EPIDEMIOLOGICAL CHARACTERISTICS OF BOIS NOIR TYPE I ("GRAPEVINE YELLOWS – CURRENT DEVELOPMENTS AND UNSOLVED QUESTIONS")

M. Maixner, M. Langer and Y. Gerhard

Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Viticulture, 54470 Bernkastel-Kues, Germany. Phone: +49-6531-971821. Mail: M.Maixner@BBA.de

Boir noir (BN) is an autochtonous and widespread grapevine yellows in Europe and the Mediterranean. In Germany it is known as Vergilbungskrankheit (VK). Three different isolates of the associated phytoplasma can be distinguished by molecular traits but also by biological characteristics such as association to different natural host plants (Langer & Maixner, 2004). All three types were found in grapevine as well as in the natural vector *Hyalesthes obsoletus..* The most predominant type in Germany is type II which is associated with *Convolvulus arvensis* (bindweed). Type I was long restricted to the Palatinate vinegrowing area where it occurred with low incidence. In the course of the last few years new outbreaks of BN have been observed in different European regions, including the German viticultural areas of Wuerttemberg, Baden and parts of Middle-Rhine, were VK has been unknown or insignificant before. These new outbreaks in Germany appeared to be associated with the formerly rare type I. We therefore studied the epidemiology of this type of VK/BN in order to understand its means of spread and the reasons for the current problems.

Disease incidence

The development of VK was compared at two locations of the Middle-Rhine region. One plot (Boppard) is traditionally affected by VK of type II and no other type has been found there so far. Disease incidence is decreasing in this region for more than six years (Fig. 1). A few vines with symptoms of GY were always present at the second location (Bacharach) which is about 30 km away from the first plot. Starting with 2002, however, the incidence increased rapidly. In a distinct focus of 300 vines it rose from 25% in 2003 to 79% in 2005. At that time, 51% of the 1250 vines of the whole vineyard showed symptoms. The incidence of VK in a nearby five year old vineyard was 17% in 2005 although approximately one third of the vines had been already replanted. No such outbreak occurred at Boppard. The characterization of 62 grapevine samples taken at Bacharach revealed an infection of 54 vines (87%) by type I, of 7 vines by type II and one simultaneous infection. The viticultural region of Wuerttemberg was virtually free from GY until we found a few infected vines at a vineyard of cv. Lemberger in 2003. One year later already 30% and in 2005 more than 50% of the vines showed symptoms (Kast, 2005). All but one of 33 grapevine samples collected all through the Wuerttemberg area (provided by M. Stark-Urnau, Weinsberg) proved to be infected by type I phytoplasma. It is evident that the recent new outbreaks of VK are caused by the previously insignificant type I, whereas type II behaves endemically in the traditionally affected areas.

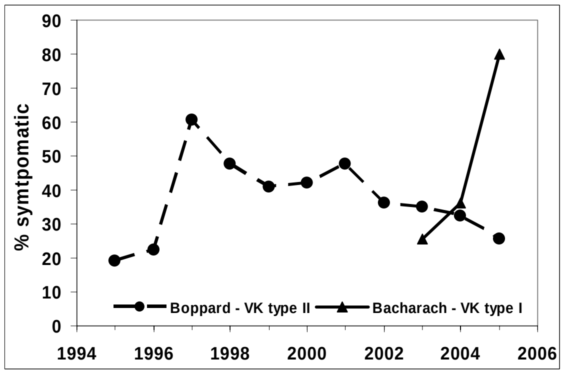

Fig. 1: Incidence of VK in a traditionally affected (Boppard) area and in a new outbreak (Bacharach).

Host plants

Regional differences in the preference of the vectoring planthopper *H. obsoletus* for host plants have long been known. *C. arvensis* is the predominant host plant in Germany (Weber & Maixner, 1998), while stinging nettle (*Urtica dioica*) is preferred, for example, in Italy (Vidano, 1988). Langer and Maixner (2004) reported that type I of the BN phytoplasma is associated to nettle and type II to bindweed, but we are still not able to consistently detect phytoplasma in nettle. Beyond this, infected nettle does not exhibit unambiguous symptoms. All locations with recent outbreaks of type I are nevertheless characterized by the presence of *U. dioica* in and/or around the affected vineyards and by the colonization of this plant by *H. obsoletus*. Progressively more vectors are now found on nettle at our long-term monitoring locations where it was not at all settled before. It is remarkable that high population densities of the vector are found on small and dispersed bushes of nettle, while large and dense stands of this plant are virtually free from the planthoppers. First sticky trap results indicate that the vectors from nettle might be more mobile than those from bindweed, thus flying from surrounding sources to the middle of vineyards.

Host specificity

The different types of VK phytoplasmas show a clear association to their specific host plants in the field. Transmission trials were carried out to test the potential of *H. obsoletus* to inoculate the major herbaceous host species with the heterologous types of phytoplasmas (Table 1). *C. arvensis, U. dioica* and *Calystegia sepium* could be inoculated with both, types I and II of VK phytoplasma although transmission to the respective homologous species was more effective. For example, 4% of *C. arvensis* became infected by type I compared to 58% infected by type II. Two nettle plants that were inoculated by 6 and 8 infected vectors, respectively, tested positive for type II two month after inoculation but negative in repeated tests thereafter. Type III whose only known alternative host is hedge bindweed *Calystegia sepium* (Langer & Maixner, 2004) could only be transmitted to this species. However, no sufficient numbers of transmission trials could be carried out so far due to the limited available number of infected vectors.

Table 1: Transmission experiments to inoculate natural host plant species with different types of VK phytoplasma.

Inoculation with VK phytoplasma (natural host)	Experimental host (infected / tested plants)		
	Urtica dioica	*Convolvulus arvensis*	*Calystegia sepium*
Type I (*U. dioica*)	4 / 8 50%	2 / 45 4%	2 / 20 10%
Type II (C. arvensis; C. sepium)	3 / 17 [a] 18% (6%) [a]	11 / 19 58%	2 / 9 22%
Type III (*C. sepium*)	0 / 2 [b]	0 / 5 [b]	2 / 5 40%

[a] two plants recovered within two weeks and stayed PCR negative thereafter

[b] test numbers too low to estimate success of transmission

Vectors

The infestation of *H. obsoletus* populations on nettle did not exceed 5% for years but has increased recently to up to 30%. The occurrence of adult vectors on nettle is delayed (Fig 2), with a maximum in mid of July compared to mid of June on bindweed. This observation corresponds well with flight data from nettle in Italy (Bertaccini *et al.*, 2003; Lessio *et al.*, 2003). Such differences in flight activity and host preference could lead to genetic isolation of the vector populations from both host plants.

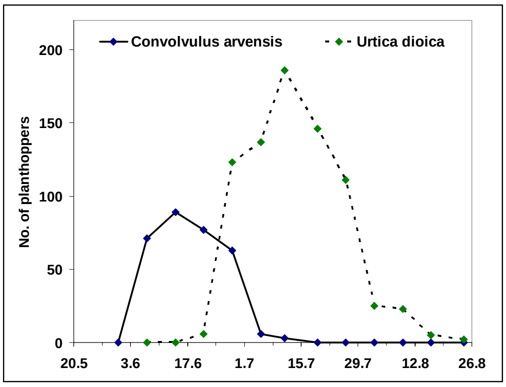

Fig. 2: Sweep net catches of *H. obsoletus* on two host plants (Bacharach, 2005).

The current outbreaks of VK type I in Germany are likely to be the result of the exploration of *U. dioica* as a new hostplant by *H. obsoletus* in this region. A high infection pressure is caused by rising population densities on nettle as well as increasing levels of infestation of these vector populations by type I phytoplasma. The reasons for this change are not yet clear. Comparative studies of the population genetics of *H. obsoletus* from different geographic regions and from different host plants are on the way. They should help to determine whether the current changes in VK epidemiology are due to local phenomena or caused by the dissemination of introduced populations of the vector and/or the BN phytoplasma.

References

Bertaccini, A., Mori, N., Botti, S., Castiglioni, A., Cavallini, G., Malossi, A., 2003. Survey on bois noir phytoplasmas spreading in vineyards of Modena province (Italy). Extended Abstracts, 14th Conference ICVG, Locorotondo, 12-17 September 2003, 89-90.

Kast, W. 2005. Personal communication.

Langer, M. & Maixner, M., 2004. Molecular characterization of grapevine yellows associated phytoplasmas of the stolbur-group based on RFLP-analysis of non-ribosomal DNA. Vitis 43, 191-199.

Lessio F., Palermo S., Tedeschi R. and Alma A.; 2003: Presence of grapevine yellows phytoplasmas vectors (Homoptera, Auchenorrhyncha) in Northwestern Italy. Extended Abstracts, 14th Conference ICVG, Locorotondo, 12-17 September 2003, 75-76.

Vidano, C., Arzone A., Alma A., and Arno C.; 1988: Auchenorrhyncha and mycoplasma diseases within the vineyard agro-ecosystem in italy. Proceedings of an international symposium Lisboa-Vila Real, Portugal, 6 to 9 June 1988, 483-488.

Weber, A. & Maixner, M., 1998. Habitat requirements of *Hyalesthes obsoletus* Signoret (Auchenorrhyncha: Cixiidae) and approaches to control this planthopper in vineyards. IOBC Bulletin 21.2, 77-78.