Vitis 33, 25–29 (1994)

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

by

C. Bauer[1]), T. F. Schulz[1]), D. Lorenz[1]), K. W. Eichhorn[1]) and R. Plapp[2])

[1]) Staatl. Lehr- und Forschungsanstalt, Abteilung Phytomedizin, Neustadt/W., Germany

[2]) Fakultät für Biologie, Lehrstuhl Mikrobiologie der Universität Kaiserslautern, Kaiserslautern, Germany

S u m m a r y : 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 sommer 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.

K e y w o r d s : Agrobacterium vitis, population dynamics, Müller-Thurgau, Riesling, crown gall.

Introduction

Crown gall on grapevines causes substantial damages every year. The most conspicious 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 (Lehoczky 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; Loubser 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 (Ophel and Kerr 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; Jäger *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; Jäger *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

S a m p l e p r e p a r a t i o n : 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; Ophel

*) 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.

Correspondence to: T. F. Schulz, VITOLAB, Bahnhofstr. 46, D-74348 Lauffen a.N., Germany

et al. 1990; BAZZI *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.

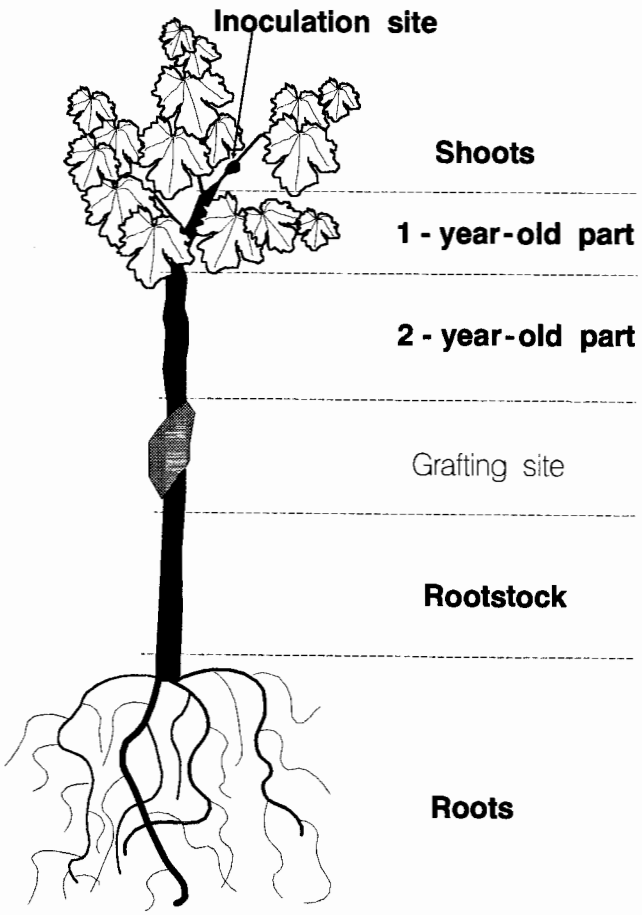

Fig.1: Inoculated grapevine. Samples were taken from shoots, from one- and two-year-old canes, from the rootstock and from the roots.

Determination of *A. vitis*: Bacterial colonies typical for *A. vitis* were taken and pured on 3 DG and YM nutrient broth. Bacteria from pured cultures were checked for pathogenicity on *Kalanchoe daigremontiana*. Test plants were wounded with sterile toothpicks and freshly cultured bacteria were subsequently placed into the wound. Inoculated test plants were monitored 4-5 weeks for tumour formation. Tumours were analyzed for the production of opines as described by OTTEN and SCHILPEROORT (1978). To determine the amount of *Agrobacterium* sp. biovar 1, the production of 3-ketolactose was examined on lactose medium (BERNAERTS and DE LEY 1963). Pathogenic isolates were additionally analyzed by detection of specific DNA sequences with the polymerase chain reaction (SCHULZ *et al.* 1993). The inoculated strain NW90 was identified by the presence of Tm4 *ipt*, AB3 IS*868*, AB3 IS*869*, and the absence of Tm4 IS*866* and S4 *vis/6b*. Bacteria identified as *A. vitis* NW90 were scored separately for every part of the plant.

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 10^6 cfu/g plant material in Riesling and 2.4 x 10^6 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 10^4 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.

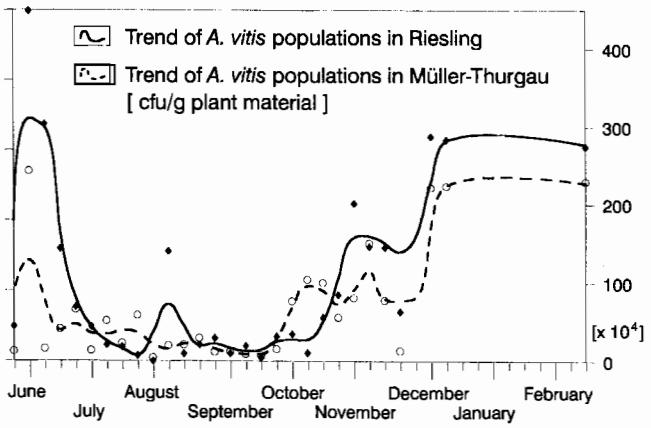

Fig. 2: Population dynamics of *A. vitis* strain NW90 in Müller-Thurgau (○) and Riesling (♦) at the inoculation site. The numbers are means of 5 samples examined weekly. The curves should indicate the trend of the *A. vitis* population and are produced by taking the previous and following value of a certain point for 50 % into account. For example: Counted bacterial cells on the first week in June (average of 5 Riesling samples): 4.5 x 10^6 bacteria per g of plant material. Previous value: 4.4 x 10^5; following value: 3.0 x 10^6. Trend of the *A. vitis* population on the first week in June: $1/2\,[(450 + (1/2\,(44 + 300))] \cdot 10^4 = 311 \cdot 10^4$ *A. vitis* bacteria per g of plant material.

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 10^3/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).

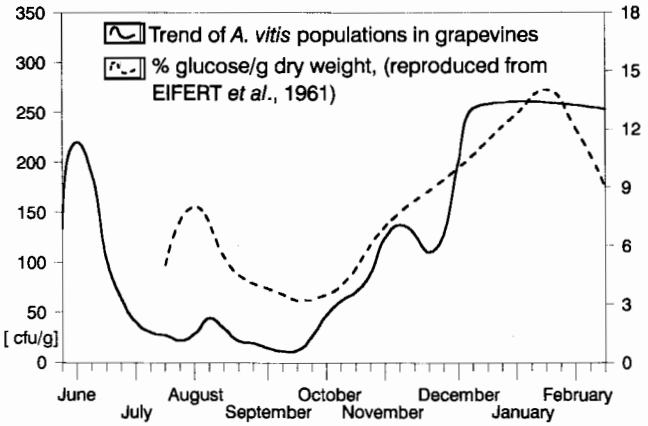

Fig. 3: Comparison of *A. vitis* population dynamics with the glucose content in grapevine canes. The trend of the *A. vitis* population in grapevine is shown as mean of the trends of the populations in Riesling and Müller-Thurgau. Data for the glucose content in grapevines are not known for the time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

Discussion

The natural habitat of *A. vitis* has been shown to be the grapevine plant itself (LEHOCZKY 1971; BURR and KATZ 1982; JÄGER *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 octopine 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.

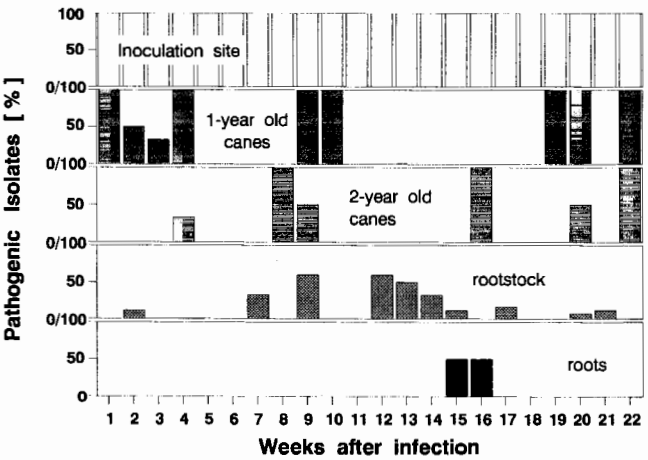

Fig. 4: Proportion of pathogenic isolates from different grapevine parts during the season.

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 BRISSET *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.

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.

References

BAZZI, C.; PIAZZA, C.; BURR, T. J.; 1987: Detection of *Agrobacterium tumefaciens* in grapevine cuttings. EPPO Bull. **17**, 105-112.

- -; STEFANI, E.; GOZZI, R.; BURR, T. J.; MOORE, C. L.; ANACLERIO, F.; 1991: Hot-water treatment of dormant grape cuttings: Its effects on *Agrobacterium tumefaciens* and on grafting and growth of vine. Vitis **30**, 177-187.

BERNAERTS, M. J.; DE LEY, J.; 1963: A biochemical test for crown gall bacteria. Nature **197**, 406-407.

BIEN, E.; LORENZ, D.; EICHHORN, K. W.; PLAPP, R.; 1990: Isolation and characterization of *Agrobacterium tumefaciens* from the German vineregion Rheinpfalz. Z. Pflanzenkrankh. Pflanzensch. **97**, 313-322.

BISHOP, A. L.; BURR, T. J.; 1986: Population dynamics of *Agrobacterium tumefaciens* biovar 3 in soil associated with host and non-host plants. [Abstr.] Phytopathology **76**, 1070.

- -; - -; MITTAK, V. L.; KATZ, B. H.; 1989: A monoclonal antibody specific to *Agrobacterium tumefaciens* biovar 3 and its utilization for indexing grapevine propagating material. Phytopathology **79**, 995-998.

- -; KATZ, B. H.; BURR, T. J.; 1988: Infection of grapevines by soilborne *Agrobacterium tumefaciens* biovar 3 and population dynamics in host and nonhost rhizospheres. Phytopathology **78**, 945-948.

BRISBANE, P. G.; KERR, A.; 1983: Selective media for three biovars of *Agrobacterium*. J. Appl. Bacteriol. **54**, 425-431.

BRISSET, M.-N.; RODRIGUEZ-PALENZUELA, P.; BURR, T. J.; COLLMER, A.; 1991: Attachment, chemotaxis, and multiplication of *Agrobacterium tumefaciens* biovar 1 and biovar 3 on grapevine and pea. Appl. Environ. Microbiol. **57**, 3178-3182.

BURR, T. J.; KATZ, B. H.; 1983: Isolation of *Agrobacterium tumefaciens* biovar 3 from grapevine galls and sap, and from vineyard soil. Phytopathology **73**, 163-165.

- -; - -; 1984: Grapevine cuttings as potential sites of survival and means of dissemination of *Agrobacterium tumefaciens*. Plant Dis. **68**, 976-978.

- -; - -; BISHOP, A. L.; 1987: Populations of *Agrobacterium* in vineyard and nonvineyard soils and grape roots in vineyards and nurseries. Plant Dis. **71**, 617-620.

- -; - -; - -; MEYERS, C. A.; MITTAK, V. L.; 1988: Effect of shoot age and tip culture propagation of grapes on systemic infestations by *Agrobacterium tumefaciens* biovar 3. Amer. J. Enol. Viticult. **39**, 67-70.

- -; OPHEL, K.; KATZ, B. H.; KERR, A.; 1989: Effect of hot water treatment on systemic *Agrobacterium tumefaciens* biovar 3 in dormant grape cuttings. Plant Dis. **73**, 242-245.

EIFERT, J.; PANCZEL, M.; EIFERT, A.; 1961: Änderung des Stärke- und Zuckergehaltes der Rebe während der Ruheperiode. Vitis **2**, 257-265.

GOODMAN, R. N.; 1988: The systemic reservoir of *Agrobacterium tumefaciens* in suckers of the grape cv. Chancellor. [Abstr.] Phytopathology **78**, 1609.

- -; BUTROV, D.; TARBAH, F.; 1987: The occurrence of *Agrobacterium tumefaciens* in grapevine-propagating material and a simplified indexing system. Amer. J. Enol. Viticult. **38**, 189-193.

- -; GRIMM, R.; FRANK, M.; 1991: Rebenunterlagen und Mauke. Schweiz. Z. Obst- Weinbau **127**, 66-68.

- -; - -; - -; 1993: The influence of grape rootstocks on the crown gall infection process and on tumor development. Amer. J. Enol. Viticult. **44**, 22-26.

- -; TARBAH, F. A.; BUTROV, D.; 1986: Rapid indexing of grape cuttings for freedom from crown gall with an inexpensive water displacement pressure chamber. [Abstr.] Phytopathology **76**, 1070

HAAS, J. H.; ZVEIBIL, A.; ZUTRA, A.; TANNE, E.; MANULIS, S.; 1991: The presence of crown gall of grape incited by *Agrobacterium tumefaciens* biovar 3 in Israel. Phytoparasitica **19**, 311-318.

JÄGER, J.; FRANKMANN, T.; LORENZ, D. H.; EICHHORN, K. W.; 1989: The indexing of grapevine propagating material latently infested by *Agrobacterium tumefaciens* biovar 3 - isolate identification by gas chromatography of whole cell fatty acids. Wein-Wiss. **44**, 177-182.

- -; LORENZ, D.; PLAPP, R.; EICHHORN, K. W.; 1990: Latent occurrence of *Agrobacterium tumefaciens* biovar 3 in grapevine (*Vitis vinifera* L.). Wein-Wiss. **45**, 14-20.

LEHOCZKY, J.; 1971: Further evidences concerning the systemic spreading of *Agrobacterium tumefaciens* in the vascular system of grapevines. Vitis **10**, 215-221.

- -; 1978: Root-system of the grapevine as a reservoir of *Agrobacterium tumefaciens* cells. Proc. 4th Int. Conf. Plant Path. Bact., Angers, France, 239-243.

- -; 1989: Inoculation experiment on trellised grapevines with *Agrobacterium tumefaciens* for study the process of crown gall disease. Acta Phytopathol. Entomol. Hung. **24**, 283-291.

LOAKE, G. J.; ASHBY, A. M.; SHAW, C. H.; 1988: Attraction of *Agrobacterium tumefaciens* C58C^1 towards sugars involves a highly sensitive chemotaxis system. J. Gen. Microbiol. **134**, 1427-1432.

LOUBSER, J. T.; 1978: Identification of *Agrobacterium tumefaciens* biotype 3 on grapevine in South Africa. Plant Dis. Reptr. **62**, 730-731.

MA, D.; YINGRUI, L.; JUAN, Z.; WANGNIAN, X.; 1985: Biotype and plasmid type of *Agrobacterium tumefaciens* isolated from the crown gall of grapevine in North China. Acta Microbiol. Sinica **25**, 45-53.

OPHEL, K.; KERR, A.; 1990: *Agrobacterium vitis* sp. nov. for Strains of *Agrobacterium* biovar 3 from grapevines. Intern.. J. Syst. Bacteriol. 40, 236-241.

- -; NICHOLAS, P. R.; MAGAREY, P. A.; BASS, A. W.; 1990: Hot water treatment of dormant grape cuttings reduces crown gall incidence in a field nursery. Amer. J. Enol. Viticult. **41**, 325-329.

OTTEN, L. A. B. M.; SCHILPEROORT, R. A.; 1978: A rapid micro scale method for the detection of lysopine and nopaline dehydrogenase activities. Biochim. Biophys. Acta **527**, 497-500.

PANAGOPOULOS, C. G.; PSALLIDAS, P. G.; 1973: Characteristics of Greek isolates of *Agrobacterium tumefaciens* (E.F. SMITH & TOWNSEND) CONN. J. Appl. Bacteriol. **36**, 233-240.

PAULUS, P.; CANADAY, J.; OTTEN, L.; 1991: Limited host range Ti plasmids: recent origin from wide host range Ti plasmids and involvement of a novel IS element, IS*868*. Mol. Plant-Microbe Interact. **4**, 190-197.

REUTHER, G.; 1975: Physiologische Kriterien der Klimaresistenz als sortenspezifische Merkmale. Angew. Bot. **49**, 75-91.

SCHULZ, T. F.; LORENZ, D.; EICHHORN, K. W.; OTTEN, L.; 1993: Amplification of different marker sequences for identification of *Agrobacterium vitis* strains. Vitis **32**, 179-182.

TARBAH, F. A.; GOODMAN, R. N.; 1986: Rapid detection of *Agrobacterium tumefaciens* in grapevine propagating material and the basis of an efficient indexing system. Plant Dis. **70**, 566-568.

Received August 12, 1993