Vitis **52** (2), 85–89 (2013)

Quantitative PCR assay for detection of Bois noir phytoplasmas in grape and insect tissue

J. Fahrentrapp[1)], G. Michl[2)] and M. Breuer[3)]

[1)] Institute of Natural Resource Sciences, ZHAW Zürich University of Applied Sciences, Departement of Life Sciences und Facility Management, Wädenswil, Switzerland
[2)] Julius Kühn-Institute (JKI), Institute for Plant Protection in Fruit Crops and Viticulture, Geilweilerhof, Siebeldingen, Germany
[3)] State Institute for Viticulture and Enology (WBI), Freiburg, Germany

Summary

In Europe's vineyards "Bois noir" (BN) is an expanding yellows disease on *Vitis vinifera*. It is associated with phytoplasmas of the stolbur group (16SrXII-A). Two subtypes are important, one is associated with *Urtica dioica* and one with *Convolvulus arvensis*. Both phytoplasma types are transmitted by the insect *Hyalesthes obsoletus*. A nucleic acid extraction method for *V. vinifera* and *H. obsoletus* was developed together with a real time PCR (qPCR) assay based on a polymorphic sequence with homology to a putative dimethyladenosine transferase. The comparison of the conventional detection method with the qPCR assay of 40 insect and 40 *V. vinifera* samples showed a 10 % higher sensitivity of qPCR in plant samples. The titer of phytoplasmas in *H. obsoletus* was 2643-fold increased in the strongest infected samples compared to the lowest ones.

The results suggest this real-time PCR as a valid and fast alternative procedure for the detection and quantification of BN phytoplasmas. The assay allows to discriminate the two phytoplasma types and to quantify phytoplasmas in *H. obsoletus*.

K e y w o r d s : Grape diseases, "Vergilbungskrankheit", phytoplasma disease, quantitative real-time PCR (qPCR).

Introduction

Yellows diseases are known all over the world. They are associated with phytoplasmas which are cell wall-less bacteria inhabiting the sieve tubes of their host plants and different tissues of their vectors (Hogenhout *et al.* 2008). "*Candidatus* Phytoplasma" belongs to the class Mollicutes and evolved from Gram-positive bacteria. They are closely related to Acheloplasmas as well as to Mycoplasmas that colonize animal tissues (Lim and Sears 1989, Lee *et al.* 2000). Phytoplasmas are transmitted by sap sucking invertebrates like planthoppers, leafhoppers and psyllids (Weintraub and Beanland 2006) but they can also be transferred to other plants by dodder or grafting (Credi and Santucci 1992). Plants infected with phytoplasmas often show symptoms such as a bushy growth due to proliferation of axillary shoots, virescence or phyllody. Phytoplasmas cause economically important diseases such as "apple proliferation disease", "flavescence dorée" (FD), and "lime witches' broom" (Bertaccini 2007). However, some economically positive examples of phytoplasma infection are described, such as for poinsettias (*Euphorbia pulcherrima*), which only branch in the desired manner when they are infected with phytoplasmas (Lee *et al.* 1997).

One grapevine yellows disease called "Vergilbungskrankheit" or "Bois noir" (BN) is widespread in Germany and other European countries. At least three different types of phytoplasmas belonging to the stolbur group 16SrXII-A (proposed as Ca. Phytoplasma solani; IRPCM 2004, Pacifico *et al.* 2009, Berger *et al.* 2009) are associated with this disease. Tuf-type a (type I) is associated with *Urtica dioica*, Tuf-type b (type II) with *Convolvulus arvensis* and Tuf-type c (type III) with *Calystegia sepium* (Langer and Maixner 2004). The latter is not important for viticulture and seems to be mainly restricted to its host plant.

The phloem-feeding cixiid *Hyalesthes obsoletus* transmits phytoplasmas from infected host plants to healthy ones. Predominantly in case of removal of their host plants, *H. obsoletus* uses alternative forage plants. For this incidence phytoplasmas can be transmitted to *V. vinifera*. Reinfection within *V. vinifera* was not reported and *V.vinifera* can be seen as dead-end host (Weintraub and Beanland 2006). At present, PCR analysis with primer set rSTOL/fSTOL is used for detection of phytoplasmas belonging to the stolbur group (Maixner *et al.* 1995). Additional RFLP analysis with the elongation factor Tu is used for the discrimination between the phytoplasma types that cause BN (Schneider *et al.* 1997). These methods are time consuming, laborious, and the risk of cross contamination during manipulation is high. Several qPCR assays detecting phytoplasmas including those of group 16SrXII-A in plant and insect tissues were developed (Hodgetts *et al.* 2009, Margaria *et al.* 2009). One qPCR assay was developed discriminating also the BN phytoplasma Tuf-type a and b in grape vine tissue (Berger *et al.* 2009).

In the following report we describe the extraction of DNA from *V. vinifera* and *H. obsoletus* tissues directly in a 96-well plate as well as the development of a subsequent qPCR assay. The qPCR enables to discriminate sequences of the two main phytoplasma strains associated with BN and to estimate the relative titer of phytoplasmas in *H. obsoletus*.

Correspondence to: Dr. J. Fahrentrapp, Institute of Natural Resource Sciences, ZHAW Zürich University of Applied Sciences, Departement of Life Sciences und Facility Management, Grüntal, 8820 Wädenswil, Switzerland. Fax: +41-58-934-5853. E-mail: johannes.fahrentrapp@zhaw.ch

Material and Methods

Insect/plant sampling and DNA extraction: In July 117 *Hyalesthes obsoletus* individuals were collected by scooping on *U. dioica* and *C. arvensis* in vineyards in Southern Germany (Baden). After freezing in liquid nitrogen, DNA was extracted in 96-deep-well plates (Qiagen, Germany). Therefore frozen *H. obsoletus* was homogenized with quartz sand in a cell mill. The disrupted tissue was solved in 400 µL of extraction buffer (1.4 M NaCl, 10 mM EDTA, 100 mM Tris / HCl (pH = 8), CTAB 2 %), incubated 20 min at 65 °C, and followed by phase separation with an equal volume of chloroform/isoamyl alcohol (24:1 v/v). Genomic DNA was precipitated with isopropanol (0.7 vol) and NaAC (0.1 vol, 3 M) at -20 °C for at least 30 min. After 20 min centrifugation at 4657 g (Multifuge 4KR, Heraeus, Germany), the precipitate was washed with ethanol (70 %), dried and dissolved in 25 µL distilled water. The DNA concentration measured by photometer (Specord®50, Analytik Jena, Germany) ranged from 30 to 150 ng·µL^{-1}. The DNA was stored in plates at -20 °C until use.

Leaves of symptomatic grapevines of different cultivars (mostly 'Pinot Noir') were collected in vineyards in Southern Germany (Baden) during end of June and beginning of July. DNA of the leaf veins of forty symptomatic leaves from forty different plants was extracted as described above with exception of the extraction buffer, which contained 1.4 M NaCl, 20 mM EDTA, 500 mM Tris / HCl (pH = 8), CTAB 2 % and ß-Mercaptoethanol 0.2 % (added directly before use).

Fourty plant and fourty insect DNA samples were used to compare qPCR and conventional PCR results and 77 samples were used to estimate relative titers of phytoplasmas in the insect.

Quantitative real time PCR: Primers and probes (TaqMan®, (LIVAK *et al*. 1995)) for qPCR were designed on a sequence named STOL4 (DAIRE *et al.* 1997). STOL4 sequences from stolbur phytoplasma Tuf-type a and b were sequenced and deposited at the GenBank (EU496867, EU496868). The forward and reverse primers were designed to amplify a 222 nucleotide sequence comprising a polymorphism between the sequences of Tuf-type a and b. The designed TaqMan® probes covered the amplified polymorphism. The probe of Tuf-type a phytoplasma at position 610 (TMStol4-tA) was 5'end labeled with FAM and 3'end quenched with BHQ 1. The probe detecting Tuf-type b phytoplasmas at position 610 (TMStol4-tB) was labeled with HEX and BHQ 1 at the 5' and 3' ends, respectively (Table). QPCR reaction with probes TMStol4 type A and -tB could be run in one reaction.

Primers that allow the amplification of DNA from *H. obsoletus* were designed from sequences obtained from touchdown PCR fragments with degenerated oligonucleotides (DON *et al.* 1991). The latter were derived from an alignment of different wnt-genes of *Apis mellifera* (XP_397473), *Tribolium castaneum* (XP_974684), *Anopheles gambiae str. PEST* (EAA14587), *Aedes aegypti* (EAT32499) and *Drosophila melanogaster* (NP_476924).

Table

Used qPCR primers and probes

Name	Forward	Reverse	Probe	Efficiency	r2	Specific for
TMStol4-tA	GATCCACCCTTCGCTTTATTT	CTTGGAATAACTGAAGCGACA	5'FAM GTCAAACACCACCTTTTATCATTCCT 3'BHQ1	102	0.99	Bois noir phytoplasma Tuf-type a
TMStol4-tB	*	*	5'HEX ATCAAAAACAACAACCTTTTATCATTC 3'BHQ1	99	0.98	Bois noir phytoplasma Tuf-type b
TMHo	TCTGTCTGCTGCGTGAACAT	ACTGTAGCTCGCCCAGGTT	5'TR TAACAAACTCCGGAAGAACATAACTCA 3'DDQ2	111	0.98	*H. obsoletus*
TMVitis18s	CCGTTGCTCTGATGATTCATGA	CGTCGCCGGCACGAT	5' FAM AACTCGACGGATCGCACGGC 3'BHQ1	105	0.99	*V. vinifera*

*same as TMStol4-tA; FAM = 6-carboxy fluorescein, BHQ1 = black hole quencher 1, HEX = 6-carboxy-2'.4.4'.5'.7.7'-hexachlorofluorescein, TR = Texas Red, DDQ2 = deep dark quencher.

The amplified and sequenced sequence of *H. obsoletus* (EU595027) was used to design a primer/probe set (TMHo) which amplified a product of 129 basepairs. Primers and probes specific for 18S rDNA from *V. vinifera* (TMVv18s) were designed on ribosomal 18S sequence of the cross *V. berlandieri* x *V. vinifera* (AF321263) (Tab. 1). Primer efficiency was evaluated on standard curves from 1:10 diluted plasmid DNA containing TA-cloned PCR fragments of stolbur phytoplasma Tuf-type a, b, *H. obsoletus* and *V. vinifera*, respectively.

The qPCR assays with the different TaqMan® probes were performed with peqGOLD Hot Start Mix 'Real Time' (PeqLab, Germany). Primer and probes were 900 and 200 nM, respectively. A volume of 0.75 µL genomic DNA (30-150 $ng \cdot \mu L^{-1}$) dissolved in distilled water was added to 10 µL reaction mixtures. The reactions were set up in duplicates on 96-well plates. QPCR reactions were run on the real time PCR cycler iQ5 (BioRad, USA) with 10 min at 95 °C followed by 40 cycles of 20 s at 95 °C and 60 s at 60 °C with plate read. Samples were counted positive for phytoplasmas with threshold cycle (Ct) < 35. The threshold line was set above the noise to 50 RFU (relative fluorescence units). A positive and negative control (kindly provided by M. Maixner) consisting of DNA extracted from *Catharanthus roseus* infected with stolbur phytoplasma Tuf-type a and b, respectively, as well as a no template control were included on every plate. Putatively failed DNA extraction was excluded by qPCR with primer pairs flanking a specific fragment of *H. obsoletus* (TMHo) and *V. vinifera* (TMVv18s), respectively.

The results obtained with qPCR were compared to the conventional detection method by PCR using the rSTOL/fSTOL primers according to Maixner *et al.* (Maixner *et al.* 1995).

The relative titers of phytoplasmas in *H. obsoletus* were estimated applying the ddCt-method (Livak and Schmittgen 2001) to the data obtained with primer/probe sets TMHo and TMStol4. Body mass and individual cell number of *H. obsoltus* were not taken into account as variables for this calculations.

Results

Sensitivity of qPCR versus conventional PCR: Four qPCR primer/probe sets based on the TaqMan® technology were developed to detect specific fragments of *V. vinifera*, *H. obsoletus* as well as Tuf-type a and b of stolbur phytoplasmas. The sequence used for designing the phytoplasma specific primers were homologous to a dimethyltransferase gene of Aster yellows witches'-broom phytoplasma AYWB and Onion yellows phytoplasma OY-W (GenBank: YP_456611 and BAF73583, respectively) with 59 and 56 % identity. The sequence of *H. obsoletus* produced with degenerated primers which was used for designing TMHo primer/probes was not homologous to any nucleotide and protein sequence of NCBI's databases. The primer efficiency was estimated to range between 98 and 111 % with an r^2 of 0.98 and 0.99 (Tab. 1). In dilution series of plasmids containing the target sequences all fragments were detectable to a concentration of 6 fg. C(t) values of phytoplasma specific primers (TMStol4-tA and -tB) ranged from 25-33 in insect samples; C(t) values of TMHo ranged from 13-20 and of TMVv18s from 17-23. Multiplex qPCR with primers TMStol4-tA and -tB could discriminate plasmids with either of the target sequences. In about half of the 40 *V. vinifera* samples phytoplasmas were detectable by qPCR. In plant tissue 19 samples were identified with qPCR to carry phytoplasmas whereas 17 samples with the conventional PCR method. Two of the positive samples were detected with qPCR to be of stolbur phytoplasma Tuf-type b whereas the other positive samples were of Tuf-type a. Ten of 40 sampled *H. obsoletus* were identified with both the conventional and the qPCR method to be infected with phytoplasmas. Additional three and six samples were detected to be infected with phytoplasmas using the conventional PCR and the qPCR, respectively. All phytoplasma detected in *H. obsoletus* samples were of Tuf-type a.

For calculation of the relative titers of phytoplasmas in insects the DNA content was normalized using the relative quantification method ddCt in which the target amplicon is quantified relative to a normalizing amplicon. The titers of 77 samples of *H. obsoletus* infected with BN phytoplasma Tuf-type a were estimated to range from one to 2643 fold of the lowest one, with a median of 40 (Figure).

Discussion

Several real-time PCR assays for different groups or subgroups of phytoplasmas have been developed focusing on the differentiation of BN and FD (Galetto *et al.* 2005, Angelini *et al.* 2007, Gori *et al.* 2007). Others are in the view of discriminating different yellows diseases and phytoplasmas of different groups (Hodgetts *et al.* 2009). The qPCR described in the present work is the first with a one-step analysis allowing detecting DNA of stolbur phytoplasma in infected plants as well as vectors. As suggested by specific amplification from plasmid DNA and control DNA samples, stolbur phytoplasma Tuf-type a and b can be discriminated. Due to its practical unimportance we did not include samples infected with stolbur phytoplasma group 16SrXII-A Tuf-type c and others in our study. Two samples of *H. obsoletus* and *V. vinifera* were detected as infected with BN phytoplasma Tuf-type b. The reported method enables to identify indirectly the phytoplasma reservoir (e.g. stinging nettle or bindweed) which can be eliminated reducing the inoculum source and new infections as previously reported (Mori *et al.* 2012).

The qPCR assay was more sensitive in detecting phytoplasmas in plant tissue when compared to the conventional PCR method. This was also found by Galetto *et al.* (2005) describing a qPCR assay targeting phytoplasmas causing FD, BN and apple proliferation disease. The lower specificity of the qPCR assay in insect sample could be explained by inhibitory substances that interact with the qPCR master mix reducing the specificity. This was also

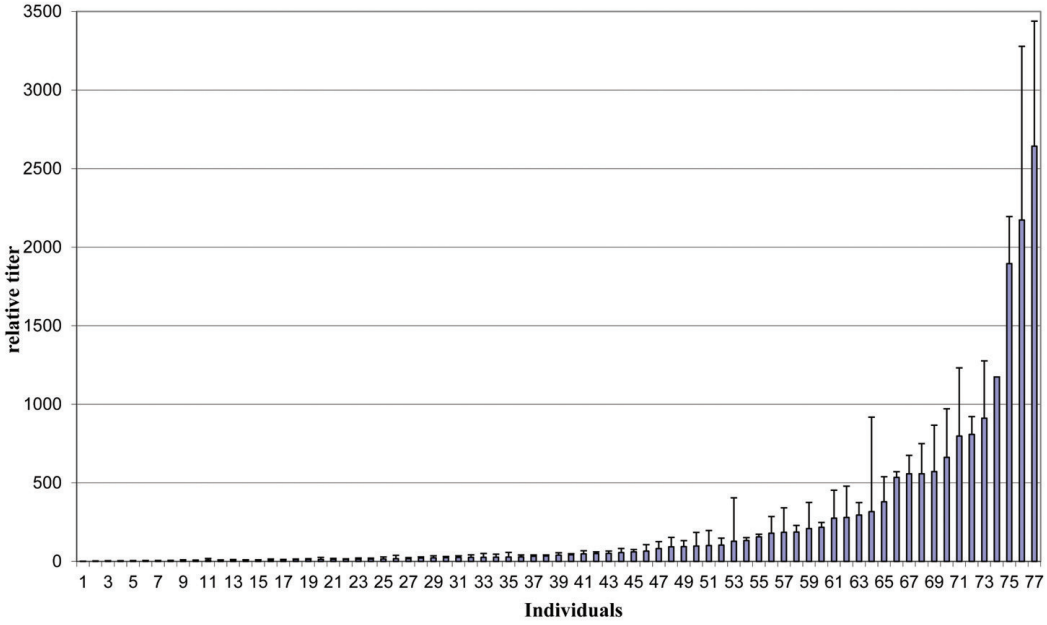

Figure: Relative Titers of infected *H. obsoletus* with stolbur phytoplasma Tuf-type a Data collected by qPCR with primers/probes sets TMHo amplifying a DNA fragment of *H. obsoletus* and TMStol4-tA specific for stolbur phytoplasma Tuf-type a associated with BN. Collected C(t)-values have been calculated to titer values using the ddC(t)-method.

suggested by Galetto *et al.* (2005) who recorded the same phenomenon in insect samples.

In other studies concentrations of phytoplasma in their vectors were quantified by means of comparing the PCR results to dilution series of plasmid DNA (Marzachí and Bosco 2005, Jarausch *et al.* 2004, Bosco *et al.* 2007). Using the ddCt method which is usually used in gene expression studies, our assay indicated titers of phytoplasmas ranging from one to about 2500. This is in accordance with two titers reported by Bosco *et al.* (2007). They found titers of 1147 and 5400 times difference in phytoplasma concentration in *Macrosteles quadripunctulatus* and *Euscelis icisus* but also very high titers of 20300 in *E. variegatus*. Bosco *et al.* (2007) also reported the multiplication of phytoplasmas in insects as a function of time after infection suggesting that tested *H. obsoletus* received phytoplasmas at different time points before sampling. This is clearly possible since the nymphs of *H. obsoltus* may feed on uninfected as well as on infected plants leading to different multiplication rates inside insects until they were sampled. Nevertheless we could not exclude, that titer differences also were caused by different DNA content of the insects depending on age, sex, body size or the presence of eggs in female individuals.

Conclusion

The established qPCR method discriminates stolbur phytoplasmas Tuf-type a and b of group 16SrXII-A in grape and insect samples. For research, the prospect to calculate titers of phytoplasmas in vector tissues will make it possible to differentiate between transmitter insects and non-transmitter but phytoplasma-carrier insects. The impact and potential of *H. obsoletus* as transmitter of BN in relation to phytoplasma titer was not studied so far. In addition, it is possible to investigate whether there are grapevine tissues in which phytoplasmas accumulate or if there are for instance preferences for phytoplasmas between old and new plant material. At present, little is known about the distribution of the BN causing phytoplasmas in plants. However, these topics will become more and more important in the struggle against BN and in the study of the multiple interactions between phytoplasmas with their host plants and transmitting vectors.

Acknowledgements

The work was supported by the Ministry of Food and Rural Affairs Baden-Württemberg, Germany. The authors are grateful to M. Maixner (Federal Research Centre for Cultivated Plants, Bernkastel-Kues, Germany) for providing the control standards of both phytoplasma types. Thanks also to Dr. T. Seibicke, who promoted the work with his ideas, J. Röcker, who was responsible for pre experiments, Dr. F. Peters for correction of the manuscript and the reviewers who made many suggestions to increase the quality of the manuscript.

References

Angelini, E.; Bianchi, G. L.; Filippin, L.; Morassutti, C.; Borgo, M.; 2007: A new TaqMan method for the indentification of phytoplasmas assosiated with grapevine yellows by real-time PCR assay. J. Microbiol. Methods **68**, 613-622.

Berger, J.; Dalla Via, J.; Baric, S.; 2009: Development of a TaqMan allelic discrimination assay for the distinction of two major subtypes of the grapevine yellows phytoplasma Bois noir. Eur. J. Plant Pathol. **124**, 521-526.

Bertaccini, A.; 2007: Phytoplasmas: diversity, taxonomy, and epidemiology. Frontiers Biosci. **12**, 673-689.

Bosco, D.; Galetto, L.; Leoncini, P.; Saracco, P.; Raccah, B.; Marzachì, C.; 2007: Interrelationships Between "Candidatus Phytoplasma

asteris" and Its Leafhopper Vectors (Homoptera: Cicadellidae). J. Econ. Entomol. **100**, 1504-1511.

Credi, R.; Santucci, A.; 1992: Dodder transmission of mycoplasma-like organism (MLOs) from grapevines affected by a flavescence dorée-type disease to Periwinkle. Phytopathol. Mediterr. **31**, 154-162.

Daire, X.; Calari, A.; Reinert, W.; Boudon-Padieu, E.; 1997: Detection and differentiation of grapevine yellows phytoplasmas belonging to the elm yellows group and to the stolbur subgroup by PCR amplification of non-ribosomal DNA. Eur. J. Plant Pathol. **103**, 507-514.

Don, R. H.; Cox, P. T.; Wainwright, B. J.; Baker, K.; Mattick, J. S.; 1991: 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. **19**, 4008.

Galetto, L.; Bosco, D.; Marzachì, C.; 2005: Universal and group-specific real-time PCR diagnosis of flavescence dorée (16Sr-V), bois noir (16Sr-XII) and apple proliferation (16SrX) phytoplasmas from field-collected plant hosts and insect vectors. Ann. Appl. Biol. **147**, 191-201.

Gori, M.; Monnanni, R.; Buiatti, M.; Goti, E.; Carnevale, S.; Da Pratto, L.; Bertaccini, A.; Biricolti, S.; 2007: Establishing a real-time PCR detection procedure of "flavescence dorée" and "bois noir" phytoplasmas for mass screening. Bull. Insectol. **60**, 255-256.

Hodgetts, J.; Boonham, N.; Mumford, R.; Dickinson, M.; 2009: Panel of 23S rRNA gene-based real-time PCR assays for improved universal and group-specific detection of phytoplasmas. Appl. Environ. Microbiol. **75**, 2945-2950.

Hogenhout, S. A.; Oshima, K.; Ammar El, D.; Kakizawa, S.; Kingdom, H. N.; Namba, S.; 2008: Phytoplasmas: bacteria that manipulate plants and insects. Mol. Plant. Pathol. **9**, 403-423.

IRPCM; 2004: International Research Programme on Comparative Mycoplasmology.

Jarausch, W.; Peccerella, T.; Schwind, N.; Jarausch, B.; Krczal, G.; 2004: Establishment of a quantitative real-time PCR assay for the quantification of apple proliferation phytoplasma in plants and insects. Acta Hort. (ISHS) **657**, 415-420.

Langer, M.; Maixner, M.; 2004: Molecular characterisation of grapevine yellows associated phytoplasmas of the stolbur-group based on RFLP-analysis of non-ribosomal DNA. Vitis **43**, 191-199.

Lee, I. M.; Davis, R. E.; Gundersen-Rindal, D. E.; 2000: Phytoplasma: phytopathogenic mollicutes. Annu. Rev. Microbiol. **54**, 221-255.

Lee, I. M.; Klopmeyer, M.; Bartoszyk, I. M.; Gundersen-Rindal, D. E.; Chou, T. S.; Thomson, K. L.; Eisenreich, R.; 1997: Phytoplasma induced free-branching in commercial poinsettia cultivars. Nat. Biotechnol. **15**, 178-182.

Lim P. O.; Sears, B. B.; 1989: 16S rRNA sequence indicates that plant-pathogenic mycoplasmalike organisms are evolutionarily distinct from animal mycoplasmas. J. Bacteriol. **171**, 5901-5906.

Livak, K. J.; Flood, S. J.; Marmaro, J.; Giusti, W.; Deetz, K.; 1995: Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. PCR Methods Appl. **4**, 357-362.

Livak, K. J.; Schmittgen, T. D.; 2001: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods **25**, 402-408.

Maixner, M.; Ahrens, U.; Seemüller, E.; 1995: Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. Eur. J. Plant Pathol. **101**, 241-250.

Margaria, P.; Turina, M.; Palmano, S.; 2009: Detection of Flavescence dorée and Bois noir phytoplasmas, Grapevine leafroll associated virus-1 and -3 and Grapevine virus A from the same crude extract by reverse transcription-RealTime Taqman assays. Plant Pathology **58**, 838-845.

Marzachì, C.; Bosco, D.; 2005: Relative quantification of chrysanthemum yellows (16Sr I) phytoplasma in its plant and insect host using real-time polymerase chain reaction. Mol. Biotechnol. **30**, 117-127.

Mori, N.; Pavan, F.; Reggiani, N.; Bacciavini, M.; Mazzon, L.; Paltrinieri, S.; Bertaccini, A.; 2012: Correlation of bois noir disease with nettle and vector abundance in northern Italy vineyards. J. Pest Sci. **85**, 23-28.

Pacifico, D.; Alma, A.; Bagnoli, B.; Foissac, X.; Pasquini, G.; Tessitori, M.; Marzachì, C.; 2009: Characterization of Bois Noir Isolates by Restriction Fragment Length Polymorphism of a Stolbur-Specific Putative Membrane Protein Gene. Phytopathology **99**, 711-715.

Panda, R. K.; 1995: Role of auxins on the eggplant infected with mycoplasma. Adv. Plant Sci. **8**, 248-252.

Prince, J. P.; Davis, R. E.; Wolf, T. K.; Lee, I. M.; Mogen, B. D.; Dally, E. L.; Bertaccini, A.; Credi, R.; Barba, M.; 1993: Molecular Detection of Diverse Mycoplasmalike Organisms (MLOs) Associated with Grapevine Yellows and Their Classification with Aster Yellows, X-Disease, and Elm Yellows MLOs. Phytopathology **83**, 1130-1137.

Schneider, B.; Gibb, K. S.; Seemüller, E.; 1997: Sequence and RFLP analysis of the elongation factor Tu gene used in differentiation and classification of phytoplasmas. Microbiology 143 (Pt 10), 3381-3389.

Weintraub, P. G.; Beanland, L.; 2006: Insect vectors of phytoplasmas. Annu. Rev. Entomol. **51**, 91-111.

Received June 18, 2012

Corrigendum

On page 86 of the manuscript:

Quantitative PCR assay for detection of Bois noir phytoplasmas in grape and insect tissue

J. Fahrentrapp, G. Michl and M. Breuer

Vitis **52** (2), 85-89 (2013)

multiple errors in the Table occurred. The primer sequences named 'TMHo' in the columns 'Forward' and 'Reverse' were wrong. The primer sequence named 'TMVitis18s' in the column 'Forward' was missing. On the right you will find the corrected Table.

The editors apologise for these errors.

Table

Used qPCR primers and probes

Name	Forward	Reverse	Probe	Efficiency	r2	Specific for
TMStol4-tA	GATCCACCCTTCGCTTTATTT	CTTGGAATAACTGAAGCGACA	5'FAM GTCAAACACCACCTTTTATCATTCCT 3'BHQ1	102	0.99	Bois noir phytoplasma Tuf-type a
TMStol4-tB	*	*	5'HEX ATCAAAAACAACAACCTTTTATCATTC 3'BHQ1	99	0.98	Bois noir phytoplasma Tuf-type b
TMHo	TCTGTCTGCTGCGTGAACAT	ACTGTAGCTCGCCCAGGTT	5'TR TAACAAACTCCGGAAGAACATAACTCA 3'DDQ2	111	0.98	*H. obsoletus*
TMVitis18s	CCGTTGCTCTGATGATTCATGA	CGTCGCCGGCACGAT	5' FAM AACTCGACGGATCGCACGGC 3'BHQ1	105	0.99	*V. vinifera*

*same as TMStol4-tA; FAM = 6-carboxy fluorescein, BHQ1 = black hole quencher 1, HEX = 6-carboxy-2'.4.4'.5'.7.7'-hexachlorofluorescein, TR = Texas Red, DDQ2 = deep dark quencher.