

# *Wolbachia* is present in the European grapevine moth

Marlon Pazian | Elisa Bacala | Fernando T. Rei | Tânia Nobre 

MED–Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Portugal

## Correspondence

Tânia Nobre, MED–Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.  
Email: tnobre@uevora.pt

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## Abstract

The European grapevine moth, *Lobesia botrana*, has high economic impact on grapevines being one of the most harmful pests of vineyards worldwide. *Wolbachia* infection has not yet been reported for this moth. We systematically look for *Wolbachia* presence in *L. botrana* from three areas within the viticultural region of Alentejo (Portugal), via *wsp* gene specific PCR, and consistently found its presence. The interaction between the grapevine, its associated moth pest and the *Wolbachia* needs to be further explored, towards its potential in the development of symbiosis-based pest management strategies.

## KEYWORDS

*Lobesia botrana*, pest management, specific PCR, symbiosis, vineyard, *wsp* gene

## 1 | INTRODUCTION

Directly associated with grapevine, the European grapevine moth—*Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae)—is actually a polyphagous insect able to develop and complete its life cycle on several host plants (e.g. Ioriatti et al., 2011; Thiéry & Moreau, 2007). Despite the diversity of possible host plants, it is on the cultivated *Vitis vinifera* that *L. botrana* encounters its main food resource, giving it the title of one of the most harmful pests of vineyards worldwide (Thiéry et al., 2018).

The impact of this pest on grapevines has been considered high, with relevant yield losses being reported in a wide geographic range (Delbac & Thiéry, 2016; Moschos, 2006; Thiéry et al., 2018). As other crops, vineyards also face the need of a drastic reduction in pesticide use, paired with the challenges arising from invasion by new pests/diseases and climate change. The use of broad-spectrum insecticides (e.g. pyrethroids, carbamates and organophosphates) are linked to high environmental impacts and its toxicological aspects are triggering the search for alternatives, such as microbial and botanical pesticides (Pertot et al., 2016), but are also fuelling the search on behaviour-modifying chemicals (Ioriatti et al., 2011) and efficient biological control agents (Thiéry et al., 2018).

Several natural enemies have been suggested for *L. botrana*, varying in time and space (e.g. Moreau, Buffenoir, Thiéry, &

Vogelweith, 2019; Moreau, Villemant, Benrey, & Thiéry, 2010; Xuéreb & Thiéry, 2006) including entomopathogenic fungi (Altimira et al., 2019; López-Plantey et al., 2019; Sammaritano et al., 2018). As to bacteria, mainly *Bacillus thuringiensis* has been used to control the European grapevine moth (Escudero, Estela, Escriche, & Caballero, 2007; Ifoulis & Savopoulou-Soultani, 2004; Ioriatti et al., 2011). However, multiple approaches are becoming available for insect pest control founded on manipulation of microbial partners (reviewed in Arora & Douglas, 2017), considering bacteria both entangled in a transient or in a persistent relationship with the insect host. One of the best-known bacterial group associated with insects with recognised potential in pest management is the *Wolbachia*, a group of obligatory intracellular and maternally inherited bacteria (Bourtzis, 2008).

*Wolbachia* presence is known to induce reproductive changes in hosts, causing decrease in fertility or even impairing reproduction—through male killing, feminisation, parthenogenesis induction or cytoplasmic incompatibility (e.g. Baldo, Lo, & Werren, 2005; Hurst et al., 1999; Kikuchi & Fukatsu, 2003; Saridaki & Bourtzis, 2010; Zchori-Fein & Bourtzis, 2011). *Wolbachia* can (but not always does) influence insects' pesticide sensitivity, altering its susceptibility (Li, Liu, & Guo, 2018; Liu & Guo, 2019). In the quest to develop efficient and target-specific pest management strategies, *Wolbachia*-based technologies have been widely discussed (e.g. Apostolaki et al., 2011;

Bourtzis, 2008; Crotti et al., 2012; Doudoumis et al., 2013; Floate et al., 2007) and one of the most iconic examples concerns the control of arboviral disease transmission by *Aedes aegypti* mosquitoes (World Mosquito Program; www.worldmosquitoprogram.org). In Lepidoptera, most references concern the moth *Ephestia kuehniella*, a cosmopolitan pest of stored products such as flour, where it was found to induce cytoplasmic incompatibility, lower fertile sperm transfer or probably male killing (e.g. Ikeda, Ishikawa, & Sasaki, 2011; Lewis, Champion De Crespigny, Sait, Tregenza, & Wedell, 2011; Sumida, Katsuki, Okada, Okayama, & Lewis, 2017; Fujii et al., 2001). Nonetheless, the first step is accessing whether the target pest population is naturally *Wolbachia*-infected or not.

For *L. botrana*, to the best of our knowledge, no systematic search for *Wolbachia* infection was performed and the only work found reported negative results (Zchori-Fein & Perlman, 2004), suggesting that this moth would not be associated with this bacterial group. Our findings, comprising *L. botrana* specimens from three sampling areas within the viticultural region of Alentejo (Portugal), show the widespread presence of *Wolbachia* in the European grapevine moth. This association needs to be further understood and we should start looking for potential effects of the interaction on *L. botrana* feeding choices, reproduction and overall fitness that might affect the grapevine as selected host plant.

## 2 | MATERIAL AND METHODS

*Lobesia botrana* was sampled in three vineyards in the Alentejo region (Figure 1a) by means of commercial sticky traps with specific pheromones (Biosani). Per sampling location, 20 morphological identified specimens were removed from the trap and conserved in ethanol 96% at  $-20^{\circ}\text{C}$ . Individuals were allowed to dry on filter paper prior to DNA extraction (NZY Tissue gDNA Isolation kit, from NZYTech, Lda). All extraction products were stored at  $-20^{\circ}\text{C}$  and later used directly in the PCR.

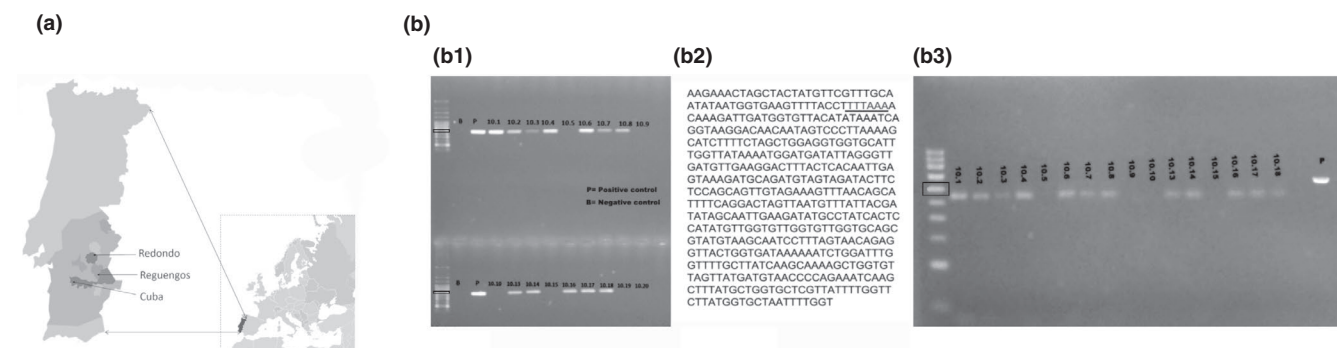
For host species confirmation, a fragment of the cytochrome c oxidase subunit I (COI) gene was amplified using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and

HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) and the protocol described in Nobre, Gomes, and Rei (2018). The purified PCR product (NZYGelpure kit from NZYTech, Lda) was commercially sequenced (Macrogen Inc.).

For *Wolbachia* presence, *wsp* gene specific PCRs were performed (2 technical-replicates) using the primers WSP1 (5'-GGG TCC AAT AAG TGA TGA AGA AAC -3') and WSP2 (5'-TTA AAA CGC TAC TCC AGC TTC TGC-3') (Zhou & Neill, 1998). PCR conditions were  $94^{\circ}\text{C}$  for 5 min; 35 cycles of  $94^{\circ}\text{C}$  for 30 s,  $55^{\circ}\text{C}$  for 30 s,  $72^{\circ}\text{C}$  for 1 min and  $72^{\circ}\text{C}$  for 10 min and 20 ng of gDNA, Buffer 1X, MgCl<sub>2</sub> 1.5 mM, dNTP 0.04 mM, primers 0.2  $\mu\text{M}$ , DNA polymerase 1U (NZYTaq II DNA polymerase, from NZYTech, Lda). For confirmation of procedure specificity and guarantees of amplification of the desired final product, two randomly selected PCR products, per sampling location, were purified and sequenced as above. PCR products of the expected size (0.6 kb) were double digested with the restriction endonucleases DraI and BseCI (Kikuchi & Fukatsu, 2003; according to producers' protocol), electrophoresed on a 2% TAE-agarose and the pattern was registered.

## 3 | RESULTS

We confirmed the analysed specimens as belonging to *Lobesia botrana* (Genbank Accession number MT233430). Not all the samples were successfully amplified; only the samples positive for COI were used in the detection of *Wolbachia*, decreasing the probability of false negatives. *Wolbachia* infection was detected in 47 of the 55 specimens examined (17/18 from Reguengos, 18/19 from Redondo and 12/18 from Cuba, see Figure 1). The *wsp* gene segments subjected to RFLP typing showed only one pattern type (Figure 1b) and the sequenced amplicons had the exact same nucleotide composition (Genbank Accession numbers MT228045, MT228046 and MT228047 for each population consensus), with the highest level of similarity with *Wolbachia* endosymbiont of *Tetranychus urticae* (but also of *Pieris rapae*, *Jalmenus evagoras* and *Paratrechina longicornis*) outer surface protein gene (100% similarity in a total query cover; GenBank BLAST).



**FIGURE 1** (a) Location of the three sampled vineyards, within the region Alentejo (darker grey); the grey shades correspond to the different wine regions. (b) Detection of *wsp* gene. (b1) Gel of *wsp* PCR specific amplicon, (b2) *wsp* consensus sequence and restriction sites of the endonucleases DraI (underlined), (b3) RFLP pattern resulting of the product digestion with the restriction endonucleases. Rectangle indicates 0.6 Kb band

## 4 | DISCUSSION

Even though it is estimated that about 80% of Lepidoptera species are hosts of *Wolbachia* (Ahmed, Araujo-jnr, Welch, & Kawahara, 2015) this is, to the best of our knowledge, the first study confirming its presence in the European grape moth, *Lobesia botrana*. It should be acknowledged, however, that the infection level here reported might be affected by false negative or positive results, as those have been reported for *wsp* gene PCR-based amplification (e.g. Carvajal, Hashimoto, Harnandika, Amalin, & Watanabe, 2019; Jeyaprakash & Hoy, 2000).

It is estimated that lepidopteran species harbour more than 90 different *Wolbachia* strains, with high incidence of identical and multiple strains of *Wolbachia* among butterflies and moths and evidence of common horizontal transmission (Ahmed, Breinholt, & Kawahara, 2016). As such, finding *Wolbachia* in the European grape moth is not surprising, but it can have an impact in terms of pest management strategies, triggering a new research line.

Ahmed et al. (2015) found no correlation between *Wolbachia* infection frequency and phylogenetic relatedness of lepidopteran hosts but a significant correlation between infection and host geography. Inter-specific, inter-familial and inter-ordinal horizontal transmission is also common (Ahmed et al., 2016). Bringing these facts together it is not surprising that the *Wolbachia* *wsp* strain here sequenced seems to be the same reported infecting the mite *Tetranychus urticae* in Portugal (Zélé, Weill, & Magalhães, 2018). Also, vector-mediated inter-specific transmission was observed in *Wolbachia* through shared food sources (e.g. Huïgens, Almeida, Boons, Luck, & Stouthamer, 2004; Oliver, Degnan, Burke, & Moran, 2010; Sintupachee, Milne, Poonchaisri, Baimai, & Kittayapong, 2006), ectoparasitic mites (Gehrer & Vorbürger, 2012; Jaenike, Polak, Fiskin, Helou, & Minhas, 2007) and parasitoids (Ahmed et al., 2015).

In Lepidoptera, *Wolbachia* is known to manipulate host reproduction through male killing, feminization and cytoplasmic incompatibility and although these strategies favour the maintenance of the endosymbiont in the host population, they also impact on host biology and evolution (reviewed in Duploup & Hornett, 2018). A highly distorted female biased sex ratio has nonetheless an obvious consequence: many females remain unmated affecting the host reproductive success. This unbalance could potentially be exploited in the developing of new, highly targeted, pest management approaches. The peculiarities of the Lepidoptera sex determination system, where the female is the heterogametic sex, leads to specificities in mechanisms and repercussions of *Wolbachia* induced reproductive manipulations (Duploup & Hornett, 2018) that need to be understood. The identification of abundant *Wolbachia* infection levels in the target pest species is just a first and needed step towards the development of new symbiosis-based pest management strategies.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Conceptualization, T.N.; methodology, M.P., E.B. and T.N.; formal analysis, M.P.; resources, F.T.R. and T.N.; writing—original draft preparation, M.P. and T.N.; writing—review and editing, M.P. and T.N.; supervision, T.N.; funding acquisition, F.T.R. All authors have read and agreed to the published version of the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Genbank® at <https://www.ncbi.nlm.nih.gov/genbank/>, with the accession numbers MT233430, MT228045, MT228046 and MT228047. Gel images will be made available from the corresponding author upon request.

## ORCID

Tânia Nobre  <https://orcid.org/0000-0002-1855-7451>

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