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Evaluation of Chestnut Susceptibility to *Cryphonectria parasitica*: Screening under Controlled Conditions

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Abstract: *Cryphonectria parasitica* (Murrill) M.E. Barr (Sordariomycetes, Valsaceae) is the causal agent of chestnut blight. This disease is a major concern for chestnut cultivation in Europe. The fungus colonizes vascular tissues and evolves generating cankers causing severe dieback and the death of the tree. Excised and debarked well-lignified shoots of 28 *C. sativa* genotypes (assay A) and of 10 progenies (assay B) were inoculated with *C. parasitica* strain FMT3bc2 (vcg: EU2). Fungal growth was measured along the longitudinal axis on the 3rd and 6th days after inoculation. Results indicated the inoculation methodology works and the results were clear after 6 days. Differences in susceptibility to chestnut blight among *C. sativa* trees of Montseny have been detected both at the individual genotype level and at the progeny level. Nineteen genotypes and four progenies showed a susceptibility to Blight not significantly different from *C. mollissima*. The methodology was easy to apply in extensive/preliminary selection screenings to assess the susceptibility of *C. sativa* materials to the Blight.

Keywords: *Castanea sativa*; *Castanea* sp.; chestnut blight; debarked budsticks; Montseny-Spain

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1. Introduction

The Ascomycete fungus *Cryphonectria parasitica* (Murrill) M.E. Barr (Sordariomycetes, Valsaceae) is the causal agent of chestnut blight, one of the most devastating diseases affecting *Castanea* sp. worldwide. The disease in Europe was first detected in Italy in 1938 (EPPO current status: Present) [1,2] from where it spread to the neighboring countries of Switzerland (1948; widespread), Slovenia (1950; restricted distribution), and France (1956; restricted distribution) [3,4]. The disease is currently present in Spain (1947; restricted distribution), Croatia (1955; widespread), Albania (1967; widespread), Serbia (1975; widespread), Portugal (1989; widespread) and Germany (1992; restricted distribution), Azerbaijan (2004; present), United Kingdom (2011; restricted distribution), and Belgium (2014; restricted distribution) [1,5]. Most of the European *C. parasitica* populations seem to have originated from single introductions since they have reduced genetic diversity [6].

Spore dispersal by air is a major dissemination mechanism of fungal pathogens [7,8]. In the case of *C. parasitica*, air-borne spores usually penetrate above-ground parts of the host through bark wounds (e.g., pruning, wind, hail, etc.) in shoots or stems. The fungus colonizes vascular tissues causing progressive wilting, and severe reduction in fruit production. Later symptoms include orangish necrotic areas in the bark [2,9]. The necrosis evolves generating cankers that usually girdle branches or even the trunk causing severe dieback, and finally the death of the tree [10,11]. Cankers harbor fruiting bodies of the fungus that disseminate their spores throughout the chestnut stands. The fungus infects *Castanea sativa*

Mill. (Europe), *Castanea dentata* (Marshall) Borkh. (America), as well as *Castanea crenata* Siebold and Zucc. and *Castanea mollissima* Blume (Asia) at any age. Among these host species, *C. sativa* and *C. dentata* are considered the most susceptible since *C. mollissima* and *C. crenata* exhibit some tolerance to the disease probably caused by ancient co-evolution with the pathogen in their native range [12,13].

This disease is a major concern for chestnut cultivation in Europe as there is currently no effective cultural or chemical control and no resistance has been found in *C. sativa* species [14]. To find a way out of chestnut blight, two main strategies are being considered: biocontrol through virus-induced fungus hypovirulence, and prevention by breeding disease-resistant genotypes. The use of RNA mycoviruses (i.e., Cryphonectria hypovirus 1; *Hypoviridae*) has resulted in highly effective in Europe [1], thus becoming one of the most successful alternatives for controlling the disease in natural stands. This biocontrol method is based on inoculation of virus-infected fungal strains belonging to the same vegetative compatibility group/s (vcg) than those causing disease in the target stand [15,16]. This infected—and therefore weakened—strain, inoculated in diseased trees, naturally transfers viruses by mycelial anastomosis to virulent strains which will become in turn hypovirulent. Mycovirus-infected strains of *C. parasitica* are not able to severely damage the tree allowing it to withstand the infection with minor symptoms [17,18]. On the other hand, breeding resistant genotypes of chestnut for planting is probably the most promising preventive measure to be implemented for woody croplands and afforestation. The transfer of resistance from Asian chestnuts to American or European species has been carried out in several programs but reaching the desired fruit quality requires several backcrosses that lengthen the selection process considerably [19,20]. Finding resistance or lower susceptibility traits in natural populations of *C. dentata*, the American chestnut, has proven possible [21] although conducting this work by inoculation of standing trees requires a great deal of work that is often not feasible. The evaluation of tolerance/resistance to biotic or abiotic injuries under controlled conditions on parts of plants/trees has proven to be an advantageous practice when many individuals/samples are to be tested. Thus, in different hardwoods similar approaches have been used, such as on *Juglans* sp. to study frost resistance [22] or more specifically on chestnut to establish levels of susceptibility to *Phytophthora* spp. [23]. Moreover, Rodríguez and Colinas [24] showed that estimating chestnut susceptibility to *C. parasitica* by means of inoculation on excised shoots was feasible and the mycelium grew as expected under controlled conditions.

In consequence, the aims of this study were: (i) to evaluate the tolerance to chestnut blight of some *C. sativa* genotypes from a Spanish local population (Montseny-Northeast of Catalonia) in excised shoots, and (ii) to study this tolerance in seedling genotypes of the same origin installed in a progeny test.

2. Materials and Methods

2.1. In Vitro Inoculation: Budstick Assay

2.1.1. Assay A

Tolerance to chestnut blight among *C. sativa* genotypes was evaluated in vitro by inoculating a virulent strain of *C. parasitica* in debarked well lignified shoots. One-year-old budsticks of 28 *C. sativa* genotypes (Table 1) were removed from healthy mother plants installed in a quarantine greenhouse of Gimenells (Lleida, 41°44′45.6″ N; 0°23′39.1″ E) at IRTA experimental station in July 2020. The harvest date was adjusted to the optimal physiological state of chestnut for the susceptibility test [24]. Budsticks (12–15 cm long) were kept at 4 °C covering the apical ends with Parafilm© while the opposite side was submerged in distilled water until processed. Moreover, lignified shoots from two genotypes of *C. mollissima* were collected at Lourizan Forest Center of Galicia (42°24′35″ N—8°00′12″ W) and sent overnight to be used as a tolerant control (Table 1).

Table 1. Description of genotypes used in the in vitro assays (A and B). G: mean daily growth of the *Cryphonectria parasitica* colony from inoculation to the sixth day of incubation along the longitudinal axis. Data for genotypes of *Castanea mollissima* in assay A and B are shown in the left and right sides of the bar (/) respectively. n: number of replicates per genotype. Mean values and standard deviation are shown.

Species	Assay	Genotype/Progeny	N Genotypes	n	Cutting Diameter (cm)	G (mm/Day)
Castanea mollissima	A/B	M2	1	8/4	0.84 ± 0.06/0.79 ± 0.14	3.9 ± 1.2/5.8 ± 2.5
		M5M	1	8/4	1.14 ± 0.05/0.60 ± 0.06	4.6 ± 1.4/4.6 ± 2.7
Castanea sativa	A	BRL02	1	8	1.41 ± 0.08	5.6 ± 1.2
	A	BRL03	1	8	0.90 ± 0.03	4.4 ± 1.7
	A	BRL06	1	8	0.98 ± 0.13	4.9 ± 1.4
	A	CNV01	1	8	0.91 ± 0.07	5.8 ± 1.2
	A	FGM01	1	8	1.21 ± 0.08	5.4 ± 1.5
	A	FGM04	1	8	1.43 ± 0.15	5.4 ± 1.8
	A	FGM11	1	8	0.93 ± 0.10	6 ± 1.2
	A	MSY03	1	8	0.95 ± 0.06	5.8 ± 1.7
	A	MSY04	1	8	0.89 ± 0.09	5.4 ± 0.9
	A	MSY08	1	8	0.91 ± 0.04	5.6 ± 2.2
	A	MSY09	1	8	0.93 ± 0.08	5.3 ± 1.8
	A	MSY10	1	8	1.06 ± 0.08	3.9 ± 1.6
	A	MSY11	1	8	1.08 ± 0.04	5.5 ± 0.7
	A	MSY12	1	8	1.16 ± 0.38	5.9 ± 0.8
	A	MSY14	1	8	1.24 ± 0.12	4.3 ± 2.0
	A	PO11	1	8	1.13 ± 0.13	4.5 ± 1.3
	A	RLL05	1	8	1.03 ± 0.12	4.5 ± 1.1
	A	RLL10	1	8	0.96 ± 0.20	6.4 ± 1.1
	A	SPV05	1	8	1.35 ± 0.06	5.9 ± 1.8
	A	VLD12	1	8	1.01 ± 0.08	5.7 ± 1.1
	A	VLD14	1	8	1.23 ± 0.10	5.4 ± 1.3
	A	VLD22	1	8	1.14 ± 0.05	5 ± 0.9
	A	VLD29	1	8	1.11 ± 0.04	5.4 ± 1.0
	A	VLD30	1	8	1.19 ± 0.10	4.4 ± 1.7
	A	VLD31	1	8	1.00 ± 0.11	6 ± 1.5
	A	VLD32	1	8	1.02 ± 0.04	5.3 ± 1.6
	A	VLD33	1	8	1.11 ± 0.06	6.1 ± 1.3
	A	VLD34	1	8	1.21 ± 0.11	5.5 ± 1.9
	B	CS-49	23	4	0.71 ± 0.13	6.7 ± 1.7
	B	CS-51	14	4	0.69 ± 0.11	6.9 ± 1.8
	B	CS-52	12	4	0.69 ± 0.11	8 ± 1.4
	B	CS-54	17	4	0.71 ± 0.09	6.9 ± 1.5
	B	CS-55	24	4	0.69 ± 0.11	7.7 ± 2.1
	B	CS-56	14	4	0.69 ± 0.12	8.1 ± 2
	B	CS-57	5	4	0.72 ± 0.14	8.8 ± 1.3
	B	CS-60	20	4	0.68 ± 0.11	7.1 ± 2.1
	B	CS-61	18	4	0.66 ± 0.12	7.2 ± 1.8
	B	CS-62	18	4	0.69 ± 0.09	6.9 ± 2

Inoculation was performed according to the methodology of Rodríguez and Colinas [24] with minor modifications. Each budstick was cut in 7 cm sections (8 replicates per genotype; 224 samples of *C. sativa* and 16 samples of *C. mollissima*) (Figure 1) in the laboratory with disinfected pruning scissors. The diameter of each cutting sample (d) was measured with a digital caliper and the section manually debarked using a sterile scalpel. Fungal cultures of *C. parasitica* strain FMT3bc2 (vcg: EU2) grown at room temperature for 5 days in PDA medium (potato-dextrose-agar 3.90% *w*/*v*; Biokar) were used as an inoculum source. More specifically, a 5 × 5 mm plug of a solid medium plug with fresh mycelium from the edge of the colony was placed in the center of each debarked shoot section with the mycelium side in touch with wood tissue (Figure 1).

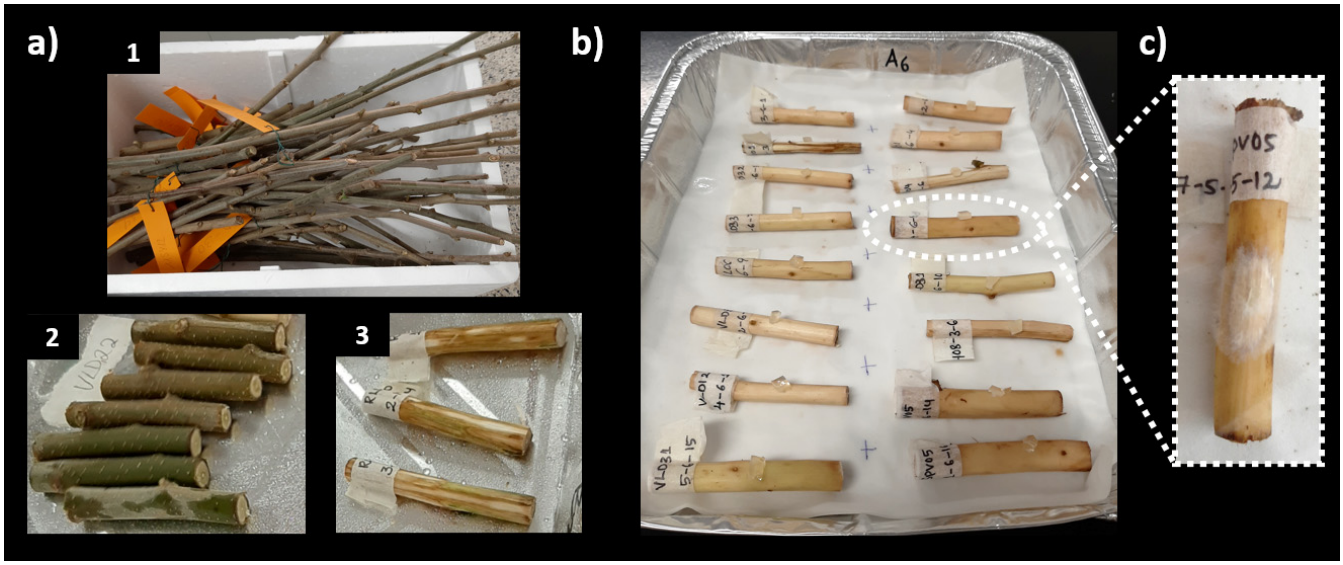

Figure 1. Outline of inoculation methodology. (**a**) inoculation protocol (1: field budsticks; 2: budstick sections; 3: debarked budsticks); (**b**) inoculated budsticks with *C. parasitica* and tray incubation; (**c**) detail of fungus growing in the debarked budstick.

Inoculated samples were labeled with white tape and randomly placed in disinfected plastic trays lined with filter paper (Figure 1). Each tray was then moistened by spraying deionized water and covered with a plastic film to maintain high relative air humidity inside the tray. The trays were protected with a sheet of paper and incubated at room temperature (24 ± 2 °C) for one week. Whenever the filter paper became dry, deionized water was sprayed in the trays. Fungal growth was measured along the longitudinal axis (mm) on the 3rd and 6th days after inoculation.

2.1.2. Assay B

In parallel to assay A, a total of 166 *C. sativa* genotypes belonging to 10 progenies (Table 1) were used for an additional in vitro study. Tree shoots (4 replicates per seedling) were collected in a progenies test orchard located in the Parc Natural i Reserva de la Biosfera del Montseny (41°45′46,6″ N; 2°25′39,1″ E) and were processed and incubated as previously described for bioassay A. The same two *C. mollissima* genotypes, four shoots of each one, were also used as control samples. The fungal growth rate was calculated as previously explained.

2.2. Statistical Analysis

Data obtained after six days of incubation were analyzed for both in vitro assays using the programming environment R [25]. We used the average value of the mean daily fungal growth rate for a six-day incubation period (G, mm/day) of both *C. mollisima* genotypes (i.e., "M2" and "M5M") as reference for model computation (control). This was tested by fitting two preliminary generalized linear models (GLMs) (one per dataset) in order to evaluate whether G significantly varied between both *C. mollissima* genotypes.

In assay A, a GLM was fitted as a null model including the mean daily fungal growth rate (G) as a response variable and the genotypes as an explanatory factor. Then, a linear mixed-effects model (LMM) was computed using the "lme4" package in R [26] including G as the dependent variable, genotype as a fixed factor and the diameter of the cutting (d) as a random factor (Table 2). Both models were compared using Akaike's Information Criteria (AIC) [27] using the "AICcmodavg" package in R [28]. The most parsimonious model was selected using AIC and the result of the model comparison using the χ^2 test.

Table 2. Results of models (GLMs and LMMs) describing the variation of mean daily lesion growth (G; mm/day) with progeny, seedling-genotype, and diameter (d; mm) in the in vitro assay. Random factors of LMMs are shown in brackets. n: number of observations used for model fitting. Selected model in bold.

Assay	Model	n	Description	Df	logLik	Deviance	AIC	ΔAIC
A	Ma0	232	G~genotype	30	−403.73	807.46	867.46	0
	Ma1		**G~genotype + (d)**	**31**	**−403.73**	**807.46**	**869.46**	**2**
B	Mbp0	664	G~progeny	12	−1355	2710	2734	0
	Mbp1		**G~progeny + (d)**	**13**	**−1351.80**	**2703.70**	**2729.70**	**−4.30**
	Mbg0		G~seedling-genotype	167	−1161.40	2322.70	2656.70	0
	Mbg1		**G~seedling-genotype + (d)**	**168**	**−1161.40**	**2322.70**	**2658.70**	**2**

In assay B two different model sets were fitted (Table 2). A null GLM was computed with G as a response variable and progeny as an explanatory factor. Then, an LMM with the same variables as well as diameter as a random factor was computed (Table 2). Analogous models (i.e., null and LMM) were fitted for the same dataset including the seedling genotypes instead of progenies as explanatory variables. Definitive models were selected following the same statistical indicators as described for assay A.

3. Results

The generalized linear model fitted for assay A showed significant variation in G among the evaluated materials (p-value = 0.03) (model Ma0). The LMM fitted to dataset A including diameter as a random factor (i.e., Ma1) retained the significance of genotype (p-value = 0.03). The most parsimonious model (Ma0) did not show conclusive differences with Ma1 according to AIC values ($\Delta AIC_{Ma1\text{-}Ma0} = 2$; Table 2), so we compared them with a χ^2 test (p-value = 1). Consequently, Ma1 was selected as the most explicative model for assay A (Table 2). The parameters of the model showed that in nineteen of the assayed genotypes (i.e., "BRL02", "BRL03", "BRL06", "FGM01", "FGM04", "MSY04", "MSY08", "MSY09", "MSY10", "MSY11", "MSY14", "PO11", "RLL05", "VLD14", "VLD22", "VDL29", "VLD30", "VLD32" and "VLD34") the daily lesion growth rate (G) was not significantly different from the control *C. mollissima* (Figure 2).

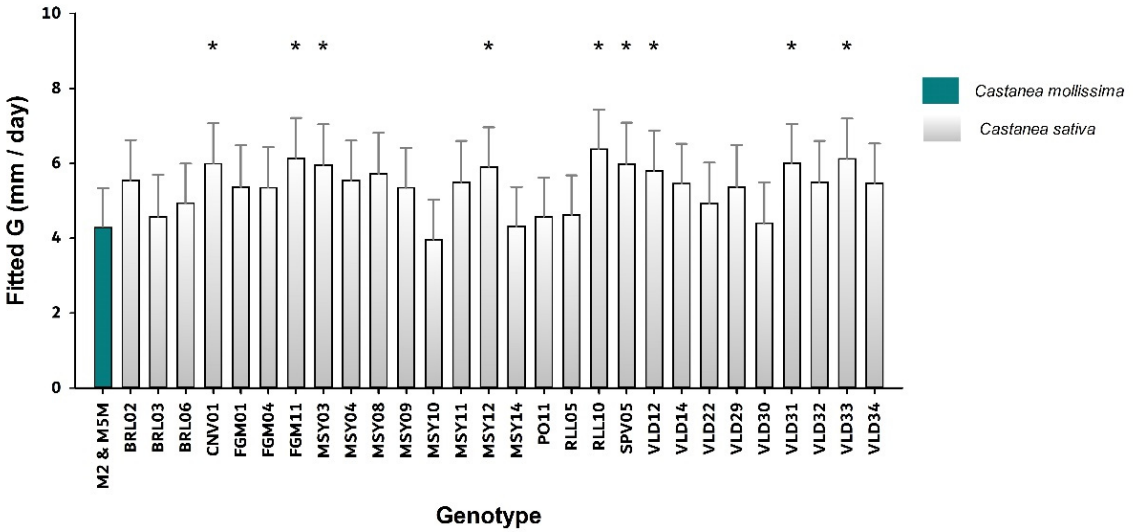

Figure 2. Values of mean daily lesion growth rate (G) of *Cryphonectria parasitica* on cuttings of *Castanea sativa* genotypes fitted by model Ma1 (Assay A). Mean values and 95% confidence error are shown. Asterisk (*) denotes significant differences in respect to *Castanea mollissima* (average value of "M2" and "M5M"). Number of repetitions: 8 ("M2" and "M5M"); 8 (all genotypes of *C. sativa*).

In the dataset of assay B, two null models (GLMs) were computed to evaluate variations of G in respect of progenies (model Mbp0) and genotypes (model Mbg0). Both

models showed a significant effect of the corresponding explicative factors (p-value < 0.01 in all cases). The LMM fitted to analyze the variation of G among genotypes including diameter as a random factor (model Mbg1) retained the significance already shown by the corresponding null model Mbg0. In addition, change in model AIC was not noticeable ($\Delta AIC_{Mbg1\text{-}Mbg0} = 2$) and both models did not differ significantly (p-value = 1). In contrast, the fitted LMM Mbp1 that included diameter as a random factor resulted more parsimonious than the corresponding null model ($\Delta AIC_{Mbp1\text{-}Mbp0} = -4.3$) and was significantly different from Mbp0 (p-value = 0.01). In consequence, either Mbp1 or Mbg1 were selected as the most explicative models for this in vitro assay (Table 2). Both models supported the lower susceptibility of *C. mollissima* against *C. parasitica* (Figures 3 and S1).

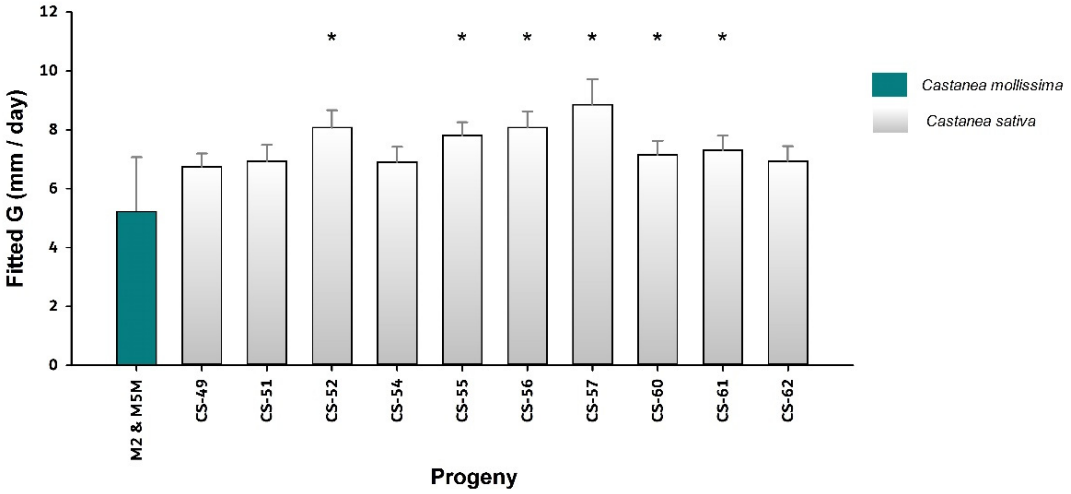

Figure 3. Values of mean daily lesion growth rate (G) of *Cryphonectria parasitica* on cuttings of *Castanea sativa* progenies fitted by model Mbp1 (Assay B). Mean values and 95% confidence error are shown. Asterisk (*) denotes significant differences in respect to *Castanea mollissima* (average value of "M2" and "M5M"). Number of repetitions: 4 ("M2" and "M5M"); see Table 1 for progenies of *C. sativa*.

According to this model, the tolerance to *C. parasitica* of progenies "CS-49", "CS-51", "CS-54", and "CS-62" is not significantly different from that of tolerant *C. mollissima* making them good candidates for further studies regarding their suitability to be planted in orchards. The use of average G values for *C. mollissima* genotypes as a reference in model fitting was supported by the lack of significant differences of G between "M2" and "M5M" in any of the datasets (p-value > 0.20 in all cases).

4. Discussion

In this assay, *C. parasitica* colonized the wood in the first six days after inoculation in contrast with other methodologies where longer periods were required [29]. The methodology used, debarking of lignified shoots, was expected to improve colonization by the fungus compared to inoculation on live seedlings [30] or on stems with bark [13]. In addition, working under controlled conditions allows to not have to worry about the potential infection and death of the plant, or part of the plant, or introducing new vcgs potentially foreign to an area. Future research involving inoculation with fungi from different populations—even in simultaneous infection—will be informative to characterize the most resistant genotypes suitable for establishing new plantations. However, an aspect to be considered is the virulence of the fungal strain used which is expected to vary among isolates [31]. In this study, we only tested one fungal strain since our main objective was to characterize host susceptibility against a strain of the EU2 vcg, the most common vcg type in the Montseny area [32].

Regarding features of plant material, Pažitný et al. [12] reported a significant effect of stem diameter on chestnut blight lesion development. This observation completely agrees

with our fitted models (Table 2) where the inclusion of stem section diameter provided a better explanation of recorded data.

Nearly all the *C. sativa* materials included in this study come from a reduced population of chestnut trees of the Montseny Natural Park, therefore, extending this study to a wider genetic base would be the first step to take in future evaluations. "PO11" is the only material not originating from this population, it is a spontaneous chestnut hybrid selected as a rootstock of *C. sativa* [33] for its resistance to *Phytophthora* spp. In this evaluation "PO11" has been included in the group whose tolerance is comparable to the one of *C. mollissima*, which was expected since this genotype carries some Asian genes [34].

It should be noted that 70% of the *C. sativa* genotypes and the 40% of progenies from Montseny inoculated with *C. parasitica* showed a response analogous to the one of *C. mollissima* in their canker development. These results are relevant because the chestnut orchards in Montseny are not described as having been introgressed with Asian materials as in other Spanish areas [35]. Their ancestry and traditional management in the last 100 years do not indicate the entry of foreign fruit varieties. Nor are there any genotypes with proper names that could suggest that they are ancient varieties of the Montseny area. Chestnut trees sampled from this area, according to Mattioni et al. [36], clustered (Nei distance) together with trees from southern Italy and Sicily but not with those of bordering areas. Southern Italy chestnuts have genetics from the Eastern Mediterranean [36]. Perhaps by going deeper into the origin of the Montseny materials, it would be possible to identify other materials to include in further *C. parasitica* susceptibility screenings.

5. Conclusions

Differences in susceptibility to chestnut blight among *C. sativa* trees have been detected both at the individual genotype level and at the progeny level. The debarked budstick-based inoculation method used here revealed nineteen genotypes and four progenies of *C. sativa* with a susceptibility to *C. parasitica* not different from that of *C. mollissima*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11111158/s1, Figure S1: Values of mean daily lesion growth rate (G) by *Castanea sativa* genotype fitted by model Mbg1 (Assay B). Mean values and 95% confidence error are shown. Asterisk (*) denotes significant differences in respect to *Castanea mollissima* (average value of "M2" and "M5M"). Number of repetitions: 4 ("M2" and "M5M"); 4 (all genotypes of *C. sativa*).

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Robin, C.; Heiniger, U. Chestnut blight in Europe: Diversity of *Cryphonectria parasitica*, hypovirulence and biocontrol. *For. Snow Landsc. Res.* **2001**, *76*, 361–367.
2. Anagnostakis, S.L. Chestnut Blight: The Classical Problem of an Introduced Pathogen. *Mycologia* **1987**, *79*, 23–37. [CrossRef]
3. Heiniger, U.; Rigling, D. Biological control of chestnut blight in Europe. *Annu. Rev. Phytopathol.* **1994**, *32*, 581–599. [CrossRef]
4. Krstin, L.; Novak-Agbaba, S.; Rigling, D.; Krajačić, M.; Ćurković Perica, M. Chestnut blight fungus in Croatia: Diversity of vegetative compatibility types, mating types and genetic variability of associated *Cryphonectria hypovirus 1*. *Plant Pathol.* **2008**, *57*, 1086–1096. [CrossRef]
5. EPPO. *Cryphonectria parasitica*. Available online: https://gd.eppo.int/taxon/ENDOPA (accessed on 10 October 2021).
6. Milgroom, M.G.; Sotirovski, K.; Spica, D.; Davis, J.E.; Brewer, M.T.; Milev, M.; Cortesi, P. Clonal population structure of the chestnut blight fungus in expanding ranges in southeastern Europe. *Mol. Ecol.* **2008**, *17*, 4446–4458. [CrossRef]
7. Rigling, D.; Prospero, S. *Cryphonectria parasitica*, the causal agent of chestnut blight: Invasion history, population biology and disease control. *Mol. Plant Pathol.* **2018**, *19*, 7–20. [CrossRef]
8. Aguayo, J.; Fourrier-Jeandel, C.; Husson, C.; Loos, R. Assessment of Passive Traps Combined with High-Throughput. *Appl. Environ. Microbiol.* **2018**, *84*, e02637-17. [CrossRef]
9. West, J.S.; Kimber, R.B.E. Innovations in air sampling to detect plant pathogens. *Ann. Appl. Biol.* **2015**, *166*, 4–17. [CrossRef] [PubMed]
10. Lione, G.; Giordano, L.; Turina, M.; Gonthier, P. Hail-induced infections of the chestnut blight pathogen *Cryphonectria parasitica* depend on wound size and may lead to severe diebacks. *Phytopathology* **2020**, *110*, 1280–1293. [CrossRef]
11. Bryner, S.F.; Prospero, S.; Rigling, D. Dynamics of *Cryphonectria hypovirus* infection in chestnut blight cankers. *Phytopathology* **2014**, *104*, 918–925. [CrossRef] [PubMed]
12. Pažitný, J.; Bolvanský, M.; Adamčíková, K. Screening for resistance of progenies derived from *Castanea sativa* × *C. crenata* and *C. crenata* to *Cryphonectria parasitica*. *For. Pathol.* **2018**, *48*, e12439. [CrossRef]
13. Cipollini, M.L.; Moss, J.P.; Walker, W.; Bailey, N.; Foster, C.; Reece, H.; Jennings, C. Evaluation of an Alternative Small Stem Assay for Blight Resistance in American, Chinese, and Hybrid Chestnuts (*Castanea* spp.). *Plant Dis.* **2021**, *105*, 576–584. [CrossRef] [PubMed]
14. Aguín, O.; Sainz, M.; Montenegro, D.; Mansilla, J.P. Biodiversidad e hipovirulencia de *Cryphonectria parasitica* en Europa: Implicaciones para el control biológico del cancro del castaño. *Recur. Rurais* **2011**, *7*, 35–47. [CrossRef]
15. Aghayeva, D.N.; Rigling, D.; Prospero, S. Low genetic diversity but frequent sexual reproduction of the chestnut blight fungus *Cryphonectria parasitica* in Azerbaijan. *For. Pathol.* **2017**, *47*, 1–7. [CrossRef]
16. Cortesi, P.; Milgroom, M.G. Genetics of vegetative incompatibility in *Cryphonectria parasitica*. *Appl. Environ. Microbiol.* **1998**, *64*, 2988–2994. [CrossRef]
17. Muñoz-Adalia, E.J.; Fernández, M.M.; Diez, J.J. The use of mycoviruses in the control of forest diseases. *Biocontrol Sci. Technol.* **2016**, *26*, 577–604. [CrossRef]
18. Xie, J.; Jiang, D. New insights into mycoviruses and exploration for the biological control of crop fungal diseases. *Annu. Rev. Phytopathol.* **2014**, *52*, 45–68. [CrossRef] [PubMed]
19. Agnagnostakis, S.L. Chestnut Breeding in the United States for Disease and Insect Resistance. *Plant Dis.* **2012**, *96*, 1392–1403. [CrossRef]
20. Clark, S.L.; Schlarbaum, S.E.; Saxton, A.M.; Baird, R. Eight-year blight (*Cryphonectria parasitica*) resistance of backcross-generation American chestnuts (*Castanea dentata*) planted in the southeastern United States. *For. Ecol. Manag.* **2019**, *433*, 153–161. [CrossRef]
21. Griffin, G.; Hebard, F.; Wendt, R.; Elkins, J. Survival of American Chestnut Trees: Evaluation of Blight Resistance and virulence of *Endothia parasitica*. *Phytopathology* **1983**, *73*, 1084–1092. [CrossRef]
22. Guàrdia, M.; Charrier, G.; Vilanova, A.; Savé, R.; Ameglio, T.; Aletà, N. Genetics of frost hardiness in *Juglans regia* L. and relationship with growth and phenology. *Tree Genet. Genomes* **2016**, *12*, 83. [CrossRef]
23. Fernández-López, F.; Díaz-Vázquez, R.; Vázquez-Ruiz, R.; Pereira-Lorenzo, S. Evaluation of resistance of *Castanea* sp. clones to *Phytophthora* sp. using excised chestnut shoots. *For. Snow Landsc. Res.* **2001**, *76*, 451–454.
24. Rodríguez, J.; Colinas, C. Resistance test for chestnut against *Cryphonectria* (*Endothia*) *parasitica*. In Proceedings of the 2nd International Symposium on Chestnut, Bordeaux, France, 19–23 October 1998; p. 494.
25. R Rcore Team. *R Core Team R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021.
26. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [CrossRef]
27. Anderson, D. *Model Based Inference in the Life Sciences: A Primer on Evidence*; Springer: New York, NY, USA, 2008.
28. Mazerolle, M.J. *AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c)*, R Package Version 2.2-1; 2019. Available online: https://rdrr.io/cran/AICcmodavg/ (accessed on 11 October 2021).
29. Lee, J.K.; Tattar, T.A.; Berman, P.M.; Mount, M.S. A Rapid Method for Testing the Virulence of *Cryphonectria parasitica* Using Excised Bark and Wood of American Chestnut. *Phytopathology* **1992**, *82*, 1454–1456. [CrossRef]
30. Dennert, F.; Rigling, D.; Meyer, J.B.; Schefer, C.; Augustiny, E.; Prospero, S. Testing the Pathogenic Potential of *Cryphonectria parasitica* and Related Species on Three Common European *Fagaceae*. *Front. For. Glob. Chang.* **2020**, *3*, 52. [CrossRef]

31. Peever, T.L.; Liu, Y.C.; Cortesi, P.; Milgroom, M.G. Variation in tolerance and virulence in the chestnut blight fungus-hypovirus interaction. *Appl. Environ. Microbiol.* **2000**, *66*, 4863–4869. [CrossRef]
32. Colinas González, C.; Rojo Sanz, M.; Argemí Relat, J.; Heras Dolander, J.; Castaño Soler, C.; Rotllan Puig, X.; Gómez Gallego, M.; Gilarte Cayuela, S.; Ustrell Juan, E.; Sarr Torras, H. El control biológico del chancro del castaño en Cataluña. In Proceedings of the 5th Congreso Forestal Español, Montes y Sociedad: Saber qué Hacer, Avila, Spain, 21–25 September 2009.
33. González, M.; Cuenca, B.; López, M.; Prado, M.; Rey, M. Molecular characterization of chestnut plants selected for putative resistance to *Phytophtora cinnamomi* using SSR markers. *Sci. Hortic.* **2011**, *130*, 459–467. [CrossRef]
34. Cuenca-Valera, B.; González-González, M.; López-Rodríguez, M.; Ferradás-Rial, Y.; González- Simón, L.; Rey-Fraile, M. Hablan japonés los castaños gallegos? Introgresión genética en el castaño del país. In Proceedings of the 6th Congreso Forestal Español, Montes: Servicios y Desarrollo Rural, Vitoria-Gasteiz, Spain, 10–14 June 2013.
35. Fernández-López, J.; Fernández-Cruz, J.; Míguez-Soto, B. The demographic history of *Castanea sativa* Mill. in southwest Europe: A natural population structure modified by translocations. *Mol. Ecol.* **2021**, *30*, 3930–3947. [CrossRef]
36. Mattioni, C.; Martin, M.A.; Chiocchini, F.; Cherubini, M.; Gaudet, M.; Pollegioni, P.; Velichkov, I.; Jarman, R.; Chambers, F.M.; Paule, L.; et al. Landscape genetics structure of European sweet chestnut (*Castanea sativa* Mill): Indications for conservation priorities. *Tree Genet. Genomes* **2017**, *13*, 39. [CrossRef]