Analysis of genotypic diversity provides a first glimpse on the patterns of spread of the wood decay fungus Perenniporia fraxinea in an urban park in northern Italy

ANALYSIS OF GENOTYPIC DIVERSITY PROVIDES A FIRST GLIMPSE ON THE PATTERNS OF SPREAD OF THE WOOD DECAY FUNGUS *PERENNIPORIA FRAXINEA* IN AN URBAN PARK IN NORTHERN ITALY

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Running title: Genotypic diversity of *Perenniporia fraxinea*

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Summary

Perenniporia fraxinea is a fungal pathogen causing wood decay in roots and bole of a wide variety of broadleaf tree species. Despite its ecological importance, little is know about the infection biology of this fungus and in particular of its ability to infect trees through the mycelia growth through root contacts. To clarify its spreading mechanisms, a genetic analysis of 20 *P. fraxinea* isolates obtained from basidiomata collected from closely located *Robinia pseudoacacia* and *Quercus robur* trees in the Vernavola Urban Park (Pavia, Italy) and in surrounding areas was performed. Random Amplified Microsatellites (RAMs) fingerprinting was conducted allowing to distinguish 19 different haplotypes. High intrapopulation diversity was confirmed by somatic incompatibility tests (SITs), which were performed by dual-culturing isolates *in vitro* in all possible combinations, resulting in detection of 16 compatibility groups. These results, together with Non-metric MultiDimensional Scaling (NMDS) analysis on genetic data, suggest that spread through root contacts is unlikely for *P. fraxinea*. In addition, a significant negative correlation between spatial distribution and kinship coefficients was observed in isolates from the Vernavola Urban Park, suggesting a limited dispersal potential of *P. fraxinea* basidiospores. This report provides a first glimpse of the primary mechanisms of spread of *P. fraxinea*.

Key words

Perenniporia fraxinea, RAMs, somatic compatibility groups, NMDS

Perenniporia fraxinea (Bull.) Ryvarden is both a saprotrophic and a pathogenic polypore affecting a broad range of host trees species as a wood decay agent (Bernicchia, 2005). *P. fraxinea* causes intense white rot in roots and in the lowest parts of tree stems (Lonsdale, 1999). Despite it is often associated with *Fraxinus* and *Robinia,* its host range is wide, with records of infection covering several broadleaved species, including those belonging to the genera *Aesculus, Acer, Castanea, Celtis, Eucalyptus, Fagus, Juglans, Olea, Malus, Platanus, Populus, Prunus, Quercus, Salix* and *Ulmus* (Kotlaba 1984; Ryvarden and Gilbertson 1994; Szczepkowski 2004; Bernicchia 2005). The fungus is reported in forests but also in urban environments, i.e., street trees, gardens and parks (Guglielmo et al., 2007).

While the role played by *P. fraxinea* in urban environment is deemed important as the fungus may result in rapid wood decay leading to tree failures (Lonsdale, 1999; Tubby and Pérez-Sierra, 2015), its infection biology and patterns of spread still need to be elucidated. In fact, little is known on whether this fungus may spread vegetatively through root contacts from adjacent infected trees and on the role of spore dispersal in the epidemiology of this fungus. One of the approaches used to elucidate the spreading mechanisms of tree pathogens include population genotyping in the field. A low genetic diversity and a low number of genotypes is observed for fungi that can spread by vegetative growth of the mycelia through host root systems, when samples deriving from nearby host plants were analyzed. Examples include the root rot basidiomycetes *Armillaria mellea* (Vahl) P. Kumm. s. l. (Korhonen, 1978), *Heterobasidion annosum* (Fr.) Bref. s. l. (Garbelotto et al., 1999; Gonthier et al., 2003), *Perenniporia subacida* (Peck) Donk (Tabata et al., 2009) and *Phellinus noxius* (Corner) G. Cunn. (Hattori et al., 1996).

Despite its wide distribution area comprising Asia (mainly Japan), Europe and North America (Szczepkowski, 2004), sites where *P. fraxinea* basidiomata are abundant appear to be uncommon, so that intrapopulation studies can be hard to perform (Guglielmo et al., 2010). In Pavia municipality (Lombardy, Italy), basidiomata are locally abundant, making this species one of the most widespread tree pathogens in this site. The present investigation is aimed at providing a descriptive picture of *P. fraxinea* genotypic diversity in this site. Results are used to infer the likely patterns of spread of *P. fraxinea* based on the fact that if identical genets are identified in closely located trees these are the likely result of the vegetative spread of the fungus through root contacts..

The study was conducted in the Vernavola Urban Park in Pavia (centroid Long. 9° 10’ 9.4” E; Lat. 45° 12’ 36.1” N). The park covers an area of approximately 35 hectares and comprises a broadleaved forest including several tree species, especially *Alnus glutinosa* (L.) Gaertn. and *Robinia pseudoacacia* L. The site is located at an elevation ranging from 72 m to 83 m a.s.l. Soil is a fluvisol

(according to FAO world reference for soil classification, Micheli et al., 2006) characterized by Holocene alluvia, namely sand and coarse sand alternated to fine gravel and sandy silt lentes.

In this study, *P. fraxinea* was isolated starting from basidiomata. Although in general the infection processes of wood decay fungi in standing trees is not correlated with the presence of emerging basidiomata (Giordano et al., 2015), *P. fraxinea* has been reported as characterized by a higher inclination to develop basidiomata compared to other fungi (Giordano et al., 2015). In addition, it was assumed that each tree was colonized by only one fungal genet, as previously documented for the congeneric species *P. subacida* (Tabata et al., 2009). Visual inspection of trees during spring-summer 2014 (March-October) allowed detection at the root collar of several putative *P. fraxinea* basidiomata based on macro- and micro-morphological characters (Bernicchia, 2005). In detail, 13 basidiomata were found, 12 of which from *R. pseudoacacia* and 1 from *Quercus robur* L. (Table 1 and Figure 1). Maximum and minimum distance of trees with emerging basidiomata was 1727 m and 1.5 m, respectively. The age of *R. pseudoacacia* trees ranged from approximately 10 to 30 years and diameter at breast height (DBH) ranged from 25 to 50 cm, while the *Q. robur* tree was approximately over 50 years old and with a DBH of 55 cm.

Additionally, seven basidiomata collected in other sites were included in the analysis, in order to be used as outgroups (Table 1). In detail, one basidioma from Bosco Giuseppe Negri (San Martino Siccomario, Pavia, Italy), one from Santa Maria della Versa (Pavia, Italy), one from Zerbolò (Pavia, Italy), three from different streets of the city of Pavia, and one from Illasi (Verona, Italy). Isolations were performed by placing fragments (approximately 2 x 2 x 1.5 cm in size) excised from the context of basidiomata in Petri dishes filled with Malt Extract Agar (MEA; Malt Extract 20 g/L, Agar 15 g/L). Dishes were incubated at 25°C. Identification was further confirmed by using the molecular diagnostic assay with taxon specific primers developed by Guglielmo et al. (2007).

In order to assess genotypic diversity of *P. fraxinea*, somatic incompatibility tests (SITs) coupled with molecular genotyping were used. SITs were performed by pairing isolates *in vitro* in all possible combinations. Pair of isolates were inoculated 2 cm apart in 6 cm Petri dishes filled with MEA additioned with citric acid (0.5 g/L) and incubated at 25°C. Self-pairings were also tested for each isolate and used as controls. The presence or absence of a barrage or a rarefaction zone between the isolates after three weeks of incubation was examined and scored as 1 (compatibility between pairs) or 0 (incompatibility between pairs; i.e. presence of a barrage or a rarefaction zone) in a binary matrix to determine the somatic compatibility groups.

Molecular genotyping was performed through the Random Amplified Microsatellites (RAMs) technique (Hantula et al., 1996), which has been successfully applied in studies of fungi (Hantula and Müller, 1997; Guglielmo et al., 2012). For the DNA extraction, the isolates were cultured in liquid

malt extract 2% for a week, harvested by vacuum filtration and lyophilized over night. DNA extraction was performed by using DNeasy Plant mini kit (QIAGEN), following manifacturer's protocol. Two 50-anchored RAMs primers were used: DDB$(CCA)_5$ and DHB$(CGA)_5$, where B ¼ C, G or T; D ¼ A, G or T; H ¼ A, C or T; V ¼ A, C or G. The PCR reactions were performed in a 25 µl volume containing 1X PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs mix, 2 mM of each primer, 0.04 U ml $\mu 1^{-1}$ of Taq polymerase (Promega, Madison, WI, USA) and 1 ng of DNA template. PCR reactions were performed using an initial denaturation at 94 °C for 3 min, followed by 35 cycles with each cycle consisting of a denaturation at 94 °C for 30 s, an annealing for 45 s at a temperature of 61 °C, an extension at 72 °C for 1 min, and one extension cycle at 72 °C for 10 min. The size of amplification products was estimated after electrophoretic migration (6 V cm^{-1}) on a gel containing 1% (w/v) of high-resolution MetaPhor (Cambrex, Rockland, Maine, USA) and 1% (w/v) of standard agarose, through the Image Lab™ version 3.0 software on the basis of the GelPilot® 100 bp (QIAGEN, Valencia, CA, USA). In order to test the repeatability of the method, all isolates were re-subjected to PCR reactions with the two RAMs primers. The presence (1) or absence (0) of each marker was scored in a binary matrix to determine the identity or diversity among isolates. Although previous studies demonstrated that RAMs markers show Mendelian segregation and could be treated as co-dominant markers (Zietkiewicz et al., 1994), here as well as in the large majority of studies (Hantula et al., 1996; Hantula and Müller, 1997; Guglielmo et al., 2012), RAMs were deemed dominant markers.

Matrices obtained from SITs and RAMs genotyping were used as inputs for cluster analysis based on UPGMA (Unweighted Pair Group Method with Arithmetic mean). The analysis was carried out by using DendroUPGMA version September 2015 (http://genomes.urv.cat/UPGMA/). Jaccard coefficient was used and 100 bootstrap replicates were performed. Shannon's index, which does not assume Hardy-Weinberg equilibrium and is generally used to estimate genetic diversity starting from band profiles (band presence/absence) of dominant markers, was calculated using GenAlEx version 6.5 (Peakall and Smouse 2012).

Non-metric MultiDimensional Scaling (NMDS) using Jaccard similarity index was performed with the software Past version 3.12 (Hammer et al., 2001). In order to analyze relationship between geographical and genetic distances, the program SPAGeDi version 1.5a (Hardy and Vekemans, 2002) was used and pairwise kinship coefficients among isolates were calculated by assuming the broad sense heritability estimates of each dominant marker (H2) as 1 (Hardy 2003) and by excluding co-segregating bands to avoid overestimation bias. In addition, to assess the association between geographical and genetic distances, both Mantel and Spearman's rank correlation tests were performed by using Past version 3.12 (Hammer et al., 2001)..

Somatic incompatibility tests (SITs) allowed to distinguish two types of interactions: (i) overlapping mycelia between the two colonies which displayed an uniform aspect; (ii) an area of inhibition (rarefaction zone), ranging from slight to strong at sites of contact between the two colonies (Figure 2). The former was assumed as a somatic compatible reaction, whereas the latter was interpreted as a somatic incompatible reaction. Sixteen somatic compatibility groups were identified, while self-pairings of isolates always resulted in compatible reactions (Figure 3).

The amplification of *P. fraxinea* DNA using RAMs primer sets produced fragments ranging in size between 225 bp and 1660 bp for DHB$(CGA)_5$ primers and between 340 bp and 1500 bp for DDB$(CCA)_5$. In total, 21 polymorphic loci were scored, including 8 polymorphic loci obtained by using DHB$(CGA)_5$ primers and 13 by using DDB$(CCA)_5$. The scoring of polymorphic loci mirroring the dissimilarity of RAMs profiles resulted in the identification of 19 distinct genets (Figure 3).

The percentage of polymorphic loci was 76.19% in samples collected in Vernavola Urban Park, and 80.95% for samples collected outside the park. Shannon index (diversity) was 0.36 (SE 0.05) and 0.45 (0.05) among Vernavola Urban Park isolates and among the other isolates, respectively. Frequencies of the presence and absence of bands*per* locus are shown in Table 2. NMDS showed that isolates from Vernavola Urban Park grouped together, with the only exception of isolate V3, whereas all the other isolates appeared dispersed (Figure 4). It should be noted that isolate V3 derived from a basidiomata collected from the *Q. robur* tree, while the other samples were collected from *R. pseudoacacia* trees. While Mantel test did not show a significant correlation between the geographical and the genetic distance matrices, a Spearman's rank correlation test between kinship coefficients and geographical pairwise distances showed a significant negative correlation (-0.25, *p*-value = 0.02).

Results of both SITs and RAMs genotyping showed the presence of high diversity among isolates, including isolates from Vernavola Urban Park. In addition, RAMs allowed to detect 19 genets vs 16 somatic compatibility groups, thus demonstrating to perform better for genotyping as previously reported (Guglielmo et al., 2012). It should be noted that the observation of compatibility reactions *in vitro* might be hard to interpreter and it might lead to an underestimation of number of genets (Worrall, 1997). NMDS allowed to discriminate the large majority of isolates of the Vernavola Urban Park from all the others, thus suggesting that they probably belong to a distinct population. This finding is supported by the reduced genetic diversity, i.e. Shannon index, among Vernavola Urban Park isolates compared to those collected outside the park. The studied population of isolates of the Vernavola Urban park included 12 genets out of 13 isolates, and distinct genets were also assigned to basidiomata collected from closely located host trees (less than 2 metres apart). Tabata et al. (2009) performed a similar analysis on isolates of *P. subacida* collected in several *Chamaerocyparis obtusa* trees in Japan and they reported that the same genet was present in different trees, thus suggesting

that *P. subacida* can spread from tree to tree through root contacts. In our study, based on molecular genotyping, distinct RAMs profiles were found in isolates from each tree, with the exception of V1 and V4. However, SITs demonstrated that V1 and V4 clearly belong to two different somatic compatibility groups and therefore they should be considered as distinct genotypes. Although RAMs genotyping perform better than SITs to discriminate genotypes, our results suggest that the two approaches are complementary and thus they may be both needed for an accurate analysis of isolates. Our findings may suggest that the spread through root contacts is unlikely for *P. fraxinea*, at least in this sampling site. The fungus may rather spread through basidiospores. The negative correlation between spatial distance and kinship coefficients could support this last hypothesis. The kinship coefficient is a measure of relatedness that represents the probability that two alleles, sampled at random from each sample, are identical by descent. In our study, a significant increase of kinship coefficients was observed for the relationship among isolates from closely located basidiomata (Table 3). A limited range of spore dispersal could be thus inferred for *P. fraxinea*, as previously documented for other wood decay fungi (Garbelotto et al., 2013). Besides, we found some positive kinship coefficients (> 0.4) among couples of isolates sampled in the range of one kilometer. This finding may be consistent with an interbreeding of genets within the Vernavola Urban Park. Interesting, kinship coefficients of isolates sampled from neighbouring trees were always lower than 0.5, with the exception of the pair V4-V4t which showed a kinship coefficient of 0.76. In the case of isolates V4 and V4t, we cannot exclude that a common homokaryotic mycelium could have colonized neighbouring trees by infecting through root contacts. According to this scenario this homokaryotic mycelium should have mated with other homokaryotic or even heterokaryotic mycelia through di-mon mating in each one of the two trees, i.e. Buller phenomenon (Buller, 1941), resulting in two different heterokaryotic genets sharing one nucleus. It should be noted that homokaryotic isolates of some wood decay Basidiomycota have been reported to be as successful as heterokaryotic ones in infecting trees (Garbelotto et al., 1997). However, a high kinship value does not necessarily indicate that a nucleus is shared between two different isolates since similar results may be observed for sib-related isolates (Malloure and James, 2013).

Although inferring the possible introduction of the *P. fraxinea* population in the Vernavola Urban Park based on genetic data may be intriguing, we deemed the number of samples insufficient to address this issue. Despite a broader sampling involving other study sites would be needed to exclude the ability of the fungus to spread through root contacts, this work provides a first glimpse on the patterns of dispersal of *P. fraxinea*.

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References

Bernicchia A., 2005. Polyporaceae s.l. Ed. Candusso, Alassio, Italy. 808 pp.

Buller, A. H. R., 1941. The diploid cell and the diploidisation process in plants and animals, with special reference to the higher fungi. *The Botanical Review* **7**: 335-387.

Decock C., Stalpers J., 2006. Proposal to conserve the name *Perenniporia* against *Physisporus* with a conserved type (Basidiomycota). *Taxon: international journal of plant taxonomy, phylogeny and evolution* **55**: 227.

Garbelotto M., Lee H. K., Slaughter G., Popenuck T., Cobb F. W., and Bruns, T. D., 1997. Heterokaryosis is not required for virulence of *Heterobasidion annosum*. *Mycologia* **89**: 92-102.

Garbelotto M., Cobb FW., Bruns TD., Otrosina WJ., Popenuck T., and Slaughter G., 1999. Genetic structure of *Heterobasidion annosum* in white fir mortality centers in California. *Phytopathology* **89**: 546-554.

Garbelotto M., Guglielmo F., Mascheretti S., Croucher PJP., Gonthier P., 2013. Population genetic analyses provide insights on the introduction pathway and spread patterns of the North American forest pathogen *Heterobasidion irregulare* in Italy. *Molecular ecology* **22**: 4855-4869.

Giordano L., Sillo F., Guglielmo F., Gonthier P., 2015. Comparing visual inspection of trees and molecular analysis of internal wood tissues for the diagnosis of wood decay fungi. *Forestry* **88**: 465-470.

Gonthier P., Garbelotto M., Nicolotti G., 2003. Swiss stone pine trees and spruce stumps represent an important habitat for Heterobasidion spp. in subalpine forests. *Forest Pathology* **33**: 191-203.

Guglielmo F., Bergemann S. E., Gonthier P., Nicolotti G., Garbelotto M., 2007. A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees. *Journal of Applied Microbiology* **103**: 1490-1507.

Guglielmo F., Gonthier P., Garbelotto M., Nicolotti G., 2010. Optimization of sampling procedures for DNA-based diagnosis of wood decay fungi in standing trees. *Letters in Applied Microbiology* **51**: 90-97.

Guglielmo F., Michelotti S., Nicolotti G., Gonthier P., 2012. Population structure analysis provides insights into the infection biology and invasion strategies of *Kretzschmaria deusta* in trees. *Fungal ecology* **5**: 714-725

Hammer Ø., Harper D.A.T., Ryan P.D. 2001., PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**: 9

Hantula J., Dusabenyagasani M., Hamelin RC., 1996. Random amplified microsatellites (RAMS)- a novel method for characterizing genetic variation within fungi. *European Journal of Forest Pathology* **26**: 159-166.

Hantula J., Müller MM., 1997. Variation within *Gremmeniella abietina* in Finland and other countries as determined by Random Amplified Microsatellites (RAMS). *Mycological research* **101**: 169-175.

Hardy O.J., Vekemans X., 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618-620.

Hardy O. J., 2003. Estimation of pairwise relatedness between individuals and characterization of isolation by distance processes using dominant genetic markers. *Molecular Ecology* **12**: 1577-1588.

Hattori T., Abe Y., Usugi T., 1996. Distribution of clones of *Phellinus noxius* in a windbreak on Ishigaki Island. *European Journal of Forest Pathology* **26**: 69-80.

Korhonen K., 1978. Interfertility and clonal size in the *Armillaria mellea* complex. *Karstenia* **18**: 31-2.

Kotlaba F., 1984. Geographical distribution and ecology of polypores Polyporales s.l. in Czechoslovakia (in Czech). Academia, Praha, 194 pp.

Lonsdale D., 1999. Principles of tree hazard assessment and management. Stationery Office Ltd, Publications Centre, UK, 388 pp.

Malloure B. D., James T. Y., 2013. Inbreeding depression in urban environments of the bird's nest fungus *Cyathus stercoreus* (Nidulariaceae: Basidiomycota). *Heredity* **110**: 355-362.

Micheli E., Schad P., Spaargared O., Dent D., Nachtergaele F., 2006. World reference base for soil resources 2006. *World Soil Resources Reports - Rome, Food and Agricultural Organization of the United Nations* **103**: 1-128.

Peakall P.E., Smouse R., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**: 2537-2539.

Ryvarden L., Gilbertson R.L., 1994. Part 2: Meripilus-Tyromyces. In: European polypores, Fungiflora A/S, 394-743.

Stenlid J., Vasiliauskas R., 1998. Genetic diversity within and among vegetative compatibility groups of *Stereum sanguinolentum* determined by arbitrary primed PCR. *Molecular Ecology* **7**: 1265-1274.

Szczepkowski A., 2004. *Perenniporia fraxinea* (Fungi, Polyporales), a new species for Poland. *Polish Botanical Journal* **49**: 73-77

Tabata M., Suyama Y., Abe Y., 2009. Distribution of genets of *Perenniporia subacida* in stands of *Chamaecyparis obtusa* (Japanese cypress) determined by AFLP fingerprints and somatic incompatibility. *Plant Disease* **93**: 826-831.

Travadon R., Smith ME., Fujiyoshi P., Douhan GW., Rizzo DM., Baumgartner K., 2012. Inferring dispersal patterns of the generalist root fungus *Armillaria mellea*. *New Phytologist* **193**: 959-969

Tubby K.V., Pérez-Sierra A., 2015. Pests and pathogen threats to plane (*Platanus*) in Britain. *Arboricultural Journal* **37**: 85-98.

Worrall JJ., 1997. Somatic incompatibility in basidiomycetes. *Mycologia* **89**: 24-36.

Zietkiewicz E., Rafalski A., Labuda D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* **20**: 176-183.

Table 1. List of isolates analysed in this study.

Isolate ID	Geographic location	Long	Lat	Host plant
V1	Vernavola Urban Park (PV)	9°10’1.8” E	45°12’14.5” N	*Robinia pseudoacacia*
V2	Vernavola Urban Park (PV)	9°10’3.9” E	45°12’21.4” N	*Robinia pseudoacacia*
V3	Vernavola Urban Park (PV)	9°10’ 3.9” E	45°12’22.1” N	*Quercus robur*
V4	Vernavola Urban Park (PV)	9°10’ 3.9” E	45°12’23.2” N	*Robinia pseudoacacia*
V5	Vernavola Urban Park (PV)	9°10’ 5.6” E	45°12’26.7” N	*Robinia pseudoacacia*
V6	Vernavola Urban Park (PV)	9°10’10.8” E	45°12’29.3” N	*Robinia pseudoacacia*
V7	Vernavola Urban Park (PV)	9°10’10.8” E	45°12’29.3” N	*Robinia pseudoacacia*
V2b	Vernavola Urban Park (PV)	9°10’10.8” E	45°12’29.3” N	*Robinia pseudoacacia*
V2t	Vernavola Urban Park (PV)	9°10’13.2” E	45°12’9.9” N	*Robinia pseudoacacia*
V4b	Vernavola Urban Park (PV)	9° 10’13.2” E	45°12’10” N	*Robinia pseudoacacia*
V4t	Vernavola Urban Park (PV)	9°10’13.2” E	45°12’10.1” N	*Robinia pseudoacacia*
V5b	Vernavola Urban Park (PV)	9°10’5” E	45°12’59” N	*Robinia pseudoacacia*
V5t	Vernavola Urban Park (PV)	9°10’13.4” E	45°12’5” N	*Robinia pseudoacacia*
RS	Pavia (Rione Scala)	-	-	*Celtis australis*
VBU	Pavia (via Baldo degli Ubaldi)	-	-	*Aesculus hippocastanum*
EFIL	Pavia (via Borgo Calvenzano)	-	-	*Platanus occidentalis*
CV	Zerbolò (PV)	-	-	*Populus alba*
BGN	San Martino Siccomario (PV)	-	-	*Populus nigra*
PZF	Santa Maria della Versa (PV)	-	-	-
VERONA	Illasi (VR)	-	-	*Olea sativa*

Table 2. Frequencies of presence (p) / absence (a) of bands and Shannon index (I) *per* locus for the Vernavola Urban Park isolates and for the isolates outside the Park.

Vernavola Urban Park isolates

Locus ID	p	a	I
CGA1	0,154	0,846	0,429
CGA2	0,692	0,308	0,617
CGA3	0,769	0,231	0,540
CGA4	0,923	0,077	0,271
CGA5	1,000	0,000	0,000
CGA6	0,154	0,846	0,429
CGA7	0,154	0,846	0,429
CGA8	0,538	0,462	0,690
CCA1	0,000	1,000	0,000
CCA2	0,231	0,769	0,540
CCA3	0,846	0,154	0,429
CCA4	0,923	0,077	0,271
CCA5	0,308	0,692	0,617
CCA6	0,077	0,923	0,271
CCA7	0,538	0,462	0,690
CCA8	0,000	1,000	0,000
CCA9	0,000	1,000	0,000
CCA10	0,231	0,769	0,540
CCA11	0,077	0,923	0,271
CCA12	0,154	0,846	0,429
CCA13	0,000	1,000	0,000

Isolates outside the Park

Locus ID	p	a	I
CGA1	0,000	1,000	0,000
CGA2	0,143	0,857	0,410
CGA3	0,857	0,143	0,410
CGA4	0,857	0,143	0,410
CGA5	0,571	0,429	0,683
CGA6	0,000	1,000	0,000
CGA7	0,000	1,000	0,000
CGA8	0,714	0,286	0,598
CCA1	0,143	0,857	0,410
CCA2	0,286	0,714	0,598
CCA3	0,429	0,571	0,683
CCA4	0,429	0,571	0,683
CCA5	0,286	0,714	0,598
CCA6	0,714	0,286	0,598
CCA7	0,714	0,286	0,598
CCA8	0,429	0,571	0,683
CCA9	0,429	0,571	0,683
CCA10	0,286	0,714	0,598
CCA11	0,143	0,857	0,410
CCA12	0,000	1,000	0,000
CCA13	0,143	0,857	0,410

370 **Table 3.** Pairwise kinship coefficient and spatial distance among isolates.

Pair		Spatial distance (km)	Kinship coefficient
V1	V2	0.280575	0.310696
V6	V7	0.257256	0.407470
V6	RS	0.765759	0.133277
V5	V7	0.152321	0.020374
V5	V6	0.379984	0.020373
V5	V5t	9.49E-05	0.439728
V5	V5b	0.002000	0.181664
V5t	V7	0.152310	-0.140920
V5t	V6	0.379974	-0.140920
V5b	V7	0.152299	0.246180
V5b	V6	0.379965	-0.076400
V5b	V5t	0.000200	0.020373
V4	V7	1.103830	0.375212
V4	V6	0.922926	0.052632
V4	V5t	1.255620	-0.173180
V4	V5b	1.255610	0.213922
V4	V5	1.255630	-0.334470
V4	V4t	0.002000	0.762309
V4	V4b	0.002000	-0.189300
V4t	V7	1.103820	0.471986
V4t	V6	0.922915	-0.173180
V4t	V5t	1.255610	-0.076400
V4t	V5b	1.255600	-0.011890
V4t	V5	1.255620	0.084890
V4b	V7	1.103840	0.165535
V4b	V6	0.922937	-0.157050
V4b	V5t	1.255630	-0.060270
V4b	V5b	1.255620	0.004244
V4b	V5	1.255640	0.101019
V4b	V4t	0.002000	0.230051
V3	V7	1.191480	-0.189300
V3	V6	0.997802	-0.189300
V3	V5t	1.342300	-0.092530
V3	V5b	1.342290	-0.028010
V3	V5	1.342310	0.068761
V3	V4t	0.111517	-0.124790
V3	V4b	0.111501	0.213922
V3	V4	0.111509	-0.221560
V2	V7	1.301930	-0.221560
V2	V6	1.104840	-0.221560
V2	V5t	1.452470	-0.124790
V2	V5b	1.452460	-0.060270
V2	V5	1.452480	0.036503
V2	V4t	0.216229	-0.157050
V2	V4b	0.216210	0.181664
V2	V4	0.216219	-0.253820
V2	V3	0.111480	0.471987
V2	V2t	0.002000	0.310696
V2	V2b	0.002000	0.084890
V2t	V7	1.301940	-0.028010
V2t	V6	1.104860	-0.028010
V2t	V5t	1.452480	0.068761
V2t	V5b	1.452470	-0.189300
V2t	V5	1.452500	0.230051

V2t	V4t	0.216239	0.036503
V2t	V4b	0.216219	0.375212
V2t	V4	0.216229	-0.382850
V2t	V3	0.111490	0.342954
V2b	V7	1.301950	0.068761
V2b	V6	1.104870	-0.253820
V2b	V5t	1.452500	0.165535
V2b	V5b	1.452480	0.230051
V2b	V5	1.452510	0.004244
V2b	V4t	0.216248	0.133277
V2b	V4b	0.216229	0.471987
V2b	V4	0.216239	0.036503
V2b	V3	0.111501	0.117148
V2b	V2t	0.002000	0.278438
V1	V7	1.580560	-0.028010
V1	V6	1.377430	-0.028010
V1	V5t	1.730530	0.068761
V1	V5b	1.730520	-0.189300
V1	V5	1.730540	0.230051
V1	V4t	0.493054	0.036503
V1	V4b	0.493034	0.375212
V1	V4	0.493044	-0.382850
V1	V3	0.392055	0.342954
V1	V2t	0.280564	0.504245
V1	V2b	0.280554	0.278438

Figure legends

Fig. 1. Distribution of the sampled trees in the Vernavola Urban Park. Triangles represent *R. pseudoacacia* trees, while the circle represent a *Q. robur* tree. Each tree is labelled with the code of the corresponding isolate.

Fig. 2. Examples of SITs results. In a and b, somatic compatibility reaction between self-pairing of isolate V5T. In c and d, rarefaction zone deriving from the pairing of V5 and V2b regarded as incompatibility reaction.

Fig. 3. UPGMA trees based on SITs (a) and RAMs genotyping (b). Only boostrap values > 50 are shown.

Fig. 4. NMDS results. Black circles represent isolates of Vernavola Urban Park, while blank triangles represent isolates outside the Park.

Figure 1

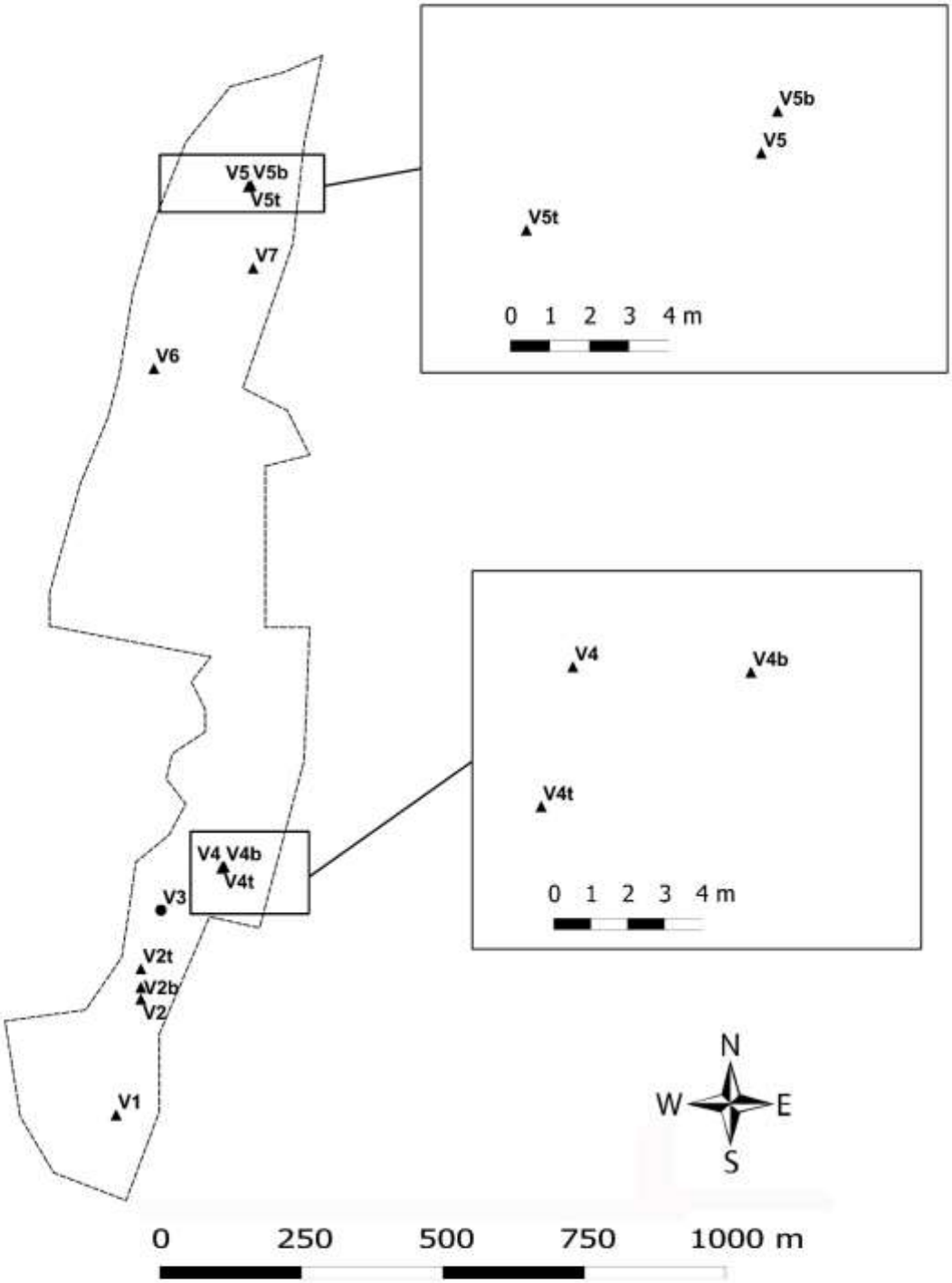

V5 V5b
V5t
V7
V6
V5b
V5
V5t
0 1 2 3 4 m
V4 V4b
V4t
V3
V2t
V2b
V2
V1
V4
V4b
V4t
0 1 2 3 4 m
N
W
E
S
0 250 500 750 1000 m

Figure 2

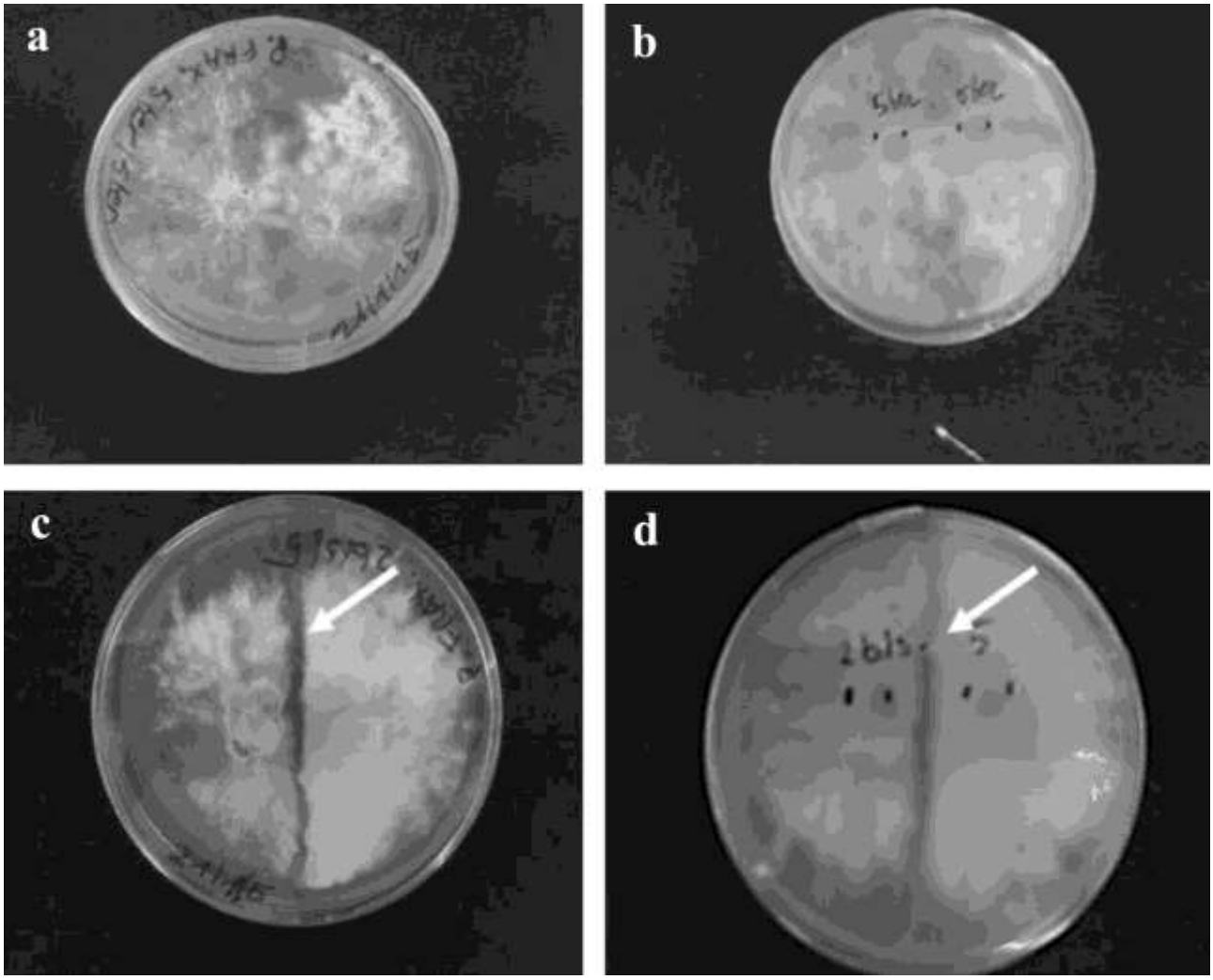


Figure 3

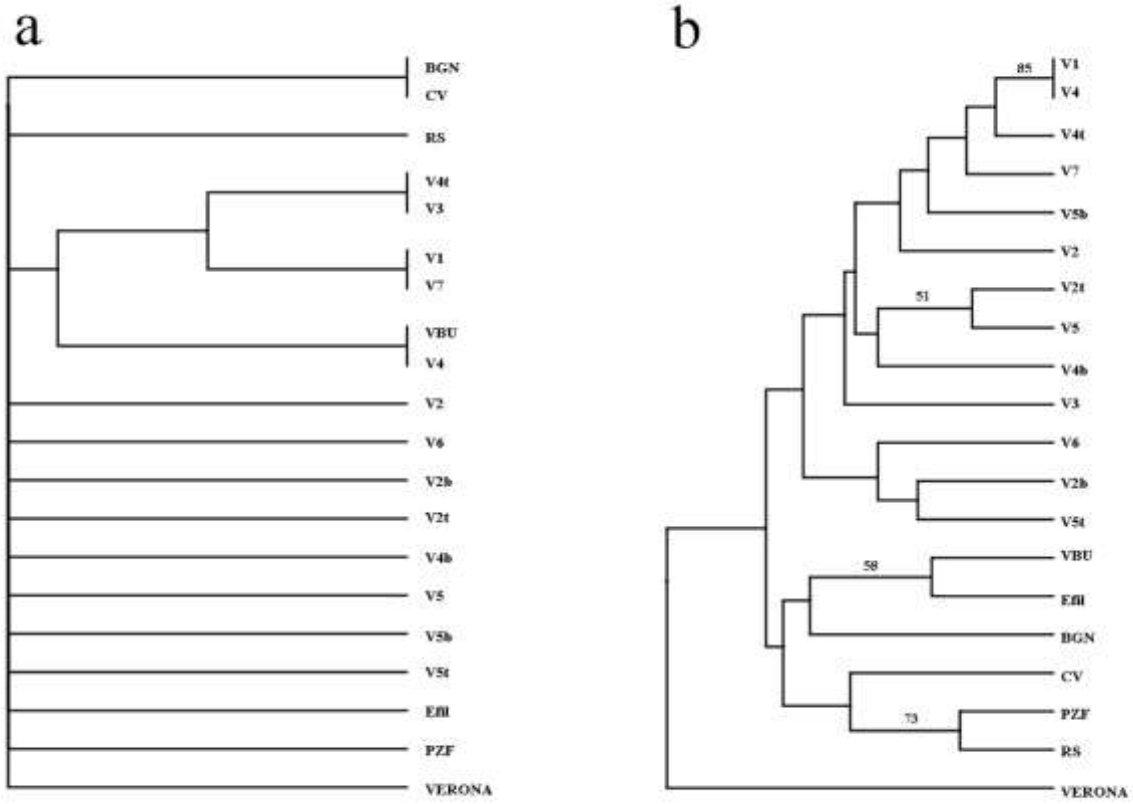
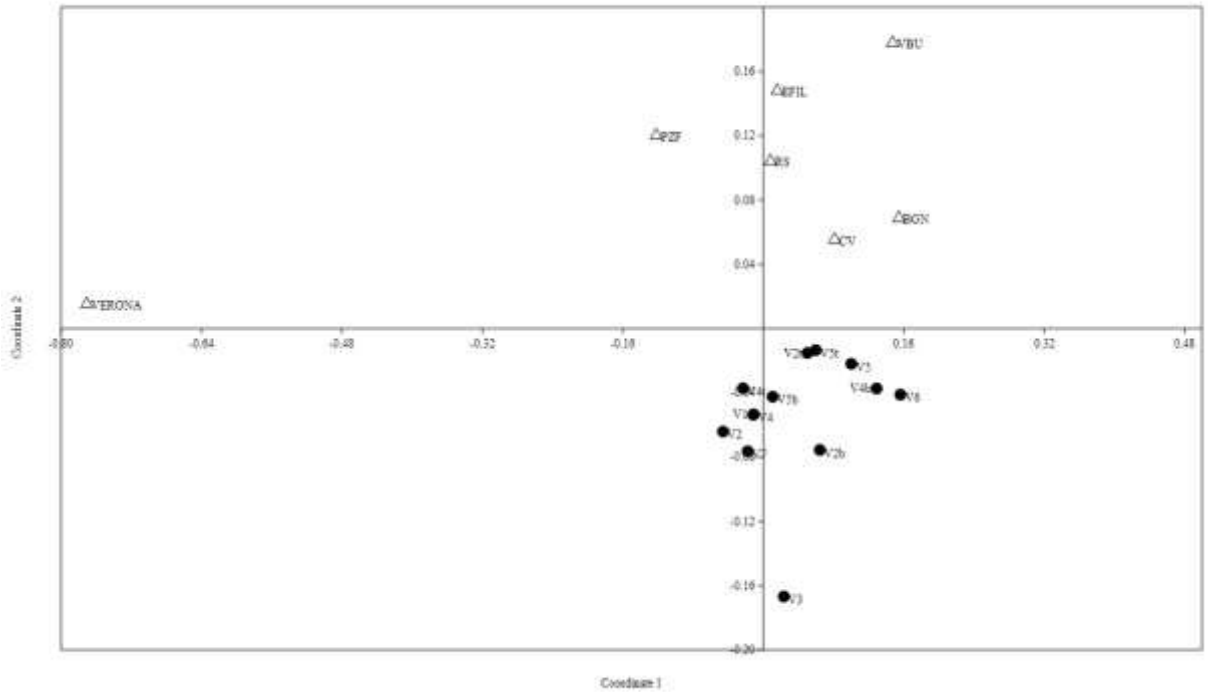

a
BGN
CV
RS
V4t
V3
V1
V7
VBU
V4
V2
V6
V2b
V2t
V4b
V5
V5b
V5t
Effl
PZF
VERONA
b
85
V1
V4
V4t
V7
V5b
V2
51
V2t
V5
V4b
V3
V6
V2b
V5t
58
VBU
Effl
BGN
CV
73
PZF
RS
VERONA

Figure 4


VBU
BFIL
PZF
BS
BGN
CV
VERONA
0.16
0.12
0.08
0.04
-0.12
-0.16
-0.20
-0.64
-0.48
-0.32
-0.16
0.16
0.32
0.48
V5
V6
V2b
V3
Coordinate 1
Coordinate 2