ANNALS OF APPLIED BIOLOGY aab

Environment-driven selection of '*Candidatus* Phytoplasma solani' strain populations associated with bois noir disease in Tuscan vineyards

Journal:	*Annals of Applied Biology*
Manuscript ID	AAB-2018-0080
Manuscript Type:	Original Article
Date Submitted by the Author:	02-Mar-2018
Complete List of Authors:	Pierro, Roberto; University of Pisa, Department of Agriculture, Food and Environment (DAFE) Passera, Alessandro; University of Milan, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA) Panattoni, Alessandra; University of Pisa, Department of Agriculture, Food and Environment (DAFE) Rizzo, Domenico; Regional Phytosanitary Service, Laboratory of Phytopathological Diagnostics and Molecular Biology Stefani, Luciana; Regional Phytosanitary Service, Laboratory of Phytopathological Diagnostics and Molecular Biology Bartolini, Linda; Regional Phytosanitary Service, Laboratory of Phytopathological Diagnostics and Molecular Biology Casati, Paola; University of Milan, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA) Luvisi, Andrea; University of Salento, Department of Biological and Environmental Sciences and Technologies Quaglino, Fabio; University of Milan, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA) Materazzi, Alberto; University of Pisa, Department of Agriculture, Food and Environment (DAFE)
Key Words:	grapevine yellows, stolbur, multi-locus sequence analysis, Vitis vinifera, Hyalesthes obsoletus

Environment-driven selection of '*Candidatus* Phytoplasma solani' strain populations associated with bois noir disease in Tuscan vineyards

Pierro R.[1], Passera A.[2], Panattoni A.[1], Rizzo D.[3], Stefani L.[3], Bartolini L.[3], Casati P.[2], Luvisi A.[4], Quaglino F.[2], Materazzi A.[1]

[1]Department of Agriculture, Food and Environment (DAFE), University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

[2]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA), University of Milan, via Celoria 2, 20133 Milano, Italy

[3]Regional Phytosanitary Service, Laboratory of Phytopathological Diagnostics and Molecular Biology, via Ciliegiole 99, 51100 Pistoia, Italy

[4]Department of Biological and Environmental Sciences and Technologies - University of Salento, via Provinciale Monteroni, 73100 Lecce, Italy

Correspondence: Fabio Quaglino, Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA), University of Milan, via Celoria 2, 20133 Milano, Italy. Tel : +39-02-50316789; fax : +39-02-50316781; e-mail: fabio.quaglino@unimi.it

Running title: Environment-driven selection of BNp strain populations

SUMMARY

Due to its complex epidemiological cycle, including several polyphagous insect vectors and host plants, and the absence of efficient control strategies, Bois noir (BN) disease of grapevine is encroaching wider territories in the main viticultural areas worldwide. Molecular approaches allowed to increase the knowledge about its etiological agent (Bois noir phytoplasma, BNp), revealing interesting features concerning BNp population structure and dynamics and transmission routes in vineyard agro-ecosystems. In the present study, a multi-locus sequence typing approach (*tufB*, *vmp1* and *stamp* genes) was utilized for describing the genetic diversity among BNp strain populations in 17 vineyards localized in five districts of Tuscany (central Italy). The results confirmed that BNp ecology in Tuscan vineyards is mainly associated to the bindweed-related host system, and allowed the identification of 13 collective BNp genotypes. Interestingly, the prevalent BNp genotype was never found in grapevines outside of Tuscany. Moreover, statistical analyses showed significant differences between the composition of BNp strain populations identified in grapevines from distinct weather condition zones (north-western and central-eastern Tuscany). These results reinforce the hypothesis that environmental conditions can drive the selection of BNp strains, also favouring the entrance of unusual genotypes, in vineyards.

Keywords: grapevine yellows; stolbur; multi-locus sequence analysis; *Vitis vinifera*; *Hyalesthes obsoletus*

INTRODUCTION

Bois Noir (BN), one of the most important diseases of the grapevine yellows (GY) phytoplasma-associated complex, causes severe crop losses in vine growing areas in Euro-Mediterranean countries (Belli *et al*., 2010), and in restricted zones of South America (Chile) (Gajardo *et al*., 2009) and Asia (China, Middle East) (Choueiri *et al*., 2002; Duduk *et al*., 2010; Salem *et al*., 2013; Mirchenari *et al*., 2015). In almost all varieties of *Vitis vinifera* L., BN induces typical GY symptoms, including desiccation of inflorescences, berry shrivel, leaf discolorations, reduction of growth and irregular ripening of wood (Belli *et al*., 2010). On the basis of unique biological properties and exclusive molecular markers within multiple genes (*16S rRNA*, *tufB*, *rplV-rpsC*, *secY*), the etiological agent of BN (BN phytoplasma, BNp) has been attributed to the species '*Candidatus* Phytoplasma solani' (subgroup 16SrXII-A) (Quaglino *et al*., 2013).

In Europe and Mediterranean basin, '*Ca*. P. solani' strains are transmitted from plant-to-plant mainly by *Hyalesthes obsoletus* Signoret (Homoptera: Cixiide), a polyphagous leafhopper living preferentially on nettle (*Urtica dioica* L.), bindweed (*Convolvulus arvensis* L.), mugworth (*Artemisia vulgaris* L.), and chaste tree (*Vitex agnus-castus* L.) (Alma *et al*., 1988; Maixner, 1994; Langer & Maixner 2004; Sharon *et al*., 2005). Further studies reported the presence of other insect vectors (e.g. *Reptalus panzeri* and *R. quinquecostatus*) and host plants (Cvrković *et al*., 2014; Landi *et al*., 2015; Marchi *et al*., 2015; Mori *et al*., 2015; Oliveri *et al*., 2015; Chuche *et al*., 2016; Kosovac *et al*., 2016), indicating that this phytoplasma species exists in varied ecosystems, where selection conceivably alters strain populations. This hypothesis implies that ecological relationships of BNp ('*Ca*. P. solani' strains associated with BN), possibly influenced by climatic and geographic features of vineyard agro-ecosystems in different regions, may be reflected in intra-species strain diversity (Quaglino *et al.,* 2009, 2017).

Sequence analysis of *tufB* gene revealed that three *tuf*-types of BNp were present in diseased grapevines, as well as in specific plant hosts, determining two natural ecologies of BNp mainly related to nettle (BNp type *tufB*-a) and bindweed (BNp type *tufB*-b) (Langer & Maixner, 2004).

Multi-locus sequence typing (MLST), based on molecular characterization of more variable genes, such as *secY*, *vmp1* and *stamp*, evidenced a large variability among BNp strains within the *tuf*-types (Foissac *et al*., 2013; Quaglino *et al*., 2016). Molecular approaches, using novel *vmp1*- and *stamp*-based molecular markers of BNp diversity, allowed to increase the knowledge of the BNp population structure and dynamics (Murolo & Romanazzi, 2015; Quaglino *et al*., 2016) and their transmission routes throughout vineyards and their surroundings (Mori *et al*., 2015; Kosovac *et al*., 2016).

In the present study, a multi-locus sequence typing approach, based on the molecular characterization of *tufB*, *vmp1* and *stamp* genes, was utilized for describing the genetic diversity among BNp strain populations in 17 vineyards localized in five districts of Tuscany. Moreover, further analyses were carried out to investigate the possible influence of environmental conditions in determining the strain composition of BNp populations.

MATERIALS AND METHODS

Field surveys

During a field surveys for grapevine yellows (GY), carried out in the late summer of 2016 in 17 vineyards located in the districts of Arezzo, Firenze, Lucca, Massa, and Siena (Tuscany Region), 75 GY-affected grapevine (*Vitis vinifera* L.) plants, cv. Chardonnay (36) and Sangiovese (37), were selected for phytoplasma detection and characterization (Table 1). For each symptomatic plant, 10-15 leaves were collected and their fresh central midribs were dissected and stored at -20°C until DNA extraction. Leaves collected in the screenhouse of the Department of Agriculture, Food and Environment (DAFE, University of Pisa, Italy) from *V. vinifera* cv. Chardonnay and Sangiovese were used as healthy control plants (HC), while leaves collected by *V. vinifera* plants, previously found infected by '*Ca*. P. solani' (subgroup 16SrXII-A) and Flavescence dorée phytoplasmas (FDp) (subgroups 16SrV-C or -D) were used as infected controls (ICs).

Phytoplasma detection

DNA was extracted with 2% cetyltrimethylammonium bromide (CTAB) based buffer from leaf veins according to the protocol described by Li *et al*. (2008), with some modifications according to Pierro *et al*. (2018).

Specific detection of phytoplasmas associated with BN (BNp) and FD (FDp), the GY mainly present in Europe, was carried out by amplification of 16S ribosomal DNA through TaqMan assay using the Rotor-Gene *Q* (Qiagen, Germany) following reaction conditions as described by Angelini *et al.* (2007). The template used in the assay was a 1:10 dilution of the DNA extracted from the samples. The grapevine chloroplast *chaperonin 21* gene and DNA extracted from HC plants and ICs were used as endogenous, negative and positive controls, respectively. Threshold cycle (Ct) < 37 was associated with the presence of GY phytoplasmas (Mori *et al*., 2015).

BNp characterization by MLST

Nucleotide sequence analyses of the non-ribosomal genomic regions *tufB*, *vmp1* and *stamp* were carried out on BNp strains detected in symptomatic grapevine plants.

Identification of the two main *tufB*-types, currently reported in Italy (*tufB* type-a and *tufB* type-b) (Mori *et al*., 2015), was performed using the TaqMan allelic discrimination assay according to Berger *et al*. (2009).

Direct PCR using StolH10F1/StolH10R1 primer pair (Cimerman *et al.*, 2009) followed by nested PCR with the TYPH10F/TYPH10R primer pair, using mixtures and PCR conditions as described by Fialová *et al.* (2009) were utilized to obtain the amplification of the *vmp1* gene in an automated thermal cycler C1000 Cycler Touch (Bio-Rad, USA). The presence of the nested PCR products were verified through electrophoresis on 1% agarose gels in Tris-borate-EDTA (TBE) buffer and then singly digested with *Rsa*I restriction enzyme (Pacifico *et al.*, 2009), according to the manufacturer's instructions (New England BioLabs, USA). Digested products were separated by

electrophoresis on 3% agarose gels in TBE buffer stained with Gel-Red (Biotum, USA) and visualized under UV transilluminator. The ΦX174 DNA-*Hae* III Digest was used as size marker. Attribution of BNp strains identified to *vmp1 Rsa*I-RFLP profiles (V-types) was determined by comparing V-types obtained in accordance with SEE-ERANET nomenclature (Foissac *et al*., 2013). *vmp1* amplicons, representative of the identified V-types, were sequenced (5X coverage per base position) by a commercial service (Eurofins Genomics, Germany). Nucleotide sequences were assembled by the Contig Assembling Program and trimmed to the annealing sites of the nested PCR primer pair in the software BioEdit, version 7.2.6 (Hall, 1999). To confirm the attribution to V-types, trimmed nucleotide sequences were virtually restricted for single nucleotide polymorphisms in recognition sites of the enzyme *Rsa*I through virtual RFLP analyses using the software Serial Cloner 2.6.1 (http://serialbasics.free.fr/Serial_Cloner.html).

Direct PCR using StampF/StampR0 primer pair followed by nested PCR with the StampF1/ StampR1 primer pair, using mixtures and PCR conditions as described by Fabre *et al.* (2011) were utilized to obtain the amplification of the *stamp* gene in an automated thermal cycler C1000 Cycler Touch. The presence of the nested PCR products were verified through electrophoresis on 1% agarose gels in Tris-borate-EDTA (TBE) buffer. *stamp* amplicons were sequenced and assembled as described above for the gene *vmp1*.

Nucleotide sequences of the genes *vmp1* and *stamp*, amplified from the BNp strains identified in the examined vineyards, were aligned using the software BioEdit in ClustalW Multiple Alignment program and analysed by Sequence Identity Matrix to estimate their genetic diversity. *vmp1* and *stamp* sequence variants, identified in the present study, were aligned and compared with representative sequences of previously defined sequence variants (updated from Pierro *et al*., 2018) (Table S1 and S2); a nucleotide sequence identity of 100% was necessary for the attribution to such sequence variants.

Based on *tufB* type, V-type, *vmp1* and *stamp* sequence variant, each BNp strains were attributed to collective *tufB*/V-type/*vmp1*/*stamp* genotypes.

BNp phylogenetic analysis

vmp1 and *stamp* gene nucleotide sequences of BNp representative strains of Vm (*vmp1*) and St (*stamp*) sequence variants, identified in this (Table 2 and 3) and in previous studies (Table S1 and S2), were aligned and used for generating unrooted phylogenetic trees by minimum evolution method carried out using the Jukes-Cantor model and bootstrap replicated 1000 times in the MEGA6 software (Tamura *et al*., 2013). Moreover, representative nucleotide sequences of *vmp1* and *stamp* genes were concatenated by BioEdit and employed for phylogenetic analyses. All positions with less than 95.0% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position.

Statistical analyses

In order to investigate which parameter(s) can influence the genetic diversity among BNp strain populations, chi square test (χ^2 test) was performed in SPSS statistical package for Windows, v. 24.0 (IBM Corporation, Armonk, NY). In detail, statistical analyses were carried out to estimate possible statistically significant differences in the distribution of *tufB* types, V-types, *vmp1* and *stamp* sequence variants, *tufB*/V-type/*vmp1*/*stamp* collective genotypes, and BNp strains grouped in distinct *vmp1*, *stamp* and *vmp1*/*stamp* phylogenetic clusters, identified in the present study, in: (i) the two grape varieties (*Vitis vinifera* cv. Chardonnay and Sangiovese); (ii) the vineyards under conventional or organic management; (iii) the geographic origins (districts of Arezzo, Firenze, Lucca, Massa, and Siena); (iv) the two statistically significant different weather condition zones [C1 (Arezzo-Firenze-Siena) and C2 (Lucca-Massa)] (Table 4), determined on the basis of data available at the website (https://www.politicheagricole.it).

RESULTS

Bois Noir phytoplasma identification and *tufB* type determination

TaqMan assay, performed using specific primer pairs for the amplification of BNp 16S rDNA in Real-time PCR, detected BNp in all the 75 symptomatic grapevine plants analysed, while FDp was not found. In details, the BN infection percentage for *Vitis vinifera* cv. Chardonnay and Sangiovese plants was 50.7% and 49.3%, respectively. Assay reliability was validated by the amplification signals of the ICs (30<Ct<32 for both), while HC and reaction mixtures gave no amplification signals.

tufB characterization analysis, carried out on the 75 grapevine plants infected by BN using the TaqMan allelic discrimination assay, revealed that the Tuscan vineyards were mainly infected by BNp strains *tufB* type-b (84.0%), while *tufB* type-a was fewer identified (16.0%) (Table 1). Chi square test analyses showed no statistically significant differences in the distribution of *tufB* types in different varieties, vineyard management strategies, and geographic origins (Table 5).

Multiple gene sequence typing of BNp strains

Among the 75 BNp strains identified, 37 yielding *vmp1* nested-PCR fragments of the expected size, amplified using the specific primer pair TYPH10F/R, were digested using the *Rsa*I enzyme. Results obtained showed the presence of five V-types. The most abundant was V11 (64.9%), followed by V3 (13.5%), V1 (10.9%), V15 (8.1%) and V4 (2.7%) (Table 1). The software Serial Cloner 2.6.1, utilized for the virtual restriction analysis selecting *Rsa*I as restriction enzyme, confirmed the presence of such profiles (Figure 1). According to V-types, TYPH10F/R nested-PCR products of 34 BNp strains were sequenced. Based on sequence identities, eight distinct *vmp1* sequence variants (VmTus1 to VmTus8) were detected (Table 2). Comparison with *vmp1* sequence variant updated dataset (Table S1) revealed that sequence variants VmTus1, VmTus2 and VmTus3 shared 100% sequence identity with previously described sequence variants Vm39, Vm41 and Vm43, respectively. Five *vmp1* sequence variants (VmTus4 to VmTus8) were described for the first time in the present study and named Vm88 to Vm92. In detail, sequence variants VmTus4 and

VmTus5 shared the best sequence identity with previously described sequence variant Vm8 (95.2% and 97.3%, respectively), VmTus6 with Vm15 (99%), VmTus7 with Vm39 (98.3%), and VmTus8 with Vm43 (97.3%). The most frequent sequence variant identified in Tuscany was VmTus3 (52.9% of the BNp strains), followed by VmTus6 (11.6%), VmTus1 and VmTus2 (8.9% for each), VmTus4 and VmTus5 (5.8% for each), VmTus7 and VmTus8 (3.0% for each) (Table 2). For each *vmp1* sequence variant, one representative nucleotide sequence was deposited to NCBI GenBank at Accession Number shown in Table 2 (named from VmTus1 to VmTus8). Chi square test analyses showed statistically significant differences in: (i) V-type distribution in the 5 districts (p = 0.001); (ii) V-type and *vmp1* sequence variants distribution in climatic areas C1 and C2 (p=0.004 and p=0.031, respectively) (Table 5; Figure 2A, B, C).

Among the 75 BNp strains identified by Real-Time PCR assays, nested-PCR reactions allowed the amplification of 63 *stamp* fragments (STAMPF1/R1) of the expected size, which were sequenced. Based on sequence identity, 5 different *stamp* sequence variants (StTus1 to StTus5) were identified (Table 3). Comparison of such sequence variants (from StTus1 to StTus5) with the updated dataset (Table S2) revealed that they were identical respectively to the previously described sequence variants St5, St9, St10, St11, and St18. The most frequent sequence variant among BNp strains identified in Tuscany was StTus3 (46.0%), followed by StTus1 (34.9%), StTus5 (12.7%), StTus4 (4.8%), and StTus2 (1.6%) (Table 3). One representative *stamp* nucleotide sequence variant was deposited to NCBI GenBank at Accession Number shown in Table 3. Chi square test showed statistically significant differences in the distribution of (i) *stamp* sequence variants in the climatic areas C1 (Arezzo-Firenze-Siena) and C2 (Lucca-Massa) (p=0.06) (Table 5; Figure 2D).

Based on the combination of *tufB* types, V-type, *vmp1* and *stamp* sequence variants, BNp strains identified in Tuscany were attributed to 17 *tufB*/V-type/*vmp1*/*stamp* genotypes (Table 1). The most abundant genotype identified in the present study was *tufB*-b/V11/VmTus3 (Vm43)/StTus3(St10), previously reported in the Chianti Classico area (Tuscany, Central Italy) (Pierro *et al*., 2018). Chi square test analyses showed no statistically significant differences in the

distribution of *tufB*/V-type/*vmp1*/*stamp* genotypes in different varieties, vineyard management strategies, and geographic origins (Table 5).

Phylogenetic analysis and selective pressure on BNp strains

The phylogenetic tree, generated from the alignment of *vmp1* nucleotide sequences of BNp strains representative of the Vm sequence variants identified in Tuscany (VmTus1 to VmTus8) and those previously described (Vm1 to Vm87) (Table S1), showed the presence of five main clusters. Sequence variant VmTus2 grouped in the cluster *vmp1*-1, variants VmTus3 and VmTus8 in the cluster *vmp1*-2, VmTus1 and VmTus7 in the cluster *vmp1*-3, VmTus4 and VmTus5 in the cluster *vmp1*-4, and VmTus6 in the cluster *vmp1*-5 (Figure 3). Chi square test analyses showed statistically significant differences in the distribution of BNp strains grouped in distinct *vmp1* clusters in the five districts (p = 0.012) and in the weather condition zones C1 and C2 (p=0.005) (Table 5; Figure 2E, F).

The alignment of *stamp* nucleotide sequences of BNp strains representative of the St sequence variants identified in Tuscany (StTus1 to StTus5) and those previously described (St1 to St58) (Table S2) was used for generating a phylogenetic tree in which four main clusters were observed. Sequence variants StTus2, StTus4 and StTus5 grouped in the cluster *stamp*-1, variant StTus1 in the cluster *stamp*-3 and variant StTus3 in the cluster *stamp*-4 (Figure 4). Chi square test analyses showed statistically significant differences in the distribution of BNp strains grouped in distinct *stamp* clusters in vineyards managed by conventional or organic strategy (p=0.048) (Table 5; Figure 2G).

The phylogenetic tree, generated from the alignment of *vmp1*/*stamp* concatenated nucleotide sequences of BNp strains representative of the *vmp1*/*stamp* types identified in Tuscany (Table 1, 2, 3) and those previously described (Vm10/St2 to Vm76/St1) (Table S3), showed the presence of five main clusters. Three *vmp1*/*stamp* types (VmTus2/StTus1, VmTus2/StTus3, and VmTus2/StTus5, sharing *tufB*-b type and V11 type) grouped in cluster *vmp1*/*stamp*-1; four *vmp1*/*stamp* types

(VmTus4/StTus1, VmTus4/StTus4, VmTus5/StTus1, and VmTus5/StTus5, sharing *tufB*-b type and V1 type) grouped in the cluster *vmp1*/*stamp*-2; three *vmp1*/*stamp* types (VmTus3/StTus1, VmTus3/StTus3, and VmTus8/StTus3, sharing V11 type and characterized by *tufB*-a and -b types) grouped in the cluster *vmp1*/*stamp*-3; two *vmp1*/*stamp* types (VmTus1/StTus1 and VmTus7/StTus1, sharing the *tufB*-b type and characterized by V15 and V4 types, respectively) grouped in the cluster 4; two *vmp1*/*stamp* types (VmTus6/StTus1 and VmTus6/StTus3, sharing V3 type and characterized by *tufB*-a and -b types) grouped in the cluster 5 (Figure 5). Further analyses revealed a difference between intra-cluster and inter-cluster genetic heterogeneity of BNp strains, calculated on both nucleotide and amino acid sequence alignments. In detail, BNp strains within each cluster (intra-cluster heterogeneity) shared a mean nucleotide/amino acid sequence identity of 98.4%/97.1% (approximately 29 SNPs/17 amino acid positions distinguishing one strain to another); on the other hand, BNp strains of distinct clusters (inter-cluster heterogeneity) shared a mean nucleotide/amino acid sequence identity of 95.1%/90.3% (approximately 90 SNPs/59 amino acid positions distinguishing one strain to another) (Table S4). Chi square test analyses showed statistically significant differences in: (i) BNp strains grouped in distinct *vmp1*/*stamp* clusters distribution in the 5 districts ($p = 0.007$) and in the climatic areas C1 and C2 ($p=0.006$) (Table 5; Figure 2H, I).

DISCUSSION

Multi-locus sequence typing analysis based on the *tufB*, *stamp* and *vmp1* genes, that are strictly correlated to biological features of '*Ca*. Phytoplasma solani' strains (Langer & Maixner, 2004; Murolo & Romanazzi, 2015; Kosovac *et al.*, 2016; Pierro *et al.*, 2018), contributes to the understanding of the ecology of this pathogen.

Considering the association of different epidemiological systems with *tufB* types (Langer & Maixner, 2004), results of TaqMan allelic discrimination assay confirmed that BNp ecology in Tuscan vineyards is mainly associated to the bindweed-related host system (*tufB* type-b = 84.0%), but revealed a lesser role of the nettle-related host system (*tufB* type-a = 16.0%). These results are

in accordance with previous studies showing a wider distribution of *tufB* type-b in central and southern Italy (Pacifico *et al.*, 2007; Marchi *et al.*, 2015; Murolo & Romanazzi 2015; Pierro *et al.*, 2018).

Within BNp strains (*tufB*-b) associated with the prevalent bindweed-related host system, multiple gene sequence typing analyses allowed the identification of 13 collective BNp genotypes and evidenced the prevalence of the genotype *tufB*-b/V11/Vm43/St10 (36.0% of collective BNp genotypes) in Tuscan vineyards, extending to Regional level the results previously obtained by Pierro *et al*. (2018) in a case study vineyard in Greve in Chianti, district of Firenze. Interestingly, based on sequence identity and phylogenetic clustering obtained by the analysis of *vmp1*, *stamp* and concatenated *vmp1*/*stamp* nucleotide sequences, this prevalent BNp genotype was strictly related to '*Ca*. P. solani' strains previously found mainly in Solanaceae hosts and in insect vectors (*H. obsoletus* and *R. quinquecostatus*) in France and central Italy (Cimerman *et al.*, 2009; Murolo & Romanazzi, 2015; Landi *et al*., 2015; Chuche *et al.*, 2016), but never in grapevines outside of Tuscany (Pierro *et al.*, 2018). Other two BNp genotypes closely related to *tufB*-b/V11/Vm43/St10, representing 10% (*tufB*-B/V11/Vm43/St10) and 4% (*tufB*-b/V11/Vm91/St10) of described collective genotypes, could share its biological features. The remaining ten BNp genotypes were sporadically found and represent, when considered all together, 38% of BNp genotypes described in Tuscany. Based on sequence identities and grouping in phylogenetic clusters obtained by the analysis of *vmp1*, *stamp* and concatenated *vmp1*/*stamp* nucleotide sequences, such BNp genotypes were closely related to those largely found in Euro-Mediterranean countries in association with plant hosts and insect vectors connected to bindweed-related host system (Figure 3, 4, 5). Within BNp strains (*tufB*-a) associated with the nettle-related host system, multiple gene sequence typing analyses allowed the identification of four collective BNp genotypes sporadically found and representing, when considered all together, 12% of BNp genotypes described in Tuscany. Sequence identity and phylogenetic analyses of *vmp1*, *stamp* and concatenated *vmp1*/*stamp* nucleotide sequences confirmed the strict relationship between these Tuscan BNp genotypes and those

previously described in Europe in association with nettle-related host system (Figure 3, 4, 5). Based on these evidences, it is reasonable to propose that BN epidemiology in Tuscany involves two BNp strain populations related to (i) bindweed and nettle host systems playing a pivotal role in BNp diffusion in European vineyards, and (ii) a BNp strain population, putatively associated with bindweed (*tufB*-b, but never found directly in *C. arvensis*), including strains identified in vineyard agro-ecosystems exclusively in Tuscany. Such BNp strains are similar to '*Ca*. Phytoplasma solani' strains previously detected in Solanaceae, *H. obsoletus* and *R. quinquecostatus* in Europe, opening new perspectives on BN epidemiological patterns.

Climatic and geographic features in agro-ecosystems may be significant, directly or indirectly, in determining the strain composition of phytoplasma populations in different regions (Cai *et al*., 2008; Wu *et al*., 2012; Quaglino *et al*., 2017). In the present study, after having described the genetic variability among BNp strain populations in Tuscan vineyards, we investigated whether the composition of BNp strain populations was influenced by some environmental parameters: in particular the analyzed variables were the (i) *V. vinifera* cv Chardonnay or Sangiovese; the (ii) vineyard management strategy, either conventional or organic; and the (iii) geographic origin of the samples, divided among the five districts of Arezzo, Firenze, Lucca, Massa, and Siena.

The distribution of the different genotypes based on the *tufB*, *stamp*, *vmp1* genes and the concatenated *vmp1*/*stamp* nucleotide sequences was compared between the different parameters with a statistical approach. The first examined parameter, (i) the cultivar, never showed a significant difference in the distribution of the genotypes, suggesting that these plant hosts have no effect on the selection of the pathogen (Table 5). This result is in line with the fact that grapevine is a dead-end host of BNp (Belli *et al*., 2010; Maixner, 2011) and, therefore, it is intuitive that the considered cultivars (Sangiovese and Chardonnay) have no effect on the distribution of this pathogen. The second parameter, (ii) the management strategy, showed significant differences in the distribution of BNp strains, grouped on the basis of *stamp* clusters, in conventional or organic vineyards (Figure 2G). Still, further studies should be carried out to confirm this experimental evidence possibly

biased by the fact that most of examined organic vineyards are limited to a single district (Massa). Moreover, the significant difference was observed by the analyses of only one gene. The last considered parameter, (iii) geographic origin, instead showed significant difference in BNp strain distribution determined on the basis of V-type, *vmp1* and *vmp1*/*stamp* clusters (Figure 2A, E, H). To understand the reason behind this observed difference, we investigated the agro-meteorological parameters of the five districts determining their attribution to two distinct weather condition zones (C1 and C2, Table S3): the C1 including the districts of Arezzo, Firenze, and Siena (central-eastern Tuscany); and C2 including the districts of Lucca and Massa (north-western Tuscany). The use of the weather condition zone as a variable for the statistical analyses revealed a significant difference in the BNp strain distribution based on V-type, *vmp1* and *stamp* sequence variants, *vmp1* and *vmp1*/*stamp* clusters between C1 and C2 (Figure 2B, C, D, F, I). These results reinforce the hypothesis that environmental conditions can play a role, directly (*i.e.* selection of most fit phytoplasma strains) or indirectly (i.e. influence on wild plant hosts and insect vectors), in shaping the composition of BNp strain populations.

Based on the overall findings of the present study, it is reasonable to theorize that the distinctive environmental conditions of Tuscany can contribute to the selection of BNp strains, favouring the entrance of an unusual '*Ca*. P. solani' genotype (*tufB*-b/V11/Vm43/St10) from the surroundings and/or other crop fields to vineyard agro-ecosystems.

REFERENCES

Alma A., Arnò C., Arzone A., Vidano C . (1988) New bio-logical r eports on Auchenorrhyncha in vineyards. In Proceedings of 6th Auchenorrhyncha Meeting, Turin, Italy, 1987, pp. 509–516. Eds C. Vidano and A. Arzone. Turin, Italy: University of Turin.

Angelini E., Bianchi G.L., Filippin L., Morassutti C., Borgo M. (2007) A new TaqMan method for the identification of phytoplasmas associated with grapevine yellows by real-time PCR assay. *Journal of Microbiological Methods*, **68**, 613–622.

Belli G., Bianco P.A., Conti M. (2010) Grapevine yellows: past, present and future. *Journal of Plant Pathology*, **92**, 303–326.

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. *European Journal of Plant Pathology*, **124**, 521–526.

Cai H., Wei W., Davis R.E., Chen H., Zhao Y. (2008) Genetic diversity among phytoplasmas infecting *Opuntia*: virtual RFLP analysis identifies new subgroups in the peanut witches'-broom phytoplasma group. *International Journal of Systematic and Evolutionary Microbiology*, **58**, 1448–1457.

Choueiri E., Jreijiri F., El Zammar S., Verdin E., Salar P., Danet J.L., Bové J., Garnier M. (2002) First report of grapevine 'Bois Noir' disease and a new phytoplasma infecting solanaceous plants in Lebanon. *Plant Disease*, **86**, 697.

Chuche J., Danet J.L., Salar P., Foissac X., Thiery D. (2016) Transmission of '*Candidatus* Phytoplasma solani' by *Reptalus quinquecostatus* (Hemiptera: Cixiidae). *Annals of Applied Biology*, **169**, 214–223.

Cimerman A., Pacifico D., Salar P., Marzachì C., Foissac X. (2009) Striking diversity of *vmp1*, a variable gene encoding a putative membrane protein of the stolbur phytoplasma. *Applied and Environmental Microbiology*, **75**, 2951–2957.

Cvrković T., Jović J., Mitrović M., Krstić Q., Toševski I. (2014) Experimental and molecular evidence of *Reptalus panzeri* as a natural vector of bois noir. *Plant Pathology*, **63**, 42–53.

Duduk B., Tian J., Contaldo N., Fan X., Paltrinieri S., Chen Q., Zha o Q., Bertaccini A. (2010) Occurrence of phytoplasmas related to stolbur and to '*Candidatus* Phytoplasma japonicum' in woody host plants in China. *Journal of Phytopathology*, **158**, 100–104.

Fabre A., Danet J.L., Foissac X. (2011) The stolbur phytoplasma antigenic membrane protein gene *stamp* is submitted to diversifying positive selection. *Gene*, **472**, 37–41.

Fialová R., Válová P., Balakishiyeva G., Danet J.L., Sâfárová D., Foissac X., Navrátil M. (2009)

Genetic variability of Stolbur phytoplsama in annual crop and wild plant species in South Moravia. *Journal of Plant Pathology*, **91**, 411–416.

Foissac X., Carle P., Fabre A., Salar P., Danet J.L., STOLBUREUROMED Consortium (2013) '*Candidatus* Phytoplasma solani' genome project and genetic diversity in the Euro-Mediterranean basin. In Torres E., Laviña A., Battle A. (ed.), Book of Abstracts of 3rd European Bois Noir Workshop; March 21-22, 2013, Barcelona, Spain.

Gajardo A., Fiore N., Prodan S., Paltrinieri S., Botti S., Pino A.M., Zamorano A., Montealegre J., Bertaccini A. (2009) Phytoplasmas associated with grapevine yellows disease in Chile. *Plant Disease*, **93**, 789–796.

Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Kosovac A., Radonjić S., Hrnčić S., Krstić O., Toševski I., Jović J. (2015) Molecular tracing of the transmission routes of bois noir in Mediterranean Vineyards of Montenegro and experimental evidence for the epidemiological role of *Vitex agnus-castus* (Lamiaceae) and associated *Hyalestes obsoletus* (Cixiidae). *Plant Pathology*, **65**, 285–298.

Landi L., Riolo P., Murolo S., Romanazzi G., Nardi S., Isidoro N. (2015) Genetic variability of stolbur phytoplasma in *Hyalesthes obsoletus* (Hemiptera: Cixiidae) and its main host plants in vineyard agroecosystems. *Journal of Economic Entomology*, **108**, 1506–1515.

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**, 191-200.

Li R., Mock R., Huang Q., Abad J., Hartung J., Kinard G. (2008) A reliable and inexpensive method of nucleic acid extraction for the PCR-based detection of diverse plant pathogens. *Journal of Virological Methods*, **154**, 48–55.

Maixner M. (1994) Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis*, **33**, 103–104.

Maixner M. (2011) Recent advances in Bois noir research. *Petria*, **21**, 17–32.

Marchi G., Cinelli T., Rizzo D., Stefani L., Goti E., Della Bartola M., Luvisi A., Panattoni A., Materazzi A. (2015) Occurrence of different phytoplasma infections in wild herbaceous dicots growing in vineyards affected by bois noir in Tuscany (Italy). *Phytopathologia Mediterranea*, **54**, 504–515.

Mirchenari S.M., Massah A., Zirak L. (2015) 'Bois noir': new phytoplasma disease of grapevine in Iran. *Journal of Plant Protection Research*, **55**, 88–93.

Mori N., Quaglino F., Tessari F., Pozzebon A., Bulgari D., Casati P., Bianco P.A. (2015) Investigation on 'bois noir' epidemiology in north-eastern Italian vineyards through a multidisciplinary approach. *Annals of Applied Biology*, **166**, 75–89.

Murolo S., Romanazzi G. (2015) In-vineyard population structure of '*Candidatus* Phytoplasma solani' using multilocus sequence typing analysis. *Infection, Genetics and Evolution*, **31**, 221–230.

Oliveri C., Pacifico D., D'Urso V., La Rosa R., Marzachi C., Tessitori M. (2015) Bois noir phytoplasma variability in a Mediterranean vineyard system: new plant host and putative vectors. *Australasian Plant Pathology*, **44**, 235–244.

Pacifico D., Alma A., Bagnoli B., Foissac X., Pasquini G., Tessitori M., Marzachì C. (2009) Characterization of Bois noir isolates by restriction fragment lenght polymorphism of a stolbur-specific putative membrane protein gene. *Phytopathology*, **99**, 711–715.

Pacifico D., Foissac X., Veratti F., Marzachì C. (2007) Genetic diversity of Italian phytoplasma and French "bois noir" isolates. *Bulletin of Insectology*, **60**, 345–346.

Pierro R., Passera A., Panattoni A., Casati P., Luvisi A., Rizzo D., Bianco P.A., Quaglino F., Materazzi A. (2018) Molecular typing of 'bois noir' phytoplasma strains in the Chianti Classico area (Tuscany, central Italy) and their association with symptom severity in *Vitis vinifera* L. cv. Sangiovese. *Phytopathology*, **108**, 362–373.

Quaglino F., Maghradze D., Casati P., Chkhaidze N., Lobjanidze M., Ravasio A., Passera A., Venturini G., Failla O., Bianco P.A. (2016) Identification and characterization of new '*Candidatus* Phytoplasma solani' strains associated with Bois noir disease in *Vitis vinifera* L. cultivars showing a range of symptoms severity in Georgia, the Caucasus Region. *Plant Disease*, **100**, 904–915.

Quaglino F., Murolo S., Zhao Y., Casati P., Durante G., Wei W., Bianco P.A., Romanazzi G., Davis R.E. (2017) Identification of new -J and -K 16SrXII subgroups and distinct single nucleotide polymorphism genetic lineages among '*Candidatus* Phytoplasma solani' strains associated with Bois noir in central Italy. *Australasian Plant Pathology*, **46**, 31–34.

Quaglino F., Zhao Y., Bianco P.A., Wei W., Casati P., Durante G., Davis R.E. (2009) New 16Sr subgroups and distinct single nucleotide polymorphism lineages among grapevine bois noir phytoplasma populations. *Annals of Applied Biology*, **54**, 279–289.

Quaglino F., Zhao Y., Casati P., Bulgari D., Bianco P.A., Wei W., Davis R.E. (2013) '*Candidatus* Phytoplasma solani', a novel taxon associated with stolbur and bois noir related diseases of plants. *International Journal of Systematic and Evolutionary Microbiology*, **63**, 2879–2894.

Salem N.M., Quaglino F., Abdeen A., Casati P., Bulgari D., Alma A., Bianco P.A. (2013) First report of '*Candidatus* Phytoplasma solani' strains associated with grapevine bois noir in Jordan. *Plant Disease*, **97**, 1505.

Sharon R., Soroker V., Wesley S., Zahavi T., Harari A., Weintraub P. (2005) *Vitex agnus-castus* is a preferred host plant for *Hyalestes obsoletus*. *Journal of Chemical Ecology*, **31**, 1051–1063.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, **30**, 2725–2729.

Wu W., Cai H., Wei W., Davis R.E., Lee I.-M., Chen H., Zhao Y. (2012) Identification of two new phylogenetically distant phytoplasmas from *Senna surattensis* plants exhibiting stem fasciation and shoot proliferation symptoms. *Annals of Applied Biology*, **160**, 25–34.

Supporting Information

Pierro *et al.* [Environment-driven selection of BNp strain populations**]**

Table S1. Sequence variants of the gene *vmp1* among '*Ca*. P. solani' strains available in GenBank (part I)

Sequence variant	Strain	Host	Location	Acc. No. *vmp1*
Vm1	S13	*Vitis vinifera*	Italy	HM008616
Vm2	Neuweier57_C	*Convolvulus arvensis*	Germany	JQ977736
Vm3	C_Boppard_C	*Convolvulus arvensis*	Germany	JQ977734
Vm4	N10	*Urtica dioica*	France	JQ977730
Vm5	N9	*Urtica dioica*	France	JQ977729
Vm6	N2	*Urtica dioica*	Germany	JQ977722
Vm7	N1	*Urtica dioica*	Germany	JQ977721
Vm8	CH1	*Vitis vinifera*	Italy	AM992105
Vm8	T2_56	*Solanum lycopersicum*	Italy	AM992104
Vm9	P74/11	*Vitis vinifera*	Italy	KJ145361
Vm10	Vv17	*Vitis vinifera*	Serbia	KC703032
Vm10	Vv21	*Vitis vinifera*	Serbia	KC703026
Vm10	Vexp Rpm5	*Reptalus panzeri*	Serbia	KC703028
Vm10	Vexp Rpg11	*Reptalus panzeri*	Serbia	KC703027
Vm10	Rpm34	*Reptalus panzeri*	Serbia	KC703024
Vm10	Rpg39	*Reptalus panzeri*	Serbia	KC703023
Vm10	Rqg31	*Reptalus quinquecostatus*	Serbia	KC703031
Vm10	Rqg60	*Reptalus quinquecostatus*	Serbia	KC703025
Vm10	STOL	*Capsicum annuum*	Serbia	AM992103
Vm11	LA6_I_C	*Convolvulus arvensis*	Germany	JQ977735
Vm12	GGY	*Vitis vinifera*	Germany	AM992102
Vm13	MK29	*Vitis vinifera*	Macedonia	KF957604
Vm14	Vv12_754	*Vitis vinifera*	Austria	KJ469734
Vm14	Vv12_751	*Vitis vinifera*	Austria	KJ469734
Vm14	Vv12_Kn6	*Vitis vinifera*	Austria	KJ469734
Vm15	60/11	*Vitis vinifera*	Italy	KJ145346
Vm15	Aa25	*Vitis vinifera*	Italy	HM008614
Vm15	Mri10	*Vitis vinifera*	Italy	HM008615
Vm15	HY.86N	*Hyalesthes obsoletus*	Italy	KM225871
Vm15	HY.80N	*Hyalesthes obsoletus*	Italy	KM225870
Vm15	Ne.10	*Urtica dioica*	Italy	KM225869
Vm15	Ho13_1006	*Hyalesthes obsoletus*	Austria	KJ469727
Vm16	N13	*Urtica dioica*	Italy	JQ977733
Vm17	N12	*Urtica dioica*	Italy	JQ977732
Vm18	N11	*Urtica dioica*	Italy	JQ977731
Vm18	Ho13_838	*Hyalesthes obsoletus*	Austria	KJ469729
Vm19	N8	*Urtica dioica*	Italy	JQ977728
Vm20	N7	*Urtica dioica*	Italy	JQ977727
Vm21	N6	*Urtica dioica*	Italy	JQ977726
Vm22	N5	*Urtica dioica*	Italy	JQ977725
Vm23	CrHo13_1183	*Hyalesthes obsoletus*	Austria	KJ469728
Vm23	N4	*Urtica dioica*	Slovenia	JQ977724
Vm24	N3	*Urtica dioica*	Slovenia	JQ977723
Vm25	MK44	*Vitis vinifera*	Macedonia	KF957605
Vm26	149/11	*Vitis vinifera*	Italy	KJ145347
Vm27	Aa16	*Vitis vinifera*	Italy	HM008602
Vm27	Bi.15	*Convolvulus arvensis*	Italy	KM225875
Vm27	HY.9B	*Hyalesthes obsoletus*	Italy	KM225874
Vm28	166/11	*Vitis vinifera*	Italy	KJ145355
Vm28	136/11	*Vitis vinifera*	Italy	KJ145354
Vm28	P10/11	*Vitis vinifera*	Italy	KJ145353
Vm28	Aaq1	*Vitis vinifera*	Italy	HM008601

Table S1. Sequence variants of the gene *vmp1* among '*Ca*. P. solani' strains available in GenBank (part II)

Sequence variant	Strain	Host	Location	Acc. No. *vmp1*
Vm28	Mca21	*Vitis vinifera*	Italy	HM008599
Vm28	B51	*Vitis vinifera*	Italy	HM008600
Vm28	Rpg47	*Reptalus panzeri*	Serbia	KC703034
Vm28	Rqg50	*Reptalus quinquecostatus*	Serbia	KC703033
Vm28	D_Bacharach_C	*Convolvulus arvensis*	Germany	JQ977738
Vm28	MK28	*Vitis vinifera*	Macedonia	KF957603
Vm28	CrAr12_722_2	*Anaceratagallia ribauti*	Austria	KJ469735
Vm28	Vv12_752	*Vitis vinifera*	Austria	KJ469735
Vm28	17-11	*Vitis vinifera*	Bosnia & Herzegovina	KP739858
Vm29	M33_F_C	*Convolvulus arvensis*	France	JQ977742
Vm30	EisHo1_C	*Convolvulus arvensis*	Italy	JQ977740
Vm31	Charente-1	*Hyalesthes obsoletus*	France	AM992098
Vm32	Moliere	*Prunus avium*	France	AM992096
Vm33	CrHo12_601	*Hyalesthes obsoletus*	Austria	KJ469730
Vm34	19-25	*Vitis vinifera*	Germany	AM992101
Vm35	PO	*Hyalesthes obsoletus*	France	AM992095
Vm36	Rqg42	*Reptalus quinquecostatus*	Serbia	KC703030
Vm37	SFRT1	*Vitis vinifera*	Italy	KJ129606
Vm38	LG	*Solanum lycopersicum*	France	AM992097
Vm39	78/11	*Vitis vinifera*	Italy	KJ145349
Vm39	B7	*Vitis vinifera*	Italy	HM008608
Vm39	HY.31B	*Hyalesthes obsoletus*	Italy	KM225862
Vm39	HY.24B	*Hyalesthes obsoletus*	Italy	KM225861
Vm39	San31_2016	*Vitis vinifera*	Italy	MF182859
Vm40	Mp49	*Vitis vinifera*	Italy	HM008607
Vm41	B2035	*Vitis vinifera*	Italy	HM008611
Vm41	C1	*Vitis vinifera*	Italy	HM008610
Vm41	San47_2016	*Vitis vinifera*	Italy	MF182860
Vm42	Mca28	*Vitis vinifera*	Italy	HM008609
Vm42	San24_2016	*Vitis vinifera*	Italy	MF182861
Vm43	ARSIA1	*Linaria vulgaris*	Italy	KJ129605
Vm43	HY.3B	*Hyalesthes obsoletus*	Italy	KM225877
Vm43	HY32.B	*Hyalesthes obsoletus*	Italy	KM225876
Vm43	San21_2015	*Vitis vinifera*	Italy	MF182856
Vm44	Mvercer2	*Vitis vinifera*	Italy	HM008612
Vm45	San2_2015	*Vitis vinifera*	Italy	MF182857
Vm45	315/11	*Vitis vinifera*	Italy	KJ145360
Vm45	P136/11	*Vitis vinifera*	Italy	KJ145358
Vm45	P75/11	*Vitis vinifera*	Italy	KJ145357
Vm45	411/11	*Vitis vinifera*	Italy	KJ145359
Vm45	Bi.47	*Convolvulus arvensis*	Italy	KM225881
Vm45	HY.48N	*Hyalesthes obsoletus*	Italy	KM225880
Vm45	HY.50B	*Hyalesthes obsoletus*	Italy	KM225879
Vm46	353/11	*Vitis vinifera*	Italy	KJ145352
Vm46	287/11	*Vitis vinifera*	Italy	KJ145351
Vm46	115/11	*Vitis vinifera*	Italy	KJ145350
Vm46	Mp46	*Vitis vinifera*	Italy	HM008606
Vm46	Ag4a	*Vitis vinifera*	Italy	HM008605
Vm46	Bi.13	*Convolvulus arvensis*	Italy	KM225866
Vm46	Vv24	*Vitis vinifera*	Serbia	KC703036
Vm46	Vv5	*Vitis vinifera*	Serbia	KC703035
Vm46	Rpm35	*Reptalus panzeri*	Serbia	KC703029
Vm46	PM1	*Solanum tuberosum*	Montenegro	KU588192
Vm47	B49	*Vitis vinifera*	Italy	HM008604
Vm48	C3	*Vitis vinifera*	Italy	HM008603
Vm49	MK19	*Vitis vinifera*	Macedonia	KF957602

Table S1. Sequence variants of the gene *vmp1* among '*Ca*. P. solani' strains available in GenBank (part III)

Sequence variant	Strain	Host	Location	Acc. No. *vmp1*
Vm50	HY.14B	*Hyalesthes obsoletus*	Italy	KM225865
Vm50	HY.5B	*Hyalesthes obsoletus*	Italy	KM225864
Vm50	HY.12B	*Hyalesthes obsoletus*	Italy	KM225863
Vm50	Ca13_RF	*Convolvulus arvensis*	Austria	KJ469732
Vm51	C6	*Vitis vinifera*	Italy	HM008618
Vm51	B4	*Vitis vinifera*	Italy	HM008617
Vm51	RA6_I_C	*Convolvulus arvensis*	Italy	JQ977737
Vm52	I_Norheim_C	*Convolvulus arvensis*	Germany	JQ977739
Vm52	Charente-2	*Hyalesthes obsoletus*	France	AM992099
Vm53	P7	*Catharanthus roseus*	Lebanon	AM992100
Vm53	Tsol89	*Vitis vinifera*	Georgia	KT184878
Vm53	Kiqu94	*Vitis vinifera*	Georgia	KT184878
Vm54	P42/11	*Vitis vinifera*	Italy	KJ145356
Vm55	T2_92	*Solanum lycopersicum*	Italy	AM992106
Vm56	36861_SLO_C	*Convolvulus arvensis*	Slovenia	JQ977741
Vm57	CrHo12_721	*Hyalesthes obsoletus*	Austria	KJ469731
Vm58	Mag1	*Vitis vinifera*	Italy	HM008613
Vm58	HY.7N	*Hyalesthes obsoletus*	Italy	KM225868
Vm58	HY.18N	*Hyalesthes obsoletus*	Italy	KM225867
Vm59	MK94	*Vitis vinifera*	Macedonia	KF957606
Vm60	CrHo12_650	*Hyalesthes obsoletus*	Austria	KJ469725
Vm61	Vv12_274	*Vitis vinifera*	Austria	KJ469726
Vm62	425/11	*Vitis vinifera*	Italy	KJ145348
Vm63	Vv12_Ill6	*Vitis vinifera*	Austria	KJ469733
Vm64	Carv1	*Convolvulus arvensis*	Georgia	KT184867
Vm65	Carv2	*Convolvulus arvensis*	Georgia	KT184868
Vm66	Char7	*Vitis vinifera*	Georgia	KT184869
Vm67	Char8	*Vitis vinifera*	Georgia	KT184870
Vm68	Sape19	*Vitis vinifera*	Georgia	KT184871
Vm69	GoMt25	*Vitis vinifera*	Georgia	KT184872
Vm70	Kisi38	*Vitis vinifera*	Georgia	KT184873
Vm71	Rkat47	*Vitis vinifera*	Georgia	KT184874
Vm71	Sape51	*Vitis vinifera*	Georgia	KT184874
Vm71	Sape62	*Vitis vinifera*	Georgia	KT184874
Vm72	Khik70	*Vitis vinifera*	Georgia	KT184875
Vm73	Amla77	*Vitis vinifera*	Georgia	KT184876
Vm74	Sabu84	*Vitis vinifera*	Georgia	KT184877
Vm75	LN-b	*Salvia miltiorrhiza*	China	KU600116
Vm75	LN-a	*Salvia miltiorrhiza*	China	KU600115
Vm75	LY-6	*Salvia miltiorrhiza*	China	KU600114
Vm75	LY-5	*Salvia miltiorrhiza*	China	KU600113
Vm75	LY-4	*Salvia miltiorrhiza*	China	KU600112
Vm75	SZ-9	*Salvia miltiorrhiza*	China	KU600111
Vm75	SZ-8	*Salvia miltiorrhiza*	China	KU600110
Vm75	SZ-7	*Salvia miltiorrhiza*	China	KU600109
Vm75	LN-3	*Salvia miltiorrhiza*	China	KU600108
Vm75	LN-2	*Salvia miltiorrhiza*	China	KU600107
Vm75	LN-1	*Salvia miltiorrhiza*	China	KU600106
Vm76	G24-13	*Vitis vinifera*	Bosnia & Herzegovina	KT766176
Vm76	05-09	*Vitis vinifera*	Bosnia & Herzegovina	KT766163
Vm76	PM2	*Solanum tuberosum*	Montenegro	KU588193
Vm77	12-11	*Vitis vinifera*	Bosnia & Herzegovina	KT766169
Vm78	HY.8B	*Hyalesthes obsoletus*	Italy	KM225878
Vm79	HY.6B	*Hyalesthes obsoletus*	Italy	KM225873
Vm79	HY.25B	*Hyalesthes obsoletus*	Italy	KM225872
Vm80	Bi.2	*Convolvulus arvensis*	Italy	KM225860
Vm80	HY.53B	*Hyalesthes obsoletus*	Italy	KM225859
Vm81	San24_2015	*Vitis vinifera*	Italy	MF182858

Table S1. Sequence variants of the gene *vmp1* among '*Ca*. P. solani' strains available in GenBank (part IV)

Sequence variant	Strain	Host	Location	Acc. No. *vmp1*
Vm82	San49_2016	*Vitis vinifera*	Italy	MF182862
Vm83	San11_2016	*Vitis vinifera*	Italy	MF182863
Vm84	San37_2016	*Vitis vinifera*	Italy	MF182864
Vm85	San5_2016	*Vitis vinifera*	Italy	MF182865
Vm86	San56_2016	*Vitis vinifera*	Italy	MF182866
Vm87	San43_2016	*Vitis vinifera*	Italy	MF182867

Supporting Information

Pierro *et al.* [Environment-driven selection of BNp strain populations**]**

Table S2. Sequence variants of the gene *stamp* among '*Ca*. P. solani' strains available in GenBank (part I)

Sequence variant	Strain	Host	Location	Acc. N. *stamp*
St1	Rqg50	*Reptalus quinquecostatus*	Serbia	KC703019
St1	11-11	*Vitis vinifera*	Bosnia & Herzegovina	KP739852
St1	115/11	*Vitis vinifera*	Italy	KJ145337
St1	17-11	*Vitis vinifera*	Bosnia & Herzegovina	KP739854
St1	20MN	*Vitis vinifera*	Montenegro	KJ926068
St1	353/11	*Vitis vinifera*	Italy	KJ145338
St1	45MN	*Convolvulus arvensis*	Montenegro	KJ926069
St1	72MN	*Vitex agnus-castus*	Montenegro	KJ926070
St1	Ag4a	*Vitis vinifera*	Italy	KJ145377
St1	B1	*Vitis vinifera*	Italy	KJ145378
St1	C45	*Convolvulus arvensis*	Macedonia	KP337319
St1	CrAr12_722_2	*Anaceratagallia ribauti*	Austria	KJ469722
St1	CrHo12_721	*Hyalesthes obsoletus*	Austria	KJ469722
St1	G21-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739856
St1	G22-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739849
St1	G23-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739846
St1	G24-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739847
St1	G4-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739853
St1	G6-13	*Vitis vinifera*	Bosnia & Herzegovina	KP739848
St1	Gb1	*Phaseulus vulgaris*	Serbia	KM977907
St1	Ho375	*Hyalesthes obsoletus*	Montenegro	KJ926071
St1	Ho66-2	*Hyalesthes obsoletus*	Montenegro	KJ926072
St1	HoC202	*Hyalesthes obsoletus*	Macedonia	KP337320
St1	Mp46	*Vitis vinifera*	Italy	KJ145379
St1	P25/11	*Vitis vinifera*	Italy	KJ145339
St1	PM1	*Solanum tuberosum*	Montenegro	KU588188
St1	PM2	*Solanum tuberosum*	Montenegro	KU588189
St1	PS8	*Solanum tuberosum*	Serbia	KP877599
St1	PS8Ho	*Hyalesthes obsoletus*	Serbia	KP877600
St1	PS8Rp	*Reptalus panzeri*	Serbia	KP877601
St1	PS9	*Solanum tuberosum*	Serbia	KP877602
St1	Rpg47	*Reptalus panzeri*	Serbia	KC703020
St1	Vv12_Ill6	*Vitis vinifera*	Austria	KJ469722
St1	Vv5	*Vitis vinifera*	Serbia	KC703021
St2	Rqg31	*Reptalus quinquecostatus*	Serbia	KC703017
St2	Br8	*Convolvulus arvensis*	Croatia	KJ573597
St2	C2_Rgg50	*Apium graveolens*	Bosnia & Herzegovina	KU295506
St2	Ho41-2	*Hyalesthes obsoletus*	Montenegro	KJ926065
St2	P10	*Capsicum annuum*	Bosnia & Herzegovina	KU295504
St2	P6	*Capsicum annuum*	Bosnia & Herzegovina	KU295502
St2	PS4	*Solanum tuberosum*	Serbia	KP877588
St2	PS4Ho	*Hyalesthes obsoletus*	Serbia	KP877589
St2	PS5	*Solanum tuberosum*	Serbia	KP877590
St2	PS5Ho	*Hyalesthes obsoletus*	Serbia	KP877591
St2	PS5Rp	*Reptalus panzeri*	Serbia	KP877592
St2	PS6	*Solanum tuberosum*	Serbia	KP877593
St2	PS6Ho	*Hyalesthes obsoletus*	Serbia	KP877594
St2	PS6Rq	*Reptalus quinquecostatus*	Serbia	KP877595
St2	Vv12_Kn6	*Vitis vinifera*	Austria	KJ469724
St2	Vv17	*Vitis vinifera*	Serbia	KC703018
St3	16MN	*Vitis vinifera*	Montenegro	KJ926073
St3	30-09	*Vitis vinifera*	Bosnia & Herzegovina	KP739851
St3	43MN	*Convolvulus arvensis*	Montenegro	KJ926074

Table S2. Sequence variants of the gene *stamp* among '*Ca*. P. solani' strains available in GenBank (part II)

Sequence variant	Strain	Host	Location	Acc. N. *stamp*
St3	79MN	*Vitex agnus-castus*	Montenegro	KJ926075
St3	Ho389	*Hyalesthes obsoletus*	Montenegro	KJ926076
St3	MK66	*Vitis vinifera*	Macedonia	KF957608
St3	P5	*Capsicum annuum*	Bosnia & Herzegovina	KU295501
St3	P7	*Catharanthus roseus*	Lebanon	FN813258
St3	PS7	*Solanum tuberosum*	Serbia	KP877596
St3	PS7Ho	*Hyalesthes obsoletus*	Serbia	KP877597
St3	PS7Rp	*Reptalus panzeri*	Serbia	KP877598
St3	Rpm35	*Reptalus panzeri*	Serbia	KC703015
St3	Vv12_751	*Vitis vinifera*	Austria	KJ469723
St4	G2	*Vitis vinifera*	Macedonia	KP337318
St4	GR328	*Capsicum annuum*	Greece	FN813253
St4	Ho10-2	*Hyalesthes obsoletus*	Montenegro	KJ926067
St4	MB11	*Zea mays*	Bosnia & Herzegovina	KU295509
St4	MB4	*Zea mays*	Bosnia & Herzegovina	KU295507
St4	MB6	*Zea mays*	Bosnia & Herzegovina	KU295508
St4	PS1	*Solanum tuberosum*	Serbia	KP877583
St4	PS1Rp	*Reptalus panzeri*	Serbia	KP877584
St4	PS1Rq	*Reptalus quinquecostatus*	Serbia	KP877585
St4	Rpg39	*Reptalus panzeri*	Serbia	KC703009
St4	Rpm34	*Reptalus panzeri*	Serbia	KC703010
St4	Rqg60	*Reptalus quinquecostatus*	Serbia	KC703011
St4	STOL	*Capsicum annuum*	Serbia	FN813261
St4	Vexp Rpg11	*Reptalus panzeri*	Serbia	KC703013
St4	Vexp Rpm5	*Reptalus panzeri*	Serbia	KC703014
St4	Vv21	*Vitis vinifera*	Serbia	KC703012
St5	215/11	*Vitis vinifera*	Italy	KJ145329
St5	287/11	*Vitis vinifera*	Italy	KJ145332
St5	315/11	*Vitis vinifera*	Italy	KJ145330
St5	425/11	*Vitis vinifera*	Italy	KJ145335
St5	78/11	*Vitis vinifera*	Italy	KJ145334
St5	Ca13_RF	*Convolvulus arvensis*	Austria	KJ469721
St5	CrHo12_601	*Hyalesthes obsoletus*	Austria	KJ469721
St5	GGY	*Vitis vinifera*	Germany	FN813256
St5	HoC205	*Hyalesthes obsoletus*	Macedonia	KP337315
St5	LA6_I_C	*Convolvulus arvensis*	Germany	JQ977720
St5	NGA9	*Hyalesthes obsoletus*	Slovenia	FN813262
St5	P136/11	*Vitis vinifera*	Italy	KJ145336
St5	P51/11	*Vitis vinifera*	Italy	KJ145331
St5	P75/11	*Vitis vinifera*	Italy	KJ145333
St5	Vv12_752	*Vitis vinifera*	Austria	KJ469721
St5	Vv12_754	*Vitis vinifera*	Austria	KJ469721
St5	San23_2015	*Vitis vinifera*	Italy	MF182869
St6	MK44	*Vitis vinifera*	Macedonia	KF957607
St6	S7	*Urtica dioica*	Slovenia	JQ977719
St7	S6	*Urtica dioica*	Italy	JQ977718
St8	49MN	*Urtica dioica*	Montenegro	KJ926078
St8	4MN	*Vitis vinifera*	Montenegro	KJ926077
St8	BN-Yan1	*Vitis vinifera*	Italy	KX151182
St8	Ho13_838	*Hyalesthes obsoletus*	Austria	KJ469720
St8	Ho13-8	*Hyalesthes obsoletus*	Montenegro	KJ926079
St8	HoU190	*Hyalesthes obsoletus*	Macedonia	KP337321
St8	S5	*Urtica dioica*	Italy	JQ977717
St8	SB5	*Vitis vinifera*	Croatia	FN813266
St9	60/11	*Vitis vinifera*	Italy	KJ145345
St9	07-11	*Vitis vinifera*	Croatia	KP274915
St9	Aa25	*Vitis vinifera*	Italy	KJ145387
St9	Aaq29	*Vitis vinifera*	Italy	KJ145388

Table S2. Sequence variants of the gene *stamp* among '*Ca*. P. solani' strains available in GenBank (part III)

Sequence variant	Strain	Host	Location	Acc. N. *stamp*
St9	Ho13_1006	*Hyalesthes obsoletus*	Austria	KJ469718
St9	Mcil	*Vitis vinifera*	Italy	KJ145385
St9	S2	*Urtica dioica*	Slovenia	JQ977714
St10	LG	*Solanum lycopersicum*	France	FN813259
St10	PO	*Hyalesthes obsoletus*	France	FN813270
St10	San21_2015	*Vitis vinifera*	Italy	MF182868
St11	19-25	*Vitis vinifera*	Germany	FN813267
St11	33MN	*Vitis vinifera*	Montenegro	KJ926080
St11	67MN	*Urtica dioica*	Montenegro	KJ926081
St11	CrHo12_650	*Hyalesthes obsoletus*	Austria	KJ469716
St11	E	*Hyalesthes obsoletus*	Germany	FN813263
St11	G1	*Vitis vinifera*	Macedonia	KP337322
St11	GBr2	*Vitis vinifera*	Croatia	KJ573590
St11	GBr4	*Vitis vinifera*	Croatia	KJ573591
St11	GVu1	*Vitis vinifera*	Croatia	KJ573592
St11	GVu2	*Vitis vinifera*	Croatia	KJ573593
St11	H17	*Hyalesthes obsoletus*	Croatia	KJ573594
St11	H18	*Hyalesthes obsoletus*	Croatia	KJ573595
St11	H21	*Hyalesthes obsoletus*	Croatia	KJ573596
St11	Ho36-8	*Hyalesthes obsoletus*	Montenegro	KJ926082
St11	HoU17	*Hyalesthes obsoletus*	Macedonia	KP337323
St11	MK94	*Vitis vinifera*	Macedonia	KF957609
St12	L646	*Lavandula angustifolia*	France	FN813265
St13	GR13	*Vitis vinifera*	Greece	FN813264
St14	C	*Solanum lycopersicum*	France	FN813260
St15	P7	*Capsicum annuum*	Bosnia & Herzegovina	KU295503
St15	Tsol89	*Vitis vinifera*	Georgia	KT184885
St15	Kiqu84	*Vitis vinifera*	Georgia	KT184885
St16	H299	*Hyalesthes obsoletus*	France	FN813254
St16	L973	*Lavandula angustifolia*	France	FN813255
St17	Ate17	*Vitis vinifera*	Italy	KJ145386
St18	266/11	*Vitis vinifera*	Italy	KJ145344
St18	Aaq1	*Vitis vinifera*	Italy	KJ145383
St18	Mdxsain	*Vitis vinifera*	Italy	KJ145384
St18	San2_2015	*Vitis vinifera*	Italy	MF182870
St19	CrHo13_1183	*Hyalesthes obsoletus*	Austria	KJ469719
St19	S3	*Urtica dioica*	Slovenia	JQ977715
St20	136/11	*Vitis vinifera*	Italy	KJ145340
St20	166/11	*Vitis vinifera*	Italy	KJ145343
St20	Ate7	*Vitis vinifera*	Italy	KJ145381
St20	Mca21	*Vitis vinifera*	Italy	KJ145382
St20	P10/11	*Vitis vinifera*	Italy	KJ145342
St20	P42/11	*Vitis vinifera*	Italy	KJ145341
St21	Aa16	*Vitis vinifera*	Italy	KJ145380
St22	Mvercer2	*Vitis vinifera*	Italy	KJ145375
St22	San24_2015	*Vitis vinifera*	Italy	MF182871
St23	Lot et Garonne	*Solanum lycopersicum*	France	FN813257
St24	HoU93	*Hyalesthes obsoletus*	Macedonia	KP337314
St24	U79	*Hyalesthes obsoletus*	Macedonia	KP337313
St25	HoU80	*Hyalesthes obsoletus*	Macedonia	KP337309
St26	G5	*Hyalesthes obsoletus*	Macedonia	KP337310
St26	HoU85	*Hyalesthes obsoletus*	Macedonia	KP337311
St27	U70	*Urtica dioica*	Macedonia	KP337312
St28	HoC68	*Hyalesthes obsoletus*	Macedonia	KP337316
St28	PS3	*Solanum tuberosum*	Serbia	KP877587
St29	Vv12_274	*Vitis vinifera*	Austria	KJ469717
St30	10MN	*Vitis vinifera*	Montenegro	KJ926066
St30	04-09	*Vitis vinifera*	Croatia	KP274914

Table S2. Sequence variants of the gene *stamp* among '*Ca*. P. solani' strains available in GenBank (part IV)

Sequence variant	Strain	Host	Location	Acc. N. *stamp*
St30	G25	*Vitis vinifera*	Macedonia	KP337317
St30	PS10Ho	*Hyalesthes obsoletus*	Serbia	KP877603
St30	PS10Rq	*Reptalus quinquecostatus*	Serbia	KP877604
St30	Vv24	*Vitis vinifera*	Serbia	KC703022
St31	BG4560	*Vitis vinifera*	Bulgaria	FN813252
St31	PS2	*Solanum tuberosum*	Serbia	KP877586
St31	Rqg42	*Reptalus quinquecostatus*	Serbia	KC703016
St32	Mp49	*Vitis vinifera*	Italy	KJ145376
St33	OSESLO2	*Hyalesthes obsoletus*	Slovenia	FN813269
St33	Rome15	*Hyalesthes obsoletus*	Italy	FN813268
St33	S4	*Urtica dioica*	Italy	JQ977716
St34	S1	*Urtica dioica*	Germany	JQ977713
St35	Carv1	*Convolvulus arvensis*	Georgia	KT184879
St36	Carv2	*Convolvulus arvensis*	Georgia	KT184880
St37	Char7	*Convolvulus arvensis*	Georgia	KT184881
St37	Kisi38	*Vitis vinifera*	Georgia	KT184881
St37	Rkat47	*Vitis vinifera*	Georgia	KT184881
St37	Sape51	*Vitis vinifera*	Georgia	KT184881
St37	Sape62	*Vitis vinifera*	Georgia	KT184881
St38	Char8	*Convolvulus arvensis*	Georgia	KT184882
St38	Sape19	*Vitis vinifera*	Georgia	KT184882
St38	GoMt25	*Vitis vinifera*	Georgia	KT184882
St39	Amla77	*Vitis vinifera*	Georgia	KT184883
St40	Sabu84	*Vitis vinifera*	Georgia	KT184884
St41	20-set	*Vitis vinifera*	Bosnia & Herzegovina	KT766177
St42	154-10	*Vitis vinifera*	Bosnia & Herzegovina	KP739855
St43	03-nov	*Vitis vinifera*	Bosnia & Herzegovina	KP739850
St44	C1_Rgg35/Rqg31	*Apium graveolens*	Bosnia & Herzegovina	KU295505
St45	Ho1152	*Hyalesthes obsoletus*	Montenegro	KM977906
St46	RQ161	*Reptalus quinquecostatus*	France	LN823951
St47	San3_2015	*Vitis vinifera*	Italy	MF182872
St48	San4_2015	*Vitis vinifera*	Italy	MF182873
St49	San6_2015	*Vitis vinifera*	Italy	MF182874
St50	San10_2015	*Vitis vinifera*	Italy	MF182875
St51	San22_2015	*Vitis vinifera*	Italy	MF182876
St52	San6_2016	*Vitis vinifera*	Italy	MF182877
St53	San8_2016	*Vitis vinifera*	Italy	MF182878
St54	San16_2016	*Vitis vinifera*	Italy	MF182879
St55	San17_2016	*Vitis vinifera*	Italy	MF182880
St56	San28_2016	*Vitis vinifera*	Italy	MF182881
St57	San29_2016	*Vitis vinifera*	Italy	MF182882
St58	San45_2016	*Vitis vinifera*	Italy	MF182883

Supporting Information

Pierro *et al.* [Environment-driven selection of BNp strain populations**]**

Table S3. *vmp1*/*stamp* types of '*Ca*. P. solani' strains available in GenBank (part I)

vmp1	*stamp*	Strain	Host	Country
Vm10	St2	Rqg31	*Reptalus quinquecostatus*	Serbia
Vm10	St2	Vv17	*Vitis vinifera*	Serbia
Vm10	St4	Rpg39	*Reptalus panzeri*	Serbia
Vm10	St4	Rpm34	*Reptalus panzeri*	Serbia
Vm10	St4	Rqg60	*Reptalus quinquecostatus*	Serbia
Vm10	St4	STOL	*Capsicum annuum*	Serbia
Vm10	St4	Vexp Rpg11	*Reptalus panzeri*	Serbia
Vm10	St4	Vexp Rpm5	*Reptalus panzeri*	Serbia
Vm10	St4	Vv21	*Vitis vinifera*	Serbia
Vm11	St5	LA6_I_C	*Convolvulus arvensis*	Germany
Vm12	St5	GGY	*Vitis vinifera*	Germany
Vm14	St2	Vv12_Kn6	*Vitis vinifera*	Austria
Vm14	St3	Vv12_751	*Vitis vinifera*	Austria
Vm14	St5	Vv12_754	*Vitis vinifera*	Austria
Vm15	St9	60/11	*Vitis vinifera*	Italy
Vm15	St9	Aa25	*Vitis vinifera*	Italy
Vm15	St9	Ho13_1006	*Hyalesthes obsoletus*	Austria
Vm18	St8	Ho13_838	*Hyalesthes obsoletus*	Austria
Vm23	St19	CrHo13_1183	*Hyalesthes obsoletus*	Austria
Vm25	St6	MK44	*Vitis vinifera*	Macedonia
Vm27	St21	Aa16	*Vitis vinifera*	Italy
Vm28	St1	Rqg50	*Reptalus quinquecostatus*	Serbia
Vm28	St1	17-nov	*Vitis vinifera*	Bosnia & Herzegovina
Vm28	St1	CrAr12_722_2	*Anaceratagallia ribauti*	Austria
Vm28	St1	Rpg47	*Reptalus panzeri*	Serbia
Vm28	St5	Vv12_752	*Vitis vinifera*	Austria
Vm28	St18	Aaq1	*Vitis vinifera*	Italy
Vm28	St20	136/11	*Vitis vinifera*	Italy
Vm28	St20	166/11	*Vitis vinifera*	Italy
Vm28	St20	Mca21	*Vitis vinifera*	Italy
Vm28	St20	P10/11	*Vitis vinifera*	Italy
Vm33	St5	CrHo12_601	*Hyalesthes obsoletus*	Austria
Vm34	St11	19-25	*Vitis vinifera*	Germany
Vm35	St10	PO	*Hyalesthes obsoletus*	France
Vm36	St31	Rqg42	*Reptalus quinquecostatus*	Serbia
Vm38	St10	LG	*Solanum lycopersicum*	France
Vm39	St5	78/11	*Vitis vinifera*	Italy
Vm39	St5	San31_2016	*Vitis vinifera*	Italy
Vm40	St32	Mp49	*Vitis vinifera*	Italy
Vm41	St5	San47_2016	*Vitis vinifera*	Italy
Vm42	St5	San23_2015	*Vitis vinifera*	Italy
Vm42	St22	San24_2016	*Vitis vinifera*	Italy
Vm43	St10	San21_2015	*Vitis vinifera*	Italy
Vm43	St54	San16_2016	*Vitis vinifera*	Italy
Vm43	St55	San17_2016	*Vitis vinifera*	Italy
Vm43	St56	San28_2016	*Vitis vinifera*	Italy
Vm44	St22	Mvercer2	*Vitis vinifera*	Italy
Vm45	St5	315/11	*Vitis vinifera*	Italy
Vm45	St5	P136/11	*Vitis vinifera*	Italy
Vm45	St5	P75/11	*Vitis vinifera*	Italy
Vm45	St18	San2_2015	*Vitis vinifera*	Italy
Vm46	St1	115/11	*Vitis vinifera*	Italy
Vm46	St1	353/11	*Vitis vinifera*	Italy
Vm46	St1	Ag4a	*Vitis vinifera*	Italy

Table S3. *vmp1*/*stamp* types of '*Ca*. P. solani' strains available in GenBank (part II)

vmp1	***stamp***	Strain	**Host**	**Country**
Vm46	St1	Mp46	*Vitis vinifera*	Italy
Vm46	St1	PM1	*Solanum tuberosum*	Montenegro
Vm46	St1	Vv5	*Vitis vinifera*	Serbia
Vm46	St3	Rpm35	*Reptalus panzeri*	Serbia
Vm46	St5	287/11	*Vitis vinifera*	Italy
Vm46	St30	Vv24	*Vitis vinifera*	Serbia
Vm50	St5	Ca13_RF	*Convolvulus arvensis*	Austria
Vm53	St15	P7	*Catharanthus roseus*	Lebanon
Vm53	St15	Tsol89	*Vitis vinifera*	Georgia
Vm53	St15	Kiqu84	*Vitis vinifera*	Georgia
Vm54	St20	P42/11	*Vitis vinifera*	Italy
Vm57	St1	CrHo12_721	*Hyalesthes obsoletus*	Austria
Vm59	St11	MK94	*Vitis vinifera*	Macedonia
Vm60	St11	CrHo12_650	*Hyalesthes obsoletus*	Austria
Vm61	St29	Vv12_274	*Vitis vinifera*	Austria
Vm62	St5	425/11	*Vitis vinifera*	Italy
Vm63	St1	Vv12_Ill6	*Vitis vinifera*	Austria
Vm64	St35	Carv1	*Convolvulus arvensis*	Georgia
Vm65	St36	Carv2	*Convolvulus arvensis*	Georgia
Vm66	St37	Char7	*Convolvulus arvensis*	Georgia
Vm67	St38	Char8	*Convolvulus arvensis*	Georgia
Vm68	St38	Sape19	*Vitis vinifera*	Georgia
Vm69	St38	GoMt25	*Vitis vinifera*	Georgia
Vm70	St37	Kisi38	*Vitis vinifera*	Georgia
Vm71	St37	Rkat47	*Vitis vinifera*	Georgia
Vm71	St37	Sape51	*Vitis vinifera*	Georgia
Vm71	St37	Sape62	*Vitis vinifera*	Georgia
Vm73	St39	Amla77	*Vitis vinifera*	Georgia
Vm74	St40	Sabu84	*Vitis vinifera*	Georgia
Vm76	St1	G24-13	*Vitis vinifera*	Bosnia & Herzegovina
Vm76	St1	PM2	*Solanum tuberosum*	Montenegro
Vm81	St22	San24_2015	*Vitis vinifera*	Italy
Vm82	St18	San49_2016	*Vitis vinifera*	Italy
Vm83	St18	San11_2016	*Vitis vinifera*	Italy
Vm84	St5	San37_2016	*Vitis vinifera*	Italy
Vm85	St10	San5_2016	*Vitis vinifera*	Italy
Vm86	St10	San56_2016	*Vitis vinifera*	Italy
Vm87	St5	San35_2016	*Vitis vinifera*	Italy
Vm87	St18	San43_2016	*Vitis vinifera*	Italy

Supporting Information

Pierro *et al*. [Environment-driven selection of BNp strain populations**]**

Table S4. Genetic diversity based on nucleotide (in white) and amino acid (in grey) sequences among *vmp1*/*stamp* types, identified in the five Tuscan districts, grouped in the same and in distinct phylogenetic clusters

vmp1/stamp																
cluster	**type**	**#**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	VmTus2/StTus1	**1**	ID	0,983	0,979	0,905	0,882	0,945	0,929	0,902	0,886	0,905	0,895	0,91	0,908	0,893
	VmTus2/StTus3	**2**	0,992	ID	0,981	0,89	0,884	0,93	0,93	0,886	0,902	0,92	0,88	0,895	0,893	0,908
	VmTus2/StTus5	**3**	0,99	0,992	ID	0,888	0,892	0,929	0,949	0,885	0,886	0,905	0,878	0,893	0,891	0,893
2	VmTus4/StTus1	**4**	0,943	0,936	0,933	ID	0,972	0,954	0,934	0,893	0,876	0,885	0,915	0,937	0,947	0,93
	VmTus4/StTus4	**5**	0,928	0,929	0,933	0,984	ID	0,928	0,938	0,867	0,868	0,877	0,889	0,911	0,921	0,922
	VmTus5/StTus1	**6**	0,975	0,967	0,965	0,965	0,951	ID	0,979	0,9	0,883	0,922	0,932	0,954	0,944	0,927
	VmTus5/StTus5	**7**	0,965	0,967	0,975	0,956	0,956	0,99	ID	0,88	0,881	0,92	0,912	0,934	0,923	0,925
3	VmTus3/StTus1	**8**	0,956	0,949	0,947	0,938	0,923	0,951	0,941	ID	0,982	0,944	0,867	0,889	0,901	0,884
	VmTus3/StTus3	**9**	0,949	0,956	0,948	0,931	0,925	0,943	0,943	0,992	ID	0,961	0,85	0,872	0,884	0,901
	VmTus8/StTus3	**10**	0,96	0,967	0,959	0,932	0,925	0,963	0,962	0,972	0,98	ID	0,883	0,905	0,886	0,903
4	VmTus1/StTus1	**11**	0,95	0,943	0,94	0,947	0,932	0,967	0,957	0,936	0,929	0,942	ID	0,977	0,918	0,901
	VmTus7/StTus1	**12**	0,958	0,951	0,948	0,958	0,944	0,978	0,969	0,948	0,94	0,953	0,987	ID	0,93	0,913
5	VmTus6/StTus1	**13**	0,955	0,948	0,946	0,962	0,947	0,971	0,961	0,952	0,945	0,942	0,958	0,965	ID	0,982
	VmTus6/StTus3	**14**	0,948	0,955	0,947	0,954	0,948	0,963	0,963	0,945	0,952	0,949	0,951	0,958	0,992	ID

Mean % nucleotide sequence identity within clusters: *vmp1*/*stamp*-1: 99.13; *vmp1*/*stamp*-2: 96.7; *vmp1*/*stamp*-3: 98.13; *vmp1*/*stamp*-4: 98.7; *vmp1*/*stamp*-5: 99.2.
Mean % nucleotide sequence identity of the cluster *vmp1*/*stamp*-1 vs: *vmp1*/*stamp*-2: 95.13; *vmp1*/*stamp*-3: 95.45; *vmp1*/*stamp*-4: 94.83; *vmp1*/*stamp*-5: 94.98.
Mean % nucleotide sequence identity of the cluster *vmp1*/*stamp*-2 vs: *vmp1*/*stamp*-3: 93.97; *vmp1*/*stamp*-4: 95.65; vmp/*stamp*-5: 95.86.
Mean % nucleotide sequence identity of the cluster *vmp1*/*stamp*-3 vs: *vmp1*/*stamp*-4: 94.13; *vmp1*/*stamp*-5: 94.75.
Mean % nucleotide sequence identity of the cluster *vmp1*/*stamp*-4 vs *vmp1*/*stamp*-5: 95.80.

Mean % amino acid sequence identity of the cluster vmp/*stamp*-1: 98.10; *vmp1*/*stamp*-2: 95.08; *vmp1*/*stamp*-3: 96.23; *vmp1*/*stamp*-4: 97.70; *vmp1*/*stamp*-5: 98.20.
Mean % amino acid sequence identity of the cluster *vmp1*/*stamp*-1 vs: *vmp1*/*stamp*-2: 91.27; *vmp1*/*stamp*-3: 89.74; *vmp1*/*stamp*-4: 89.18; *vmp1*/*stamp*-5: 89.76.
Mean % amino acid sequence identity of the cluster *vmp1*/*stamp*-2 vs: *vmp1*/*stamp*-3: 88.76; *vmp1*/*stamp*-4: 92.30; vmp/*stamp*-5: 92.98.
Mean % amino acid sequence identity of the cluster *vmp1*/*stamp*-3 vs: *vmp1*/*stamp*-4: 87.76; *vmp1*/*stamp*-5: 89.31.
Mean % amino acid sequence identity of the cluster *vmp1*/*stamp*-4 vs *vmp1*/*stamp*-5: 91.55.

TABLES

Pierro *et al.* [Environment-driven selection of BNp strain populations**]**

Table 1. BNp genotype based on *tuf, vmp1* and *stamp* genes identified in five Tuscan districts on *Vitis vinifera* cv. Chardonnay and Sangiovese (part I)

BNp strain	Vineyard	Management	District	Cultivar	*tuf* type	V-type	seq. var.	
							vmp1	*stamp*
144	Marciano della Chiana	conventional	AR	Chardonnay	b			
146	Marciano della Chiana	conventional	AR	Chardonnay	b	V15	VmTus1	StTus1
147	Marciano della Chiana	conventional	AR	Chardonnay	b			StTus5
151	Marciano della Chiana	conventional	AR	Chardonnay	b			StTus3
152	Marciano della Chiana	conventional	AR	Chardonnay	b			
149	Marciano della Chiana	conventional	AR	Sangiovese	b			StTus3
154	Marciano della Chiana	conventional	AR	Sangiovese	b			StTus3
155	Marciano della Chiana	conventional	AR	Sangiovese	b	V11	VmTus2	StTus5
189	Barberino V. Elsa	conventional	FI	Sangiovese	b			StTus1
190	Barberino V. Elsa	conventional	FI	Chardonnay	b	V11	VmTus2	StTus1
192	Barberino V. Elsa	conventional	FI	Chardonnay	b	V4	VmTus7	StTus1
193	Barberino V. Elsa	conventional	FI	Chardonnay	b	V11	VmTus3	StTus3
194	Barberino V. Elsa	conventional	FI	Chardonnay	b			StTus3
PI76	Tavernelle Val di Pesa	organic	FI	Chardonnay	b	V11		
PI77	Tavernelle Val di Pesa	organic	FI	Chardonnay	b	V11	VmTus3	StTus3
PI78	Tavernelle Val di Pesa	organic	FI	Chardonnay	b			StTus5
PI79	Tavernelle Val di Pesa	organic	FI	Chardonnay	b	V11		
184	Lastra a Signa	conventional	FI	Sangiovese	b	V11	VmTus3	StTus3
185	Lastra a Signa	conventional	FI	Sangiovese	b	V11	VmTus3	StTus3
186	Lastra a Signa	conventional	FI	Sangiovese	b	V11	VmTus8	StTus3
187	Lastra a Signa	conventional	FI	Sangiovese	b	V11	VmTus3	StTus3
188	Lastra a Signa	conventional	FI	Sangiovese	b	V11	VmTus3	StTus3
180	Lastra a Signa	conventional	FI	Sangiovese	b			StTus1
115	Lastra a Signa	conventional	FI	Sangiovese	b	V11	VmTus3	
PI41	Greve in Chianti (I)	conventional	FI	Sangiovese	b	V15	VmTus1	StTus1
PI42	Greve in Chianti (I)	conventional	FI	Sangiovese	b	V15	VmTus1	StTus1
PI43	Greve in Chianti (I)	conventional	FI	Sangiovese	a	V3	VmTus6	StTus1
PI45	Greve in Chianti (I)	conventional	FI	Sangiovese	b			StTus1
PI47	Greve in Chianti (I)	conventional	FI	Sangiovese	b	V11	VmTus2	StTus3
PI48	Greve in Chianti (I)	conventional	FI	Sangiovese	b	V11	VmTus3	StTus1
PI49	Greve in Chianti (I)	conventional	FI	Sangiovese	b	V11	VmTus3	
PI50	Greve in Chianti (I)	conventional	FI	Sangiovese	b	V11	VmTus3	
PI56	Greve in Chianti (I)	conventional	FI	Sangiovese	b	V11	VmTus3	StTus1
PI57	Greve in Chianti (I)	conventional	FI	Sangiovese	b			StTus5
PI87	Greve in Chianti (II)	organic	FI	Sangiovese	a			StTus5
PI88	Greve in Chianti (II)	organic	FI	Sangiovese	b			StTus3

Table 1. BNp genotype based on *tuf, vmp1* and *stamp* genes identified in five Tuscan districts on *Vitis vinifera* cv. Chardonnay and Sangiovese (part II)

BNp strain	Vineyard	Management	District	Cultivar	*tuf* type	V-type	seq. var.	
							vmp1	*stamp*
48	Montecarlo (I)	conventional	LU	Chardonnay	a			StTus4
49	Montecarlo (I)	conventional	LU	Chardonnay	b	V11	VmTus3	StTus3
50	Montecarlo (I)	conventional	LU	Chardonnay	b	V1	VmTus4	StTus1
52	Montecarlo (I)	conventional	LU	Chardonnay	b	V11	VmTus3	StTus1
53	Montecarlo (I)	conventional	LU	Chardonnay	b	V11	VmTus3	StTus3
54	Montecarlo (II)	conventional	LU	Chardonnay	a			StTus4
201	Montecarlo (II)	conventional	LU	Chardonnay	b	V1	VmTus5	StTus1
203	Montecarlo (II)	conventional	LU	Chardonnay	b			StTus1
258	Montecarlo (II)	conventional	LU	Chardonnay	a	V11	VmTus3	StTus1
87	Massa	organic	MS	Sangiovese	b			
90	Massa	organic	MS	Chardonnay	a			StTus1
85	Massa	organic	MS	Sangiovese	b	V1	VmTus5	StTus5
86	Massa	organic	MS	Sangiovese	b			
209	Licciana Nardi	organic	MS	Chardonnay	b	V1	VmTus4	StTus4
11	Mulazzo	organic	MS	Chardonnay	a			
31	Carrara	organic	MS	Sangiovese	b	V11	VmTus3	StTus3
106	San Gimignano	conventional	SI	Chardonnay	b			StTus3
108	San Gimignano	conventional	SI	Chardonnay	b	V11	VmTus3	StTus3
109	San Gimignano	conventional	SI	Chardonnay	b	V11	VmTus3	StTus3
110	San Gimignano	conventional	SI	Chardonnay	b			StTus3
PI21	Gaiole in Chianti (I)	conventional	SI	Chardonnay	a			StTus5
PI22	Gaiole in Chianti (I)	conventional	SI	Chardonnay	b	V3	VmTus6	StTus3
PI24	Gaiole in Chianti (I)	conventional	SI	Chardonnay	a	V3	VmTus6	StTus3
PI25	Gaiole in Chianti (I)	conventional	SI	Chardonnay	a	V3		StTus2
PI11	Gaiole in Chianti (II)	conventional	SI	Chardonnay	b			StTus1
PI12	Gaiole in Chianti (II)	conventional	SI	Chardonnay	b			StTus3
PI14	Gaiole in Chianti (II)	conventional	SI	Chardonnay	b			StTus3
PI16	Gaiole in Chianti (II)	conventional	SI	Sangiovese	b			StTus3
PI17	Gaiole in Chianti (II)	conventional	SI	Sangiovese	b			StTus5
PI20	Gaiole in Chianti (II)	conventional	SI	Sangiovese	b			StTus3
PI68	Colle Val d'Elsa	conventional	SI	Chardonnay	b			StTus3
PI69	Colle Val d'Elsa	conventional	SI	Chardonnay	b			
PI61	Colle Val d'Elsa	conventional	SI	Sangiovese	b			
212	Montepulciano	conventional	SI	Sangiovese	b			StTus3
294	Montepulciano	conventional	SI	Sangiovese	b			StTus1
295	Montepulciano	conventional	SI	Sangiovese	a			StTus3
PI6	Montepulciano	conventional	SI	Sangiovese	b			StTus1
PI9	Montepulciano	conventional	SI	Sangiovese	a			StTus1
PI7	Montepulciano	conventional	SI	Sangiovese	b	V3	VmTus6	StTus1

Table 2. *vmp1* genetic variants of BNp strains identified in the vineyards localized in five Tuscan districts in 2016, their prevalence, representative strains and sequence accession numbers deposited in NCBI GenBank.

Variant	No. of strains	Representative strain	Accession No.
VmTus1 (Vm39)	3	146	MG874657
VmTus2 (Vm41)	3	190	MG874658
VmTus3 (Vm43)	18	Pi77	MG874659
VmTus4 (Vm88)	2	209	MG874660
VmTus5 (Vm89)	2	85	MG874661
VmTus6 (Vm90)	4	Pi43	MG874662
VmTus7 (Vm91)	1	192	MG874663
VmTus8 (Vm92)	1	186	MG874664

Table 3. *stamp* genetic variants of BNp strains identified in the vineyards localized in the five Tuscan districts in 2016, their prevalence, representative strains and sequence accession numbers deposited in NCBI GenBank.

Variant	Num. Of strains	Representative strain	Accession
StTus1 (St5)	22	146	MG874665
StTus2 (St9)	1	Pi25	MG874666
StTus3 (St10)	29	Pi77	MG874667
StTus4 (St11)	3	209	MG874668
StTus5 (St18)	8	85	MG874669

Table 4. Agro-climatic conditions recorded in the five Tuscan districts considered in the present study over two years (2015-2016).

Parameters	Year	C1[a]	C2[a]	*p* value[b]
Min Temp (C°)	2015	9.1 ± 0.7	8.4 ± 0.4	*
	2016	8.8 ± 0.6	7.6 ± 0.2	
Mean Temp (C°)	2015	14.4 ± 0.4	11.8 ± 0.2	*
	2016	14.1 ± 0.5	11.0 ± 0.1	
Max Temp (C°)	2015	19.7 ± 0.2	15.2 ± 0.1	ns
	2016	19.5 ± 0.3	14.3 ± 0.1	
Rain (mm)	2015	726.7 ± 13.4	706.2 ± 4.2	ns
	2016	897.4 ± 14.5	884.5 ± 7.8	
ET (mm)	2015	967.1 ± 33.2	798.4 ± 1.0	*
	2016	836.0 ± 43.7	700.4 ± 7.5	

Data retrieved from the website https://www.politicheagricole.it

[a] The zone C1 includes the districts of Arezzo, Firenze and Siena (central-eastern Tuscany); the zone C2 includes the districts of Lucca and Massa (north-western Tuscany);

[b] * = statistically significant differences ($p < 0.05$); ns = not statistically significant differences ($p > 0.05$)

Table 5. Statistical significance (p value) of differences in the strain composition of BNp populations identified in Tuscan vineyards

Tuscan BNp strains	Differences in BNp strain composition (p value)			
	varieties	managements	districts	weather zones
tufB types	0.226	0.444	0.161	0.061
V-types	0.614	0.277	**0.001**	**0.004**
vmp1 sequence variants	0.686	0.404	0.084	**0.031**
stamp sequence variants	0.319	0.126	0.111	**0.006**
vmp1 clusters	0.455	0.332	**0.012**	**0.005**
stamp clusters	0.513	**0.048**	0.335	**0.161**
vmp1/*stamp* clusters	0.861	0.219	**0.007**	**0.006**

Significant p values ($p < 0.05$) are evidenced in bold

FIGURE LEGENDS

Pierro *et al.* [Environment-driven selection of BNp strain populations**]**

Figure 1. Virtual *Rsa*I-RFLP profiles of *vmp1* gene obtained from BNp strains identified in this study. Virtual *Rsa*I restriction profiles of *vmp1* gene were obtained digesting trimmed TYPH10F/TYPH10R fragments with the software Serial Cloner 2.6.1. Samples 209, Pi43, 192, 190 and 146 are representative of the profile V1, V3, V4, V11 and V15, respectively. M, Marker Φ174 DNA/*Hae*III (Promega).

Figure 2. Distribution of BNp strains in Tuscany. Distribution of V-types in districts (A) and weather condition zones (B); *vmp1* and *stamp* sequence variants in weather condition zones (C) and (D); BNp strains grouped in *vmp1* clusters (Figure 3) in districts (E) and weather condition zones (F); BNp strains grouped in *stamp* clusters (Figure 4) in conventional and organic vineyards (G); BNp strains grouped in *vmp1*/*stamp* clusters (Figure 5) in districts (H) and weather condition zones (I). X axis represents the number of BNp strains.

Figure 3. Unrooted phylogenetic tree inferred from *vmp1* gene nucleotide sequences of BNp strains representative of *vmp1* sequence variants previously described (Table S1) and identified in this study (Table 2); minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1,000 times. Names of strains are reported on the image. GenBank accession number of each sequence is given in parenthesis; gene sequences obtained in the present study are indicated in bold. Clusters are shown as delimitated by parentheses. Acronyms within clusters indicated phytoplasma hosts and origin. Hosts: Car, *Convolvulus arvensis*; Ho, *Hyalesthes obsoletus*; Lv, *Linaria vulgaris*; Sl, *Solanum lycopersicum*; St, *Solanum tuberosum*; Ud, *Urtica dioica*; Vv, *Vitis vinifera*. Origin: AU, Austria; B&H, Bosnia & Herzegovina; FR, France; GER, Germany; IT, Italy; MA, Macedonia; MONT, Montenegro; SLO, Slovenia.

Figure 4. Unrooted phylogenetic tree inferred from *stamp* gene nucleotide sequences of BNp strains representative of *stamp* sequence variants previously described (Table S2) and identified in

this study (Table 3); minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1,000 times. Names of strains are reported on the image. GenBank accession number of each sequence is given in parenthesis; gene sequences obtained in the present study are indicated in bold. Clusters are shown as delimitated by parentheses. Acronyms within clusters indicated phytoplasma hosts and origin. Hosts: *Apium graveolens*, Ag; Ar, *Anaceratagallia ribauti*; Can, *Capsicum annuum*; Car, *Convolvulus arvensis*; Ho, *Hyalesthes obsoletus*; La, *Lavandula angustifolia*; *Phaseolus vulgaris*, Pv; Rp, *Reptalus panzeri*; Rq, *R. quinquecostatus*; Sl, *Solanum lycopersicum*; St, *Solanum tuberosum*; Ud, *Urtica dioica*; Va-c, *Vitex agnus-castus*; Vv; *Vitis vinifera*; Zm, *Zea mays*. Origin: AU, Austria; B&H, Bosnia & Herzegovina; BU, Bulgaria; CR, Croatia; FR, France; GEO, Georgia; GER, Germany; GR, Greece; IT, Italy; MA, Macedonia; MON, Montenegro; SER, Serbia; SLO, Slovenia.

Figure 5. Unrooted phylogenetic tree inferred from concatenated nucleotide sequences of *vmp1* and *stamp* genes of BNp strains representative of *vmp1*/*stamp* types previously described (Table S3) and identified in this study (Table 1); minimum evolution analysis was performed using the neighbor-joining method and bootstrap replicated 1,000 times. Names of strains are reported on the image. GenBank accession number of each sequence is given in parenthesis; gene sequences obtained in the present study are indicated in bold. Clusters are shown as delimitated by parentheses. Acronyms within clusters indicated phytoplasma hosts and origin. Hosts: Car, *Convolvulus arvensis*; Ho, *Hyalesthes obsoletus*; Sl, *Solanum lycopersicum*; St, *Solanum tuberosum*; Ud, *Urtica dioica*; Vv, *Vitis vinifera*. Origin: AU, Austria; B&H, Bosnia & Herzegovina; FR, France; GEO, Georgia; GER, Germany; IT, Italy; MA, Macedonia; SLO, Slovenia.

Figure 1.

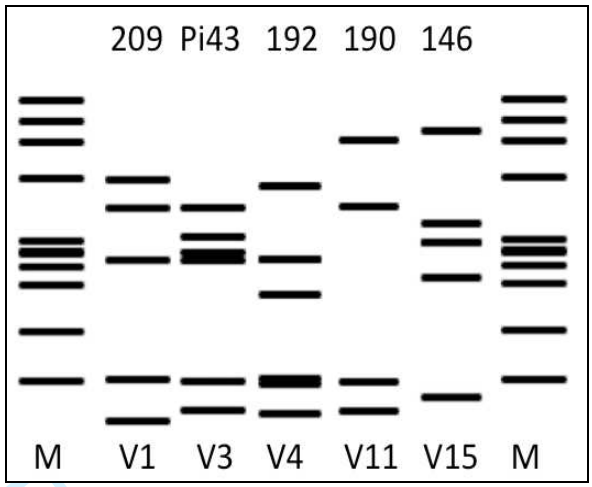
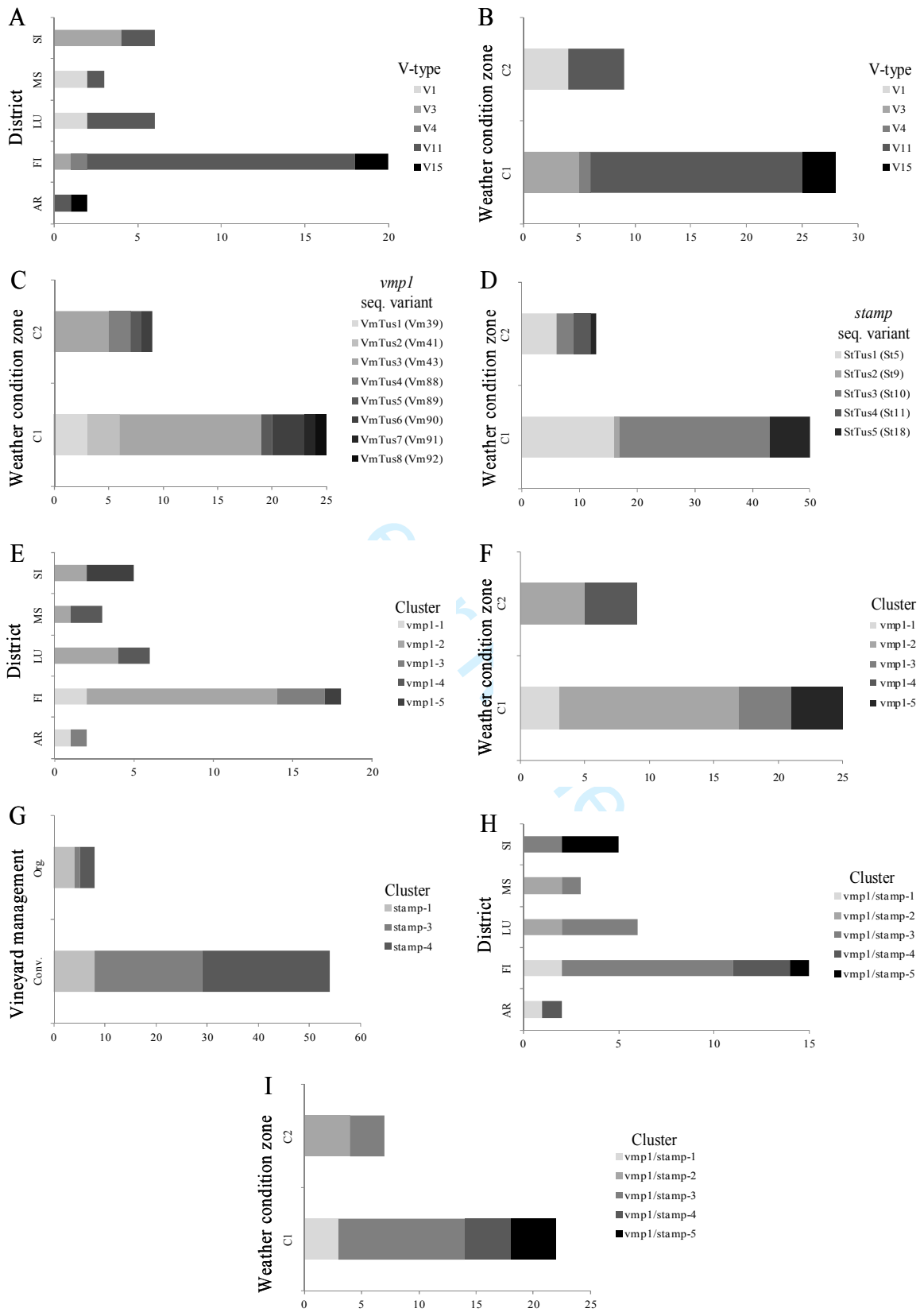
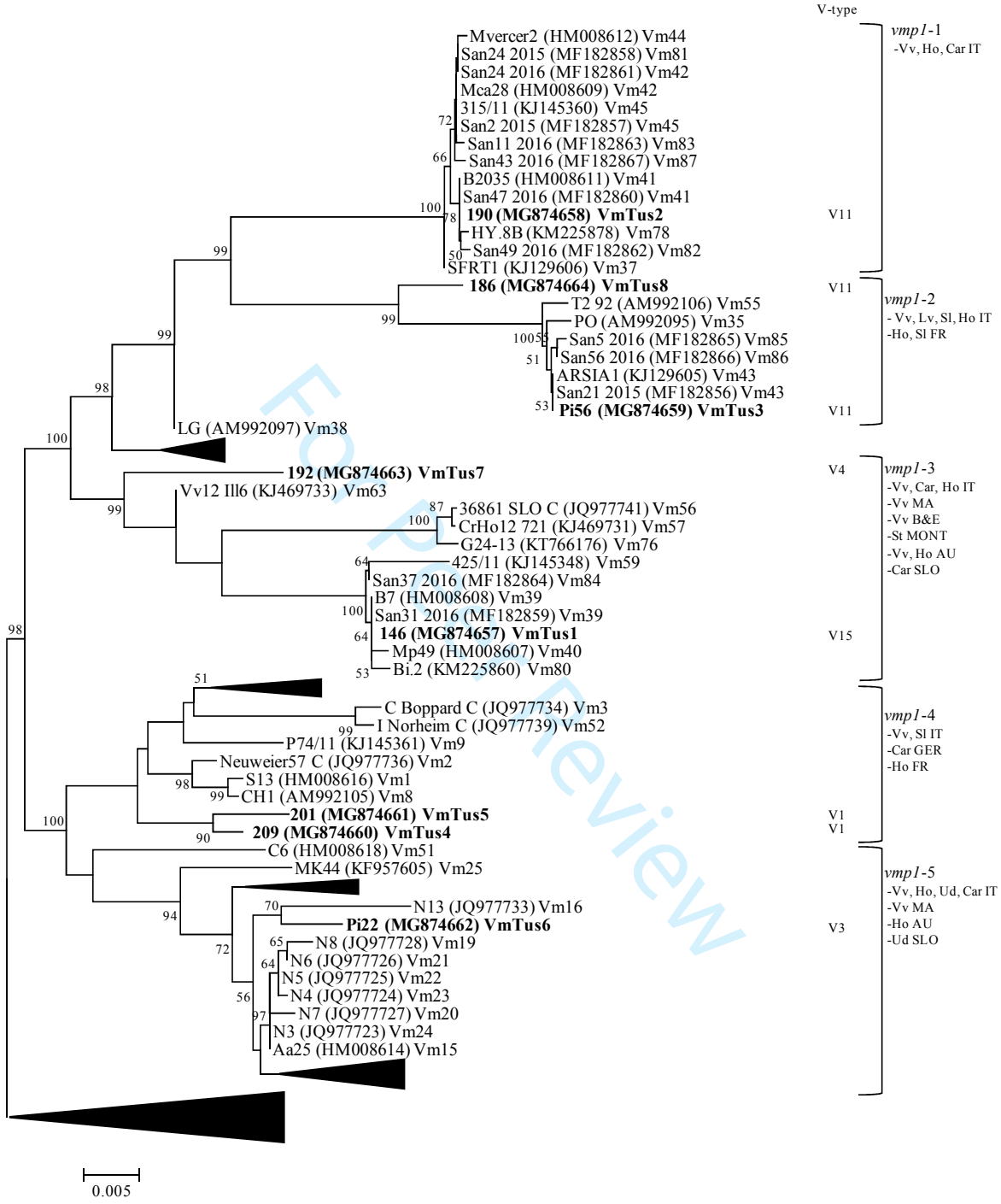


Figure 2.


A
District
SI
MS
LU
FI
AR
0
5
10
15
20
V-type
V1
V3
V4
V11
V15
B
Weather condition zone
C2
C1
0
5
10
15
20
25
30
V-type
V1
V3
V4
V11
V15
C
Weather condition zone
C2
C1
0
5
10
15
20
25
vmp1
seq. variant
VmTus1 (Vm39)
VmTus2 (Vm41)
VmTus3 (Vm43)
VmTus4 (Vm88)
VmTus5 (Vm89)
VmTus6 (Vm90)
VmTus7 (Vm91)
VmTus8 (Vm92)
D
Weather condition zone
C2
C1
0
10
20
30
40
50
stamp
seq. variant
StTus1 (St5)
StTus2 (St9)
StTus3 (St10)
StTus4 (St11)
StTus5 (St18)
E
District
SI
MS
LU
FI
AR
0
5
10
15
20
Cluster
vmp1-1
vmp1-2
vmp1-3
vmp1-4
vmp1-5
F
Weather condition zone
C2
C1
0
5
10
15
20
25
Cluster
vmp1-1
vmp1-2
vmp1-3
vmp1-4
vmp1-5
G
Vineyard management
Org.
Conv.
0
10
20
30
40
50
60
Cluster
stamp-1
stamp-3
stamp-4
H
District
SI
MS
LU
FI
AR
0
5
10
15
Cluster
vmp1/stamp-1
vmp1/stamp-2
vmp1/stamp-3
vmp1/stamp-4
vmp1/stamp-5
I
Weather condition zone
C2
C1
0
5
10
15
20
25
Cluster
vmp1/stamp-1
vmp1/stamp-2
vmp1/stamp-3
vmp1/stamp-4
vmp1/stamp-5

730 **Figure 3.**



731

732

733

734

735

736

Figure 4.

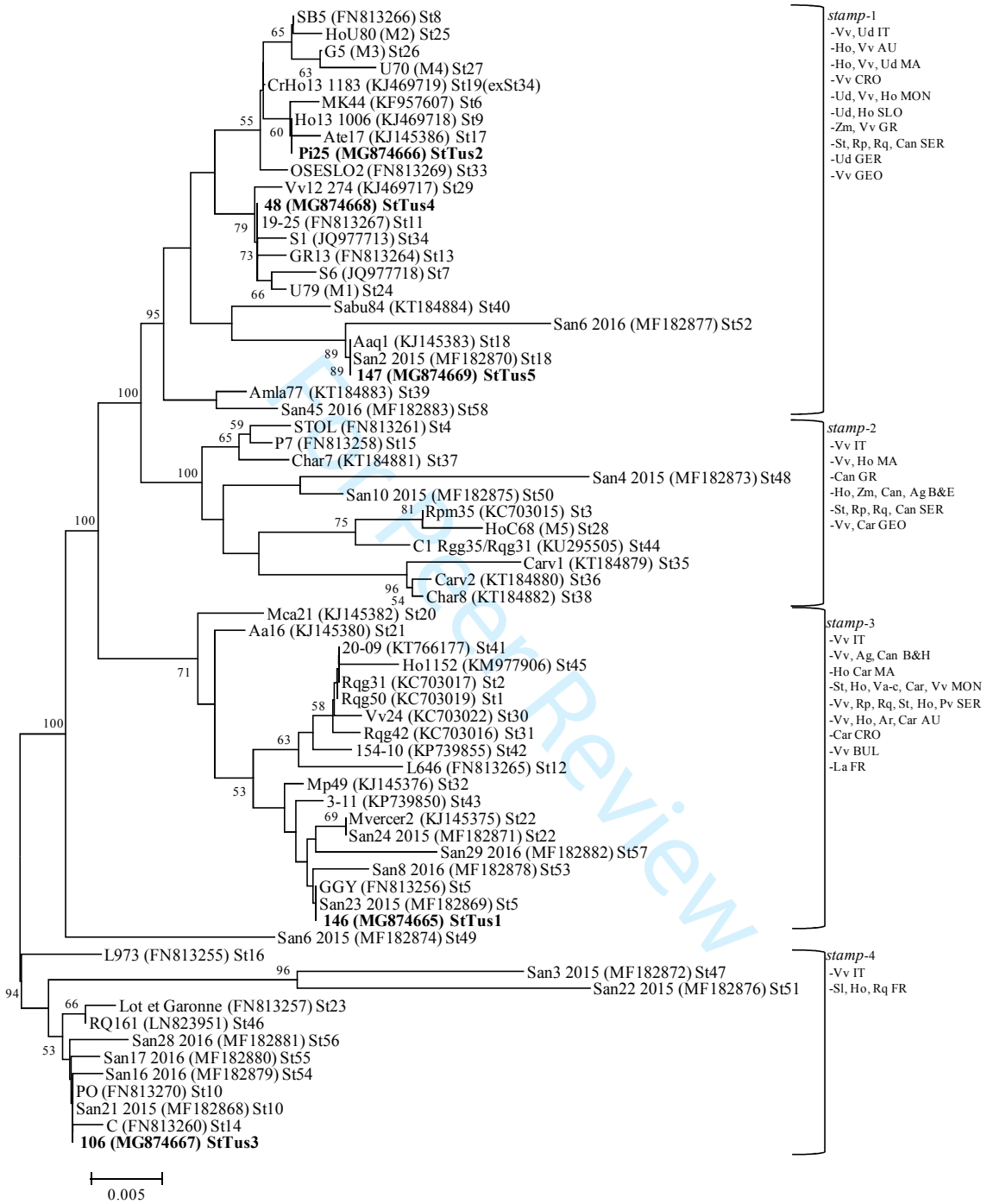


Figure 5.