FIRST DETECTION OF *TOMATO BLACK RING VIRUS* (TBRV) IN A FRENCH VINEYARD

Coralie LAVEAU[1]*, Maarten van HELDEN[2], Guillaume DARRIEUTORT[1], Daniel ESMENJAUD[3] and Gérard DEMANGEAT[4]

1 : Univ. Bordeaux, Vitinnov, ISVV, 1 cours du Général de Gaulle, 33170 Gradignan, France
2 : Univ. Bordeaux, Bordeaux Sciences Agro, ISVV, 1 cours du Général de Gaulle, 33170 Gradignan, France
3 : INRA, UMR1355 ISA, INRA-CNRS-Université de Nice Sophia-Antipolis, 06903 Sophia-Antipolis, France
4 : INRA, UMR1131 SVQV, INRA-Université de Strasbourg, 28 rue de Herrlisheim, 68021 Colmar Cedex, France

Abstract

Aim : Grapevine plants from the Bordeaux wine region (France) showing symptoms of fanleaf degeneration, but negative for the two main fanleaf viruses were screened by ELISA for other nepoviruses that could explain the symptoms.

Methods and results : ELISA tests were performed over a 3-year period (2009-2011) on leaves and woody canes. A total of 665 grapevine plants grafted with Merlot, Cabernet franc and Cabernet-Sauvignon, were found free from *Grapevine fanleaf virus* (GFLV) and *Arabis mosaic virus* (ArMV) but infected with *Tomato black ring virus* (TBRV). The Longidorid nematode species *Longidorus attenuatus*, known as the TBRV vector in grapevine, was detected from soil samples collected in the infected area.

Conclusion : Both the virus and its vector might have originated from a vegetable garden established prior to vine planting, considering that the TBRV-infected area with the most fanleaf degeneration symptoms co-localizes with this previous garden.

Significance and impact of the study : This is the first record of TBRV infection in a grapevine plot in France.

Key words : grapevine, *Tomato black ring virus* (TBRV), ELISA, *Longidorus attenuatus*, France

Résumé

Objectif : Des plants de vignes du vignoble bordelais, exprimant des symptômes de court-noué et indemnes des deux principaux virus responsables de la maladie en France, ont été testés par ELISA pour évaluer la présence d'autres népovirus afin d'expliquer les symptômes.

Méthodes et résultats : Des tests ELISA réalisés à partir de ces plants ont confirmé l'absence du *Grapevine fanleaf virus* (GFLV) et de l'*Arabis mosaic virus* (ArMV), mais ont mis en évidence la présence du *Tomato black ring virus* (TBRV). La présence du TBRV a été confirmée sur feuilles et sur bois pendant trois ans. Le nématode *Longidorus attenuatus*, connu pour transmettre naturellement le TBRV à la vigne, a été identifié dans des échantillons de sol prélevés dans la parcelle.

Conclusion : Avant la plantation de la vigne, un jardin potager, occupant la partie actuellement la plus atteinte de cette parcelle, pourrait avoir hébergé le virus et son vecteur et être ainsi à l'origine de la contamination.

Signification et impact de l'étude : Il s'agit de la première détection du TBRV sur la vigne en France.

Mots clés : vigne, *Tomato black ring virus* (TBRV), ELISA, *Longidorus attenuatus*, France

manuscript received 15th April 2013 - revised manuscript received 5th August 2013

*Corresponding author : coralie.laveau@agro-bordeaux.fr

J. Int. Sci. Vigne Vin, 2013, **47**, n°3, 191-194

INTRODUCTION

To date, more than 60 virus species have been recorded from grapevine. *Closteroviridae* and *Secoviridae* are the most economically detrimental viruses for the wine grape industry (Martelli and Boudon-Padieu, 2006). Among the *Secoviridae* family, viruses from the *Nepovirus* genus induce a degenerative disease named fanleaf degeneration, which has spread worldwide. This viral disease is responsible for symptoms such as internode shortening, leaf asymmetry, fasciation of shoots and mosaic discoloration of leaves. The degeneration leads to a decrease in grape quantity and quality through flower abortion and "millerandage" and finally to plant death.

Fifteen nepoviruses are able to induce fanleaf degeneration in grapes and eight of them have been associated with ectoparasitic nematodes for their transmission from vine to vine or from other host plants to vines (Martelli and Boudon-Padieu, 2006). *Grapevine fanleaf virus* (GFLV) is the main causal agent of fanleaf degeneration worldwide. In Europe, *Arabis mosaic virus* (ArMV) is the second most important nepovirus involved in this disease. These two nepoviruses are specifically transmitted by the dagger nematodes *Xiphinema index* and *X. diversicaudatum*, respectively.

Tomato black ring virus (TBRV) has been reported as another agent responsible for grapevine fanleaf degeneration in Europe. TBRV was first found in Germany and then in former Yugoslavia, Greece, Israel, and Turkey (Martelli and Boudon-Padieu, 2006 ; Uyemoto *et al.*, 2009). In France, TBRV has never been reported, up to now, in grapevine but is widespread in many other crops including bean, sugar beet, lettuce, raspberry, strawberry and peach (OEPP, 1990), cabbage, alfalfa, tomato and potato (http://ephytia.inra.fr). The different TBRV strains are naturally transmitted by the nematode species *Longidorus elongatus* and *L. attenuatus* but only *L. attenuatus* is able to transmit TBRV to grapevine (Martelli and Boudon-Padieu, 2006).

This research note reports the first detection of TBRV as the causal agent of fanleaf degeneration and the first detection of *L. attenuatus*, the TBRV vector, within a commercial plot of the Bordeaux wine region.

MATERIALS AND METHODS

1. Plot characteristics

The infected plot covers about 1 ha of sandy-gravelly soil and was planted with vines for the first time in 1998. The plot is divided into three sections, each planted with a different *Vitis vinifera* cultivar (Merlot, Cabernet franc and Cabernet-Sauvignon) grafted on Gravesac rootstock at 8000 plants/ha. The vine plot was formerly occupied by a house with a vegetable garden and a road. In 2009, about 30 vines showed characteristic symptoms of degeneration : a patch of 20 vines in the Cabernet franc area had low vigor (as assessed visually by internode length and cane diameter) and scattered isolated vines in areas throughout the plot exhibited mosaic leaf discoloration.

2. Sampling and Nepovirus detection by DAS-ELISA

In 2009, we first tested 665 asymptomatic vines uniformly distributed over the plot (133 batches of 5 plants) for the presence of GFLV and ArMV. Then the 30 symptomatic plants described above were sampled and tested for GFLV/ArMV and TBRV (test 1, Table 1). Thereafter, all 133 initial batches were tested for TBRV (test 2, Table 1). Finally, the plants from 24 batches positive for TBRV were re-sampled and tested individually (test 3, Table 1). In subsequent years, woody and leaf samples were collected on a varying number of previously 'positive'and 'negative'plants (tests 4 to 6, Table 1).

DAS-ELISA was carried out with crude plant extracts from leaves or woody samples. GFLV, ArMV and TBRV were detected using the reagents provided by

Table 1 - ELISA results for TBRV detection from different plant organs and sampling years.

Test	Year	Plant material	Type of sample	Total samples	TBRV-positive samples (% positive/total samples)
1	2009	Leaves	Individual plant	30	21 (70%)
2	2009	Leaves	Batch of 5 vines	133	29 (22%)
3	2009	Woody canes	Individual plant	119	43 (36%)
4	2010	Leaves	Individual plant	42	22 (52%)
5	2010	Woody canes	Individual plant	72	37 (51%)
6	2011	Leaves	Individual plant	82	35 (43%)

Bioreba AG (Switzerland). Substrate hydrolysis was recorded at 405 nm with a Dynex MRX II microplate reader.

3. Detection of the nematode vector

Soil samples were collected as described by Villate *et al.* (2008). Two-liter soil samples were taken on the side of trenches showing the most fine vine roots (at approx. 75-cm depth) at 6 locations distributed in the TBRV-infected plot among the three cultivars. Species identification used the polytomous key from Chen *et al*. (1997) and was completed with a molecular diagnostic based on specific primers developed for *Longidorus* species (Hübschen *et al.*, 2004).

RESULTS AND DISCUSSION

Among the 133 batches of leaf samples from 665 plants distributed regularly over the plot area, none reacted positively for ArMV or GFLV. Moreover, ELISA testing for GFLV and ArMV from a second sampling performed on leaves and woody canes from symptomatic grapevine plants detected none of these two viruses (data not shown). Detection was then focused on TBRV because i) it is one of the 15 nepoviruses identified in *V. vinifera*, ii) it has been reported from other crops in France (Migliori *et al.*, 1984), and iii) *L. attenuatus*, the TBRV nematode vector, has been detected in Armagnac, another south-western vineyard area (D. Esmenjaud, 1990, unpublished).

The first TBRV ELISA testing led to the detection of TBRV in 21 of the 30 symptomatic grapevine plants sampled (test 1, Table 1). Among these 21 positive plants, 14 were Cabernet franc plants located in the low vigor area. TBRV ELISA was then extended to the initial 133 batches of 5 plants that were free from GFLV and ArMV. Of these, 29 batches (22 %) were positive for TBRV (test 2, Table 1). TBRV was detected in the three cultivars but with a higher rate in Merlot. 24 of the TBRV-positive batches of 5 plants (total of 119 living plants - one plant died) were individually re-sampled on woody canes and analyzed by ELISA (test 3, Table 1). 43 of these woody samples (36 %) were TBRV-positive, which represents 6.5 % of the 665 plants initially sampled. Tests 4 to 6 (Table 1) were performed in 2010 and 2011 on samples from putative healthy or infected plants. These tests confirmed the presence of TBRV in the symptomatic area but also identified individual TBRV-positive plants outside this infected area. ELISA test conducted on woody canes from 83 grapevine plants did not show mixed infections with other nepoviruses or *Closteroviridae* (data not shown).

Visual observations of the TBRV-infected plants showed typical known fanleaf symptoms on the clusters (Martelli and Boudon-Padieu, 2006), explaining the harvest losses usually induced by nepoviruses. Indeed, clusters from infected plants were drastically smaller than clusters from TBRV-free grapevine plants. Moreover, severe “coulure” (flower abortion) symptoms were obvious on the TBRV-infected bunches as illustrated in Figure 1.

Filiform nematodes (14, 26, 1, 1 and 12 individuals) from *Longidorus* sp. were found in 5 out of the 6 2-liter soil samples. The highest number (26 individuals) was found close to the less vigorous grapevine plants. Using morphological and molecular criteria, all specimens were identified as *L. attenuatus*, the known TBRV nematode vector in grapevine.

Figure 1 - Clusters collected from TBRV-free plants (left) and symptomatic TBRV-infected plants (right) illustrating the strong yield reduction induced by the virus.

This first detection of both TBRV and its nematode vector in a commercial plot in the Bordeaux wine region in France raises the question of the origin of the contamination. To the knowledge of the owner, the infected plot had never been planted with grapevine before 1998. However, the less vigorous TBRV-infected area did match a previous vegetable garden. Vegetable crops like barley, cabbage, potato, strawberry, sugar beet and carrot are hosts of both TBRV and the nematode vector. It seems highly probable that primary grapevine infestation in this area is due to *L. attenuatus* nematodes having acquired the virus on vegetable plants from this garden. The presence of individual TBRV-positive plants scattered elsewhere in the plot could result from secondary nematode transmission. To fulfill this hypothesis, TBRV detection in nematodes is in progress.

If this scenario is right, it would imply that the absence of vines previous to planting in candidate plots for virus-free nurseries would not be sufficient to avoid transmission and infection by this virus.

Further work will focus on i) the detection of TBRV in *L. attenuatus* and the assessment of TBRV transmission to bait plants via the recovered nematodes from grapevine soil, ii) a survey to estimate the prevalence of TBRV in French vineyards, iii) the examination of the viticultural performance of TBRV-infected material, and iv) the molecular properties of this TBRV isolate as no TBRV sequence from grapevine is available up to now.

Acknowledgements : The authors thank the château where the study was carried out for assistance and financial support.

REFERENCES

Chen Q.W., Hooper D.J., Loof P.A.A. and Xu J.H., 1997. A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda : Dorylaimoidea). *Fundamental and Applied Nematology*, **20** (1), 15-28.

Hübschen J., Kling L., Ipach U., Zinkernagel V., Brown D.J.F. and Neilson R., 2004. Development and validation of species-specific primers that provide a molecular diagnostic for virus-vector longidorid nematodes and related species in German viticulture. *European Journal of Plant Pathology*, **110** (9), 883-891.

Martelli G.P. and Boudon-Padieu E., 2006. Directory of infectious diseases of grapevines and viroses and virus-like diseases of the grapevine : bibliographic report 1998-2004. Bari : CIHEAM (Options Méditerranéennes : Série B. Etudes et Recherches no. 55).

Miglori A., Marzin H., and Rana G.L., 1984. Mise en évidence du Tomato Black Ring Virus (TBRV) chez l'artichaut en France. *Agronomie*, **4** (7), 683-686.

OEPP (Organisation Européenne et Méditerranéenne pour la Protection des Plantes), 1990. Fiche informative sur les organismes de quarantaine. Préparé par le CABI et l'OEPP pour l'UE sous contrat 90/399003. http://www.eppo.int/QUARANTINE/virus/Tomato_black_ring_virus/F-tmbrxx.pdf.

Uyemoto J.K., Martelli G.P. and Rowhani A., 2009. Grapevine viruses, viruslike diseases, and other disorders. In : *Virus Diseases of Plants : Grapevine, Potato, and Wheat Image Collection and Teaching Resource* (CD-Rom). APS Press, St. Paul, MN 55121.

Villate L., Fievet V., Hanse B., Delemarre F., Plantard O., Esmenjaud D. and van Helden M., 2008. Spatial distribution of the dagger nematode *Xiphinema index* and its associated Grapevine fanleaf virus in French vineyard. *Phytopathology*, **98** (8), 942-948.