Population dynamics of Agrobacterium vitis in two grapevine varieties during the vegetation period* 

by

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Summary: In this work populations of Agrobacterium vitis were monitored within one year. Starting in the middle of May, the population density of A. vitis was screened every week in all parts of two-year-old Müller-Thurgau and Riesling grapevines which were freed from A. vitis by thermotherapy and inoculated with A. vitis NW90.

Every week, 5 plants of the two varieties were examined for A. vitis in new shoots, around the inoculation site, in one- and two-year-old parts of the stem, in the rootstock and in the roots. Beyond the inoculation site the A. vitis population density was too low for statistical evaluation of population dynamics. At the inoculation site a seasonal course of the A. vitis population was found in both grapevine varieties. The A. vitis population density was highest at the end of May, but little later it dropped to a low level during the summer months. A second maximum of population density was determined in October which reached nearly the same value as in spring. Population density of A. vitis correlated to physiological changes of the grapevine plant during the vegetation period. Though the population dynamics of A. vitis followed parallel courses in both grapevine varieties, differences in the population density and in the onset of the autumn increase were determined. This could be attributed to physiological differences of the two varieties. The migration of pathogenic bacteria from the inoculation site to the roots took at least 15 weeks.

The importance of A. vitis population dynamics in the epidemiological context is discussed.

Keywords: Agrobacterium vitis, population dynamics, Müller-Thurgau, Riesling, crown gall.

Introduction

Crown gall on grapevines causes substantial damages every year. The most conspicuous symptoms are galls and cracks that appear on grapevine trunks and shoots during the early summer. In European vine cultivation areas, tumours develop typically at the crown area around the transition from the rootstock to the grafted scion. On older plants they are also located in longitudinal cracks which can cover the whole grapevine. Another shaping of grapevine crown gall is sometimes found on roots, where the tumours form small, black-coloured spheres (Lephotzky 1971; Goodman et al. 1991, 1993). As reported from phytopathologists around the world, populations of a specialized biovar of the genus Agrobacterium are dominant in young tumours of grapevines during the season (Panagopoulos and Psallidas 1973; Lousser 1978; Burr and Katz 1983; Ma et al. 1985; Bien et al. 1990; Haas et al. 1991). Strains of this bacterium differ not only by their physiological characteristics from other Agrobacterium biovars (Ophele and Kurr 1990), but also by the restriction of their habitat to the grapevine plant itself (Burr and Katz 1983, 1984; Burr et al. 1987; Goodman et al. 1987). In non-vineyard soil, A. vitis populations decline during few months to non-detectable amounts (Bishop and Burr 1986) and are rarely isolated even from soils of galled vineyards (Bishop et al. 1988; Bien et al. 1990; Jager et al. 1990). Epidemiological studies of crown gall on grapevine indicate the vegetative propagation of infected cuttings as the most probable pathway for dissemination of A. vitis (Burr and Katz 1984; Bazzi et al. 1987).

Thus far, chemical and biological controls for crown gall of grapevine have been ineffective. It appears that a thorough understanding of the biology of A. vitis in vineyards is required for the development of effective controls. Little is known about the lifecycle of A. vitis in the grapevine plant itself. Though A. vitis can be isolated frequently from infected plants, nothing is known about the distribution and seasonal variation of bacterial populations in infected grapevines. Since the testing of propagation material is generally recommended for the production of pathogen-free plants (Tarbah and Goodman 1986, Goodman et al. 1986, 1987; Bishop et al. 1989; Jager et al. 1989), it is necessary to determine the distribution and population density of A. vitis in grapevine plants during the course of the year for selection of optimal sample material. The objective of this work was to observe the migration and dynamics of A. vitis populations in different grapevine varieties during the season.

Materials and methods

Sample preparation: New sprouts of two-year-old grapevines of Müller-Thurgau and Riesling were inoculated with A. vitis strain NW90 on 27 May. The plants had been treated by thermotherapy (Burr et al. 1989; Ophele.

*) In memoriam Prof. Dr. K. W. Eichhorn who passed away in January 1994 and his merits in science. Without him, this work would not have been possible.

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et al. 1990; Bazza et al. 1991) and were free of A. vitis. Every week, 5 plants of each variety were removed and samples of different parts of the plant (Fig. 1) were examined for A. vitis. 0.5 g of the sample material was pulverized in liquid nitrogen. The frozen powder was transferred to 1.5-ml Eppendorf tubes containing 1 ml of 150 mM glucose, 10 mM EDTA, 25 mM Tris-HCl, pH 8.0, and incubated for 2 h at 28 °C. The concentration of A. vitis bacteria was determined by plating 25 μl of undiluted and 25 μl of 1/100 diluted supernatant on 3 DG selective medium (Brisbane and Kerr 1983). Plates were incubated at 28 °C for 5 d.

**Results**

Since A. vitis was found very rarely outside the inoculation site, population dynamics could only be determined near the inoculation site. For A. vitis NW90 is shown in Fig. 2 (sample type B). During the first week after inoculation, A. vitis population reached a maximum of 4.5 x 10⁶ cfu/g plant material in Riesling and 2.4 x 10⁶ in Müller-Thurgau. The same way the population increased, it declined during the following weeks starting beginning of June. A low amount of A. vitis bacteria was found during the summer at about 1.4 - 1.6 x 10⁶ cfu/g plant material. At the beginning of October, the A. vitis population recovered and reached nearly same levels as in spring. This increase was first detected in Müller-Thurgau, and in Riesling two weeks later.

![Graph showing population dynamics of A. vitis NW90 in Müller-Thurgau and Riesling](image)

**Fig. 2: Population dynamics of A. vitis strain NW90 in Müller-Thurgau and Riesling**

The inoculated strain NW90 was identified by the presence of Tm4 ipt, AB3 IS868, AB3 IS869, and the absence of Tm4 IS866 and S4 vitis6b. Bacteria identified as A. vitis NW90 were scored separately for every part of the plant.

In both grapevine varieties the A. vitis population remained at a high level during the winter. Although the absolute numbers of A. vitis colonies differed between Riesling and Müller-Thurgau, the general changes took place in a similar manner.

Three to four weeks after inoculation the grapevines formed tumours producing octopine which is known as the main source for A. vitis nutrition in infected plants. However, we did not find any correlation between opine production and A. vitis population dynamics under these conditions. On the other hand, A. vitis populations in other parts of the plants remained at densities below 10⁶/g plant.
material, indicating the role of tumour compounds as attractants for A. vitis. Even with the increment of the A. vitis population in autumn, A. vitis could be found in higher amounts in other parts of the plants; for the first time A. vitis was also found beyond the inoculation site. Whereas apathogenic strains of Agrobacterium biovar 1 were detected in wooden parts of the plants all over the year, it took 15 weeks before A. vitis NW90 could be isolated from roots (Fig. 3).

Fig. 3: Comparison of A. vitis population dynamics with the glucose content in grapevine canes. The trend of the A. vitis population in grapevine is shown as mean of the trends of the populations in Riesling and Müller-Thurgau. Data for the glucose content in grapevines are not known for the time between May and late July.

Discussion

The natural habitat of A. vitis has been shown to be the grapevine plant itself (Lehoczyk 1971; Burr and Katz 1982; Jaeger et al. 1990). However, several questions remain on the ecological behaviour of this bacterial species. While epidemiological aspects are nearly resolved, the events within an infested grapevine plant and their effects to the pathogen and its host are still unknown.

In this work, populations of A. vitis strain NW90, characterized as an opine inciting strain with a small TA region and limited host range (Paulus et al. 1991), were monitored within one season. Starting in the middle of May, the A. vitis population density was screened weekly in all parts of inoculated Müller-Thurgau and Riesling grapevines. It followed characteristic courses showing two maxima in spring and autumn/winter, while the population decreased to a very low level during the summer. In Fig. 4 the A. vitis population dynamics in Riesling is plotted against the glucose content of grapevines from August to March (Eifert et al. 1961). The parallel curves indicate that A. vitis populations first respond to changes in the physiological conditions of their host. This also explains the differences in A. vitis populations between the two grapevine varieties. In Riesling, which is a late-maturing variety, the A. vitis population rose even in November. In Müller-Thurgau, the autumn increase of the A. vitis population started 2-3 weeks before, but did not reach such high levels as in Riesling.

The sugar content in grapevines may play a major role in A. vitis population dynamics. On the one hand, sugars might be important for A. vitis nutrition; on the other hand, they could serve as a sign for the disposition of the grapevine during the annual course. The role of the sugar content in grapevines for A. vitis is also reflected by the cultivar-dependent population density, which in general in Riesling was twice as high than in Müller-Thurgau. Because Riesling is known as highly frost resistant, the higher amount of A. vitis in Riesling could be due to a higher vine sugar concentration (Reuther 1975). Changes in the A. vitis population did not correlate to meteorological data like temperature or precipitation during the season. There might be an indirect influence of these conditions to A. vitis by their effect on the vitality of the grapevine host and the resulting physiological changes of the plant, which should be proved in a follow-up investigation.

Though A. vitis were concentrated around the infection site, the opine production of the tumour tissue did not influence the changes in A. vitis population. On the other hand, the finding of only few A. vitis bacteria in other parts of the plant could be due to the role of opines or other tumour compounds as attractants for pathogenic agrobacteria (Loake et al. 1988; Brisset et al. 1991). This thesis is supported by the increase of A. vitis in shoots and canes after the vegetation period when the tumour tissue died out and opine production failed (data not shown).

A. vitis was detected in roots for the first time 15 weeks after infection (Fig. 3). Because the average distance from the inoculation site to the roots was about 50-60 cm, we assume a migration velocity of ca. 4-6 mm/d for A. vitis in the vine under natural conditions. These results correspond to those measured by Brisser et al. (1991), who found migrations of 6-9 mm in 48 h for A. vitis in a semisolid medium. The migration of A. vitis in infected plants may be affected by additional factors like temperature, attractants from wounded plant cells, or seasonal conditions; these aspects require further investigation. The results presented here indicate that the spreading of A. vitis in grapevines during the season is an active process and not forced by passive transition within the vascular system of the plant.

Fig. 4: Proportion of pathogenic isolates from different grapevine parts during the season.
Whereas no pathogenic bacteria could be isolated in apical parts of the infected grapevines during the vegetation period, they could frequently be isolated from wooden parts of the plant, and from new shoots after the maturing process in autumn (data not shown). Similar results have been reported by Burr et al. (1988), who isolated A. *vitis* in shoots not before late August.

As mentioned above, the *A. vitis* population in infected grapevines was concentrated around the infection site during the vegetation period. Only few pathogenic bacteria could be isolated from other parts of the plant during that time. In fall, *A. vitis* spread into other parts of the plant (data not shown) accompanied by a general increase of the population density which stayed at a high level until the last examinations were performed in February. Since the study stopped in February, there are no data on *A. vitis* population dynamics prior to and during bud-burst.

Lehoczky (1978, 1989) and Goodman (1988, 1991) assume the root system and the suckers as reservoirs for *A. vitis* bacteria and conclude a seasonal flush of *A. vitis* into aerial parts of the grapevine forced by the root pressure in springtime. The results presented here favour the alteration of physiological conditions of the grapevine plant for changes in *A. vitis* populations during the year.

It is likely that *A. vitis* population densities have to be at a high level during the time of possible infection of injured plant cells. If one assumes that wounded plant cells will occur mostly by frost injury, *A. vitis* should build up its population prior to this event as shown here by the increase of the population towards winter. On the other hand, the flush of *A. vitis* from roots and suckers could contribute to sufficient concentrations of pathogenic bacteria at wound sites of the aerial parts of the plant.

Further investigations are necessary to elucidate the processes within the host plant prior to actual infection. Knowledge of the natural preconditions for host and pathogen leading to tumour initiation could help to understand the irregularities of crown gall events in vineyards and to introduce appropriate control measures.

**Conclusions**

We have shown that the population density of *A. vitis* in infested grapevines is influenced by physiological changes of its host during the year. The population density decreases down to a low level during the summer, increases in fall and stays at a high level during the winter. Therefore, the sampling of plant material for indexing of *A. vitis* should be restricted from October to April/May to get reliable results of possible latent infections.

Since the *A. vitis* population density differs in grapevine varieties, the quantity of *A. vitis* cells found in a plant should be proportioned to the respective grapevine variety. It was not possible to exclude, whether the size of the *A. vitis* population in the plant effects on the sensitivity and the frequency of crown gall in different grapevine varieties; this requires further investigations.

If tumour formation has already taken place, the majority of the *A. vitis* population seems to remain around the tumour location until the production of opines stops at the end of the season. The bacteria spread from infection sites into other parts of the plant by migration rates of ca. 5 mm per day. For examination of different parts of galled grapevine plants, the distance of the sample to the tumour and the time necessary for spreading of *A. vitis* bacteria into these locations should be taken in consideration for deciding on the right sampling time and material. Isolation of *A. vitis* bacteria can be difficult from canes from already galled plants (Jäger, pers. com.). Therefore, the use of plant material from galled grapevines as positive controls for indexing latent infections of *A. vitis* in propagation material should be restricted to parts near the site of the tumour to avoid false negative reactions.

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