GRAPE RESEARCH NEWS

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Promising New Methods for Controlling Crown Gall in Grapes

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Introduction

Crown gall is a serious disease of grapevines worldwide and causes substantial losses of nursery stock in many other plant species. The most obvious symptom of this disease in grapevines is a fleshy callus-like swelling (a gall) which is most often observed in the crown region of the trunk, just above the soil line (Figure 1).

The pathogens which cause crown gall are in the genus *Agrobacterium.* More than 200 plant species are reported to be susceptible to crown gall and this disease is economically important in a wide variety of crops. For most crops other than grape, the greatest losses occur in the nursery where plants having gall symptoms must be destroyed. Among the crops most affected by crown gall are grapes, stone fruits, roses, pears, and walnuts.

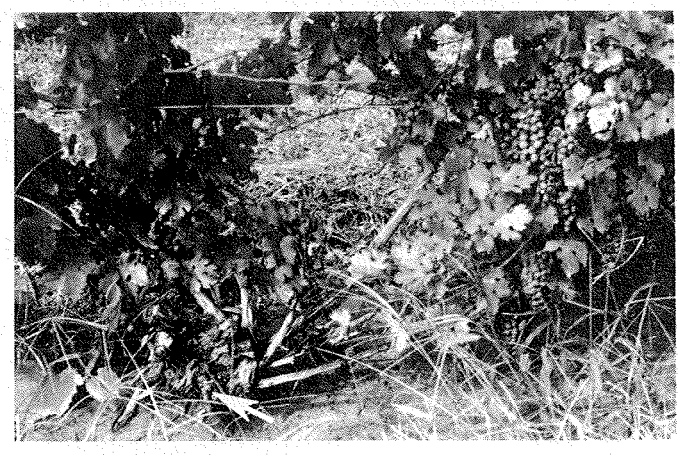

Figure 1. Crown gall in grapevines is characterized by fleshy callus-like swellings (galls) in the crown region of the trunk, just above the soil line (arrow).

Symptoms of this disease are observed only after the crown gall bacterium inserts some of its own genes (DNA) into the plant cell nucleus. Transfer of bacterial genes is triggered by exposure of the pathogen to wounded plant cells and can only occur if cells are actively dividing. These bacterial genes cause high levels of plant hormones to be produced. High levels of plant hormones affect plant development resulting in the disorganized growths recognized as galls. After bacterial genes are inserted into the plant cell nucleus, they are replicated along with plant genes as cells divide. As a result, gall tissue can continue to grow even if all of the pathogen is killed. The soft gall tissue often desiccates during dry or cold periods and may slough off, but new galls may develop the following year at the same site. A single plant cell transformed by pathogen genes may be enough to produce a crown gall, although in grapes it appears that galls tend to be bigger when more cells receive bacterial genes directly from the pathogen. As an aside, this ability of *Agrobacterium* to move genes into plant cells is used to "genetically engineer" plants after the disease-causing genes are replaced by genes that may improve plant performance.

Crown Gall in Grape

Crown gall in vineyards is most serious when young plants are affected. The growth of gall tissue can partially girdle young vines and interfere with vine growth. The result is weak plants that are less productive and often less likely to

survive additional stress. Reduction in yield and vine growth is greatest in vines that are more than 50% girdled by crown gall. However, one study, performed in California, found that vines with only a small amount of gall tissue can perform as well as healthy vines. When mature plants develop crown gall, the resulting damage is usually less significant since gall development at this stage will not disrupt the older vascular tissue already present in the plants.

The strains of *Agrobacterium* associated with a great majority of grape crown galls are distinct from pathogens causing crown gall in other crops. There are several reasons why this is important to viticulturists. The strains which cause crown gall in most plant species, other than grapes, are common in soils worldwide. These strains are so widespread that crown gall is thought to develop after soil bacteria are splashed onto plant wounds. In contrast, no detectable levels of the grape crown gall pathogen, designated *Agrobacterium vitis*, have been found on sites where grapes have not been grown. In addition, *Agrobacterium vitis* (or *AV*) has been shown to exist systemically (throughout plant tissues) within many apparently healthy vines, including some nursery stock, so it is now thought that infected grape nursery stock is the principal source of crown gall inoculum in new vineyards. Pathogenic *Agrobacterium* has been shown to move systemically in a few host species, but this appears to be especially common in grapevines. When crown gall-infected vines are introduced into a vineyard, *AV* then infests the soil by living in association with roots and sloughed-off gall tissue. These bacteria can survive in the soil for many years, even after living vines are removed. Uninfected susceptible vines can become readily infected by the soil-borne pathogen. Unlike other *Agrobacterium* strains, the grape crown gall pathogen produces lesions on grape roots that may assist entry of the bacterium into the vines.

Our current understanding of crown gall in grapes is that *AV* is likely to be systemically established in the vine either before the plant leaves the nursery or after exposure to soil-borne *AV* in a previously infested vineyard. In regions where vines are winter-damaged, wounding from cold injury appears to trigger the transfer of bacterial genes into the plant cells, so that more galls develop in the spring following severe winter temperatures.

A discussion of controlling crown gall in grapes must include two topics: 1) techniques to identify or produce "clean" vines, which may permit avoidance of crown gall in new vineyard sites, and 2) methods for dealing with a vineyard that is already infested with *Agrobacterium vitis*.

AV-free Vines

Crown gall may be dramatically reduced in new vineyard sites by simply using *AV*-free nursery stock, since it appears that significant populations of grape *Agrobacterium* aren't found in non-vineyard sites. Historically, nursery stock with obvious crown gall has been discarded, but the systemic nature of grape *Agrobacterium* permits infected material to pass visual inspection. Techniques have been developed to screen nursery stock for *Agrobacterium* infection. Typically, water is flushed through shoots and then plated on bacterial culture medium that is selective for *AV*. Unfortunately, this method is not very sensitive and material must be indexed repeatedly to confidently declare it pathogen-free. We have found that freezing shoots prior to testing greatly enhances the recovery of *Agrobacterium* using this flushing technique. If this procedure simulates events that occur in the vineyard, it is possible that movement of *AV* from older infected wood into the current season's growth may be enhanced by exposure to freezing. This suggests that the *AV* levels in propagation wood may be greatly reduced by collecting canes prior to the onset of freezing temperatures.

Several techniques have been studied for eliminating *AV* from grapevines. Tom Burr, in the Plant Pathology Department of the Agricultural Experiment Station at Geneva, has shown that shoot-tip culture eliminates this pathogen from grapevines. A few commercial organizations are using this method to produce crown gall-free nursery stock. Chateau Saint Michelle in Washington has been planting *AV*-free vinifera produced through shoot-tip culture and has greatly reduced the incidence of crown gall on new planting sites.

Another method that may be perfected is the heat treatment of dormant cuttings to eliminate *AV*. Immersion of cuttings in 50°C (122°F) water for 60 minutes kills more than 95% of the *AV* present, but so far no reliable procedure has been developed to completely eradicate *AV* without also killing the cuttings. Pretreatment of dormant canes to increase heat tolerance may resolve this problem.

Use of *AV*-free material may also reduce the risk of crown gall when vines are replanted into infested sites. However, experiments have shown that susceptible vines exposed to soil *AV* can become readily infected. What can be done to protect clean vines in a replant situation?

Controlling Crown Gall with Protective Strains

One interesting approach is pre-inoculation of plants with non-pathogenic *Agrobacterium* which then will produce antibiotics to prevent growth of other bacteria. This technique has been used for many years for stone fruit nursery stock; however, the protective strain used for stone fruits is not effective against all crown gall pathogens and does not control *AV*. Strains that prevent *AV* growth in the laboratory have been identified and several groups are testing these strains. A non-pathogenic *AV* strain isolated in South Africa, F2/5, has been shown to reduce the growth of most pathogenic *AV* strains when tested in the laboratory. Greenhouse studies by Tom Burr indicate that treatment with this strain usually reduces crown gall severity when grapevines are inoculated with pathogenic *AV*.

Controlling Crown Gall with Resistant Rootstocks

Grape species vary widely in their susceptibility to crown gall. In our research (Ed Stover, Harry Swartz at the University of Maryland and Tom Burr), we decided to see if potential rootstocks could be identified that would prevent or reduce passage of soil-borne *AV* into

crown gall-susceptible scions. While varieties that are not normally grafted are susceptible to crown gall, vinifera varieties which are grafted are among the most susceptible to this disease. Use of crown gall-resistant rootstocks with *AV*-free nursery material may greatly reduce the threat of crown gall in replant situations.

Initially, we screened over 50 grape genotypes for susceptibility to crown gall. We found two different resistance groups among the grapevines we tested: 1) the closely related North American species *Vitis riparia* and *V. rupestris*, and 2) some Asian *Vitis* species, most notably *V. amurensis*. These two resistance sources responded differently to the strains of *AV* that we used, which suggests that they might have different mechanisms of resistance. When we studied steps in the disease process to see where resistance occurs in these genotypes, wounded tissue from crown gall-resistant grapevines was as effective as the crown gall-susceptible in triggering the transfer of bacterial genes. Only rarely were the bacterial genes successfully incorporated into the resistant plant nuclei. Interestingly, the resistant varieties were also much less susceptible to the *AV*-induced tissue disruption that causes root lesions.

When crown gall-resistant grapevines were inoculated with *AV*, the pathogen population was suppressed compared to crown gall-susceptible vines. These observations support the idea that use of resistant rootstocks might suppress the development of crown gall when *AV*-free material is planted on *AV*-infested sites. One of the pleasant surprises in this work was the discovery that some of the most crown gall-resistant grapevines are rootstock varieties that we are already using (Table 1). Couderc 3309, and Riparia 'Gloire' were among the most resistant types. A few of the rootstocks that are widely used elsewhere, such as Richter 110 and 420A Mgt, are as susceptible to crown gall as the highly susceptible vinifera cultivar 'Cabernet Sauvignon'. In the spring of 1995, Tom Burr, Bob Pool and I will initiate a field study to test whether crown gall-resistant rootstocks are more effective than crown gall-susceptible rootstocks in protecting susceptible scions when *AV*-free vines are planted on *AV*-infested sites. Tom Burr hopes to conduct field tests using an *AV* suppressive strain of *Agrobacterium* that has been very promising in greenhouse tests.

Eradication of Existing Galls

A number of techniques have been used to try to either eliminate *Agrobacterium* from galls that have already formed or kill gall tissues before vines are girdled. In some species application of antibiotics, kerosene, or proprietary disinfectants has been reported to reduce the incidence of renewed gall development. In grapes, where *AV* develops systemically, these treatments have generally been ineffective.

Managing an Infected Vineyard

Cultural practices are used by many viticulturists to manage crown gall. Most grapevines in the Northeast are grown with multiple trunks in the hope that crown gall or severe winter-injury will not affect all of the trunks and that reasonable canopy area can be developed from trunks that are undamaged. Hilling of soil over the graft union is sometimes practiced to prevent cold damage and resultant crown gall at the graft union which could prevent establishment of healthy renewal trunks. In areas where cold-injury and associated crown gall are particularly severe, some growers will pull vines down from the trellis and bury them in the fall. These management practices to deal with crown gall in our vineyards are labor intensive and do not eliminate economic damage from this disease. We hope that current research on control of crown gall in grapes will reduce the effort and expense we devote to this disease in our vineyards.■

Table 1. Crown gall formation of *Vitis* genotypes and their hybrids after inoculation with three strains of *Agrobacterium vitis*. Galls were evaluated three months after inoculation and characterized by mean percentage of inoculated sites forming galls, mean size of galls when produced, and largest gall per plant.

Vitis Genotype	% with Galls		Gall Size (mm)		Largest Gall (mm)	
V. riparia X *V. rupestris* C3309	5.7	a*	2.4	ab*	3.1	ab*
V. riparia 'Gloire'	8.1	a	1.2	a	1.3	a
V. longii X *V. riparia* C1616	11.8	ab	6.1	cdef	6.6	abc
V. berlandieri X *V. riparia* T5C	12.5	abc	3.4	abcd	6.2	abc
V. rupestris GVIT 279	13.3	abc	2.2	ab	2.8	ab
V. rupestris GVIT 277	14.8	abc	1.3	ab	1.8	ab
V. riparia Manitoba 37 GVIT 401	21.3	bcd	4.6	bcde	6.6	abc
V. riparia X *V. rupestris* 101-14 Mgt	21.4	bcd	3.8	abcd	5.9	abc
V. rupestris GVIT 202	22.0	bcd	2.7	ab	3.2	ab
V. rupestris 'St. George'	23.0	bcde	2.8	abc	4.6	ab
V. berlandieri X *V. riparia* 5BB	23.9	cde	2.5	ab	3.4	ab
V. amurensis GVIT 1295	25.7	bcde	1.2	ab	1.5	ab
V. amurensis GVIT 689	28.9	de	3.3	abcd	5.3	abc
V. riparia Geisenheim 1	30.6	de	7.5	ef	10.7	c
V. riparia Quebec GVIT 612	34.4	e	6.1	def	7.7	bc
V. berlandieri X *V. rupestris* 110R	83.1	f	8.2	f	17.8	d
V. champinii 'Dog Ridge'	86.8	f	8.4	f	18.8	d
V. vinifera 'Cabernet Sauvignon'	89.3	f	9.2	fg	21.5	d
V. berlandieri X *V. riparia* 420A	89.5	f	12.0	g	22.4	d

*Within a column, means followed by the same letter are not significantly different using paired t-tests, probability level 5%.

FROM THE EDITOR

Martin Goffinet

Well, here it is the middle of the growing season again. By now most growers know just how much winter injury their vineyards sustained in New York State and what it means to their potential crop this season. One of the many concerns after such a winter is the increased likelihood of crown gall showing up in vineyards this summer. Ed Stover, now at Cornell's Hudson Valley Laboratory, earned his recent Ph.D. while researching grapevine crown gall. I have asked him to submit an article on this bacterial disease, with an emphasis on research perspectives for its control.

Each year I try to give our readers some idea of the types of research done on grapes, wine and juice in New York state. Much of this research relies on grant money administered by the New York Wine and Grape Foundation. The Foundation also matches research dollars generated by others for grapevine research, such as the New York Grape Production Research Fund and Mr. John Dyson. These dollars provide for research in grape breeding, culture, pest and disease control, and juice and wine quality at Cornell University, New York State's land-grant university. Such funding is critical to the research needed to keep our grape industry competitive. Grape researchers in other states will tell you that New York has the best overall grape research programs in the country. The table below summarizes Cornell's grape research projects supported by matching funds from the New York Wine and Grape Foundation for 1994.

Some last-minute funding to the Foundation from the legislature will allow some additional research this year.

It also bears repeating each year that the Wine Analytical Laboratory, Department of Food Science and Technology, New York State Agricultural Experiment Station, is also subsidized by the Foundation to minimize costs to wineries submitting samples for analysis. The wine lab will taste wine samples and then recommend the next step. Contact Thomas Henick-Kling, Department of Food Science & Technology, for information on wine analysis. Phone: 315-787-2277. Finally, this very newsletter is funded by Foundation dollars so that growers are knowledgeable about recent grape research programs.

Cornell grape-related research projects funded for 1994 by the New York Wine and Grape Foundation.

Researcher	Cornell Department	Projects
Christopher Becker and Harvey Hoch	Plant Pathology, Geneva	Epidemiology and control of black rot
Thomas Burr	Plant Pathology, Geneva	Cultural and biological methods of controlling crown gall
Lee Creasy	Fruit & Vegetable Sciences Ithaca	Resveratrol in grapes
Richard Derksen	Agricultural & Biological Engineering, Ithaca	Vineyard spray application systems
William Edinger and Thomas Henick-Kling	Food Science & Technology, Geneva	Survey for available nitrogen for yeast growth in NY grape musts
David Gadoury	Plant Pathology, Geneva	Developing a practical model for management of powdery mildew
Martin Goffinet	Horticultural Sciences, Geneva	Investigations of bud development and crop potential
Dennis Gonsalves	Plant Pathology, Geneva	Eliminating of rupestris stem-pitting virus from vines and diagnosing the virus using nucleic acid probes
Wesley Gunkel	Agricultural & Biological Engineering, Ithaca	Robotic grapevine pruning research
Alan Lakso	Horticultural Sciences, Geneva	Pruning and irrigation interactions on vine performance and vineyard economics
Brigitte Martineau, T. Henick-Kling and Terry Acree	Food Science & Technology, Geneva	Production and metabolism of diacetyl by yeast and malolactic bacteria in NY wines
Timothy Martinson	Entomology, Geneva	Reducing insecticide use for leafhoppers using *Anagarus epos* as a biological control
Robert Pool	Horticultural Sciences, Geneva	Improving Concord production efficiency via canopy spacing and mechanization Testing vinifera clones and varieties and their suitable rootstocks for New York production
Bruce Reisch	Horticultural Sciences, Geneva	Statewide evaluation of new grapevine varieties. Genetic technology to improve vine disease resistance
Gilbert Stoewsand	Food Science & Technology, Geneva	Wine and health

Grape Extension Publications and Educational Materials

Update of Other States' Grape Publications

The following listings are in addition to those published in the Summer 1993 issue of "Grape Research News." The entire listing can be requested from the editor using the "Question" form at the back of this newsletter, or send a note to: Martin Goffinet, Dept. Horticultural Sciences, NY State Agricultural Experiment Station, Geneva, NY 14456.

Arkansas:	Extension Communications, University of Arkansas, Little Rock, AR 72203. Phone: 501-569-2117.
EL246	Spraying grapes
EL648	Plant growth regulators for tree fruits and grapes
California	University of California, Agricultural Sciences Publications, University of California, Berkeley, CA 94720. Phone: 510-642-2431.
No. 4105	Grape pest management
Indiana:	Horticulture Extension Office, 1165 Horticulture, Purdue University, West Lafayette, IN 47907-1165. Phone: 317-494-1349.
No.10169	Indiana commercial small fruit and grape spray guide
FS1	Home winemaking guide
Other Pubs:	Growing grapes in Indiana (contact: Bruce Bardelon, Dept. of Horticulture, Purdue University, West Lafayette, IN 47907)
	Proceedings fruit and berry wine symposium, 1992 (contact: Dr. Richard Vine, Dept. Food Science, 1160 Smith Hall, Purdue University, West Lafayette, IN 47907)
Michigan:	Cooperative Extension Bulletins Center, Michigan State University, East Lansing, MI 48824. Phone: 517-355-0240.
E1899	Grape varieties for Michigan's vineyards
E1935	Pruning grapevines in Michigan
Mississippi:	MAFES, Dept. of Information Services, Box 5446, Mississippi State University, Mississippi State, MS 39762. Phone: 601-325-2262.
#510	Common insects and diseases of bunch grapes
#1444	Fruit and nut review: bunch grapes and blackberries
Missouri:	Missouri Cooperative Extension Publications, University of Missouri–Columbia, 2800 Maguire Blvd., Columbia, MO 65211. Phone: 314-882-7216.
#6085	Home fruit production: grape varieties and culture
#6090	Home fruit production: grape pruning systems
#6160	Establishing a vineyard in Missouri
#6161	Pruning and training grapevines
#6162	Training systems for Missouri vineyards
	For the following, contact: State Fruit Experiment Station, Rt. 3, Box 63, Mountain Grove, MO 65711. Phone: 417-926-4105.
MS-2	Before establishing a vineyard — plan
MS-7	Management of major grape diseases in Missouri
MS-8	Field evaluation of fungicides for control of grape diseases
MS-10	Initial report on French hybrid wine grape research
MS-11	The grape phylloxera
MS-14	Training and pruning small fruit crops in Missouri
MS-18	Growing fruit for home use

MS-19	Missouri grape pest control guide (current year)
MS-21	Fruit cultivars released by the state fruit experiment station
MS-22	1987 Missouri grape acreage survey
MS-24	Quality evaluation of Missouri wines
MS-26	Laboratory manual for wine and must analysis
MS-27	Laboratory techniques
Bull. #35	Grape growing in Missouri
Bull. #41	Cold hardiness of grapes: a guide for Missouri growers
Unnumbered	Proceedings of the Missouri Small Fruit Conference (1981-93)
Unnumbered	Proceedings of the Midwest Regional Grape And Wine Conference (4th, 5th, 6th)
Newsletter	Vineyard and Vintage View
New Jersey:	Publications Distribution Center, Cook College, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903. Phone: 908-932-9762.
FS594	Grape Tissue Analysis
Unnumbered	NJ Commercial Vineyard Series I. Site selection
Unnumbered	NJ Commercial Vineyard Series II. Establishing the planting
Unnumbered	NJ Commercial Vineyard Series III. Training and maintenance
Unnumbered	NJ Commercial Vineyard Series IV. Vineyard liming and fertilization
Unnumbered	Proceedings "Grape Expectations – A Viticultural and Enological Symposium" (1989 to present)
North Carolina:	Agricultural Communications, Box 7603, North Carolina State University, Raleigh, NC 27695. Phone: 919-515-2800.
Bull. 480	Winegrape reference for North Carolina (NC Ag. Res. Ser. Bull. 480)
Bull. 470	A guide to the identification and biology of insects feeding on Muscadine and bunch grapes in North Carolina
Ohio:	Extension Publications, Ohio Cooperative Extension Service, The Ohio State University, Columbus, OH 43210. Phone: 614-292-1607.
OCES Bull. 458	Fertilizing fruit crops
OCES Bull. 506B	Ohio Commercial small fruit spray guide
OCES Bull. 815	Grape production, management and marketing (1991)
Bull. 730	Insect and mite pests of grapes in Ohio. 25 pp.; color plates. (1986)
Bull. 815	Grapes: Production, management and marketing. 61 pp.; color plates. (1991)
HYG-2140-88	Japanese beetle—a major grape pest. Home, Yard and Garden Facts. OCES/OSU Fact Sheet (1988)
HYG-2146-89	Biology and behavior of the rose chafer, *Macrodactylus subspinosus* (F.). Home, Yard and Garden Facts. OCES/OSU. Fact Sheet (1989)
HYG-2600-92	Grape phylloxera. Home, Yard and Garden Facts. OSES/OSU. Fact Sheet (1992)
Misc.	Proceedings Ohio Grape and Wine Shortcourses (1991–93)
Virginia:	Virginia Cooperative Extension Division Distribution Center, 112 Landsdowne St., Blacksburg, VA 24061. Phone: 703-231-6192.
Pub.423-011	Dormant pruning and training of grapevines in Virginia
Pub.456-017	Pest management guide for horticultural crops
Pub.463-007	Grapevine nutrition
Pub.463-016	Site selection for commercial vineyards
Pub.463-018	A guide to commercial vineyard establishment
Pub.463-019	Commercial grape varieties for Virginia
Pub.463-001	Controlling microbial growth in wine
Pub.463-012	Protein fining agents
Pub.463-013	A review of potassium bitartrate stabilization in wine
Pub.463-014	Bentonite fining of juice and wine
Pub.463-015	Protein stability determination
Pub.463-017	A review of *Methode Champenoise* production
Other:	"Vineyard and Winery Information" series. For subscription contact: Dr. Tony Wolf, VPI and SU, 2500 Valley Ave., Winchester, VA 22601. ■

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This newsletter and the extensive grape research it is based on are made possible by funding from the **New York Wine & Grape Foundation.** The Foundation's budget depends totally on private sector contributions which are matched by the State of New York. And now extensive cuts in State funding have made these private sector contributions more vital than ever.

If the Foundation's research and promotional programs are to continue, we need your support through modest dues—a rate schedule and membership application are below. (Wineries and juice manufacturers have already made financial contributions of up to $15,000 each to support the effort.) Please join your neighbors and industry associates in forging a more productive and profitable future. **(Join using this form and we'll send you a "Best of the Bunch!" T-shirt.)**

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Question: _____

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Save yourself a long distance phone call. Put it in writing on the back of form below, cut it out, and send it to us.

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