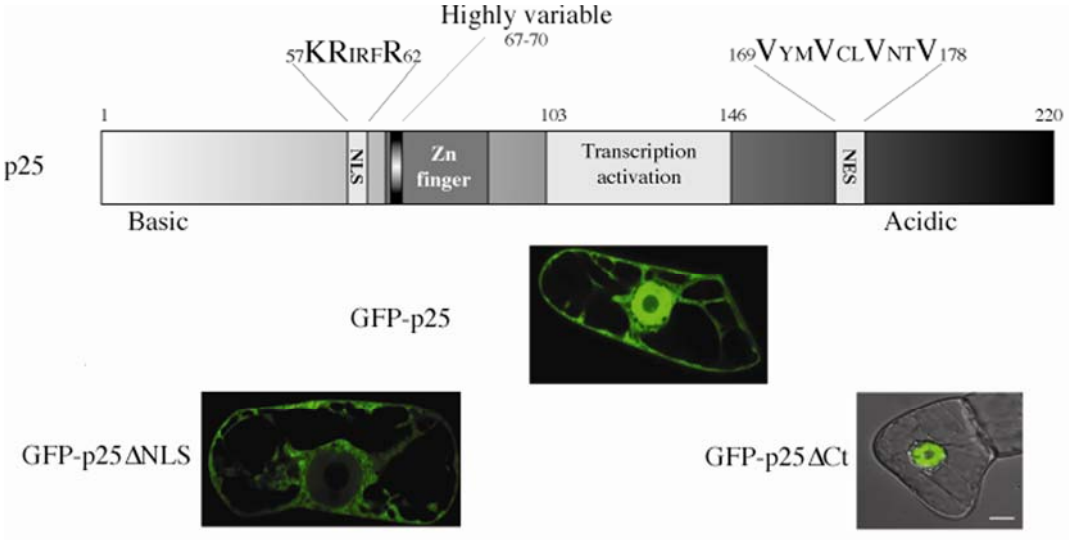

Fig. 4 Characteristics of the RNA-3-encoded p25: Line drawing representation of the p25 protein and of its known functional domains. (Top) The basic N-terminal domain contains a nuclear localization signal (NLS, $_{57\text{-}62}$), the highly variable 'Tetrad' sequence (67-70) and a Zinc finger domain. The acidic C-terminal part of the protein encompasses a domain responsible for the transcription activation (103-146) and the nuclear export sequence (NES, $_{169\text{-}178}$). (Center) Transient expression of a GFP-p25 fusion leads to the localization of the protein in the nucleus and in the cytoplasm of the cell, whereas removal of the NLS (GFP-p25ΔNLS, bottom left) prevents the nuclear addressing of the fusion protein. Deletion of the C-terminal domain that contains the NES motif leads to the accumulation of the fusion protein in the nucleus (GFP-p25ΔCt, bottom right).

synthesis of a 6.8 kDa protein that has been found expressed only from RNA-3 truncated forms, fused or not with p25 ORF (Jupin *et al.* 1992). In such situations, expression of the protein induces severe necrosis on *T. expansa* and *C. quinoa* leaves (Jupin *et al.* 1992). Fused to GFP protein, p6.8 is localized to cortical reticulum even in the absence of viral infection (David Gilmer, unpublished). However, its expression has never been detected under natural conditions, leading to the hypothesis that this ORF is cryptic. The last ORF encodes potentially for a 4.6 kDa polypeptide (p4.6). Such protein could be expressed from the so-called RNA-3 subgenomic species (RNA-3 sub) that corresponds to the last 550 nts of the RNA (Balmori *et al.* 1993). The functions of this short RNA and its encoded peptide, if it is expressed, are still unknown.

A particular function for RNA-3

BNYVV RNA-3 has been used to map 5′ and 3′ *cis* acting elements required for its replication and encapsidation and for the characterization of their secondary structure in solution (Jupin *et al.* 1990; Gilmer *et al.* 1992b, 1993; Lauber *et al.* 1997, 1999). However, such RNA possesses another function, which seems independent of its expression. Onto *B. macrocarpa*, RNA-3 is absolutely required for systemic spread of the virus. The RNA-3 sequence required for such systemic movement was located between nts 1033 and 1257 (Lauber *et al.* 1998). Curiously, complementation of BNYVV Stras12 strain, which contains only RNA-1 and -2, with BSBMV RNA-3 allows systemic movement of the viral particles (Claudio Ratti and David Gilmer, unpublished). A common sequence of 20 nts is found between both RNA species. Complementary experiments will allow deciphering the exact mechanism that permits the long distance movement.

RNA-4 is 1467 nts long and encodes for a 31 kDa protein (p31), which is essential for the efficient transmission of the virus by *P. betae* (Tamada and Abe 1989; Rahim *et al.* 2007). RNA-4-encoded protein could act in synergy with the minor p75 coat protein. P31 modulate foliar symptoms, and has been recently described as a root specific silencing suppressor (Rahim *et al.* 2007).

RNA-5 is present only within particular isolates (see below) and is 1350 nts long. It encodes a 26 kDa protein (p26), which induces severe rhizomania symptoms, reducing sugar yields (Heijbroek *et al.* 1999). Full-length infectious cDNA clone allowed the analysis of RNA-5 expression upon viral pathogenesis and the obtention of a new viral expression vector (Link *et al.* 2005; Schmidlin *et al.* 2005). Viral expression of p26 induces severe necrosis onto *C. quinoa* leaves. P26 protein is localized in the nucleus and in the cytoplasm of infected cells. Moreover, when p26 is fused to GAL4DB or LexA, the fusion proteins are able to strongly activate the transcription in yeast (Link *et al.* 2005).

Sequence variations

Restriction fragment length polymorphism, single-strand conformation polymorphism, sequence analyses and the presence or the absence of a fifth RNA in viral isolates permitted to classify BNYVV in different groups that are serologically identical (Kruse *et al.* 1994; Koenig *et al.* 1995). The distinction between A and B-type is possible by the characterization of amino acid residues variations of the RNA-2-encoded CP in position 62, 103 and 172 (**Table 1**). Eight variable residues within RNA-5-encoded p26 permit the distinction between European (P-type) or Asian (J-type) RNA-5 (**Table 1**). European P-type RNA-5s have a shorter 5'UTR and their coding region is four codons longer than J-type RNA-5s (**Table 1**). Such composition and size differences do not interfere on the known properties of p26 (Link *et al.* 2005): both proteins induce similar foliar symptoms, localize in the nucleus and in the cytoplasm and are able to activate the transcription in yeast (Link *et al.* 2005) (David Gilmer, unpublished). It should be noted here that

Table 1 Sequence variation within BNYVV RNA-2 encoded structural protein CP and RNA-3 and -5 encoded pathogenicity factors p25 and p26, respectively. Δ, deletion; * and ** refer to the presence of P-type or J-type respectively. (adapted from Schirmer *et al.* 2005)

CP[1]										
Position	*62*	*103*	*172*							
B-type	**S**	**N**	**F**							
A-type	**T**	**S**	**L**							
p25 'Tetrad'[1]										
Position	*67*	*68*	*69*	*70*						
A-type	**A**	**C**	**H**	**G**						
A-type	**A**	**F**	**H**	**G**						
A-type	**A**	**F**	**H**	**R**						
B-type	**A**	**H**	**H**	**R**						
A-type	**A**	**H**	**H**	**G**						
A-type	**A**	**L**	**H**	**G**	*					
A-type	**A**	**Y**	**F**	**G**						
B-type	**A**	**Y**	**H**	**R**						
A/B-type	**A**	**Y**	**H**	**G**	**	C. Bragard, unpublished				
A-type	**A**	**Y**	**P**	**R**		A. Schirmer, unpublished				
A-type	**A**	**Y**	**R**	**V**	**					
A-type	**S**	**Y**	**H**	**G**	*					
A-type	**T**	**Y**	**H**	**G**	*	(Ward *et al*. 2007)				
A-type	**V**	**C**	**H**	**G**						
A-type	**V**	**L**	**H**	**G**						
p26[1]										
Position	*30*	*69*	*77*	*103*	*142*	*146*	*149*	*174*	*200*	*227-229*
P-type	**N**	**L**	**D**	**G**	**R**	**F**	**G**	**S**	**A**	**NNN**
J-type	**H**	**F**	**Δ**	**D**	**C**	**H**	**R**	**N**	**S**	**Δ**

[1] Amino acid sequence variation within the coat protein that permit the distinction between A- or B-type of BNYVV.
[2] Amino acid sequence variation observed within the hypervariable Tetrad sequence of the BNYVV p25 protein. Some of those sequence variations are specific to A or B-type strains.
[3] Differences observed between Asian (J-type) and P-type p26 proteins.

RNA-5-containing isolates could harbour an A or B-type RNA-2 (Miyanishi *et al.* 1999; Koenig and Lennefors 2000; Schirmer *et al.* 2005). A-type viruses are found in European countries, Iran, USA, China and Japan. B-type is particularly present in France, Germany, Switzerland, Sweden as well as in China and Japan (Miyanishi *et al.* 1999; Suarez *et al.* 1999; Lennefors *et al.* 2000; Sohi and Maleki 2004). RNA-5 containing isolates were discovered in Japan (Tamada *et al.* 1989; Kiguchi *et al.* 1996), France (Koenig *et al.* 1997), China (Dawei *et al.* 1999), Kazakhstan (Koenig and Lennefors 2000) and in England (Harju *et al.* 2002; Ward *et al.* 2007). Phylogenetic studies were performed on RNA-3 (Schirmer *et al.* 2005). Characterisation of RNA-3-encoded p25 sequences permitted the identification of a highly variable sequence motif between position 67 and 70 named 'tetrad' (**Table 1**) (Schirmer *et al.* 2005). Recently, new tetrad motifs have been found (Ward *et al.* 2007) (Audrey Schirmer, unpublished). RNA viruses endorse strong mutation rates that allow them to adapt rapidly to various situations (Domingo 2000), keeping their genetic robustness and their infectivity even in the presence of mutations (Drake and Holland 1999). Analysis of selective pressure on p25 sequences revealed that 'tetrad' sequence as well as amino acid residue 198 were submitted to high positive selection pressures (Schirmer *et al.* 2005) similar to those described for animal viruses bypassing mammalian immunity system (Fares *et al.* 2001). Such a positive selection could explain the adaptation of BNYVV to resistance genes (Schirmer *et al.* 2005) and has recently been validated for the tetrad motif (Acosta-Leal *et al.* 2008).

PATHOGENICITY

Root alterations occur similarly when A- or B-type viruses set the infection; however, isolates that contain RNA-5 are more aggressive, particularly onto resistant crops, accumulate at higher concentration and induce severe rhizomania

symptoms (Heijbroek *et al.* 1999). Such a pathogenicity was retrieved onto host plants with artificial isolates (Link *et al.* 2005). However, the presence of RNA-5 is not sufficient to explain the increased virulence of some isolates as RB occurred in the U.S., where RNA-5 is absent (Liu *et al.* 2005). Molecular analyses of RB isolates revealed the presence of amino acid variation within the p25 tetrad sequence (Acosta-Leal and Rush 2007; Liu and Lewellen 2007) as well as amino acid residue 135 (Acosta-Leal and Rush 2007). Using artificial isolates that mimics such changes, virologists will be able to conclude if tetrad motif by itself is sufficient or requires another viral partner (e.g. encoded by RNA-1 or -2) to overcome *Rz* effect. Some preliminary experiments performed onto p25 revealed the importance of the tetrad motif upon the protein properties. Changing a B-type p25 tetrad sequence by one of the identified motifs drastically modifies the foliar symptoms, transcription activation and multimerization properties of p25 (Klein *et al.* 2007). Klein *et al.* suggested that p25 could behave as an avirulence gene product that could modify host responses. Chiba *et al.* (2008) recently demonstrated that p25 is indeed an *Avr* protein in *B. vulgaris*.

The *Polymyxa betae* vector

Besides BNYVV, three other viruses are also linked with rhizomania, namely the *Beet soil-borne mosaic virus* (BSBMV), the *Beet soil-borne virus* (BSBV) and the *Beet virus Q* (BVQ) (Meunier *et al.* 2003). All these viruses are transmitted to beet by the vector *P. betae* Keskin, an obligate parasite that develops in the epidermal and cortical root cells. The *Polymyxa* genus is represented by two economically very significant species: *P. graminis* Ledingham is specific to cereals (rice, wheat, barley) and *P. betae* to the *Chenopodiaceae* (*Beta vulgaris*, *Spinacia oleracea*, *Atriplex patula*, *Chenopodium bonus-henricus*, *C. hybridum and C. polyspermum*) and species like *Amaranthaceae*, *Caryophyllaceae* and *Portulaceae* (Abe and Tamada 1986; Barr 1988; Legrève *et al.* 2000; Legrève *et al.* 2005). Recent molecular studies of the characterization of the ITS (internal transcribed spacers) regions of the ribosomal DNA support species separation (Legrève *et al.* 2002). *P. betae* is ubiquitous. Its distribution covers all beet-growing areas (Rush 2003). *P. betae* belongs to the *Plasmodiophoridae*, a monophyletic group including ten genera. For a long time ranked among the lower fungi, it is now classified as a protist (Archibald and Keeling 2004). The host range of *P. betae*, long considered to be narrow, now appears to be wider. *P. betae* was recently detected by RT-PCR in the roots of some species of *Brassicaceae*, *Papaveraceae*, *Poaceae* and *Urticaceae* (Anne Legrève, unpublished). Although only a low level of infection was detected in these species, it could facilitate viral recombination or help to maintain the viral load in the vector.

P. betae cycle

The viruses transmitted by *Polymyxa* spp. are internalised by the vector (Chen *et al.* 1991; Rysanek *et al.* 1992; Campbell 1996; Verchot-Lubicz *et al.* 2007). The biological cycle of the viral diseases they transmit thus depends on the vectors life cycle (**Fig. 5**). This vectors great capacity for survival explains, firstly, the recurrence of viral diseases in contaminated fields and, secondly, their dissemination either through the soil adhering to agricultural machinery and produce or via drainage and irrigation water. In the absence of host plant radicles, *P. betae* survives in the soil in the form of sporosores (which may be viruliferous). Sporosores are spore clusters of 5 to 7 µm in diameter with a very thick, strong wall that can live for many years in the soil (Adams 1990; Maraite 1991). In the presence of host plants, resting spore germination releases biflagellated zoospores 4-5 µm in size which move through the free water in the soil before encysting on a root hair or epidermal cell of a host plant root. In order to penetrate the cell, a dagger-like structure called "Stachel (sting)" is formed in the encysted zoospore (Keskin and Fuchs 1969) and a sticky tube develops between the cyst and the plant cell wall. The Stachel, which is injected into the host cell through the tube, pierces the cell wall and the plasma membrane and allows the zoospore content to be transferred into the cytoplasm of the host cell (Barr 1988). Cruciform nuclear divisions indicative of mitosis lead to the formation of a multinucleate plasmodium surrounded by a thin membrane. In the sporangial phase this plasmodium develops into a multi-lobed zoosporangium enclosed by a thin wall within which the secondary zoospores are formed. The latters are released outside the root, or sometimes into the deeper root cells, by small plasmodial cells, which dissolve a hole in the cell wall (Barr 1988). In the sporogenic phase non-cruciform nuclear divisions are observed, with the formation of synaptonemal complexes characteristic of meiosis (Braselton 1988). The plasmodium divides into mononucleate cells by forming membrane layers within the cytoplasm. A four to five-layer wall is then deposited between the cells, with adjacent spores remaining connected by bonds between the two outermost layers

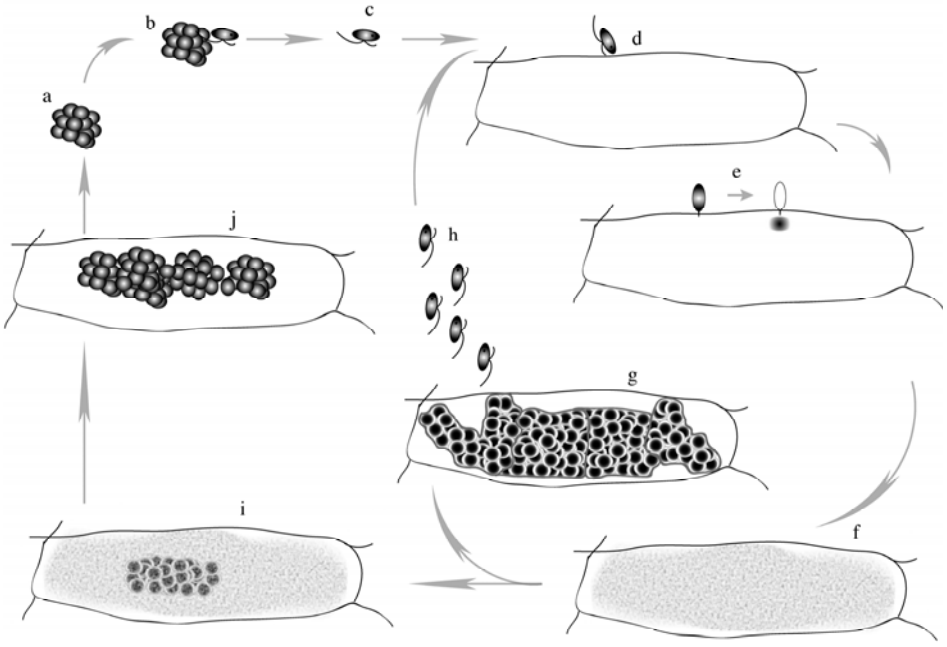

Fig. 5 Drawing of the *Polymyxa* spp. life cycles and its developing states. (a) sporosore; (b) germinating zoospore; (c), swimming zoospore to a (d) cortical or epidermal cell; (e) the zoospore encyst on the cell and injects its contents through the cell wall and the cellular membrane via the "satchel"; (f), developing plasmode that will tend to a zoosporangium (g) that will issue either (h) the secondary zoospores able to infect new cells or (i) to the sporogenous plasmod (j) leading to new sporosores. Such sporosores will be further released in soil after root decomposition.

(Chen *et al.* 1998). The sporosores formed remain in the root debris and are released into the soil by root decomposition. Within this life cycle the moments of cell fusion and karyogamy have not yet been pinpointed. Observation of double size quadriflagellate zoospores (Ledingham 1939) suggests fusion of two zoospores, but the moment of nuclear fusion is not known. The determinants for development of the various phases are likewise unknown (Braselton 1995). However, the host and the degree of root colonisation appear to be significant (Legrève *et al.* 1998). Three conditions are essential for root infection by this parasite: (1) presence of a host plant; (2) presence of free water in the soil to promote germination and facilitate zoospore access to the roots, and (3) a sufficiently high temperature between 10 and 30°C, ideally between 20 and 25°C according to the origin of the strains (Legrève *et al.* 1998 ; Webb *et al.* 2000). Hot, wet springs are therefore propitious to early infections, as are light, sandy, well-drained soils as these heat up faster than heavy organic soils with high retention capacity (Webb *et al.* 2000). The soil pH and calcium content also affect vector activity. Spore germination and root infection by zoospores are affected by acid pH conditions (Abe and Tamada 1987). They are promoted in neutral or alkaline pH soils, especially if the calcium and magnesium levels are greater than 350 and 20 mg/100 g of soil respectively (Goffart and Maraite 1991). Managed liming and practices for regulating soil pH and calcium levels would probably result in better control of rhizomania.

Vector detection

Plant infection by *P. betae* is not apparent from any particular symptoms. Until the early 1990s this parasite was detected in roots by observing the roots under the microscope following colouring with Lactophenol Cotton Blue. This fairly laborious method was initially used to study the vectors ecological requirements and the soil infectious potential levels by the most probable number technique, growing trap crops on soil dilutions (Tuitert 1990). Molecular techniques (DNA probes, PCR, nested PCR, ELISA) have greatly facilitated rapid detection of *P. betae* in roots and even in soil (Mutasa *et al.* 1996; Kingsnorth *et al.* 2003; Ward *et al.* 2005). Multiplex RT-PCR enables the presence of *P. betae*, BNYVV, BSBV and BVQ to be revealed in a single test using a root RNA extract (Meunier *et al.* 2003).

Virus-vector interactions

Modes of virus acquisition and transmission by plasmodiophorids have been described (Rochon *et al.* 2004). These viruses survive in the spores in a form that has not yet been clarified. However, accumulated RNA and movement proteins of *Soil-borne wheat mosaic virus* (SBWMV) and capsid proteins of *Wheat spindle streak virus* (WSSMV) within the resting spores of the vector, *P. graminis*, probably denote the presence of ribonucleoprotein complexes (Driskel *et al.* 2004). In the case of BNYVV, both the sporosores and the plasmodia of *P. betae* are labelled using antibodies against the viral capsid protein (Doucet 2006). Structural and non-structural proteins have been detected in *P. betae* spores and zoospores. These results suggest that this vector could also be a BNYVV host (Verchot-Lubicz *et al.* 2007). When transmitted by the viruliferous zoospores, the virus is present in particle form. Virus transmission by plasmodiophorids was for many years regarded as a passive mechanism, which occurred during mixing of plant cell cytoplasms and the protozoan, prior to membrane formation (Campbell 1996). However, recent research has revealed the special role played by some viral proteins in the process of transmission by the vector. The BNYVV capsid protein readthrough (RT) domain plays an important part in the transmission process, since deletions in the C-terminal portion of this domain are correlated to loss of virus transmission. Substituting the four KTER amino acids located in position 553 to 556 of the RT domain by the ATAR motif completely blocks transmission (Tamada *et al.* 1996). A comparative analysis of the viral genomes transmitted by plasmodiophorids, which do not have the same genomic organisation, has identified the presence of two complementary transmembrane domains in the RT domains of the capsid protein of *Beny-*, *Furo-* and *Pomovirus* and in the P2 proteins of *Bymovirus* (Adams *et al.* 2001). Deletion or substitution of the second domain also blocks transmission by the vector. The molecular model is not yet detailed, but the transmembrane helical sequences may perhaps determine a particular structure facilitating membrane invagination and virus movement through the membrane of the vector (Adams *et al.* 2001). Although the molecular mechanism of the interaction is far from fully explained, the involvement of protein p31, coded by RNA-4, is strongly suspected. Two independent studies of comparative transmission of wild or mutated viruses in the coding sequence for protein p31 have in fact shown a big decrease in protein p31 mutant transmission (Rahim *et al.* 2007) (Anne Legrève, pers. comm.). In addition to its role in transmission, protein p31 is also thought to play a part both in inducing symptoms in *Nicotiana benthamiana* and in suppressing RNA silencing in roots, without affecting viral RNA accumulation (Rahim *et al.* 2007).

Genetic resistance strategies used against rhizomania disease

One of the major goals of the sugar beet industry is to stabilize the sugar yields. Due to the large area covered by rhizomania infested fields, calculated to span *approx.* 610,000 ha in 2000 (Richard-Molard and Carriolle 2001), a qualitative and quantitative high yielding sugar beet production is only given by growth of resistant cultivars. Since the first attempts in achieving rhizomania resistant breeding material, resistance sources were found in germplasms or wild type *B. vulgaris* ssp. *maritima*, which all confer phenotypes of quantitative resistance (**Table 2**). Involvement of additional minor genes for the expression of the rhizomania resistance trait is most likely (Biancardi *et al.* 2002). Nowadays, hybrid varieties (**Table 2**) that do not or poorly develop symptoms, display reduced virus content have replaced susceptible varieties.

The first selection of partial resistant BNYVV sugar beet genotypes started at the end of the seventies. Sugar beets characterized by reduced virus symptoms, increased white sugar content and increased processing quality were subsequently selected (De Biaggi 1987; Lewellen *et al.* 1987). Although these sugar beet genotypes were also in-

Table 2 Characteristics of the *Rz* resistance genes and transgenic sequences (underlined) used in sugar beet crops. All *Rz* genes are located on Chr. III; PDR: pathogen-derived resistance.

Hybrid variety	Origin	Resistance gene	Nature	Resistance level
Holly	*B. vulgaris* ssp. *vulgaris* (California)	*Rz1*	Dominant	Low < good depending on inoculum
WB42	*B. vulgaris* ssp. *maritima* (Denmark)	*Rz2*	Dominant	Rz2 > Rz1
WB41	*B. vulgaris* ssp *maritima* (Denmark)	*Rz3*	Partialy dominant Allelic to Rz2?	Variable
R36	*B. vulgaris* ssp. *maritima*	*Rz4*	Allelic to other Rz?	Partial resistance
4D6834	*B. vulgaris*	*Movement protein*	PDR	Excellent
G018	*B. vulgaris*	*Replicase*	PDR/PTGS	Excellent
-	*B. vulgaris*	*CP*	PDR	Good

fected with BNYVV, the symptoms were less pronounced and plants displayed a better performance in field trils (Bürcky 1987). In 1983, the US-American Holly Sugar Company identified the "Holly" resistance representing the first described BNYVV resistance source (Lewellen *et al.* 1987; Biancardi *et al.* 2002). 'Holly' carryies the resistance gene *Rz*, which was renamed *Rz*1 (Scholten *et al.* 1999). Holly does not show rhizomania symptoms, but allows the virus to replicate as shown by the viral titer contents in such varieties (Scholten and Lange 2000). 'Rizor' was the first diploid hybrid partially resistant to rhizomania (Richard-Molard 1985; De Biaggi 1987). In the eighties, further resistance sources were identified in Danish tests of *Beta vulgaris* ssp. *maritima* (L.) Arcang wild-beet germplasms leading to WB41 and WB42 sources (**Table 2**) representing *Rz*3 and *Rz*2 resistance genes, respectively (Lewellen *et al.* 1987; Whitney 1989). Greenhouse tests performed applying severe infestation conditions have revealed the better performance of *Rz*2 versus *Rz*1 resistance genes suggesting a different origin of both genes (Scholten *et al.* 1999). However, these genes are present within ChrIII at a distance range of 20 cM (Scholten *et al.* 1994; Scholten *et al.* 1999) to 35 cM (Amiri *et al.* 2003). *Rz*3 resistance gene (**Table 2**) has been also mapped on ChrIII at about 5 cM from *Rz*1, suggesting that *Rz*2 and *Rz*3 might be allelic (Gidner *et al.* 2005). Recently, the use of the hybrid line R36 permitted the identification of a new QTL named *Rz*4 (**Table 2**), which confers partial resistance to BNYVV infection (Grimmer *et al.* 2007). Rz1 or Rz2 carrying cultivars reduce viral titer up to 6.10^4 fold when compared to susceptible lines (Acosta-Leal *et al.* 2008). However, their systematic use might favor genetic drift of viral population that seems to accumulate nucleotide variation twice rapidly, leading to high probability for the emergence of RB isolates (Acosta-Leal *et al.* 2008).

Growing of BNYVV resistant sugar beet cultivars is generally accepted as the only strategy to keep sugar beet cultivation in infested fields profitable. Reports about increased aggressiveness of BNYVV P-type (France) and appearance of new BNYVV A-types (USA: Imperial Valley, California and Minnesota) have been published (Liu *et al.* 2005; Liu and Lewellen 2007). These interesting studies show the viral overcome of *Rz*1 carrying cultivars in the field and measure increased BNYVV levels in hair-roots of *Rz*1 plants in greenhouse resistance tests.

Artificially generated resistance represents an alternative to the natural resistance (**Table 2**). The transgenic expression of virus-derived sequences that form double-stranded RNA (dsRNA) has been obtained and lead to BNYVV resistance (Lennefors *et al.* 2008). Expression of dsRNA induces RNA silencing (Baulcombe 2004, 2005; Filipowicz *et al.* 2005), an innate defense mechanism against invasive nucleic acids that leads to the sequence specific degradation of RNA. High levels of resistance to rhizomania were obtained in sugar beets expressing a 0.4 kb inverted repeat construct based on a partial BNYVV replicase gene derived sequence (**Table 2**) (Lennefors *et al.* 2008).

Transgenic sugar beets expressing the BNYVV coat protein (Scholten and Lange 2000), or a mutated form of one of the tree movement protein TGB-p3 (Lauber *et al.* 2001) or the sequence derived from RNA-1 (Lennefors *et al.* 2006) both generate higher protection levels than *Rz*1.

CONCLUSIONS

BNYVV RNA-3 and in particular the p25 protein constitutes the rhizomania disease keystones. New emerging isolates tend to bypass resistance genes by the modification of the p25 gene sequence. Sequence variations within p25 combined with the post-translational modifications make the study of this protein rather difficult. Further studies will aim to characterize the major functions of p25 protein and the cellular genes involved in the root proliferation and the parasitic infection. Thereby, such studies will be performed within and out viral context. The characterization of the p25-deregulated cellular functions should allow the identification of cellular markers that in term could help breeders for the selection of resistant crops. Before the extensive use of such new resistant varieties, prophylactic treatments are used to limit the infection. Indeed, the presence of *P. betae* in soils, crop transportation and scattering of soil by the mean of farming activities are the main factors that ensure the persistence and the spread of the virus. Even with some adapted crop rotations, the presence of highly resistant viruliferous vector spores prevents any reduction of the infection. Soil fumigation with methyl bromide reduces inoculum intensity but the Montreal protocol prevents its use as it depletes the ozone layer. Crop selection and the use of new tolerant varieties having many resistant genes (i.e. for the virus and the vector) and also the use of efficient pathogen-derived resistance may represent the most powerful alternative to grow sugar beet and maintain sugar yields.

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REFERENCES

Abe H, Tamada T (1986) Association of *Beet necrotic yellow vein virus* with isolates of *Polymyxa betae* keskin strains in rhizomania infested soils of sugar beet in Japan. *Annals of the Phytopathological Society of Japan* **52**, 235-247

Abe H, Tamada T (1987) A test tube culture system for multiplication of *Polymyxa betae* and *Beet necrotic yellow vein virus* in rootlets of sugar beet. In: *Proceedings of the Sugar Beet Research Association, Japan* **29**, 34-38

Acosta-Leal R, Fawley MW, Rush CM (2008) Changes in the intraisolate genetic structure of beet necrotic yellow vein virus populations associated with plant resistance breakdown. *Virology* **376**, 60-68

Acosta-Leal R, Rush CM (2007) Mutations associated with resistance-breaking isolates of *Beet necrotic yellow vein virus* and their allelic discrimination using Taqman technology. *Phytopathology* **97**, 325-330

Adams MJ (1990) Epidemiology of fungally-transmitted viruses. *Soil Use and Management* **6**, 184-189

Adams MJ, Antoniw JF, Mullins JG (2001) Plant virus transmission by plasmodiophorid fungi is associated with distinctive transmembrane regions of virus-encoded proteins. *Archives of Virology* **146**, 1139-1153

Amiri R, Moghaddam M, Mesbah M, Sadeghian SY, Ghannadha MR, Izadpanah K (2003) The inheritance of resistance to *Beet necrotic yellow vein virus* (BNYVV) in *B. vulgaris* subsp. *maritima*, accession wb42: statistical comparisons with holly-1-4. *Euphytica* **132**, 363-373

Archibald JM, Keeling PJ (2004) Actin and ubiquitin protein sequences support a cercozoan/foraminiferan ancestry for the plasmodiophorid plant pathogens. *Journal of Eukaryotic Microbiology* **51**, 113-118

Balmori E, Gilmer D, Richards K, Guilley H, Jonard G (1993) Mapping the promoter for subgenomic RNA synthesis on beet necrotic yellow vein virus RNA 3. *Biochimie* **75**, 517-521

Barr DJS (1988) Zoosporic plant parasites as fungal vectors of viruses: Taxonomy and life cycles of species involved. In: Cooper JI, Asher MJ (Eds) *Developments in Applied Biology II Viruses with Fungal Vectors*, Association of Applied Biologists, Wellesbourne, UK, pp 123-137

Baulcombe D (2004) RNA silencing in plants. *Nature* **431**, 356-363

Baulcombe D (2005) RNA silencing. *Trends in Biochemical Sciences* **30**, 290-293

Biancardi E, Lewellen RT, Biaggi MD, Erichsen AW, Stevanato P (2002) The origin of rhizomania resistance in sugarbeet. *Euphytica* **127**, 383-397

Bleykasten C, Gilmer D, Guilley H, Richards KE, Jonard G (1996) *Beet necrotic yellow vein virus* 42 kDa triple gene block protein binds nucleic acid *in vitro*. *Journal of General Virology* **77**, 889-897

Bouzoubaa S, Niesbach-Klosgen U, Jupin I, Guilley H, Richards K, Jonard G (1991) Shortened forms of beet necrotic yellow vein virus RNA-3 and -4: Internal deletions and a subgenomic RNA. *Journal of General Virology* **72**, 259-266

Bouzoubaa S, Quillet L, Guilley H, Jonard G, Richards K (1987) Nucleotide sequence of beet necrotic yellow vein virus RNA-1. *Journal of General Virology* **68**, 615-626

Braselton JP (1988) Karyology and systematics of plasmodiophoromycetes. In: Cooper JI, Asher MJ (Eds) *Developments in Applied Biology II Viruses with Fungal Vectors*, Association of Applied Biologists, Wellesbourne, UK, pp 139-152

Braselton JP (1995) Current status of the plasmodiophorids. *Critical Review in Microbiology* **21**, 263-275

Bürcky K (1987) BNYVV-resistenz indizierende merkmale und deren mögliche nützung zur selektion rizomaniatoleranter zuckerrüben. In: International Institute for Beet Research (IIBR) (Ed) *Proceedings of the 50th Congress of the IIRB*, 11-12 February 1987, IIRB, Brussels, pp 3-7

Campbell RN (1996) Fungal transmission of plant viruses. *Annual Review of Phytopathology* **34**, 87-108

Canova A (1959) On the pathology of sugarbeet. *Informatore Fitopatologico* **9**, 390-396

Chen JP, Swaby AG, Adams MJ, Ruan YL (1991) *Barley mild mosaic-virus* inside its fungal vector, *Polymyxa-graminis*. *Annals of Applied Biology* **118**, 615-621

Chen JP, Wang ZQ, Hong J, Collier CR, Adams MJ (1998) Ultrastructural studies of resting spore development in *Polymyxa graminis*. *Mycological Research* **102**, 687-691

Chiba S, Miyanishi M, Andika IB, Kondo H, Tamada T (2008) Identification of amino acids of the *Beet necrotic yellow vein virus* p25 protein required for induction of the resistance response in leaves of *Beta vulgaris* plants. *Journal of General Virology* **89**, 1314-1323

Dawei L, Jialin Y, Chenggui H, Tao L, Shucai Q, Koenig R, Yi L (1999) Detection and nucleotide analysis of RNA5 of *beet necrotic yellow vein virus* isolated in China. *Chineese Journal of Biotechnology* **15**, 253-257

De Biaggi M (1987) Méthodes de sélection, un cas concret. In: International Institute for Beet Research (IIBR) (Ed) *Proceedings of the 50th Congress of the IIRB*, 11-12 February 1987, IIRB, Brussels, pp 157-163

Domingo E (2000) Viruses at the edge of adaptation. *Virology* **270**, 251-253

Doucet D (2006) Characterization of the pecluvirus movement in plant roots (development of a model and comparison with a benyvirus). PhD thesis, Université Catholique de Louvain, 226 pp

Drake JW, Holland J (1999) Mutation rates among RNA viruses. *Proceedings of the National Academy of Sciences USA* **96**, 13910-13013

Driskel BA, Doss P, Littlefield LJ, Walker NR, Verchot-Lubicz J (2004) Soilborne wheat mosaic virus movement protein and RNA and wheat spindle streak mosaic virus coat protein accumulate inside resting spores of their vector, *Polymyxa graminis*. *Molecular Plant-Microbe Interactions* **17**, 739-748

Dunoyer P, Pfeffer S, Fritsch C, Hemmer O, Voinnet O, Richards KE (2002) Identification, subcellular localization and some properties of a cysteine-rich suppressor of gene silencing encoded by *Peanut clump virus*. *The Plant Journal* **29**, 555-567

Erhardt M, Dunoyer P, Guilley H, Richards K, Jonard G, Bouzoubaa S (2001) Beet necrotic yellow vein virus particles localize to mitochondria during infection. *Virology* **286**, 256-262

Erhardt M, Morant M, Ritzenthaler C, Stussi-Garaud C, Guilley H, Richards K, Jonard G, Bouzoubaa S, Gilmer D (2000) P42 movement protein of *Beet necrotic yellow vein virus* is targeted by the movement proteins p13 and p15 to punctate bodies associated with plasmodesmata. *Molecular Plant-Microbe Interactions* **13**, 520-528

Erhardt M, Vetter G, Gilmer D, Bouzoubaa S, Richards K, Jonard G, Guilley H (2005) Subcellular localization of the triple gene block movement proteins of *Beet necrotic yellow vein virus* by electron microscopy. *Virology* **340**, 155-166

Fares MA, Moya A, Escarmis C, Baranowski E, Domingo E, Barrio E (2001) Evidence for positive selection in the capsid protein-coding region of the Foot-and-mouth disease virus (FMDV) subjected to experimental passage regimens. *Molecular Biology and Evolution* **18**, 10-21

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (Eds) (2005) In Virus Taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses, Elsevier/Academic Press, London, 1162 pp

Filipowicz W, Jaskiewicz L, Kolb FA, Pillai RS (2005) Post-transcriptional gene silencing by siRNAs and miRNAs. *Current Opinion in Structural Biology* **15**, 331-341

Gidner S, Lennefors BL, Nilsson NO, Bensefelt J, Johansson E, Gyllenspetz U, Kraft T (2005) QTL mapping of BNYVV resistance from the WB41 source in sugar beet. *Genome* **48**, 279-285

Gilmer D, Allmang C, Ehresmann C, Guilley H, Richards K, Jonard G, Ehresmann B (1993) The secondary structure of the 5′-noncoding region of beet necrotic yellow vein virus RNA 3: Evidence for a role in viral RNA replication. *Nucleic Acids Research* **21**, 1389-1395

Gilmer D, Bouzoubaa S, Hehn A, Guilley H, Richards K, Jonard G (1992a) Efficient cell-to-cell movement of *Beet necrotic yellow vein virus* requires 3' proximal genes located on RNA 2. *Virology* **189**, 40-47

Gilmer D, Richards K, Jonard G, Guilley H (1992b) Cis-active sequences near the 5'-termini of beet necrotic yellow vein virus RNAs 3 and 4. *Virology* **190**, 55-67

Goffart JP, Maraite H (1991) Soil and agronomic factors affecting the inoculum potential of *Polymyxa betae* keskin in Belgium. *Parasitica* **47**, 165-192

Grimmer M, Trybush S, Hanley S, Francis S, Karp A, Asher M (2007) An anchored linkage map for sugar beet based on AFLP, SNP and RAPD markers and QTL mapping of a new source of resistance to *Beet necrotic yellow vein virus*. *Theoretical and Applied Genetics* **114**, 1151-1160

Haeberle AM, Stussi-Garaud C (1995) In situ localization of the non-structural protein p25 encoded by beet necrotic yellow vein virus RNA 3. *Journal of General Virology* **76**, 643-650

Haeberle AM, Stussi-Garaud C, Schmitt C, Garaud JC, Richards KE, Guilley H, Jonard G (1994) Detection by immunogold labelling of p75 readthrough protein near an extremity of beet necrotic yellow vein virus particles. *Archives of Virology* **134**, 195-203

Harju VA, Mumford RA, Bockley A, Boonham N, Clover GRG, Weekes R, Henry CM (2002) Occurence in the united kingdom of beet necrotic yellow vein virus isolates which contain RNA 5. *Plant Pathology* **51**, 811

Hehn A, Bouzoubaa S, Bate N, Twell D, Marbach J, Richards K, Guilley H, Jonard G (1995) The small cysteine-rich protein p14 of *Beet necrotic yellow vein virus* regulates accumulation of RNA 2 in *cis* and coat protein in *trans*. *Virology* **210**, 73-81

Hehn A, Fritsch C, Richards KE, Guilley H, Jonard G (1997) Evidence for *in vitro* and *in vivo* autocatalytic processing of the primary translation product of beet necrotic yellow vein virus RNA 1 by a papain-like proteinase. *Archives of Virology* **142**, 1051-1058

Heijbroek W, Musters PMS, Schoone AHL (1999) Variation in pathenogenicity and multiplication of *Beet necrotic yellow vein virus* (BNYVV) in relation to the resistance of sugarbeet cultivars. *European Journal of Plant Pathology* **105**, 397-405

Jupin I, Guilley H, Richards KE, Jonard G (1992) Two proteins encoded by beet necrotic yellow vein virus RNA 3 influence symptom phenotype on leaves. *The EMBO Journal* **11**, 479-488

Jupin I, Richards K, Jonard G, Guilley H, Pleij CW (1990) Mapping sequences required for productive replication of beet necrotic yellow vein virus RNA 3. *Virology* **178**, 273-280

Keskin B (1964) *Polymyxa betae* n.Sp., a parasite in the roots of *Beta vulgaris* tournefort, particularly during the early growth of the sugar beet. *Archiv für Mikrobiologie* **19**, 348-374

Keskin B, Fuchs WH (1969) The process of infection by *Polymyxa betae*. *Archiv für Mikrobiologie* **68**, 218-226

Kiguchi T, Saito M, Tamada T (1996) Nucleotide sequence analysis of RNA-5 of five isolates of *Beet necrotic yellow vein virus* and the identity of a deletion mutant. *Journal of General Virology* **77**, 575-580

Kingsnorth CS, Asher MJC, Keane GJP, Chwarszczynska DM, Luterbacher MC, Mutasa-Gottgens ES (2003) Development of a recombinant antibody ELISA test for the detection of *Polymyxa betae* and its use in resistance screening. *Plant Pathology* **52**, 673-680

Klein E, Link D, Schirmer A, Erhardt M, Gilmer D (2007) Sequence variation within beet necrotic yellow vein virus p25 protein influences its oligomerization and isolate pathogenicity on *Tetragonia expansa*. *Virus Research* **126**, 53-61

Koenig R, Haeberle AM, Commandeur U (1997) Detection and characterization of a distinct type of beet necrotic yellow vein virus RNA 5 in a sugarbeet growing area in Europe. *Archives of Virology* **142**, 1499-1504

Koenig R, Jarausch W, Li Y, Commandeur U, Burgermeister W, Gehrke M, Luddecke P (1991) Effect of recombinant *Beet necrotic yellow vein virus* with different RNA compositions on mechanically inoculated sugarbeets. *Journal of General Virology* **72**, 2243-2246

Koenig R, Lennefors BL (2000) Molecular analyses of european A, B and P type sources of *Beet necrotic yellow vein virus* and detection of the rare P type in Kazakhstan. *Archives of Virology* **145**, 1561-1570

Koenig R, Luddecke P, Haeberle AM (1995) Detection of beet necrotic yellow vein virus strains, variants and mixed infections by examining single-strand conformation polymorphisms of immunocapture RT-PCR products. *Journal of General Virology* **76**, 2051-2055

Kruse M, Koenig R, Hoffmann A, Kaufmann A, Commandeur U, Solovyev AG, Savenkov I, Burgermeister W (1994) Restriction fragment length polymorphism analysis of reverse transcription-PCR products reveals the existence of two major strain groups of *Beet necrotic yellow vein virus*. *Journal of General Virology* **75**, 1835-1842

Lauber E, Guilley H, Richards K, Jonard G, Gilmer D (1997) Conformation of the 3'-end of beet necrotic yellow vein benevirus RNA 3 analysed by chemical and enzymatic probing and mutagenesis. *Nucleic Acids Research* **25**, 4723-4729

Lauber E, Guilley H, Tamada T, Richards KE, Jonard G (1998) Vascular movement of *beet necrotic yellow vein virus* in *Beta macrocarpa* is probably dependent on an RNA 3 sequence domain rather than a gene product. *Journal of General Virology* **79**, 385-393

Lauber E, Janssens L, Weyens G, Jonard G, Richards KE, Lefebvre M, Guilley H (2001) Rapid screening for dominant negative mutations in the beet necrotic yellow vein virus triple gene block proteins p13 and p15 using a viral replicon. *Transgenic Research* **10**, 293-302

Lauber E, Jonard G, Guilley H, Gilmer D (1999) Effects of structural modifications upon the accumulation *in planta* of replicons derived from beet necrotic yellow vein virus RNA 3. *Archives of Virology* **144**, 1201-1208

Ledingham GA (1939) Studies of *Polymyxa graminis*, n. Gen. N. Sp., a plasmodiophoraceous root parasite of wheat. *Canadian Journal of Research* **17**, 38-51

Lee L, Telford EB, Batten JS, Scholthof KB, Rush CM (2001) Complete nucleotide sequence and genome organization of *Beet soilborne mosaic virus*, a proposed member of the genus *Benyvirus*. *Archives of Virology* **146**, 2443-2453

Legrève A, Delfosse P, Maraite H (2002) Phylogenetic analysis of polymyxa

species based on nuclear 5.8s and internal transcribed spacers ribosomal DNA sequences. *Mycological Research* **106**, 138-147

Legrève A, Delfosse P, Vanpee B, Goffin A, Maraite H (1998) Differences in temperature requirements between *Polymyxa* sp. of Indian origin and *Polymyxa graminis* and *Polymyxa betae* from temperate areas. *European Journal of Plant Pathology* **104**, 195-205

Legrève A, Schmit JF, Bragard C, Maraite H (2005) The role of climate and alternative hosts in the epidemiology of rhizomania. In: Rush CM (Ed) *International Working Group on Plant Viruses with Fungal Vectors*, 5-7 September 2005, American Society of Sugar Beet Technologists, Bologna, Italy, pp 125-128

Legrève A, Vanpee B, Delfosse P, Maraite H (2000) Host range of tropical and sub-tropical isolates of *Polymyxa graminis*. *European Journal of Plant Pathology* **106**, 379-389

Lennefors BL, Lindsten K, Koenig R (2000) First record of A and B type *Beet necrotic yellow vein virus* in sugar beets in Sweden. *European Journal of Plant Pathology* **106**, 199-201

Lennefors BL, Savenkov EI, Bensefelt J, Wremerth-Weich E, Roggen P, Tuvesson S, Valkonen JP, Gielen J (2006) DsRNA-mediated resistance to beet necrotic yellow vein virus infections in sugar beet (*Beta vulgaris* L. ssp. *vulgaris*). *Molecular Breeding* **18**, 313-325

Lennefors BL, van Roggen PM, Yndgaard F, Savenkov EI, Valkonen JP (2008) Efficient dsRNA-mediated transgenic resistance to *Beet necrotic yellow vein virus* in sugar beets is not affected by other soilborne and aphid-transmitted viruses. *Transgenic Research* **17**, 219-228

Lewellen RT, Skoyen IO, Erichsen AW (1987) Breeding sugar beet for resistance to rhizomania: Evaluation of host-plant reactions and selection for and inheritance. In: International Institute for Beet Research (IIBR) (Ed) *Proceedings of the 50th Congress of the IIRB*, 11-12 February 1987, IIRB, Brussels, pp 139-156

Link D, Schmidlin L, Schirmer A, Klein E, Erhardt M, Geldreich A, Lemaire, Gilmer D (2005) Functional characterization of the beet necrotic yellow vein virus RNA-5-encoded p26 protein: Evidence for structural pathogenicity determinants. *Journal of General Virology* **86**, 2115-2125

Liu H-Y, Lewellen RT (2007) Distribution and molecular characterization of resistance-breaking isolates of *Beet necrotic yellow vein virus* in the United States. *Plant Disease* **91**, 847-851

Liu HY, Sears JL, Lewellen RT (2005) Occurence of resistance-breaking *Beet necrotic yellow vein virus* of sugar beet. *Plant Disease* **89**, 464-468

Maraite H (1991) Transmission of viruses by soil fungi. In: Beemster ABR, Bollen GH, Gerlagh M, Ryuissen MA, Schipers B, Tempels A (Eds) *Developments in Agricultural and Managed-Forest Ecology: Biotic Interactions and Soil-Borne Diseases*, Elsevier Science Publishers, Amsterdam, NL, pp 67-82

Meunier A, Schmit JF, Stas A, Kutluk N, Bragard C (2003) Multiplex reverse transcription-PCR for simultaneous detection of *Beet necrotic yellow vein virus*, *Beet soilborne virus*, and *Beet virus Q* and their vector *Polymyxa betae* keskin on sugar beet. *Applied and Environmental Microbiology* **69**, 2356-2360

Miyanishi M, Kusume T, Saito M, Tamada T (1999) Evidence for three groups of sequence variants of beet necrotic yellow vein virus RNA 5. *Archives of Virology* **144**, 879-892

Morales FJ, Ward E, Castaño M, Arroyave JA, Lozano I, Adams MJ (1999) Emergence and partial characterization of *Rice stripe necrosis virus* and its fungus vector in South America. *European Journal of Plant Pathology* **105**, 643-650

Morozov SY, Solovyev AG (2003) Triple gene block: Modular design of a multifunctional machine for plant virus movement. *Journal of General Virology* **84**, 1351-1366

Mutasa ES, Chwarszczynska DM, Asher MJ (1996) Single-tube, nested PCR for the diagnosis of *Polymyxa betae* infection in sugar beet roots and colorimetric analysis of amplified products. *Phytopathology* **86**, 493-497

Niesbach-Klosgen U, Guilley H, Jonard G, Richards K (1990) Immunodetection *in vivo* of beet necrotic yellow vein virus-encoded proteins. *Virology* **178**, 52-61

Pollini CP, Giunchedi L (1989) Comparative histopathology of sugar beets that are susceptible and partially resistant to rhizomania. *Phytopathologia Mediterranea* **28**, 16-21

Pollini CP, Masia A, Giunchedi L (1990) Free indole-3-acetic acid in sugar-beet root of rhizomania-susceptible and moderately resistant cultivars. *Phytopathologia Mediterranea* **29**, 191-195

Putz C (1977) Composition and structure of *Beet necrotic yellow vein virus*. *Journal of General Virology* **35**, 397-401

Quillet L, Guilley H, Jonard G, Richards K (1989) *In vitro* synthesis of biologically active beet necrotic yellow vein virus RNA. *Virology* **172**, 293-301

Rahim MD, Andika IB, Han C, Kondo H, Tamada T (2007) RNA4-encoded p31 of *Beet necrotic yellow vein virus* is involved in efficient vector transmission, symptom severity and silencing suppression in roots. *Journal of General Virology* **88**, 1611-1619

Richard-Molard M (1985) Rhizomania: A world-wide danger to sugar beet. *Span* **28**, 92-94

Richard-Molard M, Carriolle M (2001) Biotic and abiotic stress and genetic improvement in France. In: International Institute for Beet Research (IIBR) (Ed) *Proceedings of the 64th Congress of the IIRB*, 26-27 June 2001, IIRB, Bruges, pp 153-158

Rochon D-A, Kakani K, Robbins M, Reade R (2004) Molecular aspects of plant virus transmission by olpidium and plasmodiophorid vectors. *Annual Review of Phytopathology* **42**, 211-241

Rush CM (2003) Ecology and epidemiology of *Benyviruses* and plasmodiophorid vectors. *Annual Review of Phytopathology* **41**, 567-592

Rysanek P, Stocky G, Haeberle AM, Putz C (1992) Immunogold labelling of beet necrotic yellow vein virus particles inside its fungal vector, *Polymyxa betae* k. *Agronomie* **12**, 651-659

Schirmer A, Link D, Cognat V, Moury B, Beuve M, Meunier A, Bragard C, Gilmer D, Lemaire O (2005) Phylogenetic analysis of isolates of *Beet necrotic yellow vein virus* collected worldwide. *Journal of General Virology* **86**, 2897-2911

Schmidlin L, De Bruyne E, Weyens G, Lefebvre M, Gilmer D (2008) Identification of differentially expressed root genes upon rhizomania disease. *Molecular Plant Pathology* **9**, 741-751

Schmidlin L, Link D, Mutterer J, Guilley H, Gilmer D (2005) Use of a beet necrotic yellow vein virus RNA-5-derived replicon as a new tool for gene expression. *Journal of General Virology* **86**, 463-467

Schmitt C, Balmori E, Jonard G, Richards KE, Guilley H (1992) *In vitro* mutagenesis of biologically active transcripts of beet necrotic yellow vein virus RNA 2: Evidence that a domain of the 75-kda readthrough protein is important for efficient virus assembly. *Proceedings of the National Academy of Sciences USA* **89**, 5715-5719

Scholten OE, Bock TSMD, Klein-Lankhorst RM, Lange W (1999) Inheritance of resistance to *Beet necrotic yellow vein virus* in *Beta vulgaris* conferred by a second gene for resistance. *Theoretical and Applied Genetics* **99**, 740-746

Scholten OE, Lange W (2000) Breeding for resistance to rhizomania in sugar beet: A review. *Euphytica* **112**, 219-231

Scholten OE, Paul H, Peters D, Van Lent JW, Goldbach RW (1994) *In situ* localisation of *Beet necrotic yellow vein virus* (BNYVV) in rootlets of susceptible and resistant beet plants. *Archives of Virology* **136**, 349-361

Sohi H, Maleki M (2004) Evidence for presence of types A and B of *Beet necrotic yellow vein virus* (BNYVV) in Iran. *Virus Genes* **29**, 353-358

Steven A, Trus B, Putz C, Wurtz M (1981) The molecular organization of *Beet necrotic yellow vein virus*. *Virology* **113**, 428-438

Suarez MB, Grondona I, Garcia-Benavides P, Monte E, Garcia-Acha I (1999) Characterization of beet necrotic yellow vein furovirus from spanish sugar beets. *International Microbiology* **2**, 87-92

Tamada T (1999) *Benyviruses*. In: Webster R, Granoff A (Eds) *Encyclopedia of Virology* (2nd Edn), Academic Press, New York, N.Y., pp 154-160

Tamada T, Abe H (1989) Evidence that beet necrotic yellow vein virus RNA-4 is essential for transmission by the fungus *Polymyxa betae*. *Journal of General Virology* **70**, 3391-3398

Tamada T, Baba T (1973) *Beet necrotic yellow vein virus* from rhizomania-affected sugar beet in Japan. *Annals of the Phytopathological Society of Japan*, 325-332

Tamada T, Schmitt C, Saito M, Guilley H, Richards K, Jonard G (1996) High resolution analysis of the readthrough domain of beet necrotic yellow vein virus readthrough protein: A KTER motif is important for efficient transmission of the virus by *Polymyxa betae*. *Journal of General Virology* **77**, 1359-1367

Tamada T, Shirako Y, Abe H, Saito M, Kigushi T, Harada T (1989) Production and pathogenicity of isolates of *Beet necrotic yellow vein virus* with different numbers of RNA components. *Journal of General Virology* **70**, 3399-3409

Tamada T, Uchino H, Kusume T, Saito M (1999) RNA 3 deletion mutants of *Beet necrotic yellow vein virus* do not cause rhizomania disease in sugar beets. *Phytopathology* **89**, 1000-1006

Tuitert G (1990) Assessment of the inoculum potential of *Polymyxa betae* and *Beet necrotic yellow vein virus* (BNYVV) in soil using the most probable number method. *Netherlands Journal of Plant Pathology* **96**, 331-341

Valentin C, Dunoyer P, Vetter G, Schalk C, Dietrich A, Bouzoubaa S (2005) Molecular basis for mitochondrial localization of viral particles during beet necrotic yellow vein virus infection. *Journal of Virology* **79**, 9991-10002

Van Regenmortel MH, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB (Eds) (2000) Virus taxonomy: VIIth report of the International Committee on Taxonomy of Viruses, Academic Press, NewYork, 1162 pp

Verchot-Lubicz J, Rush C, Payton M, Colberg T (2007) *Beet necrotic yellow vein virus* accumulates inside resting spores and zoosporangia of its vector *Polymyxa betae* BNYVV infects *P. betae*. *Virology Journal* **4**, 37

Vetter G, Hily JM, Klein E, Schmidlin L, Haas M, Merkle T, Gilmer D (2004) Nucleo-cytoplasmic shuttling of the beet necrotic yellow vein virus RNA-3-encoded p25 protein. *Journal of General Virology* **85**, 2459-2469

Ward E, Kanyuka K, Motteram J, Kornyukhin D, Adams MJ (2005) The use of conventional and quantitative real-time PCR assays for *Polymyxa graminis* to examine host plant resistance, inoculum levels and intraspecific variation. *New Phytologist* **165**, 875-885

Ward L, Koenig R, Budge G, Garrido C, McGrath C, Stubbley H, Boonham N (2007) Occurrence of two different types of RNA-5-containing *Beet*

necrotic yellow vein virus in the UK. *Archives of Virology* **152**, 59-73

Webb CR, Gilligan CA, Asher MJC (2000) Modelling the effect of temperature on the development of *Polymyxa betae*. *Plant Pathology* **49**, 600-607

Weiland JJ, Van Winckle D, Edwards MC, Larson RL, Shelver WL, Freeman TP, Liu H-Y (2007) Characterization of a US isolate of *Beet black scorch virus*. *Phytopathology* **97**, 1245-1254

Whitney ED (1989) Identification, distribution, and testing for resistance to rhizomania in *Beta maritima*. *Plant Disease* **73**, 287-290

Wisler GC, Lewellen RT, Sears JL, Wasson JW, Liu HY, Wintermantel WM (2003) Interactions between *Beet necrotic yellow vein virus* and *Beet soilborne mosaic virus* in sugar beet. *Plant Disease* **87**, 1170-1175

Xie Q, Frugis G, Colgan D, Chua NH (2000) Arabidopsis NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. *Genes and Development* **14**, 3024-3036