



SHORT COMMUNICATION

Xiphinema index-resistant grapevine materials derived from muscadine are also resistant to a population of *X. diversicaudatum*

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ABSTRACT

Grapevine is severely affected by two major nepoviruses that cause grapevine degeneration: the grapevine fanleaf virus (GFLV) and the arabis mosaic virus (ArMV), specifically transmitted by the dagger nematodes *Xiphinema index* and *X. diversicaudatum*, respectively. While natural resistance to *X. index* has been shown to be a promising alternative for controlling *X. index* and GFLV transmission, the resistance interaction between *X. diversicaudatum* and grapevine has not yet been documented. In the present study, we evaluated the host suitability to *X. diversicaudatum* in materials previously characterised for their resistance to *X. index*. Two *X. index*-resistant accessions VRH8771 (F1 hybrid) and Neadex Alain Bouquet (BC1 hybrid) derived from muscadine, together with the *X. index*-susceptible reference accession *V. vinifera* cv. Cabernet-Sauvignon and the *X. index*-resistant reference accession *V. riparia* '10128', were challenged with a *X. diversicaudatum* population obtained from woody host plants and a reference isolate of *X. index*. The reproduction factors of *X. diversicaudatum* and its numbers per gram of roots paralleled those of *X. index*, showing a resistance interaction to the population of the former species and suggesting that resistance determinants to both nematode vectors might be the same or linked. Nevertheless, these two criteria illustrated a poorer host suitability of grapevine materials to this *X. diversicaudatum* population than to *X. index*.

KEYWORDS: *Muscadina rotundifolia*, host suitability, plant-nematode interaction, plant resistance

INTRODUCTION

Grapevine is severely affected worldwide by two major nepoviruses that cause grapevine degeneration: the grapevine fanleaf virus (GFLV) and the arabis mosaic virus (ArMV), which are specifically transmitted by the nematodes *Xiphinema index* and *X. diversicaudatum*, respectively (Hewitt *et al.*, 1958; Hooper, 1974; Pitcher *et al.*, 1974; Taylor and Brown, 1997; Esmenjaud, 2008). Both closely related *Xiphinema* nematode vectors are migratory ectoparasitic species that live in the soil and transmit viral particles when feeding on plant root tips. *Xiphinema index* is a meiotic parthenogenetic species with very rare males (Nguyen *et al.*, 2020) and rare cognate sexual reproduction events (Villate *et al.*, 2010; Nguyen *et al.*, 2021), while *X. diversicaudatum* reproduces sexually with female and male stages found in equivalent numbers in the soil (Pitcher *et al.*, 1974). In contrast to *X. index*, which has a distribution area matching the worldwide distribution of its major host, grapevine (Nguyen *et al.*, 2019), *X. diversicaudatum* has a wider host range (Thomas, 1970), including woody species of which many plants belong to the Rosaceae family, as well as annual crops and weeds (Pitcher *et al.*, 1974). Even though its natural distribution is more northern than that of the native *X. index*, *X. diversicaudatum* has been presumably disseminated worldwide from Europe with clonally propagated crops, such as strawberry and rose (Pitcher *et al.*, 1974; Winfield, 1974). Strawberry and raspberry are good hosts that are frequently attacked in Northern European countries, such as England (Cotten, 1977), Scotland (Thomas, 1969; Thomas, 1970) and Ireland (Staunton and Moore, 1967). Based on data obtained in Spain in the wild, *X. diversicaudatum* is considered to be a typically-Atlantic native species (Navas *et al.*, 1988). Grapevine also acts as a *X. diversicaudatum* host to which it can transmit ArMV particles and induce fanleaf degeneration with fanleaf-type symptoms, such as leaf deformation and discoloration, shortened internodes and abnormal branching. ArMV can greatly reduce grape yield by causing poor berry set and yield reductions by up to 77 % (Rudel, 1985; Legin *et al.*, 1993).

The control of these ectoparasitic nematode vectors used to rely on highly toxic nematicides that are becoming banned in an increasing number of countries; therefore, environmentally friendly alternatives for the control of the vector nematode and/or the virus are urgently needed (Fuchs and Lemaire, 2017; Claverie *et al.*, 2022). Among these alternatives, planting fallow crops between two successive grapevine plantings and ensuring natural rootstock resistance to the vector are promising methods, as they help reduce nematode numbers and the correlative viral dissemination in the vineyard. The search for resistance to the nematode *X. index* has been ongoing in France and the U.S. since the 1970's. Resistance (R) has been discovered in accessions of several *Vitis* species, notably in *Vitis arizonica* (Van Zyl *et al.*, 2014), but the highest R levels have been revealed in muscadine grape (*Muscadinia rotundifolia*) (Esmenjaud and Bouquet, 2009; Ollat *et al.*, 2016). In particular, resistance has been studied in France in

plant materials derived from the muscadine source NC184-4: these are the F1 accession 'VRH8771' (named 8771) and the BC1 accession 'Nemadex Alain Bouquet' (named NAB) (Esmenjaud *et al.*, 2010; Nguyen *et al.*, 2020). Both accessions have drastically decreased nematode numbers under controlled conditions. The accession NAB, registered as a *X. index*-resistant rootstock, has also been shown to significantly delay GFLV in the vineyard (Ollat *et al.*, 2011; Nguyen, 2018).

In contrast to *X. index*, the host suitability of different grapevine accessions to *X. diversicaudatum* has never been studied and no resistance to this species has yet been reported. Indeed, resistance experiments with *X. index* have benefitted from its ease of multiplication on fig as a substitution host, but such a favourable host for the production of high numbers of *X. diversicaudatum* is currently lacking. Here, we used a *X. diversicaudatum* population multiplied on plum plants to evaluate the host suitability of the two *X. index*-resistant accessions 8771 and NAB derived from muscadine, together with a *X. index*-susceptible reference accession *V. vinifera* cv. Cabernet-Sauvignon and a *X. index*-resistant reference accession *V. riparia* '10128'. We showed that the multiplication and densities of *X. diversicaudatum* paralleled those of *X. index*. Moreover, numbers of *X. diversicaudatum* were lower than those of *X. index*, illustrating that grapevine is a poor host, at least for the *X. diversicaudatum* population used.

MATERIALS AND METHODS

1. The population of *X. diversicaudatum* and the reference isolate of *X. index*

A *X. diversicaudatum* population designated 'La Valette', sampled in 1994 from a glasshouse rose crop located in La Valette-du-Var (Provence, France), was introduced to the collection of UMR ISA (INRAE, Sophia Antipolis, France) and grown on the rose accession *Rosa indica* cv. Major in a glasshouse. Its species-specific identification was performed using morphological and morphometrical characteristics (Luc and Dalmasso, 1974) confirmed by a multiplex PCR with SCAR markers (Wang *et al.*, 2003).

A *X. index* isolate that had already been developed and characterised for previous diagnostic and resistance experiments was used in the present study (Nguyen *et al.*, 2019; Nguyen *et al.*, 2020; Nguyen *et al.*, 2021). This isolate obtained from the population 'Sharekord' (Sharekord, Iran) is referred to as 'Ir-Sh' in the phylogeographical study conducted by Nguyen *et al.* (2019). Nematodes from this isolate were reared on fig plants in a glasshouse at UMR ISA as described in Wang *et al.* (2003).

2. History and multiplication of the *X. diversicaudatum* population on woody plant hosts

After its introduction in 1994, the population 'La Valette' was maintained at ISA in 5-litre rose pots (*R. indica* cv. Major) until 2002. Then soil from these pots was carefully

added to and mixed with the sterile soil of four 8-litre pots for multiplication on woody host plants: i) the rose accession ‘*R. indica* cv. Major’ (rose), ii) and iii) two full-sib brothers accessions from the plum *Prunus cerasifera* carrying (plum R) or lacking (plum S) the *Ma* gene for resistance to root-knot nematodes (Claverie *et al.*, 2011; Duval *et al.*, 2019), and iv) the grapevine accession ‘*V. rupestris* cv. du Lot’ (grapevine). Plants obtained from hardwood cuttings were grown for three years (2003–2006) before the evaluation of nematode numbers in the soil. A representative sample (2 litres) was recovered from the total soil content of each pot and nematode extraction was performed using the elutriation method adapted from Oostenbrink (Verschoor and de Goede, 2000). Numbers were lowest on the grapevine, intermediate on the rose and highest on the two plum accessions (equivalent numbers) (Table S1). Consequently, the soil from the two pots of plum, the most suitable host, was used for the production of nematodes in two 20-litre containers, in each of which a new plum plant was planted. Those two plum plants were grown and maintained as a reservoir of soil inoculum for a preliminary experiment and for the experiment reported here.

3. Grapevine accessions

We evaluated four accessions with different host suitabilities to *X. index*. These comprised two muscadine-derived accessions (Figure 1): i) ‘VRH 8771’ (named 8771), the resistant F1 accession (*V. vinifera* x *M. rotundifolia* cv. NC184-4), and ii) ‘Némadex Alain Bouquet’ (named NAB), the resistant BC1 accession [F1 hybrid VRH 8773 x (*V. berlandieri* x *V. rupestris* cv. 140 Ru)]. The other two accessions were: iii) ‘Cabernet-Sauvignon’ (named CS), the susceptible reference accession (*V. vinifera* cv. Cabernet-Sauvignon) (Nguyen *et al.*, 2020), and iv) *V. riparia* ‘10128’ (named Rip10128), a resistant accession used as a pure *Vitis* reference. In accession 8771, resistance to *X. index* is controlled by three independent QTLs designated *XiR2*, *XiR3* and *XiR4* (Rubio *et al.*, 2020).

4. Experimental design and nematode inoculation in the resistance experiment

Hardwood cuttings (two nodes) of the four accessions were sampled in February 2021 from dormant grapevine mother plants in the repository at UMR EGFV (INRAE, Villenave d’Ornon, France). The cuttings were rooted individually without hormones in 150-ml cells filled with a fine-sand substrate. The plants were transferred to UMR ISA (Sophia Antipolis) and re-potted individually in 2-litre containers in early May. For *X. diversicaudatum*, there were 7 replicates of CS, NAB and 8771 and 5 replicates of Rip10128. For *X. index*, there were 5 replicates of all accessions. Pots from each nematode species were placed on adjacent benches separated by splash screens and distributed in randomised experimental designs.

The plants were left to grow for 6 weeks until inoculation in mid-June. The duration of the experiment was 6.5 months between inoculation and harvest. The pots were drip-irrigated daily, and the air temperature was regulated to reach a maximum of 30 °C. The inoculum levels were 300 individuals per plant for *X. diversicaudatum*, and 750 individuals per plant for *X. index*. In a preliminary experiment (3 replicates) with 250 *X. diversicaudatum* individuals per plant and 3 months between inoculation and harvest, the ‘reproduction factor’ (RF = ratio between final and initial total numbers) of *X. diversicaudatum* was estimated as 1.29 for the *X. index* susceptible accession CS, and as 0.06 and 0.17 for the *X. index*-resistant accessions 8771 and NAB, respectively. The retained period of 6.5 months was based on the hypothesis that the life cycle length of *X. diversicaudatum* under controlled conditions is equivalent to that of *X. index*; i.e., 2 to 3 months (from our experience and from Cohn and Mordechai (1969) and Wyss (2014)), which corresponds to approx. 2–3 cycles in our experiment. The inoculum level of 300 *X. diversicaudatum* individuals per plant – which was equivalent to that of our preliminary experiment – was also expected to prevent intraspecific competition, given that the actively growing plants re-potted 6 weeks previously had a high rootlet density resulting in appropriate nematode dispersal from the inoculation date. The 750 individuals of

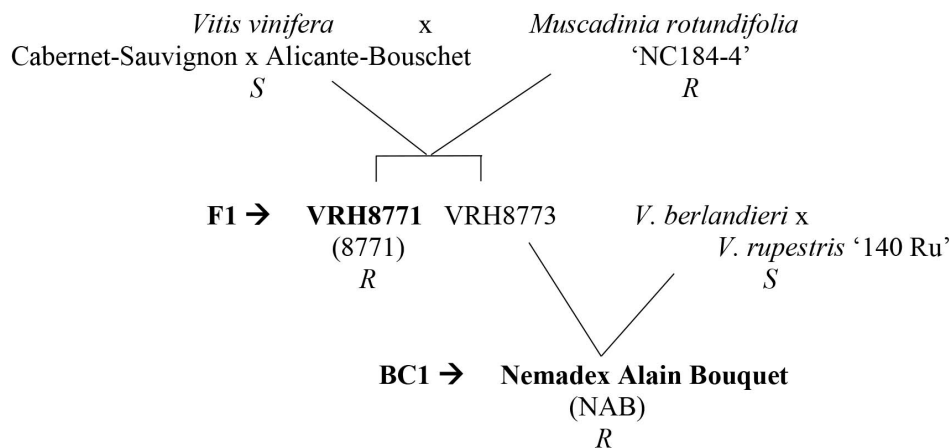


FIGURE 1. Pedigree of the two muscadine-derived accessions (in bold) used in the study. R = resistant to *X. index*, S = susceptible to *X. index*.

X. index inoculated per plant correspond to the level that has been classically used in previous studies on resistance genetics with this nematode species (Rubio *et al.*, 2020).

For *X. diversicaudatum*, the inoculation was performed using soil sampled from plum containers. The sampled soil was carefully mixed and nematode numbers from three 2-litre aliquots were extracted by elutriation (Verschoor and de Goede, 2000) and evaluated. For *X. index*, the same procedure was performed in parallel using soil from fig containers. Considering the mean nematode density evaluated for each species, soil amounts were diluted as needed to obtain the appropriate number of nematodes in 150 ml of soil per plant. Soil was added to the surface of each pot. Light irrigation without water basal leaking was then performed.

5. Criteria of resistance evaluation

At harvest date, the aerial part of each plant was cut at the collar and each container was hermitically placed into a double plastic bag and transferred to a climatic chamber at 6 °C until soil nematode extraction. The extractions were performed sequentially; i.e., one replicate after another. Total nematode numbers (from all the developmental stages) were taken into account and evaluated from the entire 2-litre soil volume of each container. The fresh root weight of each plant was also recorded. Resistance was evaluated using the criterion ‘reproduction factor’ (RF) of the nematode (Esmenjaud *et al.*, 2010; Fourie *et al.*, 2010). The RF of each accession was the mean value of all its replicates. Accessions were classified as resistant (R) when their RF was < 1 and susceptible (S) when their RF was ≥ 1. The ‘number of nematodes per gram of roots’ (Number/g) was also calculated as a complementary criterion for resistance evaluation.

6. Statistical analysis of the data

The rating criteria analysed were RF and Number/g. In the first step, data from the plants inoculated with *X. diversicaudatum* and *X. index* were considered together. A two-factor ANOVA was carried out on data from combinations of the factors ‘accession’ and ‘nematode species’ using XLSTAT software (version 2014.5.03; Addinsoft, Paris, France). F values and probabilities $Pr > F$ for each factor are reported in Supplementary data (see Results section). A multiple comparison analysis was performed on the data using the Fisher LSD multiple range test at $P \leq 0.05$. Next, the four accessions were analysed separately for *X. diversicaudatum* and *X. index* using a single-factor ANOVA completed by the multiple-comparison analysis and the Fisher LSD multiple range test at $P \leq 0.05$. Finally, for each grapevine accession, we analysed the differences in the reproduction factor of each species using a t test (Student) at $P \leq 0.05$ (XLSTAT software).

RESULTS

We first considered altogether the RF and Number/g criteria for the population of *X. diversicaudatum* and the isolate of *X.*

index on the four accessions 8771, NAB, Rip10128 and CS. The overall synthesis of ANOVA data showed that each of the ‘nematode’ and ‘accession’ factors had a highly significant effect on both criteria (Tables S2AB and S3AB). When all the data were taken together, there were overall differences in RF values between *X. diversicaudatum* and *X. index*, with the former species reproducing significantly less than the latter (Table S2C). From the same mixed set of data, there were also differences between the two nematodes in terms of Number/g, with significantly lower nematode densities in *X. diversicaudatum* than in *X. index* - a result that was to be expected, given the lower inoculum number for the former species than for the latter species (Table S3C). Furthermore, with all data taken as a whole, the accession CS had significantly higher RF and Number/g values than those of 8771, NAB and Rip10128. Overall, neither of the criteria in 8771, NAB and Rip10128 were significantly different (Tables S2C and S3C).

We then considered the two nematode species separately in a more detailed analysis (Tables S4 and S5). RFs of the nematode *X. diversicaudatum* were 0.18, 0.36 and 0.46 in the resistant accessions 8771, Rip10128 and NAB, respectively, and they reached 1.49 in the susceptible accession CS. Differences between CS and the other accessions were highly significant (Figure 2A and Table S4A). For the ‘Number/g’ criterion, all four accessions were classified in the same order as for the ‘RF’ criterion, even though the value for NAB was intermediate and no longer significantly different from 8771 and Rip10128 on the one hand, and CS on the other (Figure 3A and Table S4B). As expected for *X. index* (Esmenjaud *et al.*, 2010; Nguyen *et al.* 2020), RFs were low and equivalent in accessions 8771 (0.36), NAB (0.54) and Rip10128 (0.69) and high in the CS accession (7.03). Differences between CS and the other accessions were highly significant (Figure 2B and Table S5A). Nematode densities (Number/g) of *X. diversicaudatum* were also lower (highly significant differences) than in accession CS and non-significantly different between the three resistant accessions (Figure 3B and Table S5B). Thus, RFs of *X. diversicaudatum* in the three *X. index*-resistant accessions remained under the threshold of 1, which indicates that they are all resistant to the population used in our study. The *X. index*-susceptible CS accession, with an RF above 1, is also susceptible to *X. diversicaudatum*.

We finally compared the reproduction factors and the nematode numbers per gram of roots between the two nematode species in each accession (Table 1). For the RF criterion, the t test showed that differences were highly significant in CS and Rip10128, and significant in NAB and 8771. For the criterion Number/g, the two nematode species had also highly significant differences in CS and Rip10128 and a significant difference in 8771. In NAB, the number of individuals per gram of roots for *X. diversicaudatum* was lower than for *X. index*, but the difference was non-significant.

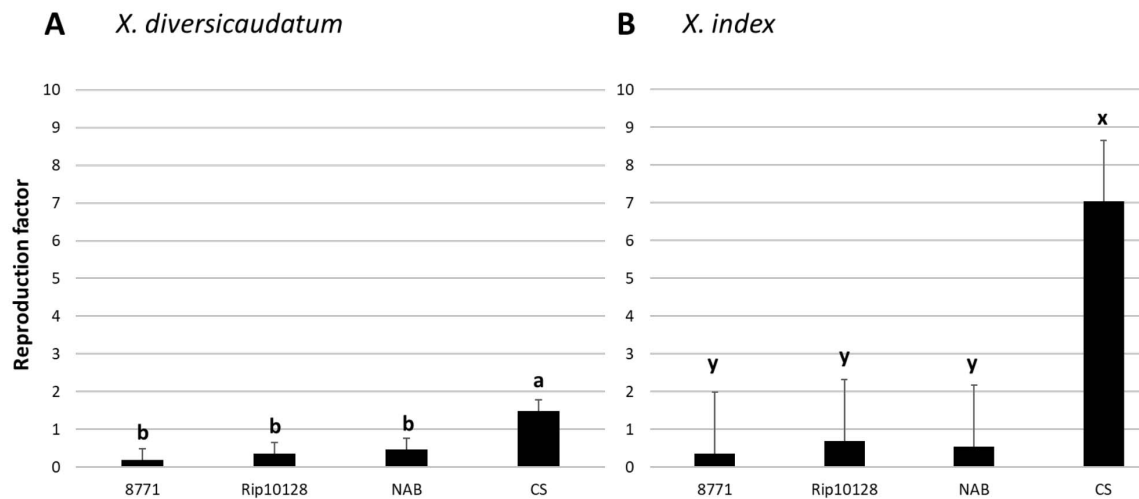


FIGURE 2. Reproduction factors (RFs) of *X. diversicaudatum* (A) and *X. index* (B) in the F1 accession 8771, the BC1 accession NAB, the *X. index* susceptible control accession CS and the *X. index* resistant control accession Rip10128. Data are means (\pm SE) of 7 replicates (8771, NAB and CS) and 5 replicates (Rip10128) for *X. diversicaudatum* and of 5 replicates (all accessions) for *X. index*. Bars with different letters significantly differ according to the Fisher LSD test ($P \leq 0.05$).

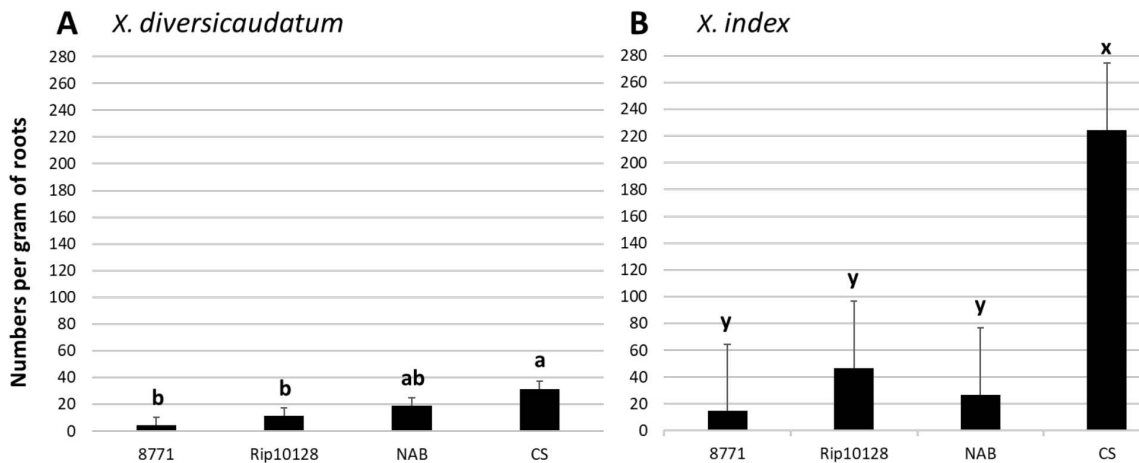


FIGURE 3. Number of nematodes per gram of roots (Number/g) for *X. diversicaudatum* (A) and *X. index* (B) in the F1 accession 8771, the BC1 accession NAB, the *X. index* susceptible control accession CS and the *X. index* resistant control accession Rip10128. Data are means (\pm SE) of 7 replicates (8771, NAB and CS) and 5 replicates (Rip10128) for *X. diversicaudatum* and of 5 replicates (all accessions) for *X. index*. Bars with different letters significantly differ according to the Fisher LSD test ($P \leq 0.05$).

DISCUSSION

In our experiments, we inoculated the plants using nematode-infected soil rather than a suspension of previously extracted nematodes. This is because when working with *X. index* we have found that nematodes survive and reproduce better using the former method, presumably because they are not subject to the stress of the extraction, storage and dilution steps preceding inoculation; we therefore hypothesised that this is also the case for *X. diversicaudatum*. This protocol is also used to evaluate the transmission efficiency of GFLV variants (Marmonier *et al.*, 2010; Schellenberger *et al.*, 2010; Andret-Link *et al.*, 2017) or to evaluate plant resistance to GFLV infection (Hemmer *et al.*, 2018; Djennane *et al.*, 2021). Interestingly,

with this inoculation method, our results have provided the first data illustrating different host suitabilities to *X. diversicaudatum* amongst grapevine accessions. However, the alternative option of suspending nematodes in water should also be evaluated in the future, because it could allow the number of nematodes used for inoculation to be better controlled and modulated. While numerous studies have relied on natural resistance to *X. index* in grapevine, our work has shown for the first time the existence of resistant grapevine materials to *X. diversicaudatum*. We have illustrated that the *X. index*-resistant accessions, 8771, NAB and Rip10128, are also resistant to at least the population of *X. diversicaudatum* used in our experiment. It is highly plausible that this resistance to *X. diversicaudatum*, in particular for the muscadine-derived F1 and BC1

TABLE 1. P-values from t tests (Student) for comparison of the reproduction factor (RF) and the number per gram of roots (Number/g) of *X. diversicaudatum* and *X. index* in each accession

	CS	NAB	8771	Rip10128
RF	0.003**	0.046*	0.015*	0.000**
Number/g	0.004**	0.374 NS	0.022*	0.006**

** Significant at $P \leq 0.01$; * Significant at $P \leq 0.05$; NS Non-significant.

materials tested, is associated with a mechanism similar to the hypersensitive-like response induced by *X. index* (Esmenjaud and Banora, unpublished data). As in *X. index*/GFLV, lower numbers of *X. diversicaudatum* would likely result in less ArMV transmission to grapevine.

RF values have been shown to be inversely related to the initial number of individuals for root-knot nematodes *Meloidogyne* spp. (Fourie *et al.*, 2010; Lopez-Gomez and Verdejo-Lucas, 2017) and this is likely the same for dagger nematodes *Xiphinema* spp. Even though we did not apply several inoculum levels per nematode species to monitor the evolution of the RF values in our materials, we can hypothesise that those applied were low enough to prevent intraspecific competition, a major factor in the resistance relationship. Having the same inoculum level for both species would have been preferable, even though the biology of both species is different (with contrasting reproduction modes). Nevertheless, as the inoculum was higher for *X. index* than for *X. diversicaudatum* (750 versus 300 individuals/pot), we would have observed even higher *X. index* RFs, if the grapevine materials had received the same number of this nematode as *X. diversicaudatum*. Consequently, the *X. index* RF was certainly underestimated in our study and grapevine has undoubtedly a better host suitability for *X. index* than for *X. diversicaudatum*.

Nevertheless, our results regarding the natural resistance of grapevine materials were obtained with the population 'La Valette', a population initially sampled from rose. We do not know whether 'La Valette' is representative of the behaviour of the *X. diversicaudatum* species taken as a whole or whether an intraspecific variability exists among populations for resistance. The sexually-reproducing species *X. diversicaudatum* is more polymorphic than the mainly parthenogenetic species *X. index* (Villate, 2008; Villate *et al.*, 2010; Nguyen, 2018; Nguyen *et al.*, 2021); thus we cannot exclude the fact that another population would behave differently. As reported in our Material and methods section, the multiplication of inoculum by three woody hosts all sensitive to ArMV inoculation by *X. diversicaudatum* (Thomas, 1970) suggested that plum has a better host suitability to 'La Valette' than rose and grapevine (Table S1). The availability of a population detected in high numbers from a grapevine plot and expected to express a better adaptation to/reproduction in this plant (i.e., with a higher aggressiveness than 'La Valette') would help increase knowledge about the putative population-specificity of resistance.

A non-population-specific resistance would open the way to the study of the genetics of resistance to *X. diversicaudatum* in grapevine. It is plausible that the resistance factors controlling *X. index* in the muscadine source used here (Rubio *et al.*, 2020) also act on *X. diversicaudatum*. As ArMV is often found together with GFLV in the grapevine plots, a common genetic determinism to *X. diversicaudatum* and *X. index* would facilitate the control of both nematodes and their respective transmitted viruses. This could provide additional support for the creation of multi-vector nematode-resistant rootstocks. Major resistance genes have been identified in another economically important nematode genus, the root-knot nematodes (RKNs) *Meloidogyne* spp.; these genes are used to control a more or less wide spectrum of RKN species in annual crops, such as tomato (Williamson, 1998) and pepper (Djian-Caporalino *et al.*, 2001), and in perennial crops (Saucet *et al.*, 2016; Duval *et al.*, 2019), such as *V. cinerea* (Smith *et al.*, 2018), plum (Claverie *et al.*, 2011), peach (Duval *et al.*, 2014) and almond (Van Ghelder *et al.*, 2018).

By using a *X. diversicaudatum* population from grapevine with a better reproduction it would also be possible to obtain more reliable results when working on the ArMV transmission in this crop, given that the efficiency of the viral transmission has been shown to depend on the original host plant of the nematode vector (Brown, 1986a; Brown, 1986b; Brown and Trudgill, 1983; Taylor and Brown, 1997). Even though grapevine appears to be a poor host for *X. diversicaudatum*, the vectored ArMV has a high detrimental impact on this crop (Rudel, 1985; Legin *et al.*, 1993). This impact may be partly due to the long survival of *X. diversicaudatum* in the soil (McNamara, 1980), as well as to its cognate and possibly long ArMV retention time. Actually, it is probable that this retention time is similar to the one of *X. index*, which retains GFLV for more than four years (Demangeat *et al.*, 2005).

CONCLUSION

Our study allowed to get the first data regarding the host suitability of different grapevine accessions to *X. diversicaudatum*. We evaluated the host suitability of *X. index*-resistant material derived from muscadine together with *X. index*-resistant and -susceptible reference accessions, to a population of *X. diversicaudatum* obtained from woody host plants. We showed that the host suitability of these materials paralleled that of *X. index*, which suggests that resistance determinants to both nematode vectors might

be the same or linked. Our results illustrated a poorer host suitability of grapevine materials to the *X. diversicaudatum* population used than to *X. index*. In order to take into account the higher diversity of *X. diversicaudatum* than *X. index*, other populations of the former species, and in particular a population obtained from grapevine, will have to be evaluated.

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