

Emergence Of Stolbur Tuf-type-A Phytoplasma in Western Europe Relative to Dispersal of its Vector *Hyalesthes Obsoletus*: A Summary

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The dissemination of vector transmitted plant pathogens is determined by survival and dispersal of the vector, the vector's ability to transmit the pathogen and how the pathogen affects the infected plant and vector. The polyphagous planthopper *Hyalesthes obsoletus* is the major vector of stolbur phytoplasma (16SrXII-A group), which are responsible for yellows diseases in grapevine (bois noir), maize (maize redness) and various Solanaceous crops in Europe. The epidemiology of bois noir is coupled to the infection of herbaceous weeds, not to grapevine, as it is a dead-end host for stolbur and not a nymphal substrate of *H. obsoletus*. Stolbur has two major strains, defined by variants of the tuf gene (Langer and Maixner 2004). The tuf-type-a strain is associated with stinging nettle (*Urtica dioica*) while the second strain, tuf-type-b, has a broader range of natural host plants among which field bindweed (*Convolvulus arvensis*) is a dominant host. While the use of field bindweed has been recognised since stolbur was first characterised, the system bound to stinging nettle has emerged as a major source of infection, particularly in western parts of Europe. In Germany, the use of stinging nettle was observed for the first time about 20 years ago. The vector's host shift and the dissemination of two stolbur strains suggest two independent transmission cycles. Johannesen et al. (2008) showed that vector populations in Germany originated east of the European Alps but that a new immigration of vectors had reached the southern most Germany west of the Alps via France. In this Extended Abstract, we summarise data from three investigations (Imo et al. submitted; Maniyar et al. submitted; Johannesen et al. submitted) that were initiated to explore the emergence and spread of stolbur tuf-type-a in Western European viticultural regions and the vector's influence on this process.

MATERIAL AND METHODS

We studied host plant fidelity and dispersal of stolbur and *H. obsoletus* in Western Europe in a co-dispersal context using comparative gene genealogies and genotype/allele frequency distributions among regional populations. Both organisms were analysed from the two putative ancestral ranges of *H. obsoletus* (relative to Germany): 1) Italy and southern France, and 2) Slovenia and Croatia; from 3) the contact area between these two lineages in Switzerland and from 4) Germany where vectors are thought to consist of two discrete host-plant related populations. Host plant fidelity was analysed at syntopic sites in each region. Sequence diversity in stolbur tuf-type-a was characterised at four genes, *Stol-11*, *SecY*, *VMP1* and *Stamp*. The genes were amplified from DNA extracted from infected *H. obsoletus* caught on stinging nettle or field bindweed. We analysed about 70 tuf-type-a isolates with 10-16 isolates per gene per region. In *H. obsoletus*, we quantified genetic variation at seven microsatellite loci (> 1000 individuals) and for partial mtDNA sequences of COII and ND1 (175 individuals). Thus, all tuf-type-a genotypes were related to *H. obsoletus* mtDNA and microsatellite genotypes.

RESULTS AND DISCUSSION

Host plant fidelity. Tuf-type-a analysed from the four regional populations was monophyletic relative to tuf-type-b at all four genes, thus confirming previous findings of host plant specificity in this strain. By contrast, host plant specificity (microsatellites) in *H. obsoletus* was observed only in German and probably northern Swiss populations. Populations in central Switzerland, southern France, Italy and Slovenia, were not differentiated relative to host plant using the markers in this study. The specificity of *H. obsoletus* in Germany was caused by genetic divergence of stinging nettle-associated populations. These populations were genetically deprived relative their syntopic German field bindweed-associated populations and at the same time more related to these than to geographically distant syntopic host-plant populations. This and the finding that German field bindweed-associated

populations are genetically as polymorphic as ancestral populations in Italy and Slovenia strongly indicates that reduced genetic diversity in German stinging nettle-associated populations was caused by a founder effect during colonisation from field bindweed to stinging nettle and not by stepping-stone dispersal during geographic range expansion of southern nettle-associated populations. This interpretation is corroborated by the distribution of mtDNA haplotypes where both German populations have the derived haplotype “aa”.

Geographic range expansion of *tuf*-type-a and its vector. The diversity of three polymorphic *tuf*-type-a genes, *SecY* (3 genotypes) *VMP1* (13 genotypes) and *Stamp* (6 genotypes), was highest in Italy and decreased significantly towards both the eastern and western range borders. The number of *tuf*-type-a *SecY*, *VMP1* and *Stamp* genotypes in Italy was 3, 10 and 6, compared to 2, 2 and 3 in Slovenia and Croatia, and 1, 2 and 1 in both Switzerland and Germany, which had identical genotypes and were practically monomorphic. Nucleotide diversity in *VMP1* was c. 20 times higher in Italy (0.00979) than in Slovenia and Croatia (0.00050) and c. 10 times higher in *stamp* (0.01028 vs. 0.00117). The general finding that genetic diversity is highest in ancestral populations corroborated the phylogenetic analysis of both *VMP1* and *Stamp* for which basal genotypes were observed in Italy. The regions Germany/Switzerland and Slovenia/Croatia did not share genotypes. The German/Swiss *tuf*-type-a genotypes were most related to French isolates in both genes. However, the phylogenetic relationships of *VMP1* and *Stamp* genotypes differed within three Italian isolates. The difference was partly caused by diversifying selection (i.e. rates) on both genes but evidence also suggests within-strain and potentially between-strain hybridisation. Positive selection and/or hybridisation might question the phylogenetic rigor of the basal position of Italian genotypes. Despite this quandary, selection was not creating random phylogenetic signals at the geographic level because the genealogical and the geographic associations were correlated.

Mitochondrial DNA diversity in *H. obsoletus* was highest in Italy and decreased towards the west in France/Switzerland as well as the east in Slovenia/Croatia, becoming monomorphic in Germany (which was originally colonised from the east). The former region was dominated by the derived haplotype “bb” (frequency 0.85) while the latter region was dominated by the derived haplotype “aa” (0.50-0.93), as mentioned above. The frequency of the two haplotypes in Italy was 0.30 and 0, respectively, while the basal haplotype “ab” (Johannesen et al. 2008) had the frequency 0.41. Demographic analyses for *H. obsoletus* in these three regional populations (Italy, France/Switzerland, Slovenia/Croatia/Germany) indicated significant population growth for the western (Fu's $F = -3.14$, $P < 0.05$) and the Italian (Fu's $F = -1.25$, $P < 0.01$) populations but not for the eastern one (Fu's $F = -0.02$). The microsatellite data supported a recent expansion of *H. obsoletus* from Italy into France and Slovenia but not into Germany (see above).

Summary. The combined data for *tuf*-type-a and its vector *H. obsoletus* showed a common origin south of the European Alps but also that the two organisms had incongruent co-dispersal histories. The emergence of *tuf*-type-a in Germany was explained by a secondary migration west of the Alps of genetically undifferentiated vectors carrying *tuf*-type-a, which were likely transferred to nettle-specialised vector populations of the eastern mtDNA lineage. The emergence of *tuf*-type-a in Germany was neither explained by resident vectors transferring *tuf*-type-a from field bindweed to stinging nettle in the course of a host-plant shift nor by primary co-migration from the resident vector's historical area of origin in Slovenia. Thus, the rapid dissemination of *tuf*-type-a in Germany depends on the vector's host shift but the vector's host-plant specialisation is independent of the introduction of *tuf*-type-a. In Western Europe as a whole, dissemination of *tuf*-type-a is likely related to a general range expansion of the vector with newly acquired *tuf*-type-a pathogens that might be of hybrid origin.

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