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THE ROLE OF *VITEX AGNUS-CASTUS* AND ASSOCIATED *HYALESTHES OBSOLETUS* IN THE EPIDEMIOLOGY OF *BOIS NOIR* IN MEDITERRANEAN VINEYARDS

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'Candidatus Phytoplasma solani' (CPs), a 16S rRNA XII-A subgroup belonging phytoplasma (Quaglino et al., 2013), endemic to Europe and the Mediterranean area, causes the most widespread grapevine yellows disease in Europe - Bois noir (BN). Severe damage in grapevine biomass and consequential agroeconomic losses following epidemiological outbreaks (Johannesen et al., 2008) emphasize the importance of clarifying the complex dynamics among inoculum source, pathogen and the vector. *Hyalesthes obsoletus* Signoret, 1865 (Hemiptera: Cixiidae) is the primary vector of CPs to grapevine (Maixner, 1994; Sforza et al., 1998) and as a polyphagous planthopper the species occurs in vineyard ecosystems throughout the Mediterranean basin up to southwestern Germany to the north and Asia Minor in the southeast (Hoch and Remane, 1985). While *Urtica dioica* and *Convolvulus arvensis* are the most frequently recorded hosts of *H. obsoletus* in west and central Europe (Langer and Maixner, 2004; Kessler et al., 2011; Johannesen et al., 2012; Imo et al., 2013), in the Mediterranean several plants characteristic for this bio-geographic area are noted as food source for the adults: *Vitex agnus-castus, Olea europaea, Tamarix sp., Quercus ilex,* etc. (Hoch and Remane, 1985). Among these, *V. agnus-castus* stands out as host plant that provides a niche for larval development (Sharon et al., 2005). Records of *H. obsoletus* occurrence in association with this aromatic woody shrub throughout Greece, Turkey and Israel (Hoch and Remane, 1985; Sharon et al., 2005) prompted the research on its potential role in CPs epidemiology in the Mediterranean littoral, both as a host for the vector populations and as well as pathogen inoculum source.

We investigated in Montenegro, a Mediterranean grape growing country with records of BN (Radonjić et al., 2009), whether *V. agnus-castus* has a role in disease epidemiology and whether it interferes with pathways associated with *U. dioica* and *C. arvensis* (Kosovac et al., 2016). The aim was to employ molecular epidemiology and experimental transmission assays to identify the infection incidence of focal reservoir plants and corresponding vector populations, and to trace transmission pathways of the CPs genotypes from their inoculum source through associated vector populations to symptomatic grapevine (dead-end host).

The *tuf/stamp/vmp1* based multilocus typing revealed 12 genotypes in total, and confirmed a direct, independent pathway of transmission from *U. dioica* by associated *H. obsoletus* populations to grapevine (Langer and Maixner, 2004). Among CPs isolates associated with nettle-sourced cycles some had the typical tuf-a genotype, however some show the intermediate tuf-ab type previously found in Austria (tuf-b2; Aryan et al., 2014). In relation to this, the genotype tuf-b/Rqg50/V17, recently also detected in *H. obsoletus* in BN-diseased vineyards in Austria (CPsM4_At10; Aryan et al., 2014) was found

in grapevine and in two inoculum source plants: *C. arvensis* and *V. agnus-castus* along with their corresponding insect populations, revealing epidemiological routes that overlap and possibly intermix. Based on this finding and assuming that both CPs and *H. obsoletus* are of Mediterranean origin, it is reasonable to suspect that *V. agnus-castus* could be the original host plant of this genotype. Congruently, the same genotype was just recently identified in infected grapevine in Mediterranean vineyards of Bosnia and Herzegovina (Delić et al., 2016). In laboratory controlled transmission assays *Vitex*-affiliated populations of *H. obsoletus* successfully transmitted CPs genotypes to experimental grapevines, thus confirming its vector role. Furthermore, the data unambiguously indicate on natural occurrence of BN in association with *V. agnus-castus* in this region.
Combined results of field collected and experimentally obtained data confirm the role of *V. agnus-castus* in the epidemiology of BN and point out to the importance of this dual host plant as symptomless inoculum source and vector host-plant. This encourages further research in elucidating its occurrence, infection incidence and epidemiological significance, as some of the most important grape growing regions in the world are located in the coastal zone of Spain, France, Italy and Croatia.

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