Scientific Papers, Series B, Horticulture, Vol. LVI, 2012
ISSN Online 2286-1580, ISSN-L 2285-5653

PREVENTING AND LIMITING THE SPREAD OF CROWN GALL IN VINEYARDS

Diana E. VIZITIU (BĂLĂȘOIU)[1], Liviu C. DEJEU[1], Ion RĂDULESCU[2], Carmen F. POPESCU[2]

[1] University of Agronomic Sciences and Veterinary Medicine - Bucharest, Faculty of Horticulture, 59 Mărăşti Blvd., Sector 1, Postal Code 011464, Bucharest, Romania, vizitiud@yahoo.com, liviudejeu@gmail.com
[2] National Research and Development Institute for Biotechnology in Horticulture, Stefanesti, Bucharest Street, No. 37, Postal Code 117715, Tel/Fax: 0248/266814, e-mail: radulescuion56@yahoo.com, carm3n_popescu@yahoo.com, Arges, Romania

Corresponding author email: vizitiud@yahoo.com

Abstract

Crown gall, produced by Agrobacterium vitis and Agrobacterium tumefaciens is a very dangerous disease that significantly reduces the growers' income. The pathogen attacks the roots, trunks and arms of vines, reduces plant vigour and finally decreases yield. Systemic survival of bacteria in vines and its spread into tissues plants represent the main difficulties to control this disease in vineyards. The aim of this review was to present the main feature of this pathogen and preventive measures recommended to be applied in vineyards and grapevine nurseries aiming to reduce its spread. In order to avoid bacteria invasion by grafting or cuttings is compulsory to detect and identify the presence of the pathogen in plants and soil (nurseries, plantations). The phytosanitary inspection in mother plantations is the safest procedure to prevent the spread of crown galls of grapevines. After removing the infected plants and their burning, is absolutely necessary to disinfect the soil to destroy the survival bacteria.

Keywords: *Agrobacterium tumefaciens, bacteria, grapevine nurseries, Rhizobium vitis, soil*

INTRODUCTION

The disease occurs most frequently by the appearance of small swellings on the root, on the stem near the soil line, or on aerial portions of the plant. Young tumors (resemble often with the callus tissue that results from wounding) are soft, somewhat spherical and white to cream colored even rose in some cases (Photo1-6). In time, the shape of tumors changed becoming irregular and also the color turning to brown or black. Tumors may be connected to the host surface by a small piece of tissue, or may appear as a swelling of the stem, not distinctly separated. Several tumors may appear on the same plant and may fall from the surface of the plant completely or partially, but may occur again in the same area, season after season. Other tumors become persistent, and every year, become increasingly larger. [2], [20], [36]. After the removing vines from the vineyard, the pathogenic bacteria survive in soil and plant debris for at least 2 years. So, *Agrobacterium* cells remain viable and active in soil and could infect the new planting material [6]; [14].

Photo 1. Vine roots necrosis produced by *Agrobacterium vitis* [6]

Photo 2. Crown gall of grape [4]

Photo 3. Crown gall formed on canes of grapevines [24]

Photo 4. Crown gall causing rough-surfaced swelling on a grape trunk [22]

Photo 5. Galls at graft unions [5]

Photo 6. *Agrobacterium vitis [51]*

Taxonomy

The pathogen that causes crown gall of grapevines belongs to the genus *Agrobacterium,* family *Rhizobiaceae*, the order *Rhizobiales*, class *Alphaproteobacteria*, Division *Proteobacteria*, kingdom *Bacteria*.

The most known species of *Agrobacterium* are: *Agrobacterium tumefaciens, Agrobacterium vitis, Agrobacterium radiobacter, Agrobacterium rubi, Agrobacterium larrymoorei*, *and Agrobacterium rhizogens.* Among these, the most distributed specia are *A. tumefaciens* (synonym biovar 1, *Rhizobium radiobacter), A. rhizogens* (synonym biovar 2; *Rhizobium rhizogens)* and A. *vitis* (synonym biovar 3, *Rhizobium vitis)* [1], [26]. Strains of *Agrobacterium* are classified in the three biovars based on their in vitro predominant nutrition with different carbohydrates and other biochemical tests applied for their detection.

The other species *Agrobacterium rubi* (*Rhizobium rubi*) and *Agrobacterium larrymoorei (Rhizobium larrymoorei)* are considered as minor pathogen [3], [50].

For the first time, the bacteria was isolated in 1897 by Cavara F. at the Laboratorio di Botanica del Recherci Instituto Forestale di Vallom Drosa in Naples, Italy and called it *Bacterium tumefaciens*. After ten years, Smith E. F. and Townsend C.O. in the United States isolated the same bacterium from *Chrysanthemum* and called it *Phytomonas tumefaciens*. The same authors subsequently changed the name in *Agrobacterium tumefaciens* [24].

Lately, some strains of *Agrobacterium* have been used in genetic engineering for gene transfer. Thus, *Agrobacterium*-mediated genetic transformation has become the preferred

method to generate transgenic plants [35]. Great progress has been made for *Agrobacterium*-mediated transformation of maize, wheat, sorghum, rice, cotton, soybean or ornamental plants as a key element in the process of varietal improvement [28].

The pathogen

Agrobacterium is a Gram-negative bacterium, rod-shaped, non-spore-forming, motile, having one to six peritrichous flagella. At the infected plants, virulent strain of *Agrobacterium* causes abnormal cell proliferation which results in tumor formation. At an optimum growth temperature of 25–28˚C, the bacteria metabolize a wide range of mono- and di-saccharides and salts of organic acids.

Crown gall produced by *Rhizobium vitis* is the most important bacterial disease of the grapevine in the world [6], [9], [39] [47] It is considered as the predominant tumorigenic specie-specific to *Vitis* spp. [48], but has been occasionally isolated from other hosts, such as *Actinidia* [40]. *Agrobacterium vitis* appears to be unique among pathogenic *Agrobacterium* species in being associated with roots decay symptoms [7].

Rhizobium rhizogenes was isolated from tumors that developed at the grapevines in Hungary [43] and Spain [29].

As a general aspect, the infection is a four-step process: injury the host plants; bacterial cells attach to the surface plant cells in wounded areas; Ti plasmid of bacteria is transferred into the host cells; the Ti-DNA integrates into the host cell genome. So, is well established that the plant injury is an essential step for the transformation process and also for attachment of bacteria to the plant surface cells, necessary for tumor initiation (fig. 1).

Infected planting material is the main source of pathogens. In the mother plantations dedicated for producing canes could be present infected plants without noticeable symptoms from which, material is harvested for grafting. Bacteria survive in canes, during the grafting process and also during growing seasons of vineyards, from which new pathogenic cells infect the surrounding soil. The bacteria can remain dormant for several years or cause galls to the grafting point and in areas where plants have been injured.

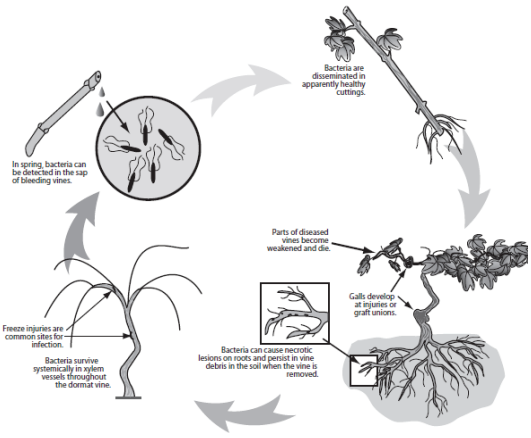

Fig. 1. Disease Cycle of Grape Crown Gall [6], [14]

The period of incubation for bacteria cells into the plant tissues varies, depending on plant age and environmental conditions. At a temperature of 20-25°C the incubation period is of 13-14 days, while at a lower temperature of 10-15°C are necessary 27-28 days for bacteria cell incubation. Infection potential is increased by a higher relative humidity of 80-90%, and decreased by light intensity. The disease is also favored by wet and compact soils, frost damage of plants, nitrogen fertilizers, low affinity between scion and rootstock, injury produced by hail, or attack of nematodes [41].

The *A. tumefaciens* bacteria cells are naturally present in the rhizosphere of woody plants and also of numerous herbaceous weeds. So, this pathogen is very easy spread during cultivation practices or disseminated as infected plant material with the soil, or with cultivation equipment when galls are removed manually with the same cutting tools used in pruning.

Biological control

Some strains of *A. tumefaciens* are sensitive to the agrocin antibiotic produced by *A. radiobacter*, a closely related bacterium that does not infect plants.

Knowing that *A. radiobacter* produced an analog of the opine, agrocinopine A (Agrocin-84) that inhibits DNA replication and also bacterial cell growth of *A. tumefaciens*, this feature was used for biological control [24]. So, a 1:1 ratio with cells of *A. tumefaciens* and A. *radiobacter* strain K84 suspended in water is used to treat seeds, seedling or cuttings before

planting. The Agrocin-84 acts only as a preventative treatment to protect any wound sites against pathogenic invasion, not to cure bacterial infections.

In the last years, has proposed utilization of non-pathogenic *A. vitis* strain F2/5 for biological control of virulent strains [10], [6]. This strain, like *Rhizobium leguminosarum* bv *trifolii*, produces an antibiotic (trifolitoxin - TFX) toxic to many *A. vitis* strains *in vitro*, reducing the number of galas, their size and in some cases killing pathogenic bacteria. Nonpathogenic *A. vitis* strains F2/5 may be applied on the injured tissues of the grapevines to prevent appearance of crown gall [23].

Another nonpathogenic strain of *A. vitis* (VAR03-1) was used by Kawaguchi and his team [25] as biological control agent against crown gall of grapevine plants. According to their data, by applying 1:1 ratio of pathogen/non-pathogenic strain suspension at tomato, sunflower and vines, were obtained a lower incidence of number of tumors. The authors considered this method as an effective one and recommended it to control crown gall of grapevine caused by tumorigenic *A. vitis, A. rhizogenes*, and *A. tumefaciens*.

Strain HX2 of *Rahnella aquatilis* was reported by Chen et al., [12] as a potential biological control agent for crown gall of grapevine. Antibacterial substance produced by this strain has a bactericidal effect against the virulent strain of *A. vitis*, both in vitro and in vivo conditions. *Rahnella aquatilis* HX2 was isolated from soil samples and has demonstrated to have a significant inhibitory effect in tumor growing at grapevines. By immersing the basal ends of grape cuttings in HX2 cell suspension was induced inhibition or completely prevented crown gall formation in plant material artificial infected with the virulent strain *A. vitis* K308. Further studies in vineyards revealed a normal plant growing and no microflora degradation on soil as result of HX2 cell suspension treatment [13].

For the control of *A. tumefaciens* pathogen were tested biological preparations of paurin and tumarin, obtained from *Pseudomonas fluorescens* cultures. Before planting, vines are dipped in solutions of paurin and tumarin for 10-15 minutes, or their roots are sprayed with these biological compounds to prevent further infection [27]

Chemical control

Studies on *Agrobacterium* pathogen infection in grapevines proved for the moment an ineffective effect of chemical compounds upon bacterial cells inside plant tissues, but benefic effects could be obtained by using different chemical solutions for treatment the infected soil. For example, it was established that antibiotics and copper compounds kill bacteria from galas, but do not destroy any pathogenic bacteria from plant tissues. As result, the pathogens are surviving and maintained through vascular system.

In the past was used methyl bromide with and without chloropicrin for pre-plant soil fumigation aiming to control of soilborne pathogens and weeds. Due to its dangerous effect on ozone layer of the upper atmosphere, the methyl bromide was forbidden since 2005. The researchers identified as alternatives to methyl bromide treatment a combinations of 1,3-dichloropropene, chloropicrin, and metam sodium. Other chemicals alternatives have been also proposed to replace the methyl bromide. One of these is acrolein (2-propenal), which has been formulated and registered for use as an aquatic herbicide in irrigation systems. This product was proved to have an efficient effect to control the *A. tumefaciens* in soil. It is also used to control microorganisms and bacteria in oil wells, liquid hydrocarbon fuels, cooling-water towers and water treatment ponds [18], [19]. The interaction between plants and pathogens was studied by Pu and Goodman [37] in vineyards established with indexed *Agrobacterium* free grapevines plants, but on *Agrobacterium*-infested vineyard soil. After 16 months, the bacteria were detected in grapevine plants. In early spring, when the sap begins to flow into the trunk was detected a high level of sap infection, revealing that the primary source of pathogens was the soil. So, was tested the influence of fumigation of the soil with Vorlex. The repeated analysis with the same trunks proved the decrease of initial infection level and also a lower frequency of tumours development as results of fumigation.

Other chemical substances, such as creosote-based compounds, copper-based solutions, and

strong oxidants (sodium hypochlorite) were tested to eradicate the crown galls. Every time the effect of applied treatment was a transient one. Eradication procedures with chemicals proved to be very laborious, need an adequate application, at a proper time, but with short time effects. Moreover, these type of treatments are costly and with unpredictable effect to the environment. The superficial treatments are ineffective against systemically infected plants. Generally, at the moment, chemicals are rarely used for control crown gall in grapevine plantation [24].

Possibilities to prevent the bacteria spread

A general acceptance is that there is no cure for crown gall disease in grapevine. Different methods were tested to remove the infected plants, or the surrounding infested soil, and parts of plants roots. Further are presented the main roles recommended to prevent the spread of *A. tumefaciens* bacteria:

- Grapevine propagation material must be provided from healthy mother plantations;
- Avoid setting up plantations in soil infected with virulent Agrobacterium spp. and / or with nematodes; do not establish a new plantation in clay soils, with poor drainage, in cold areas, wet, and northern exposure, or with low in nutrient or organic matter [46];
- Use frost resistant grapevine varieties for the new vineyards;
- Avoid the establishment of vineyards with susceptible varieties to crown gall, such as: 'Afuz Ali', 'Ceaus roz', 'Ceaus roşu', 'Italia', 'Merlot', 'Muscat de Hamburg', 'Regina viilor', 'Cardinal', 'Perla de Csaba', 'Cabernet Sauvignon', 'Chardonnay', 'Riesling italian', 'Baco Noir', 'Cabernet Franc', 'Chancellor', 'Gewürtztraminer', 'Limberger', 'Pinot Blanc', 'Pinot Gris', 'Pinot meunier', 'Pinot noir', 'Sauvignon Blanc' [22], [41];
- Use for the establishment of new vineyards less susceptible cultivars to crown gall, such as: 'Feteasca regala', 'Furmint', 'Coarna neagra', 'Pinot gris', 'Zghihara de Husi', 'Cascade', 'Catawba', 'Concord', 'Delaware', 'Einset Seedless', 'Foch', 'Fredonia', 'Ives', 'Steuben', 'Vanessa', 'Ventura' [22], [33], [41];
- Use also as rootstocks only resistant or less susceptible varieties to crown gall, such as: 'Riparia Gloire', 'C 3309', 'SO 4', '101-14 Mgt', 'NAZ1', 'NAZ2', 'NAZ4', 'NAZ5' and 'NAZ6' [21], [31], [42], [43]. These rootstocks do not prevent infection but they have resistance to transformation. Rootstocks can greatly affect the severity of crown gall infection of grapevine [6]; [38]; [45];
- Strong recommendation is crop rotation in the vines nursery;
- Before planting, vines have to be carefully selected, sorted and excluded infected plants; in order to assure a good plant nutrition is recommended to supply the soil with nutrients and lime to avoid vine stress due to poor nutrition or low pH;
- Mud for sink vines roots before planting will be prepared with fungicide that provide protection against infections with bacteria, for example: copper sulphate 1%, Captadin 50 PU 1% Topas EC-0,025% [32] or Kasumin, Potassium salt 0,5%, Rovral, Mikal, Saprol in higher doses than to prevent fungal pathogens [49];
- All the equipments and tools used for cuttings and forcing the grapevines will be disinfected with formalin 2-5%, sodium hypochlorite 1-3%, before and during working;
- Disinfection of canes before storage;
- The planting material (scions and rootstocks) used for multiplication could be treated before grafting by: a) bathing for 15 minutes in formalin solution 0,3-1%; b) immersion in hot water at a temperature of 50-52°C, for 30-60 minutes [8] [11]; [16]; [30]; c) spraying or bathing with Chinosol W 0,5% or Solvochim 0,5%; d) dipping for 10-15 minutes in Captan 0,2% or copper sulphate 1% solution;
- After planting is important to avoid mechanical injury of the plants; for winter period is recommended to protect the trunks against frost, because any injury of the trunk as result of cold effect represent a gateway for bacteria entrance;
- Avoid as much as possible a supplementary nitrogen fertilization because could represent a food source for pathogenic bacteria cells;
- A supplementary potassium fertilization is recommended to improve vines resistance to cold, and also to obtain a better resistance of the canes to virulent species of Agrobacterium [6];
- Use the double or multiple trunk system of training. This system may be useful for

minimizing losses due to crown gall; if one trunk is infected, remove it. The remaining trunk can be pruned leaving a full number of buds until the second trunk can be renewed;

- Adopt a low or high management forms on the arms with periodic replacement;
- Avoid a prolong vegetation which is detrimental to cane maturation;
- Burying the mature grapevines canes for the winter period to avoid injury due to frost;
- Apply a correct treatment and in good time for Plasmopara viticola and Uncinula necator pathogens for a complete maturation of the wood [15];
- Treat the soil for nematodes presence; the nematodes injure the roots and stems of the grapevines and in the same time favor the penetration of bacteria into plant tissues [46];
- Apply specific treatment to kill all larvae and insects with chewing device, because they are passive carriers of the bacterium;
- From May to August are recommended treatments with products based on copper, such as: Turdacupral 50 PU 0,4 %; Funguran OH 50 WP 0,3%; Champion 50 PU 0,3%; Captadin 50 PU 0,2%; Captan 50WP 0,2% - to stop the proliferation of bacteria [32];
- Avoid cold water irrigation.
- Avoid plants mechanical injury during cultural practices;
- Remove the infected plants from nurseries and mother plantations;
- Diseased plant material will be collected and put into sealed packages to prevent the spread of infection to other plants or surrounding soil [32];
- All infected plants, or their debris will be burned;
- The soil have to be disinfected by steam; 2% formalin (10 l/m² especially in greenhouses) or leave gaps in the plantations for at least 3 years; the same procedure is applied for soil in greenhouses, with steam (82˚C for at least 30 minutes) or fumigants after removing all plant material. For soil fumigant are carefully followed all the manufacturer's directions and precautions;
- Weed control by mechanical work or/and with total herbicides is strongly recommended;
- Use the biological control methods to protect plants from possible infections with Agrobacterium tumefaciens - Galltrol-A, Nogall, Diegall and Norbac 84C before planting [17];
- Utilization products based on Bacillus subtilis for: disinfection of scion and rootstock strings for the production of grafted vines [32]
- Careful disinfection of spaces for grafting and forcing; tools used for cutting, or soil grinding in vine nursery have to be also disinfected periodically to prevent infection of healthy plants.

CONCLUSIONS

The best way to prevent the spread of crown galls in the vineyards is to use healthy planting material and to avoid soil contamination with the bacteria. So, in a mother plantations as source of producing scion and rootstock canes is compulsory to perform regularly phytosanitary inspections aiming to detect infected individuals, to remove these plants and then to destroy them. So, only healthy vine material, free of *Agrobacterium* virulent strains will be maintained in the vineyards and used as planting material. With grapevine, like any other crop plant, the most effective way is to use very efficient procedures to control and prevent the diseases. If the pathogen is detected in vineyards with valuable planting material are indicated chemicals and biological products treatments. These will stop, or reduce the spread of bacteria in the surrounding areas, but will not destroy the pathogen.

ACKNOWLEDGEMENTS

These results were obtained through POS-DRU/88/1.5/S/52614 project. The authors are thankful to the National Research and Development Institute for Biotechnology in Horticulture for the technical support.

REFERENCES

[1] Bautista-Zapanta J., Arafat H.H., Tanaka K., Sawada H., Suzuki K., 2007. *Variation of 16S-23S internally transcribed spacer sequence and intervening sequence in rDNA among the three major Agrobacterium species*. Microbiol. Res., 2009;164(6):604-12.

[2] Bobeş I., Comes I., Drăcea A., Lazăr A., 1972. *Fitopatologie*. Editura Didactică şi Pedagogică, Bucureşti, p.408.

[3] Bouzar H., Jones J.B., 2001, *Agrobacterium larrymoorei sp. nov., a pathogen isolated from aerial*

tumours of Ficus benjamina. Int J Syst Evol Microbiol 51, 1023–1026.
[4] Burr T.J., Crown Gall Disease – *Viticultural Information*. Crown Gall Disease – University of California, UC Integrated Viticulture, http://ucanr.org/sites/intvit/?uid=1&ds=351.
[5] Burr T.J., Bazzi C., Brissett M.N., 1991. *Crown gall, Agrobacterium tumefaciens* (Smith and Townsend) Conn. Integrated Pest Management.
[6] Burr T.J., Bazzi C., Sule S., Otten L., 1998. *Crown Gall of Grape: Biology of Agrobacterium vitis and the Development of Disease* Control Strategies. Plant Disease 82:1288-1297.
[7] Burr T.J., Bishop A.L., Katz B.H., Blanchard L.M., C. Bazzi, 1987. *A Root-specific decay of grapevine caused by Agrobacterium tumefaciens* and *Agrobacterium radiobacter biovar 3*. Phytopathology 77: 1424–1427.
[8] Burr T.J., Ophel K., Katz B.H., Kerr A., 1989. *Effect of hot water treatment on systemic Agrobacterium tumefaciens Biovar 3 in dormant grape cutting*. *Plant Disease* 73(3), 242–245.
[9] Burr T.J., Otten L., 1999. *Crown gall of grape: biology and disease management*. Annu Rev Phytopathol 37:53–80.
[10] Burr T.J., Reid C.L., 1994. *Biological Control of Grape Crown Gall with Non-tumorigenic Agrobacterium vitis Strain F2/5*. Am. J. Enol. Vitic. 45:2:213-219.
[11] Burr TJ, Reid CL, Splittstoesser DF, Yoshimura M., 1996. *Effect of heat treatments on grape bud mortality and survival of Agrobacterium vitis in vitro and in dormant grape cuttings*. *American Journal of Enology and Viticulture* 47, 119–123.
[12] Chen F., Guo Y.B., Wang J.H., Li JY, Wang H.M., 2009. *Biological control of grapevine crown gall: purification and partial characterisation of an antibacterial substance produced by Rahnella aquatilis strain HX2*. Plant Pathology, Volume 124, Number 3, 427-437.
[13] Chen F., Guo Y.B., Wang J.H., Li JY, Wang H.M., 2007. *Biological control of grape crown gall by Rahnella aquatilis HX2*. Plant Disease 2007 Vol. 91 Nr. 8 p. 957-963
[14] Creasap J.E., Burr T.J., 2006. *Grape Crown Gall. Integrated Pest Management*. http://nysipm.cornell.edu/factsheets/grapes/diseases/crown_gall.pdf.
[15] Dami I.E., 2005. *Mildwest grape production guide*. Ohio State University Extension - http://ohioline.osu.edu/b919/pdf/b919.pdf
[16] Dula T., Kölber M., Lázár J., Szegedi E. 2007. *Production of healthy grapevine propagating material: pathogens and methods*.
http://www.oiv2007.hu/documents/viticulture/Szegedi_OIV_2007_text.pdf
[17] Fravel D., Larkin R., 1996. *Availability and application of biocontrol products*.
http://wenku.baidu.com/view/4cfa12c34028915f804dc2c7.html
[18] Gerik J.S., Wang D., 2008. *Dose response of Agrobacterium tumefaciens to soil fumigants*. Page 110 in: Proc Annu. Intl. Res. Conf. Methyl Bromide Alternatives Emissions Reductions. http://mbao.org/2008/Proceedings/110GerikJmbao2008.pdf.
[19] Gerik S.J., Wang D., 2008. *Control of Agrobacterium tumefaciens with soil fumigants*. USDA/ARS San Joaquin Valley Agricultural Sciences Center, Parlier, CA, http://mbao.org/2008/110Gerik.pdf.
[20] Gheorghieş C., Cristea S, 2002. *Fitopatologie*. Vol.I, Editura Ceres, Bucuresti.
[21] Goodman R.N., Drimm R., Frank M., 1993. *The influence of grape rootstocks on the crown gall infection process and tumor development*. Am. J. Enol. Viti. 44:22-26.
[22] Hartman J., 2007. *Grape Crown Gall. Plant Pathology Fact Sheet*. www.ca.uky.edu/agcollege/plantpathology/ext_files/PPFShtml/PPFS-FR-S-11.pdf.
[23] Herlache T.C., Triplett E.W., 2002. *Expression of a crown gall biological control phenotype in an avirulent strain of Agrobacterium vitis by addition of the trifolitoxin production and resistance genes*. *BMC Biotechnology*, 2:2doi:10.1186/1472-6750-2-2, www.biomedcentral.com/1472-6750/2/2.
[24] Kado C.I., 2002. *Crown gall. The Plant Health Instructor*, DOI:10.1094/PHI-I-2002-1118-01, http://www.apsnet.org/edcenter/intropp/lessons/prokaryotes/Pages/CrownGall.aspx.
[25] Kawaguchi A., Inoue K., Sawada H., Nasu H., 2008. *Studies on diagnosis and biological control of grapevine crown gall*. Journal of Plant Pathology, 90 (2, Supplement), S2.81-S2.465.
[26] Kuykendall L.D., Young J.M., Martínez-Romero E., Kerr A., Sawada H., 2005. *Order Rhizobiales (new) Family Rhizobiaceae Genus Rhizobium. In Brenner, Krieg, Staley and Garrity (Eds), The Alpha-, Beta-, Delta- and Epsilonproteobacteria, The Proteobacteria, Part C, Bergey's Manual of Systematic Bacteriology, 2nd. Ed., Vol. 2*, Springer, New York, NY, pp. 324-340.
[27] Lemanova N.B., 1990. *Biological preparations for protection of grapes against bacterial canker*. Sadovodstvo i Vinogradarstvo 1990 No. 11, 30-32
[28] Li R., Qu R. 2010. *High throughput Agrobacterium-mediated switchgrass transformation. Biomass and Bioenergy*. doi:10.1016/j.biombioe.2010.11.025 http://www.cropsci.ncsu.edu/personnel/Qu_Ron/documents/Ruyu_B&B10.pdf
[29] López M.M., Palacio-Bielsa A., González-Abolafio R., Santiago R., Salcedo C.I., Penyalver R., 2008. *Tumorigenic Agrobacterium rhizogenes (biovar 2) isolated from grapevine in Spain*. Plant Pathol, 57:367.
[30] Mahmoodzadeh H., Nazemieh A., Majidi I., Paygami I., Khalighi A., 2003. *Effects of Thermotherapy Treatments on Systemic Agrobacterium vitis in Dormant Grape Cuttings*. Journal of Phytopathology, Volume 151, Issue 9, 481–484.
[31] Mahmoodzadeh H., Nazemieh A., Majidi I., Paygami I., Khalighi A., 2004. *Evaluation of crown gall resistance in Vitis vinifera and hybrids of Vitis spp*. Vitis 43 (2), 75–79, http://www.vitis-vea.de/admin/ volltext/e049975.pdf.
[32] Mihu G. et. al., 2010. *Solutii biologice de combatere a bacteriozelor patogene la plantele horti-viticole*. Editura Do-MinoR: Rawex Coms, Bucuresti.
[33] Naqvi SAMH, 2004. *Diseases of Fruits and Vegetables*. vol. 2, Kluwer Academic Publishers.

[34] Pacurar D.I., 2009. *Model organisms – a journey from the dawn of biological research to the post-genomic era Rom. Biotechnol. Lett.*, Vol. 14, No. 1, 4087-4094.
[35] Pacurar D.I., Thordal-Christensen H., Păcurar M.L., Pamfil D., Botez C., Bellini C., 2011. *Agrobacterium tumefaciens: From crown gall tumors to genetic transformation.* Physiological and Molecular Plant Pathology, Volume 76, Issue 2, 76-81.
[36] Pârvu M. 2010. Ghid *practic de fitopatologie*, Ediţia a III-a, Editura Presa Universitară Clujană, p. 386.
[37] Pu XA, Goodman R.N. 1993. *Effects of Fumigation and Biological Control on Infection of Indexed Crown Gall Free Grape Plants***.** Am. J. Enol. Vitic. 44:3:241-248.
[38] Ronald S.J., 2008. *Wine Science*. Editura Academic Pres, Ediţia a III-a.
[39] Sawada H., Ieki H., Takikawa Y., 1990. *Identification of grapevine crown gall bacteria isolated in Japan*. Ann Phytopathol Soc Jpn 56, 199–206.
[40] Sawada H., Ieki H., 1992. *Crown gall of kiwifruit caused by Agrobacterium tumefaciens in Japan*, Plant. Dis., 76-212.
[41] Severin V., Dejeu L., 1994. *Bolile şi dăunătorii viţei de vie*. Editura Ceres Bucuresti, p.124.
[42] Stover E.W., Swartz H.J., Burr T.J. 1997. *Crown gall formation in a diverse collection of Vitis genotypes inoculated with Agrobacterium vitis*. *Am. J. Enol. Vitic*. 48, 26-32.
[43] Stover E.W. 1993. *Resistance to crown gall in Vitis: Studies directed toward the identification of crown gall resistant rootstocks*. Maryland University, College park. Ph.D. Diss.
[44] Sule S. 1978. *Biotypes of Agrobacterium tumefaciens in Hungary*. J Appl Bacteriol 44, 207-213.
[45] Sule S., Burr T.J. 1998. *The influence of rootstock resistance to crown gall (Agrobacterium spp.) on the susceptibility of scions in grape vine cultivars*. Plant Pathology 47, 84-88.
[46] Sule S, Lehockzy J., Jenser G., Nagy P., Burr T.J. 1995. *Infection of grapevine roots by Agrobacterium vitis and Meloidogyne hapla.* J. Phytopathology 143, 169-171.
[47] Szegedi E., Bottka S., Mikulás J., Otten L., Sule S. 2005. *Characterization of Agrobacterium tumefaciens strains isolated from grapevine*. Vitis 44 (1), 49–54.
[48] Thies K.L., Griffin D.E., Graves C.H., Hegwood C.P., 1991. *Characterization of Agrobacterium isolates from muscadine grape*. Plant Dis. 75, 634–637.
[49] Tomoiagă L., 2011. *Şi viile încep să sufere de cancer.* Lumea Satului Nr.1, 1-15 Ianuarie 2011, http://www.lumeasatului.ro/si-viile-incep-sa-sufere-de-cancer_l918.html.
[50] Young J.M., Kerr A., Sawada H. 2005. *Genus II. Agrobacterium*. In: Bergey's manual of Systematic Bacteriology. Second Edition, Vol. 2 The Proteobacteria, Part C, Springer, New York, 340–345.
[51] h*ttp://*www.calspl.com/pdf/grapevine_crowngall.pdf (20.08.2011).