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STUDY ON THE REACTION OF SOME BULGARIAN AND INTRODUCED GRAPE VARIETIES TO CROWN GALL *AGROBACTERIUM* SPP

Two experimental trials were carried out in order to determine the reaction of different varieties to crown gall. Aartificial inoculations were performed using two strains of A. vitis and one A. tumefaciens. The reaction of different varieties was reported on by counting the plants with tumors and measuring the size and the weight of tumors. The results showed that among widespread local and introduced varieties there are not resistant to inoculation with strains of A. vitis. Some of the varieties do not form tumors when infected with a strain of A. tumefaciens, and react with formation of relatively smaller size and weight of tumors with inoculation with strains of A. vitis. The susceptibility of vine varieties to crown gall, under field conditions, depends also on their reaction to low temperatures, a factor which has a great importance for the development of crown gall, than their immunity to strains of Agrobacterium spp.

***Key words*:** grapevine, crown gall, *Agrobacterium*, cultivar susceptibility.

The crown gall of grapevine is known as one of the most important bacterial disease all over the world. The disease is common in almost all vine-growing countries, but it causes serious damages on grapevines in geographic latitudes characterized by more severe and cold climate (Burr and Otten, 1999). The disease influences not only the yield, but also the vineyards persistence, hence causes economic loses to the nurserymen, the grapevine growers and the wine industry as a whole.

The beginning of grapevine crown gall appearance in Bulgaria is unknown, but it was reported for the first time by Valachev (1902) and later by Malkov (1903) (Malenin, 1980). In Bulgaria the disease is known under the name “bacterial cancer”, because of its similarity to human and animal tumors. The most severe spread of the disease has been noted in the 1960s and 1970s of the past century, which has incited more extensive studies of the causative agent. More recently the disease was studied and isolates from Bulgarian vineyards were phenotypicaly characterized and genotyped by PCR analyses (Genov, 2012).

The grapevine crown gall is predominantly caused by the phytopathogenic *Agrobacterium vitis* (Ophel and Kerr 1990) = *Rhizobium vitis* (Ophel and Kerr 1990) Young et al. (2001), and more rarely - *A. tumefaciens* (Smith and Townsend, 1907) Conn (1942). The representatives of *Agrobacterum* cause crown gall on over 600 plant species belonging to more than 300 genera and 90 families (De Cleene and Deley, 1976). The causal agent can subsists in the vascular system of the grapevine (Lehoczky, 1968; Malenin, 1970) and in plant residues from diseased grapevines in soil (Burr and Katz, 1984)

At present there are no efficient means of chemical protection and control. The systemic nature of the disease and its latent states favor its spread by propagative material (Malenin, 1970, Burr and Katz, 1984). In this connection, the studies in the last years have been directed to development of sufficiently reliable methods for diagnostics, identification of the causal agent *Agrobacterium* spp. (Bini et al. 2008; Eastwell et al.; 1995), as well as of schemes for control and production of certified grapevine planting material. One of the strategies for prevention of the disease suggests the planting of relatively resistant varieties as a means of tackling the disease, but experience shows that these varieties are often ignored by the producers of quality wines at the expense of highly susceptible varieties such as Merlo, Cabernet Sauvignon and Chardonnay (Burr et al., 1998). Even in 1910 Hedgcock tested vine varieties for susceptibility to crown gall (Malenin 1982, Burr et al., 1998). Similar studies on the reaction of varieties of *Vitis* spp. were conducted at the end of the past century (Malenin, 1973, 1982, Ferreira & van Zyl, 1986)

Stover et al (1997) examined the susceptibility of the 47 genotypes to a range of different strains of *A. vitis* and found none of the tested genotypes were immune to crown gall. There was a significant interaction in the system "strain x genotype". *V. amurensis* has been particularly susceptible to one of the strains of *A. vitis*. Some genotypes resistant to certain strains, but formed large tumors when inoculated with different strains. Therefore, the susceptibility of the vine to crown gall is determined by genetic determinants of the plant and the pathogen (Burr et al., 1998).

One of the directions in the breeding program of the IVE - Pleven, under way since the seventies of the past century is obtaining varieties with improved resistance to stress by abiotic (mainly low winter

temperatures) and biotic (phylloxera, mildew, powdery mildew and crown gall) factors (Ivanov, 2009, Kostadinova et al., 2007). The objective of this study was to determine the reaction of some Bulgarian and introduced grape varieties to crown gall (*Agrobacterium* spp.)

Material and methods.

Two experimental trials were performed to determine the reaction of different varieties to crown gall. The first was determination of reaction of 21 grape varieties to inoculation with two strains of *A. vitis*. For this purpose, one-bud cuttings of the varieties were potted. The plants were grown in a growth chamber WTB-Binder Labortechnik at 20-26 ˚ C, relative humidity 80% and photoperiod (High / Low) 16/8h. Three plants of each variety were inoculated by pricking the shoots in a rich smear of a 48 hour culture of each of the two *A. vitis* strains (IVE-2 and IVE-4) and two plants (controls) who were prick without smears bacterium (Burr et al., 1983). The results were recorded visually in a three-level scale for 6 weeks after inoculation. According to the presence and size of the generated tumors were taken following indications: (-) did not form tumors (+) small (1-2 mm), (+ +) medium (2-5 mm), and (+ + +) large (> 5 mm).

The second experiment was aimed studding the reaction of 20 Bulgarian and introduced grape varieties, and it was held on one-bud rooted cuttings, grown in a greenhouse at a temperature of 20-26˚ C, relative humidity 70-80% and natural lighting. The artificial inoculation was performed by the above described methods, as were two strains of *A. vitis*, IVE-2 and IVE-13, and one of *A. tumefaciens* - IVE-203/1. The reaction of different varieties was reported on by counting the plants with tumors and measuring the size and the weight of tumors. The infected grapevines were recorded and biometrical characterizations of the tumours were made by determining their sizes and weight. The significance of differences between the average values of tumour size and weight by variants was determined by means of t-test.

Results and discussion.

The studies of the reaction of 21 varieties to infection with two pathogenic strains of *A. vitis* (Table 1) showed that with the exception of a variety Rkatziteli, others react with tumor formation. Varieties differ in the size of tumors formed by table varieties form larger tumors than the wine varieties. This dependence is manifested in both strains used, except for variety Muscat Ottonel in which the response to strain IVE-4 was negative. Although the use of other methods, the results for the reaction of varieties are one-way with studies of Malenin (1973, 1982) on the susceptibility of the same variety.

Table 1

Reaction of different vine varieties to infection with *A. vitis* strain IVE-2 and strain IVE-4

№	Variety	Reaction to strain		Control*
		IVE-2	IVE-4	
1	Italia	+++	+++	-
2	Bolgar	+++	+++	-
3	Cardinal	+++	+++	-
4	Pleven	+++	+++	-
5	Rusalka	+++	+++	-
6	Senzo	+++	+++	-
7	Muscat Hamburg	++	++	-
8	Pomoriyski Biser	++	++	-
9	Gamza	++	++	-
10	Buket	++	++	-
11	Chardonney	++	++	-
12	Mavrud	++	++	-
13	Dimyat	++	++	-
14	Ugni Blan	++	++	-
15	Naslada	+	+	-
16	Merlo	+	+	-
17	Riseling Rain	+	+	-
18	Cabernet Sauvignon	+	+	-
19	Sauvignon	+	+	-
20	Muscat Othonel	+	-	-
21	Rkatziteli	-	-	-

* control – puncture without inoculation with bacteria; size of tumors: (-) no tumor formation (+) small (1-2 mm); (++) medium (2-5 mm); (+++) large; (> 5 mm)

"The same trend

Results of the second experiment showed that all tested varieties form tumors at the site of inoculation with strains IVE-2 and IVE-13 of *A. vitis*. Variety Rubin, Muscat Ottonel, Marselan, Petit Manseng and Chardonnay do not form tumors when infected with strain IVE-203/1 of *A. tumefaciens*.

With regard to the average size of the tumors, differences were found between studied variants, but they are too small and significant only between individual varieties. An exception is the cultivar Italia, wherein the mean particle size (7.67 mm) is distinguished significantly from the tumors of all other plants infected with strain IVE-2 (Table 2 and Fig. 1). The same trend was observed in the tumor weight score (Table 2). In IVE-13 strain of *A. vitis* not observed distinct differences in the response of varieties. The average size and weight of tumors are greater of the variants infected IVE-2, which indicates a different reaction for the varieties depending on the strain (Tables 2 and 3, Figure 2).

Table 2

Average tumor size at the inoculation place with *Agrobacterium* spp

№	Variety	Strain		
		IVE-2 (mm)	IVE-13 (mm)	IVE-203/1 (mm)
1	Bolgar	2.90 ± 1.01	5.47 ± 0.38	1.87 ± 0.08
2	Brestovitsa	3.37 ± 0.17	5.10 ± 0.80	3.70 ± 0.86
3	Italia	7.67 ± 0.38	4.67 ± 0.77	1.07 ± 0.08
4	Palieri	2.00 ± 0.76	4.43 ± 1.53	n. t.
5	Pleven	3.00 ± 0.32	6.40 ± 0.38	1.47 ± 0.54
6	Super Ran Bolgar	2.50 ± 0.46	3.23 ± 0.88	0.93 ± 0.47
7	Muscat Hamburg	3.07 ± 0.24	4.05 ± 1.35	4.07 ± 0.97
8	Sauvignon blanc	1.30 ± 0.61	3.73 ± 1.05	0.00 ± 0.00
9	Chardonnay	2.73 ± 0.09	4.27 ± 0.64	0.00 ± 0.00
10	Muskat Ottonel	1.33 ± 0.81	2.37 ± 0.22	0.00 ± 0.00
11	Pinot Noir	1.73 ± 0.55	5.10 ± 1.78	1.13 ± 0.43
12	Rubin	0.93 ± 0.62	2.10 ± 0.70	0.00 ± 0.00
13	Viognier	3.60 ± 0.71	n. t.	3.00 ± 0.10
14	Cabernet Franc	4.40 ± 0.40	2.83 ± 0.23	2.20 ± 0.32
15	Carmener	3.03 ± 0.49	3.63 ± 0.53	2.13 ± 0.19
16	Colombard	2.07 ± 0.30	4.67 ± 0.37	1.63 ± 0.64
17	Marselan	1.70 ± 0.95	5.43 ± 3.14	0.00 ± 0.00
18	Pinot Blanc	1.80 ± 0.30	2.93 ± 0.17	2.65 ± 0.32
19	Petit Verdot	3.57 ± 0.64	4.47 ± 0.45	1.95 ± 0.66
20	Petit Manseng	1.57 ± 0.41	n. t.	0.00 ± 0.00

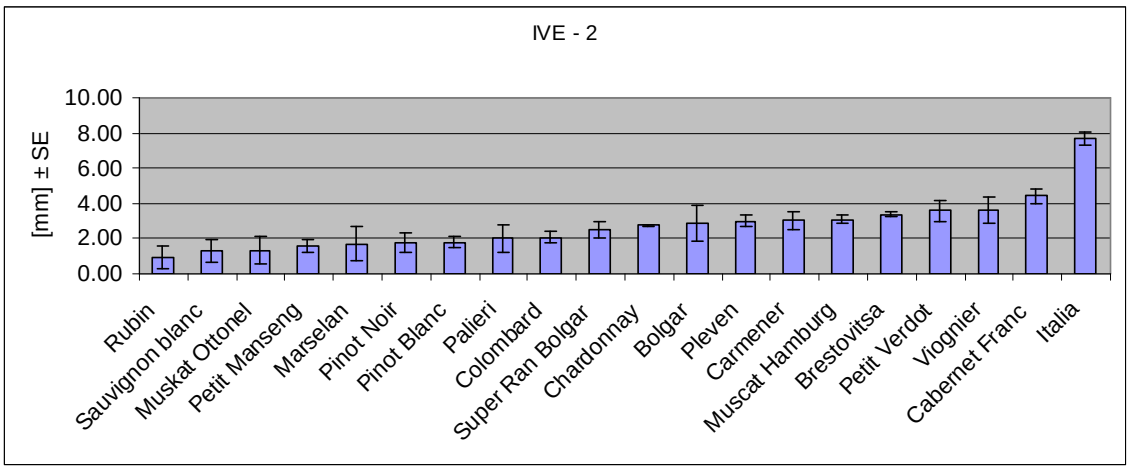

Fig. 1. Average tumor size (mm) in variants infected with strain IVE-2.

Average of 4 repetitions ± standard error (SE); n.t. - not tested

The results of the experiments for susceptibility of vine varieties to the cause of crown gall showed that none of the tested varieties is completely immune to the disease under artificial inoculation Variety Muscat Ottonel and Rubin, that did not form tumors when infected with strain IVE-203/1 (Figure 3) react with the formation of relatively small size and weight tumors when infected with strains IVE-2 and IVE-13 . The trend of our previous study, that the experimental plants of table varieties form a relatively large size of the tumors than wine varieties was confirmed.

Table 3

Average tumor weight at the inoculation place with *Agrobacterium* spp

№	Variety	Strain		
		IVE-2 (mg)	IVE-13 (mg)	IVE-203/1 (mg)
1	Bolgar	13.00 ± 8.73	113.60 ± 13.25	1.87 ± 0.08
2	Brestovitsa	3.37 ± 0.17	158.40 ± 38.63	3.70 ± 0.86
3	Italia	176.97 ± 14.06	120.40 ± 30.36	1.07 ± 0.08
4	Palieri	38.63 ± 21.14	168.37 ± 72.37	n. t.
5	Pleven	9.55 ± 1.55	202.33 ± 53.81	1.47 ± 0.54
6	Super Ran Bolgar	7.70 ± 2.06	29.63 ± 5.05	3.13 ± 2.21
7	Muscat Hamburg	25.43 ± 4.34	70.10 ± 1.20	4.07 ± 0.97
8	Sauvignon blanc	16.95 ± 13.25	21.83 ± 2.49	0.00 ± 0.00
9	Chardonnay	22.70 ± 4.62	28.13 ± 8.79	0.00 ± 0.00
10	Muskat Ottonel	27.30 ± 19.59	8.53 ± 2.19	0.00 ± 0.00
11	Pinot Noir	0.00 ± 0.00	122.43 ± 99.46	1.13 ± 0.43
12	Rubin	0.00 ± 0.00	23.70 ± 4.53	0.00 ± 0.00
13	Viognier	3.60 ± 0.71	n. t.	3.00 ± 0.10
14	Cabernet Franc	20.13 ± 3.01	31.93 ± 4.33	2.20 ± 0.32
15	Carmener	8.17 ± 4.52	60.10 ± 20.57	2.13 ± 0.19
16	Colombard	5.80 ± 1.87	71.33 ± 7.72	1.63 ± 0.64
17	Marselan	1.70 ± 0.95	26.4 ± 12.4	0.00 ± 0.00
18	Pinot Blanc	1.80 ±0.30	26.07 ± 5.02	2.65 ± 0.32
19	Petit Verdot	3.57 ± 0.64	44.30 ± 15.58	1.95 ± 0.66
20	Petit Manseng	1.57 ±0.41	n. t.	0.00 ± 0.00

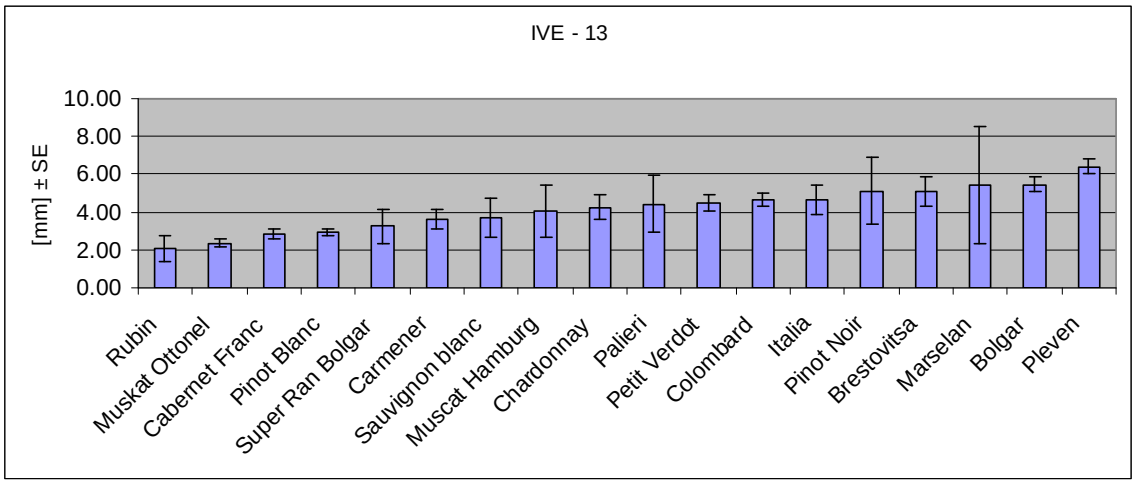

Fig. 2. Average tumor size (mm) in variants infected with strain IVE-13.

Conclusions.

The results for the susceptibility of some of the varieties under controlled conditions coincide with the observed relatively low spread of crown gall in these varieties. The conducted experiments showed that in

our widespread local and introduced varieties there are not resistant to inoculation with strains of *A. vitis*. The varieties Muscat Ottonel and Rubin, that do not form tumors when infected with a strain of *A. tumefaciens*, react with formation of relatively smaller size and weight of tumors with inoculation with strains of *A. vitis*. Among the newly bred varieties are not observed completely resistant genotypes also, but they are differently susceptible to the artificial inoculation depending on the species of the used strain (Genov and Ivanov, 2008).

The immune response of the varieties, under controlled conditions, depends on both the genotype of variety and the genotype of the strain used for inoculation. However, the susceptibility of vine varieties to crown gall, under field conditions, depends on their reaction to other factors, the most important of which is climate (Malenin, 1972; Genov, 2007). For example the variety Muscat Kaylashki form tumors under controlled inoculation in greenhouses, but under natural conditions show a relative resistance to the disease because of its increased resistance to low temperatures. Most likely, the tolerance of such varieties to low temperatures is a factor which has a great importance for the development of crown gall, than their immunity to strains of *Agrobacterium* spp. Using these varieties except that it can prevent direct crop losses due to damage from low temperatures, may be limited and prerequisites for the development of crown gall and death of whole vines.

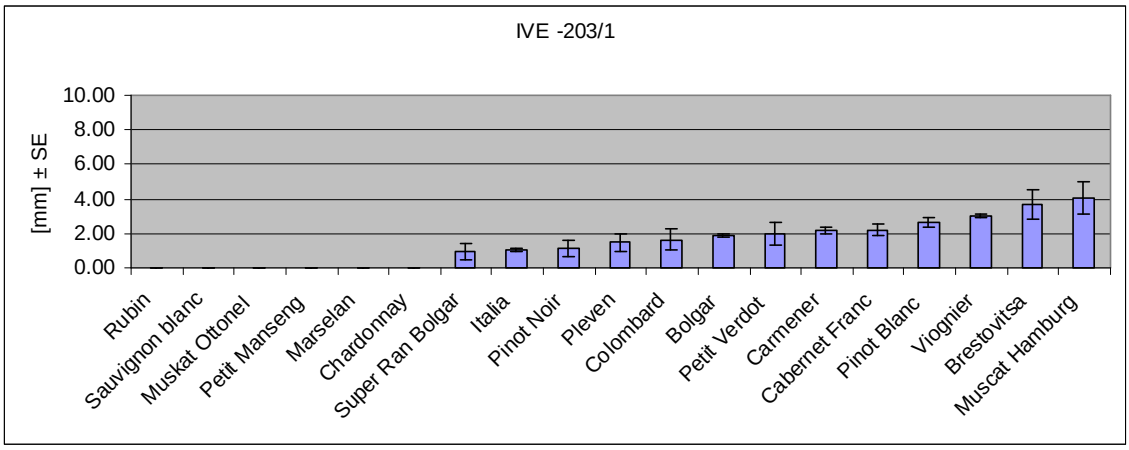

Fig. 3 Average tumor size (mm) in variants infected with strain IVE-203/1.

Reference

1. Bini F., Kuczmog A., Putnoki P.,Otten L., Bazzi C.,Burr T., Szegedi E., 2008. Novel pathogen-specific primers for the detection of *Agrobacterium vitis* and *Agrobacterium tumefaciens. Vitis* 47, 181-189 .
2. Burr T., Bazzi C., Sule S, Otten L. 1998. Crown gall of grape: biology of *Agrobacterium vitis* and the development of disease control strategies. *Plant Disease* 82:1288–97
3. Burr T. and Katz B. 1984. Grapevine cuttings as potential sites of survival and means of dissemination of *Agrobacterium tumefaciens*. Plant Disease 68:976-978.
4. Burr T. and Otten L. 1999. Crown gall of grape: Biology and Disease Management. Annu.Rev. Phytopathol. 37:53-80.
5. De Cleene M. and Deley J. 1976. The host range of crown gall. Bot. Rev. 42:389-460.
6. Eastwell J.C., Willis L.G. and Cavileer T.D. 1995. A rapid and sensitive method to detect *Agrobacterium vitis* by polymerase chain reaction. Plant Disease 79:822-827.
7. Ferreira J., van Zyl F., 1986. Susceptibility of grape-vine rootstocks to strains of *Agrobacterium tumefaciens* biovar 3. S. Afr. J. Enol. Vitic. 7:101-104.
8. Genov, N., 2007. Influence of some factors on the development and spatial distribution of crown gall in the vineyard. *Proceedings of Scientific Confference* "Stable development of the Viticulture and Enology based of the knowledge", 29-30 Aug 2007 Pleven, 124-131
9. Genov, N., 2012. Studies on Crown gall of grapevine in Bulgaria and the causative agents *Agrobacterium* spp. PhD thesis, Plovdiv, 132p..
10. Ivanov, M. 2009. Ampelographic characteristic of new breed table varieties with increased resistance to stress factors. Lozarstvo i vinarstvo, 2, 44-47
11. Kostadinova, M., T. Lyubenova, N. Genov, 2007. Reaction of table grape cultivars grown in Bulgaria to diseases and pests. Lozarstvo i vinarstvo, № 6, Annex - 21-30.

12. Lehoczky J. 1968. Spread of *Agrobacterium tumefaciens* in the vessels of the grapevine after natural infection. Phytopathology. 63:239-246
13. Malenin I. 1970. Study on the spreading of *Agrobacterium tumefaciens* in the vessels of grapevine and formation of secondary tumors. Horticultural and viticultural science, vol. 7, 5:127-133.
14. Malenin, I., 1972. Influence of the ecological factors on the infection of grapevine with bacterial cancer under field conditions. Gradinarska i Lozarska Nauka., т. IX, № 6, 129-135
15. Malenin, I. 1973. Studies on the resistance of some varieties of bacteria to cancer. Horticultural and viticultural science, so X, № 1, 101-104.
16. Malenin I. 1980. Geographic spread of bacterial cancer of grapevine. Bulletin of IVE, vol. VI: 11-22.
17. Malenin, I. 1982. Studies on the resistance of some varieties of *Vitis vinifera*, direct cultivars and rootstocks to bacterial cancer. Reports of the Second National Symposium on immunity of plants, Plovdiv - 1982, Volume III. 217-225
18. Stover E, Swartz H., Burr T. 1997. Crown gall formation in a diverse collection of Vitis genotypes inoculated with *Agrobacterium vitis*. Am. J. Enol. Vitic. 48:26–32
19. Young, J., Kuykendall, L., Martı´nez-Romero, E., Kerr, A. & Sawada, H., 2001. A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et al. 1998 as new combinations: *Rhizobium radiobacter, R. rhizogenes, R. rubi, R. undicola,* and *R. vitis. Int J Syst Evol Microbiol* 51, 89–103

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Исследование реакции некоторых болгарских и интродуцированных сортов винограда к бактериальному раку Agrobacterium SPP.

Два экспериментальных опыта были проведены с целью изучения реакции различных сортов к бактериальному раку. Искусственная инокуляция проводилась с использованием двух штаммов A. vitis и одного A. tumefaciens. Реакция различных сортов определялась путем подсчета растений с опухолями, измерениями размеров и веса опухолей. Результаты показали, что среди местных и интродуцированных сортов нет устойчивых к инокуляции штаммом A. vitis. Некоторые из сортов не образуют опухолей при заражении штаммом A. tumefaciens, и реагируют образованием относительно меньшими размерами и весом опухоли с инокуляцией штаммом A. vitis. Восприимчивость сортов винограда к бактериальному раку в полевых условиях зависит также от их реакции на низкие температуры - фактор, имеющий большое значение для развития болезней, чем их иммунитет к штамму Agrobacterium SPP.

Ключевые слова: виноградная лоза, бактериальный рак, Agrobacterium, сорт восприимчивости.