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Host plant specificity of *Hyalesthes obsoletus* and consequences for “bois noir” epidemiology

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Abstract

The complex epidemiological system of grapevine “bois noir” (BN) consists of stolbur (16SrXII-A) phytoplasma associated with the disease, the vectoring planthopper *Hyalesthes obsoletus,* and wild herbaceous host plants of the pathogen and the vector. Severe outbreaks of BN that occurred in Germany since 2004 are assumed to be related to the exploitation of a new host plant by the vector and the spread of a host-specific type of the phytoplasma. This paper confirms the association of tuf-type I of the BN phytoplasma with the recent BN outbreaks in Germany and the restriction of different BN-types to specific host plants. Mitochondrial DNA analysis of *H. obsoletus* from different European countries and Israel demonstrated a recent demographic expansion and circum-alpine immigration of *H. obsoletus* to Germany, and RAPD analysis revealed differences between host plant populations.

Key words: “Bois noir”, phytoplasma, epidemiology, vector, host races.

Introduction

“Bois noir” (BN), a grapevine yellows disease associated to phytoplasmas of the 16SrXII-A group and transmitted by the planthopper *Hyalesthes obsoletus* Signoret, has become a major problem in European viticulture. Different strains of the BN-phytoplasma (BNp) can be distinguished by RFLP-analysis of the tuf-gene (Langer and Maixner, 2004). Tuf-type I (VK-I) is associated to stinging nettle (*Urtica dioica*) while tuf-type II (VK-II) is found with bindweed (*Convolvulus arvensis*) as natural hosts. The current rise of BN incidence and its spread to new areas is thought to be related to the proliferation of tuf-type I. Nettle and bindweed are also the major host plants of *H. obsoletus,* but until recently only bindweed was utilized in Germany. *H. obsoletus* used to be restricted to xerothermic habitats, mainly vineyards on slopes of river valleys. About a decade ago the planthopper started to exploit nettle in Germany as a new host plant. These nettles often occur in less favorable habitats where the planthopper before was unable to live on *C. arvensis.*

The epidemiology of BN is determined by a complex interaction between the phytoplasma, herbaceous host plants, and the vector; grapevine is only an accidental host of the vector and a dead end host of the phytoplasma. The present investigation was carried out with the objective to study the role of *H. obsoletus* populations on nettle and the ‘nettle-type’ I of BNp for the current outbreaks of BN. We investigated whether genetic host races of the vector exist and if the recent spread of BN is related to a geographic and demographic expansion of *H. obsoletus* in Europe.

Materials and methods

Samples of symptomatic grapevines and *H. obsoletus* were collected in German and other European viticultural areas. At one location, Bacharach, where different host-populations of *H. obsoletus* coexist, the vectors were collected from both hosts. At Bernkastel, where *H. obsoletus* traditionally occurred on bindweed only, an isolated nettle stand was checked since 2001 for the presence of the vector. Additional samples were collected in Germany by M. Breuer, A. Schartl and M. Stark-Urnau. Specimens from eight European countries and Israel were provided by E. Angelini, A. Batlle, E. Boudon-Padieu, A. Bressan, P. Kuntzmann, G. Pasquini, H. Reisenzein, G. Seljak, R. Sharon and Z. Der. Phytoplasma detection and tuf-type affiliation was carried out according to Langer and Maixner (2004).

Mitochondrial DNA including four partial gene sequences (1,180 bp) was amplified from 111 specimens by PCR and sequenced. Haplotype mtDNA genealogies were constructed, and molecular diversity indices, pairwise differences (pd) and nucleotide diversity were calculated for individual samples and for regional host-plant populations. The association of vector haplotypes from Germany and Slovenia to host plants was checked with the χ^2 test. Potential immigration pathways of *H. obsoletus* into Germany were analyzed by isolation by distance (IBD) analysis.

RAPD analysis was performed on 347 *H. obsoletus* from 6 bindweed and 10 nettle populations using 5 arbitrary decamer primers. Host plant fidelity was based on the absence/presence of RAPD bands. Levels of variability were compared among host affiliated populations and a principal coordinate analysis (PCO) was carried out for all, for northern, and for the Bacharach individuals. A maximum likelihood phylogenetic analysis based on RAPD allele frequencies was carried out to identify host plant and/or geographic clusters.

Results and discussion

The survey of BN diseased grapevines confirmed the close association of tuf-type I with the new outbreaks of the disease in Germany. Where the disease occurred only recently, 87% of PCR-positive vines were infected by tuf-type I. Where BN was already present but increased rapidly, 79% of the vines were infected by this type compared to only 7% at locations with BN history but without considerable changes in infestation rates and bindweed as the still predominant host plant. The corresponding proportions of tuf-type I in PCR-positive *H. obsoletus* were 87%, 81%, and 2%, respectively.

At Bacharach, *H. obsoletus* were collected separately from *C. arvensis* and *U. dioica.* About 18% of *H. obsoletus* from bindweed and 29% from stinging nettle were infected. The proportion of tuf-type I in the population on nettle reached 95%, while 97% of infected *H. obsoletus* from *C. arvensis* carried tuf-type II. A complete correspondence between host plant and BN type cannot be expected since adult vectors move around.

First specimens of *H. obsoletus* were found on an isolated nettle stand at Bernkastel in 2003. A total of 118 vectors were caught in 2005 and 151 specimens in 2006. After first infected vectors occurred in 2005 (12.5%) the infestation increased to 26% in 2006. Only tuf-type I was found. The BN tuf-type I type epidemiological system seems to develop in three steps. First, nettle stands are colonized by a few individuals of *H. obsoletus,* followed by a rapid population growth and a subsequent increase of the infestation of populations by tuf-type I.

The data strongly suggest the ongoing colonization of nettle by *H. obsoletus* as the reason for the recent outbreaks of BN and the spread of tuf-type I in Germany. However, additional factors leading to increased infestation of nettle populations must be involved. At Ungstein in the Palatinate area, for instance, *H. obsoletus* was first detected on nettle in 1995 but the infestation of this population remained less than 5% until 2000. Corresponding to the general spread of BN tuf-type I in Germany it increased from 7% in 2001 to 23% in 2005.

To explain the recent exploitation of nettle by *H. obsoletus* at its northern range border and the change in BN epidemiology we tested whether genetic host races exist and nettle-affiliated genotypes could have immigrated from the south. The mtDNA analysis revealed 12 haplotypes. Diversity was highest in Italy (6 haplotypes; pd=0.97) followed by Slovenia, Spain and France, Hungary and Israel. It was lowest in Germany (2 haplotypes; pd=0.33). Insects from Israel differed highly from European samples. Within Europe, an eastern sub-lineage in Hungary and a western one in western and northern Europe could be distinguished. A basal western haplotype is rooted to the Hungarian haplotypes and distributed throughout south-western Europe. Two haplotypes derived from the basal western haplotype were found on alternative sides of the classical Italian/Slovenian limestone biogeographic divide. They meet at a contact zone in southern Germany. These data suggest a recent circum-alpine invasion of *H. obsoletus* into wine-growing regions of Germany and northern France from the northern Balkans or Italy. Since the predominant haplotype in Germany and the western basal haplotype were found on nettle and field bindweed, no host plant-haplotype affiliation was observed based on mtDNA.

RAPD analysis revealed a total of 56 polymorphic fragments, but none was diagnostic for host plant affiliation. Bindweed populations were on average more polymorphic. PCO analysis found host plant affiliation for the northern and the Bacharach *H. obsoletus* populations, although axis-scores were very low, but did not discriminate affiliation in the total data set. Maximum likelihood phylogenetic analysis based on RAPD allele frequencies found three significant splits representing host plant and geographic divisions. These were field bindweed populations, Slovenian and Italian nettle populations, and German nettle populations.

The results of the genetic analyses strongly suggest that *H. obsoletus* immigrated recently to the northern viticultural regions by a circum-alpine range expansion. Only the eastern haplo-lineage is dispersed in all German viticultural areas and on both host plants. The western haplo-lineage was found only in the south-western area of Baden adjacent to the French Alsace region.

The recent immigration of *H. obsoletus* is likely the reason for the occurrence of BN in Germany since the 1960s, but it alone cannot explain the contemporary spread of the disease. *H. obsoletus* was restricted to bindweed until the end of the 1990s although nettle was available in its habitats. The spread of BN in Germany is correlated to increased utilization of nettle. Moreover, by using nettle *H. obsoletus* is expanding its range into less suitable sites. The mtDNA analysis suggests that it is intrinsically able to utilize both host-plant species, yet the RAPD data indicate a subtle but significant host plant diversification that is likely to be promoted by ecological differences between host populations; for example, flight activity of the *H. obsoletus* host races is not synchronised. What enabled the host shift of *H. obsoletus* is not known. Changing climate could play a role, either allowing local populations of the planthopper to use a hitherto suboptimal host or enabling nettle adapted southern populations to immigrate.

The current study contributes to a better understanding of BN epidemiology by shedding light on the vector-host plant relationship. However, it does not explain the affiliation of different types of the BN phytoplasma with those host plants. This important element of the complex BN epidemiology needs to be further investigated.

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References

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