

Genetic diversity and population structure of *Botryosphaeria dothidea* and *Neofusicoccum parvum* on English walnut (*Juglans regia* L.) in France

Marie Belair¹, Adeline Picot¹, Olivier Lepais², Cyrielle Masson³, Marie-Neige Hébrard⁴, Aude Moronvalle⁵, Gwénaëlle Comont⁶, Victor M. Gabri Martin⁷, Sylvie Tréguer¹, Yohana Laloum⁵, Marie-France Corio-Costet⁶, Themis J. Michailides⁷, Juan Moral⁸, Gaétan Le Floch¹, Flora Pensec^{1,*}

¹ Univ Brest, INRAE, Laboratoire Universitaire de Biodiversité et Écologie Microbienne, F-29280 Plouzané, France

² Univ. Bordeaux, INRAE, BIOGECO, 33610 Cestas, France

³ Station d'expérimentation Nucicole Rhône Alpes, 38160 Chatte, France

⁴ Station Expérimentale de Creysse, Perrical 46600 Creysse, France

⁵ Centre Technique Interprofessionnel des Fruits et Légumes, Centre opérationnel de Lanxade, 24130 Prignonrieux, France

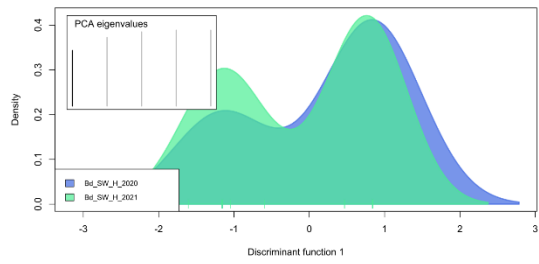
⁶ INRAE, UMR Santé et Agroécologie du Vignoble, ISVV, Labex Cote, CS 20032, 33882 Villenave d'Ornon, France

⁷ University of California Davis, Department of Plant Pathology, Kearney Agricultural Research and Extension Center, Parlier, CA, 93648, USA

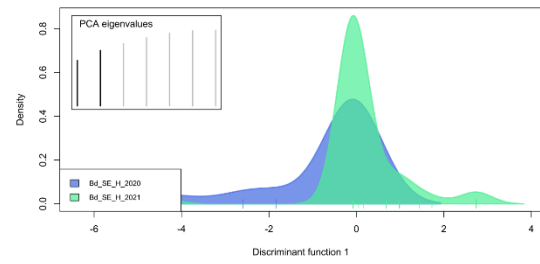
⁸ Department of Agronomy (Maria de Maetzu Excellence Unit), University of Córdoba, Campus de Rabanales, 14071 Córdoba, Spain

* corresponding author : flora.pensec@univ-brest.fr

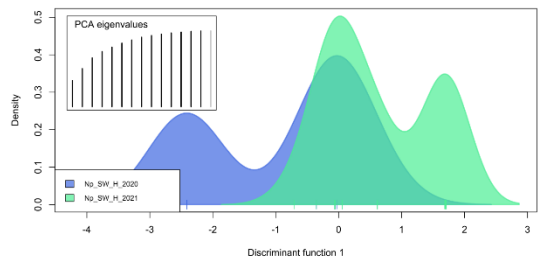
A



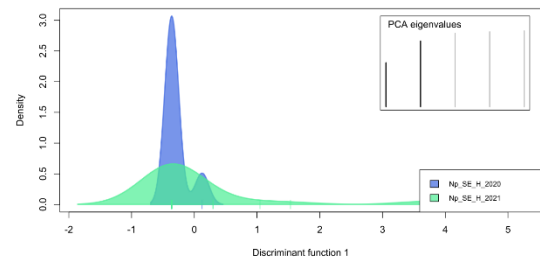
B



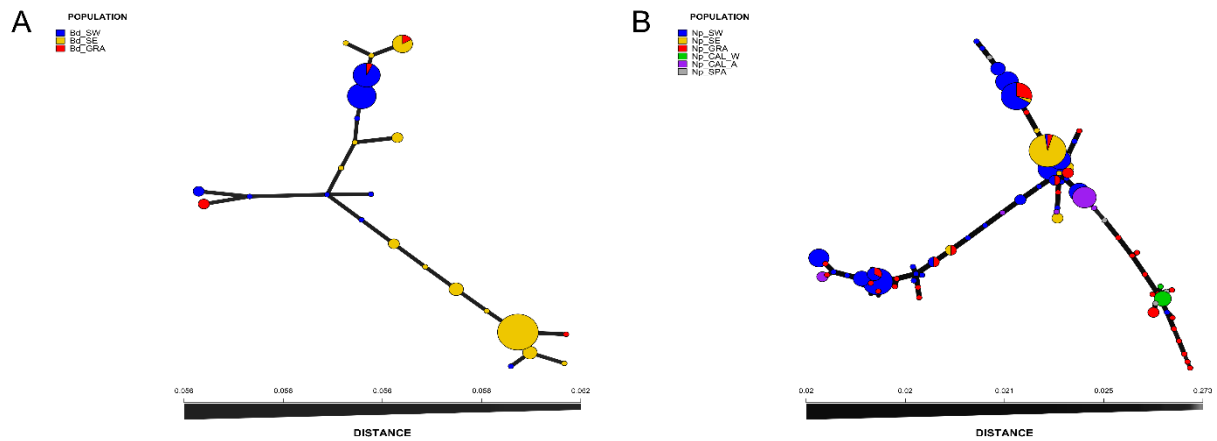
C



D



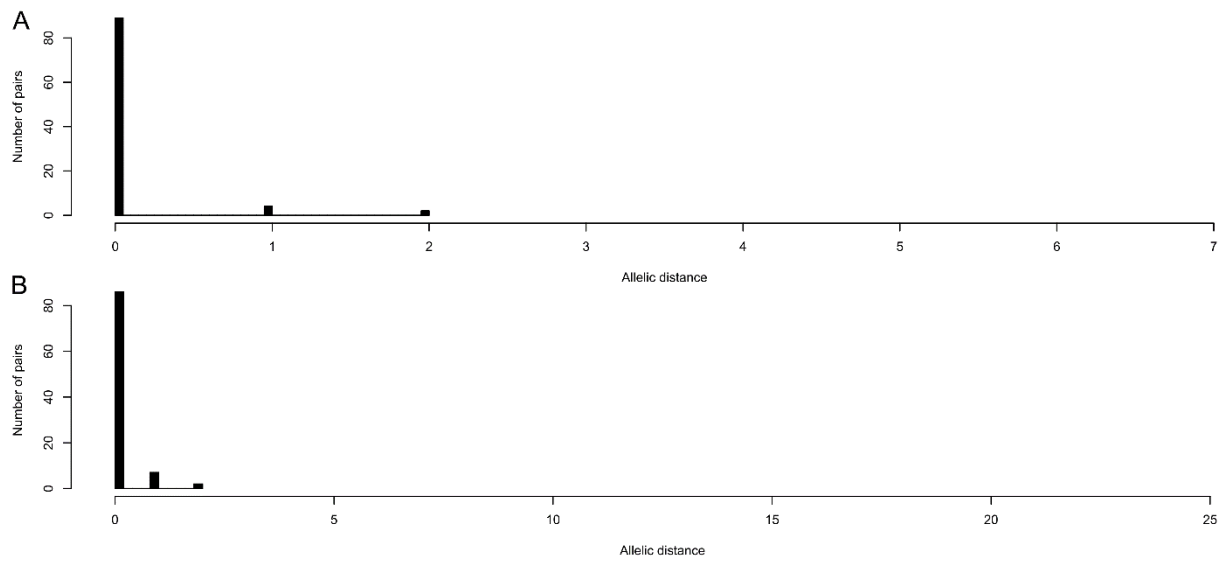
Supplementary Figure S1. Discriminant Analysis of Principal Components (DAPC) of *B. dothidea* (A and B) and *N. parvum* (C and D) populations. Populations were distinguished according to the geographical origin (South-West _SW, A and C; and South-East _SE, B and D) and the year of sampling (2020 and 2021) of the walnut husks (H).



Supplementary Figure S2. Minimum spanning networks of MLGs of *B. dothidea* (A) and *N. parvum* (B) populations. The networks were based on Bruvo's distance and were generated for the southwestern (SW) and the southeastern (SE) French walnut-producing areas, the French vineyard population (GRA), the Californian walnut (CAL_W) and almond (CAL_A) trees and the Spanish walnut trees (SPA) populations. MLGs are represented by nodes (circles) and edges (lines) representing the genetic distance between individuals. The size of the nodes corresponds to the number of individuals sharing the same MLG.



Supplementary Figure S3. Maximum-Likelihood phylogenetic consensus tree based on ITS, EF1- α and TUB loci of fungal species belonging to Botryosphaeriaceae. Maximum-likelihood bootstrap supports (> 60%) and Bayesian posterior probabilities are represented at nodes (left and right, respectively). The tree was rooted with *Neoscytalidium dimidiatum* CBS 499.66. Red isolates: French walnut trees collection; Green isolates: French vineyards collection; Blue isolates: Californian and Spain collection.



Supplementary Figure S4. Allelic distance between pairs of *B. dothidea* (A) and *N. parvum* (B) individuals sequenced twice.

Supplementary Table S1. Analysis of molecular variance (AMOVA) for *B. dothidea* and *N. parvum* strains among and within regions.

Species	Source of variation	Uncorrected dataset				Clone-corrected dataset			
		Degree of freedom	Sum of squares	Variance components	Explained variation (%)	Degree of freedom	Sum of squares	Variance components	Explained variation (%)
<i>B. dothidea</i>	Between regions	1	107.81	1.93	64.66	1	8.58	0.58	20.74
	Between individuals within orchards	131	138.63	1.06	35.34	20	44.51	2.23	79.26
	Total	132	246.44	2.99	100	21	53.09	2.81	100
<i>N. parvum</i>	Between regions	1	62.82	0.89	22.31	1	7.94	0.31	6.61
	Between individuals within orchards	154	476.42	3.09	77.69	38	165.97	4.37	93.39
	Total	155	539.24	3.98	100	39	173.91	4.68	100

Supplementary Table S2. Descriptive statistics and indices of genetic diversity of *B. dothidea* and *N. parvum* populations based on the organ type (Husk _H and twig _T) in the two main French walnut-producing areas (South-West _SW and South-East _SE).

Species	Population	Nb of individuals	AR	uh (s.d.)	P(%)	MLG	eMLG
<i>B. dothidea</i>	Bd_SW_H	22	2.1	0.102 (0.043)	54.55	7	6.05
	Bd_SW_T	17	2.0	0.083 (0.031)	45.45	5	5
	Bd_SE_H	76	1.7	0.087 (0.032)	72.73	12	4.55
	Bd_SE_T	18	1.5	0.030 (0.016)	27.27	4	3.83
<i>N. parvum</i>	Np_SW_H	46	2.3	0.179 (0.037)	85.19	17	7.44
	Np_SW_T	61	3.2	0.124 (0.033)	51.85	24	9.15
	Np_SE_H	37	1.6	0.041 (0.014)	37.04	7	3.17
	Np_SE_T	12	1.4	0.037 (0.014)	22.22	2	2.00

s.d.: standard deviation; AR: allelic richness; uh: unbiased haploid genetic diversity; P(%): percentage of polymorphic loci; MLG: number of observed multilocus genotypes; eMLG: number of multilocus genotypes expected at the smallest sample size.

Supplementary Table S3. Analysis of molecular variance (AMOVA) for *B. dothidea* and *N. parvum* strains among and within organs.

Species	Source of variation	Uncorrected data set				Clone-corrected data set			
		Degree of freedom	Sum of squares	Variance components	Explained variation (%)	Degree of freedom	Sum of squares	Variance components	Explained variation (%)
<i>B. dothidea</i>	Between organs	1	4.55	0.05	2.76	1	1.03	-0.11	-4.81
	Between individuals within organs	131	241.89	1.85	97.24	26	61.00	2.35	104.81
	Total	132	246.44	1.90	100	27	62.03	2.24	100
<i>N. parvum</i>	Between organs	1	8.30	0.06	1.78	1	2.27	-0.10	-2.21
	Between individuals within organs	154	530.95	3.45	98.22	46	215.96	4.70	102.21
	Total	155	539.25	3.51	100	47	218.23	4.60	100

Supplementary Table S4. Geographical and plant origin of isolates of *B. dothidea* and *N. parvum*.

Host	Country	Number of isolates	
		<i>B. dothidea</i>	<i>N. parvum</i>
Grapevines	France (Grand-Est)	1	10
	France (Nouvelle-Aquitaine)	3	26
	France (Occitanie)	1	7
	Total	5	43
Walnut trees	USA (California)	-	5
	Spain	-	5
	Total	-	10
Almond trees	USA (California)	-	14
	Total	-	14