Crown Gall of Grape

Biology of *Agrobacterium vitis* and the Development of Disease Control Strategies

Several early descriptions of crown gall on grape from different European countries and the United States were thoroughly reviewed by Hedgecock (29). In 1897, Cavara (20) first demonstrated the infectious nature of a bacterium causing crown gall of grape (*Bacillus ampelopsorae*) in Italy. The crown gall bacterium was first isolated in the United States from Paris daisy by Smith and Townsend in 1907 (54). By inoculating several plants, they demonstrated the tumorigenic nature of the bacterium. Hedgecock then isolated strains of a bacterium from grape crown gall and demonstrated their tumorigenicity on peach and apricot (29). Today, *Agrobacterium vitis* is recognized as the predominant species causing grape crown gall. Within the past 25 years, grape crown gall has been reported from China, Japan, and South Africa and from several other countries in Europe, the Middle East, and North and South America. In a recent investigation carried out in 1996 by the O.I.V. (Office International de la Vigne et du Vin, France), the sporadic occurrence of grape crown gall was highlighted in France, Spain, Germany, Italy, Chile, and Israel; it is endemic in South Africa, with recurrent and severe attacks. We will discuss how the pathogen survives and spreads, how it infects plants, and various strategies that are being used commercially or are being researched for disease management.

Dr. Burr's address is: Department of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456; E-mail: tjb1@cornell.edu

Thomas J. Burr
NYSAES, Cornell University
Geneva, NY

Carlo Bazzi
Institute of Plant Pathology, University of Bologna
Bologna, Italy

Sandor Süle
Plant Protection Institute of Hungarian Academy of Sciences
Budapest, Hungary

Leon Otten
C.N.R.S. Institute of Plant Molecular Biology
Strasbourg, France

tion of the bacterium in vines or the sensitivity of the methods used for detection of the bacterium. Stover et al. (58) demonstrated that by prefreezing dormant cuttings, the number of *A. vitis* cells detected using a vacuum flushing method (discussed below) was greatly increased. In this case, it appears that the physical effects of freezing facilitated the increased numbers of cells flushed from the cuttings. Related research done by Bauer et al. showed

The Disease Cycle

Survival in vines. A key point in the disease cycle of grape crown gall (Fig. 1) is the systemic survival of *A. vitis* in grape. Lehoczy presented the first direct evidence that *A. vitis* survives systemically in grape and induces crown gall at injuries caused by twisting and other wounding of canes (38). This information has been particularly important for explaining the induction of crown gall following vine injuries as well as the systemic nature of the pathogen and how it is disseminated in propagation material. Further studies by Lehoczy and others demonstrated that *A. vitis* could be isolated from sap of bleeding vines (39,40,42). Samples of sap sometimes yielded nearly pure cultures of *A. vitis*, whereas other samples from the same or other infected vines yielded none, demonstrating an uneven distribution of the bacterium. Lehoczy hypothesized that the pathogen overwinters primarily in the root system and suggested that under moist conditions in the spring, root pressure causes xylem fluids to "sweep" bacterial cells from the roots upward, where they may be attracted to wounds. *A. vitis* has been isolated directly from 1-year-old canes, but the percentage of cuttings from which *A. vitis* is detected is often highly variable. This may reflect uneven distribu-

tion of *A. vitis* were greatest at inoculation sites on shoots of vines and that population curves were similar for Riesling and Müller-Thurgau cultivars, with the highest numbers of bacterial cells being detected in the spring and fall (1). The greatest populations were detected in Riesling, and it required at least 15 weeks for *A. vitis* to move internally from inoculation sites to the root system.

Translocation of *A. vitis* in green shoots was demonstrated by Tarbah and Goodman (64). They inoculated shoots by submerging their basal ends in a bacterial suspension and then monitoring the movement of the bacterium. Bacteria translocated up to 30 cm from the point of entry within 24 h. Lehoczy indirectly demonstrated the presence of *A. vitis* in green shoots by grafting plants with shoots from infected vines and finding that 6.75% of the grafted vines became infected (41). The stage at which current-season shoots are invaded by *A. vitis* (moving from woody cane tissue) may be related to the development of vasculature between canes and shoots. Burr et al. (13) did not detect the bacterium in green shoots on grapevines growing in a crown gall-infested vineyard until late in the growing season (when shoots were becoming lignified) and never detected *A. vitis* in shoot tips. More recently, Szegedi

Fig. 1. Grape crown gall disease cycle.

and Nemeth (63) also did not detect *A. vitis* in green shoots of crown gall-infected grapevines.

Although most research has focused on survival and translocation of *A. vitis* in xylem tissue, Jager et al. provided convincing evidence that the bacterium persists in the rind (layer of tissue directly beneath bark) of canes (32). Therefore, the specific survival sites of *A. vitis* within vines are not clearly defined and may vary with cultivar and time of year.

Soil survival and root necrosis. The genus *Agrobacterium* is a common soil inhabitant, but the vast majority of strains that are detected in soil are nontumorigenic. Where soils have been analyzed specifically for *Agrobacterium* spp., *A. vitis* was not detected (8,9). The potential for *A. vitis* to persist in grape tissue debris in soil was investigated (18). Strains of *A. vitis* remained tumorigenic in vine debris and were detected over the 2-year course of the study. In this case, tumorigenic *A. vitis* persisted in a saprophytic phase, since no galls were formed on any of the grape tissues. These findings indicate that once a vineyard becomes contaminated with *A. vitis*, the bacterium will survive in grape debris for years after vines are removed. Therefore, the effectiveness of eradicating

the pathogen from vineyard sites by removing infected vines and leaving soil fallow or planting to nonhosts will vary depending on the amount of grape debris in soil and its rate of decomposition.

A. vitis does not typically cause crown gall on roots of grape but instead induces a localized necrosis (Fig. 2) (11). A mutant strain that no longer produces polygalacturonase was found to cause a reduced level of necrosis and tumorigenicity on grape (50). In this case, mutant CG50 did not induce necrosis when inoculated to Concord grape seedling crowns at a concentration of 10^6 CFU/ml, whereas the wild type strain CG49 did. At higher concentrations (10^8 CFU/ml), CG50 induced necrosis, indicating that polygalacturonase is only one factor associated with necrosis.

Wild grape species as hosts for *A. vitis*. The ability of *A. vitis* to survive in *Vitis* spp. growing in the wild is being investigated in Italy and in the United States. Root samples of *V. riparia* were collected at several locations near and far-removed (more than 150 km [100 miles]) from commercial vineyards in New York. *A. vitis* strains were commonly isolated (49); they were identified based on colony morphology on the medium of Roy and Sasser (RS) (12), the testing scheme of Kerr and

Fig. 2. Necrosis of grape roots caused by *Agrobacterium vitis*.

Panagopoulos (35), reaction to a species-specific monoclonal antibody (6), production of polygalacturonase (50), and ability to induce necrosis of grape seedling roots (11). All strains from wild grape were found to be nontumorigenic.

The Italian study involved numerous samples of *V. vinifera silvestris* cuttings and roots from viticultural and nonviticultural areas (C. Bazzi, unpublished). Fifty-five *A. vitis* strains were isolated and identified by enzyme-linked immunosorbent assay (ELISA). All were nontumorigenic on indicator plants and did not generate polymerase chain reaction (PCR) products from tumor-inducing (Ti) plasmid-derived primer sets, as described by Schulz et al.

(53). It is interesting that, thus far, all strains from wild grape species are nontumorigenic and crown gall has not been observed on these feral vines. The potential role of these strains in the spread and development of crown gall disease is being investigated. Can they acquire Ti plasmids, thus becoming tumorigenic? At least some nontumorigenic *A. vitis* are able to inhibit gall formation by tumorigenic strains on grape, as discussed below.

How *A. vitis* infects. Vineyards often remain crown gall-free for several years until conditions conducive to infection (generally freeze injuries) occur. This was the case during the winter of 1984-85, which was followed by severe outbreaks on several susceptible cultivars in Italy. In the United States, the frequency of occurrence and severity of the disease have increased dramatically as wine industries expand into regions where vines are exposed to injuries caused by freezing temperatures.

Crown gall infections are initiated at plant injuries for reasons that will be discussed below. Whereas in the majority of grape-growing regions, freeze injuries provide sites for initiating crown gall, in South Africa and Israel, high temperatures and humidity are considered equally important. Crown galls commonly form on the trunks of vines (Fig. 3); however, they even develop on 1-year-old canes (Fig. 4). Crown gall can also be significant in grape nurseries. Galls develop at wounds made by disbudding, at the base of rooted cuttings, and at grafts (Fig. 5). However, a more significant consideration for nurserymen is that propagation material may be systemically infested with *A. vitis*. Infected vines often produce inferior growth, and in some cases, the disease may cause partial or complete vine death. The importance of crown gall in reducing the vigor and yield

of the cultivar Zinfandel was quantitatively shown by Schroth et al. (52). Another less common expression of the disease may develop when established vines are top-grafted or budded with new scion cultivars. In this case, severe crown gall may form at grafting and budding sites (Fig. 6), resulting in poor growth or death of scion shoots.

Infection of plants by *Agrobacterium* is a multistage process (71,72). It represents the only known example of DNA transfer from a bacterium to a plant in nature. It has been known for several years that a part of the bacterial Ti plasmid (the T-DNA) is transferred to the plant cell and expressed in the plant genome. Not all steps of the infection process for *A. vitis* have been identified, but many are known to be very similar to those of *A. tumefaciens*. The first steps are chemotactic attraction toward wounded plant cells and attachment of the bacterium to them. In the case of grapes, freeze injuries often provide the wounded cells that are considered to be highly susceptible to infection by *A. vitis*. Certain phenolic and other compounds released by wounded cells induce the virulence genes of *Agrobacterium*. Acetosyringone is a common inducer that is studied in the *A. tumefaciens* infection system, and methyl syringate was identified as a signal compound from grape (55). A protein encoded by the bacterial Ti plasmid, VirA, detects the chemical signals from the plant. When a phenolic compound of the right structure binds to VirA, the complex acquires the capacity to phosphorylate another protein, VirG, which is inside the bacterium, and this in turn leads to the activation of various other genes that make up the virulence (or vir) region of the Ti plasmid.

Although much is already known about the functions of the virulence genes, certain important points remain to be eluci-

dated. Basically, the *virD2* gene codes for the enzyme VirD2, which liberates a linear fragment of the T-DNA (the T-strand), or T-DNAs in some strains. VirD2 becomes firmly attached to the T-strand and pilots it out of the bacterium and into the plant cell nucleus, where it gets incorporated into one of the plant chromosomes. The *virB* genes (which make at least 11 different proteins) probably enable the bacterium to construct a porelike structure through which the T-strand passes. It is remarkable that similar proteins are used in several bacterial conjugation systems where plasmid DNA passes from one bacterium into another. It has therefore been proposed that in the course of evolution, the *Agrobacterium* plant infection process originated from a bacterial conjugation system (47). Other vir genes (*virE* and *virF*) play a role in protecting the T-strand on its way to the plant cell chromosome. Once inserted in a plant chromosome, the T-strand will be transcribed. This leads to the synthesis of various proteins, which can be divided into two functional groups: those involved in opine synthesis and those that stimulate cell growth, resulting in crown gall disease.

Plant gall-inducing genes (oncogenes) on the T-DNA turn on the rapid multiplication of plant cells. The oncogenes encode auxin synthesis (a two-step process requiring the *iaaM* and *iaaH* genes) and cytokinin synthesis (*ipt* gene), and include various genes that induce or modify the growth of crown galls. The infected cells also produce opines, thereby further benefiting the survival of the pathogen.

Opines are small molecules that are secreted from the plant crown gall cells and then are utilized by *Agrobacterium* living in the vicinity of galls. Many types of opines have been described; all result from the combination of two small, abundant

Fig. 3. Severe crown gall on multiple trunks of a *Vitis vinifera* vine.

Fig. 4. Crown gall on a 1-year-old cane that is wrapped around the trellis wire.

plant cell compounds like keto acids, amino acids, or sugars. The combination into larger opine compounds makes it more difficult for most microorganisms to use them; *Agrobacterium* carries special enzymes that are able to degrade opines. In this way, *Agrobacterium* selectively exploits opines synthesized following crown gall infections. Within the genus *Agrobacterium*, and even within a given species, there is fierce competition for the opines around plant galls, as is evident from the existence of different types of opine synthesis and degradation genes within these groups. We will discuss the Ti plasmids and T-DNAs found so far in *A. vitis* strains.

Diversity of *Agrobacterium vitis*. It was assumed for many years that grape crown gall was caused by strains of *A. tumefaciens*, the cause of crown gall on numerous dicotyledonous plants. However, in 1977, Kerr and Panagopoulos classified tumorigenic *Agrobacterium* strains from grape as biovar 3 of *A. tumefaciens* (35). In subsequent years, researchers in several parts of the world identified strains from grape as biovar 3. Further taxonomic studies by Ophel and Kerr resulted in the distinction of biovar 3 as a separate species, *A. vitis* (43). *A. vitis* is now recognized as the predominant species causing grape crown gall; however, tumorigenic strains of biovars of *A. tumefaciens* are also infrequently isolated from grape.

The first Ti plasmid and T-DNA of an *A. vitis* strain were described in 1984 (10). Since then, many strains have been investigated, and several Ti and T-DNA types have been defined. The *A. vitis* group is the only *Agrobacterium* species that has been systematically investigated with regard to T-DNA, Ti plasmid, and chromosome structures. These studies were made possible because the natural host range of *A. vitis* is restricted to grape and because *A.*

vitis strain collections exist all over the world.

Three basic T-DNA structures have been defined in *A. vitis* (Fig. 7). Each differs in the number of T-DNAs and in the makeup of oncogenes. The most abundant structure (found in about 60% of the strains) consists of two independent T-DNAs (TA-DNA and TB-DNA) on the Ti plasmid. The TA region contains oncogenes 5, TA-*iaaM*, TA-*iaaH*, *ipt*, *6b*, and opine genes *acs* and *ocs* (the latter two coding for synthesis of agropinopine and octopine). The TB region contains oncogenes TB-*iaaM*, TB-*iaaH*, and opine genes *acs* and *cus* (for cucumopine synthesis). This TA/TB T-DNA makeup has been called the octopine/cucumopine type. Detailed studies of cloned and sequenced TA and TB regions have revealed the existence of numerous variants. Remarkably, these variants differ not so much in nucleotide sequence as by insertion and deletion of so-called insertion (IS) elements (mobile DNA fragments that can insert randomly in the bacterial genome, thereby leading to mutations). One such mutant (having a small TA region) no longer carries TA-*iaaM*, TA-*iaaH*, or *ipt* but still induces tumors through the activity of genes *6b* and TB-*iaaM* and TB-*iaaH*. This is exemplified by strain AB3 in Figure 7. Functional studies of the different oncogenes (31) have shown that the TA/TB oncogene combination is to a certain extent redundant (some of the genes in TA have the same function as genes in TB). This probably explains the existence of the variants. These variants are recent on the scale of evolution and represent either random mutations that provide different selective advantages to *A. vitis* or mutants that have already passed through the sieve of natural selection, allowing them to occupy special niches in nature. Only competition experiments that compare mixtures of variants

under controlled conditions can answer these questions.

The second T-DNA structure (about 30% of strains) is the nopaline type, having a single T-DNA with oncogenes 5, *iaaM*, *iaaH*, *6b*, and 3', and opine genes *acs* and *nos* (nopaline synthase). No variants have yet been detected, indicating that this group either is very recent, contains less-active IS elements, or does not easily generate viable mutants.

The third group (about 10% of the strains) is the vitopine type, which carries three independent T-DNAs, a situation so far only found in *A. vitis*. T1 carries *6b* and *vis* (vitopine synthesis), T2 has *iaaH* and *iaaM*, and T3 has *ipt* and a mutated, inactive *vis* gene (19). Why these genes, which normally occur together, are distributed over three different T-DNAs is unknown. It may represent a primitive condition occurring before T-DNA genes were combined into a single structure.

Interestingly, we have recently detected a new T-DNA form in strain CG474. This form is related to the TA region of o/c strains but is clearly different from it. This exceptional strain may have acquired its Ti plasmid and T-DNA from an *A. tumefaciens* strain. It is not known whether the different strain types are specialized for specific grape cultivars or they have adapted to particular environments. However, the exhaustive molecular knowledge of *A. vitis* now allows us to carry out such ecological studies and to follow the fate of the different types over time.

Although T-DNA structures are eminently important to the infection process, the remainder of the bacterial DNA should

Fig. 5. Crown gall that developed at graft unions on vines growing in a nursery.

Fig. 6. Crown gall at a site where a mature trunk was budded.

not be forgotten. Little is known about the Ti plasmid virulence genes of the different *A. vitis* subgroups. Whereas the *vir* region of the o/c strains seem to be similar to that found in classical *A. tumefaciens* strains, the vitopine strains have a different *vir* region organization and possibly additional *vir* genes (26). Ti plasmids have been cloned and physically mapped to identify major types, but they have not been fully analyzed. Other genes that influence grape infection, such as the opine utilization genes and plasmid replication genes, remain to be studied. A further complication is the difference in chromosome structure; although most crown gall induction genes are on the Ti plasmid, the chromosome also contains virulence genes that may be important for survival in the soil and in the grape plant. It is therefore important to know the overall extent of chromosomal variation in *A. vitis*. This problem has been addressed recently using PCR to amplify specific chromosomal regions of *A. vitis*. The intergenic space between the 16S and 23S rRNA genes was digested with different endonucleases, and DNA fingerprints were generated (46). Several chromosomal groups have been defined and their phylogenetic relationships established; however, more work is needed to relate these forms to pathological properties. The chromosomal data show that most Ti plasmids, with their characteristic T-DNAs, are associated with a particular chromosomal background, suggesting that Ti plasmid exchange or transfer is rare under natural conditions. In practice, this means that chromosomal markers can be used to predict the Ti plasmid type.

Among the potential *A. vitis* host range factors, two have been studied in considerable detail; the polygalacturonase gene (*pehA*), which codes for an enzyme associated with grape tissue necrosis, and the tartrate degradation (*tar*) genes. *PehA*-minus mutants are less pathogenic on grapevine but not on other hosts (50).

The TAR region codes for tartrate utilization and is found in most *A. vitis* strains (45). Three different types have been isolated and sequenced; their basic structures and functions are the same, but they are found on different plasmids. Once again, the vitopine strains are exceptional in that their TAR region seems to be quite different. Competition experiments between a TAR-plus strain and a TAR-minus mutant showed that the TAR system confers a clear advantage to the bacterium on grapevine but not on other hosts and is therefore a major host range determinant (L. Otten, unpublished data).

Management of Crown Gall

Varietal susceptibility. Evaluations of grape cultivars for crown gall susceptibility were done as early as 1910 (29). In more recent years, many *Vitis* species, rootstocks, and scion cultivars have been evaluated in different ways for crown gall susceptibility (25,27,58,61). *V. vinifera* cultivars, including Chardonnay, Riesling, Merlot, Cabernet Sauvignon, and many others, are highly susceptible, as determined by field observation and controlled inoculations. In contrast, *V. riparia* clones (such as Riparia Gloire) are generally resistant to *A. vitis*, although some are susceptible. *V. labrusca* and hybrid cultivars

are generally more resistant than *V. vinifera*, although some (such as Chancellor and Niagara) can become severely infected with crown gall. Ferreira and van Zyl evaluated 40 rootstocks for crown gall susceptibility using five *A. vitis* strains (25). Although some discrepancies exist between their results and those of Stover et al. (58), it is evident that certain rootstocks used worldwide, such as Courderc 3309, 101-14 Mgt, and Riparia Gloire, are resistant; whereas Teleki 5C and 110 Richter are susceptible. A summary of crown gall susceptibility ratings for various scion and rootstock cultivars from different countries is given in Table 1. Complicating the resistance screening process is the fact that *A. vitis* strains are genetically variable (as previously described), affecting their tumorigenicity on different *Vitis* germ plasm. For example, in the papers cited, various rootstocks and *Vitis* spp. were screened against five strains of *A. vitis*. Not all strains caused the same numbers and sizes of galls on the same genotypes. Also, some genotypes were resistant to certain strains but developed large galls when inoculated with other strains. Therefore, crown gall susceptibility of grape appears to be determined by genetic determinants of the plant and pathogen.

The strategy of planting relatively resistant cultivars and rootstocks as a means of disease management has been suggested. This is not practical for situations where susceptible cultivars are preferred by the producer, who is growing them to make premium wine. Recently, however, it was demonstrated that under field conditions, crown gall was significantly reduced

Fig. 7. T-DNA gene arrangements in *Agrobacterium vitis* strains. TM4 and AB3 are O/C (octopine/cucumopine) strains with large and small TA regions, respectively. Strain AB4 has a NOP (nopaline) T-DNA arrangement, and S4 has a VIT (vitopine) T-DNA. Flag symbols denote border sequences of T-DNAs. Genes are described in text. Regions where IS elements IS866, IS868, and IS869 have inserted are denoted.

when susceptible *V. vinifera* scion cultivar Blue Frank was grafted onto resistant rootstock Riparia Gloire compared with grafting on susceptible Teleki 5C (59). This difference became more noticeable over a 6-year period in the field. The apparent increased resistance of the scion cultivar may result from reduced survival of *A. vitis* in the resistant rootstock or because compounds inhibitory to the bacterium are translocated from the resistant rootstock to the susceptible scion. It was previously shown that resistant grape genotypes support lower populations of *A. vitis* than do susceptible genotypes (57).

The only studies thus far on genetic inheritance of crown gall resistance were done by Szegedi and Kozma (62). Resistant clones of *V. amurensis* and hybrids were crossed with susceptible cultivars of various sources, and the progeny were inoculated with *A. vitis* strain AT-1. It was determined that a Mendelian dominant inheritance exists for resistance. Further studies of this type using a diverse collection of *A. vitis* strains may lead to the identification of useful resistance genes in grape.

Production of *A. vitis*-free vines. Hot water treatments. In recent years, hot water treatments have been evaluated as a means of eradicating *A. vitis* from dormant cuttings. Although initial experiments were promising, further studies in the United States and in Italy showed that treatments of 50°C for 30 or 60 min greatly reduced but did not eliminate *A. vitis* cells from grape cuttings (16). Wample (66) demonstrated that it was possible to treat dormant grape cuttings collected in Washington State at temperatures greater than 50°C without causing significant bud death. Therefore, such treatments may have improved effectiveness for eradicating *A. vitis*. Experience in New York showed that although a treatment of 50°C was not harmful to fully dormant cuttings, higher temperatures caused bud injury and therefore are not recommended. Growing conditions and wood hardness may be related to the apparent differences in heat sensitivity of the cuttings treated in Washington and in New York.

Although hot water treatment cannot be relied on to completely eradicate *A. vitis*, it is still being used in some commercial nurseries in different regions of the world. Researchers (4) and nurserymen have noticed reduced crown gall in treated vines as well as improvement in plant growth. Reasons for improved plant growth have not been determined.

Shoot tip propagation. As mentioned previously, natural populations of *A. vitis* have not been detected in young green shoots. This has led to the strategy of eliminating the pathogen from vines by shoot tip propagation. The efficacy of this method has been evaluated for several years now. Impressive results have been

observed where shoot-tip-propagated vines were planted in soils that had not grown grapes previously. After 7 years of growth under cold climate conditions, vines still remain free of crown gall (T. J. Burr, unpublished data).

Indexing vines for *A. vitis*. Over the last 20 years, various methods have been developed for detecting *A. vitis* in dormant grape cuttings. One of the first methods developed by Lehoczy (39), and later modified, involves callusing cuttings in sterile perlite for 4 to 6 weeks, triturating callus tissue in sterile water, and plating suspensions of the tissue on RS medium. Colonies with morphologies resembling *A. vitis* after 5 to 7 days are transferred to potato dextrose medium and identified with an ELISA procedure using a species-specific monoclonal antibody (6).

Another rapid method for the detection of the pathogen in vessels of cane tissues was developed by Tarbah and Goodman (65). Vascular fluids are collected by flushing water through the cuttings and plating the fluid collected on RS. During this same period, a serological procedure for detection of *A. vitis* in xylem extracts was developed by Bazzi et al. (3). The method consists of extracting vascular fluids from 1-year-old dormant cuttings, concentrating cells by centrifuging, and identifying *A. vitis* by indirect immunofluorescence staining (IFAS) with conventional polyclonal antibodies or by direct

isolation on RS. Only about 12% of *A. vitis* cells that were artificially infiltrated into cuttings were detected by this method, indicating a lower sensitivity than desirable for the method.

A serological study was done (2) to compare IFAS with indirect solid-phase immunosorbent assay (ELISA) on a collection of *Agrobacterium* strains, including *A. vitis*. The specificities of the monoclonal antibody described above and two conventional polyclonals (anti-*A. vitis* and anti-*A. tumefaciens* biovar 1) were compared. In IFAS, the anti-*A. vitis* polyclonal antibodies were quite specific, reacting strongly with nine of 12 strains. The monoclonal gave very faint positive reactions and was not suitable for this assay. With microtiter plate ELISA, the monoclonal was highly specific for *A. vitis* and gave no cross reactions to other biovars, thus providing a significant improvement over previous attempts to produce biovar-specific (in this case species-specific) antisera.

Further improvements in indexing procedures continue to be made by employing tools of molecular biology. Genetic probes consisting of T-DNA genes (14) and specific sequences from octopine/cucumopine *A. vitis* strains (44) have been effective for identifying tumorigenic strains. More recently, PCR-based methods were developed for identifying strains of *A. vitis* (22,2328,51,53). Sawada et al. (51) developed a universal primer set (VCF/VCR)

Table 1. Susceptibility of selected grape scion and rootstock cultivars to crown gall^a

Country	Susceptible	Resistant
Scion cultivars		
South Africa	Muscat d'Alexandrie	
Chile	Pumard, Pais, Pedro Jimenez, Torontel, Muscatel negra	Chardonnay, Semillon, Riesling, Carignan, Blanca ovoide
Italy	Merlot, Lambrusco, Albana, Muscat Hambourg, Cardinal, Italia, Perla Csaba	Regina
Israel	Muscat d'Alexandrie, Dabouki, other local varieties	
U.S. ^b	Chardonnay, Merlot, Riesling, Cabernet Sauvignon, Grenache, and other vinifera	Several <i>V. labrusca</i> cultivars and some hybrids
Rootstocks		
South Africa ^c	110-Richter, 99-Richter, Jacques, Ramsey	Paulsen 775, Freedom, Couderc 3309, 101-14 Mgt, Kober 5BB
Spain	161-49	
Italy	Teleki 5C, 17-37, Paulsen 775, Kober 5BB	
Hungary ^d	Kober 5BB, Teleki 5C, Teleki 8B, Paulsen 775, 287C	Riparia Gloire, 101-14 Mgt
Israel	Ruggeri 140	1103 Paulsen
U.S. ^e	110-Richter, Teleki 5C	Couderc 3309, Riparia Gloire, 101-14 Mgt

^a Ratings were reported in 1996 by the O.I.V. (Office International de la Vigne et du Vin, France) except where noted.

^b Ratings are based on field observations and greenhouse inoculations.

^c For a more complete assessment see ref. 25.

^d See ref. 59.

^e See ref. 58.

based on the sequences of the *virC* operon. In another study, primer sets derived from conserved regions of the *virD2* and *ipt* genes were used in multiplex PCR to differentiate between tumorigenic and nontumorigenic *A. tumefaciens* biovars 1 and 2 and *A. vitis* (28). For indexing of dormant grape cuttings, PCR primer sets were derived from conserved regions of the *A. vitis* genome (including oncogenes, opine genes, and IS elements) that amplify characteristic products from octopine/cucumopine, nopaline, and vitopine strains (53). Another PCR-based method developed by Eastwell et al. (23) involves direct lysis of *A. vitis* cells in cuttings by infiltration of lysis buffer and repeated microwave-cooling, followed by purification of the DNA from grapevine exudates by ion exchange chromatography (Qiagen tip-20 columns). One primer set was derived from the *pehA* gene (30) that encodes polygalacturonase in tumorigenic and nontumorigenic *A. vitis* strains. More recently, a very promising indexing protocol was developed based on immunocapture cultivation (with selective medium 3DG) followed by PCR (primer set derived from the *6b* gene) (33). This procedure proved to be effective for detecting *A. vitis* in tumors as well as from extracts from systemically colonized symptomless plants.

Biological control of grape crown gall. Effective biological control of crown gall with nontumorigenic *A. rhizogenes* strain K84 has been proven in many regions of the world and remains one of the most successful examples of biocontrol for a plant disease (34). K84 is not effective against all strains of *Agrobacterium* spp., however, and does not prevent infection by *A. vitis*. This has stimulated research to identify strains that will control grape crown gall.

Several bacteria have been shown to provide some level of control of grape crown gall (Table 2). These include *A. tumefaciens* strain J73 (67), which produces an agrocin that is active against many strains of *Agrobacterium* spp., including *A. vitis*. Bell et al. demonstrated that certain endophytic bacteria of grape can reduce populations of *Agrobacterium* in situ and provide significant biocontrol of

crown gall (5). In China, nontumorigenic biovar 1 strains MI15 (69) and HLB-2 (68) and nontumorigenic *A. vitis* strain E26 (70) were found to be effective for inhibiting grape crown gall. Pu and Goodman (48) demonstrated that the suppression of tumor formation was effective when HLB-2 was applied either before or with a tumorigenic strain and at a cell concentration higher than that of the pathogenic strain. In field experiments, they demonstrated that combined treatments of HLB-2 and the fumigant Vorlex (methyl isothiocyanate) had a synergistic effect on crown gall control. Visible galls were observed on only 9% of the treated vines compared with 100% of the controls. Staphorst et al. (56) in South Africa determined that nontumorigenic *A. vitis* strain F2/5 inhibited growth of many *A. vitis* strains in vitro as well as gall formation on grapevines. Burr and Reid (15) also demonstrated that control was most effective on vines when wounds were inoculated with F2/5 before or at the same time as the pathogen. At least one tumorigenic strain (CG78) was sensitive to the F2/5 agrocin in vitro but was not prevented from causing galls on plants. More recent experiments with F2/5 (17) show that agrocin is not the primary control factor and that pathogenic strains appear to attach to grape cells as efficiently as F2/5.

In Italy, experiments were done in the greenhouse and field with strains HLB-2, F2/5, CG1077 (an agrocin-minus F2/5 mutant), and CG523 (a nontumorigenic and non-agrocin-producing *A. vitis* strain from *V. riparia*) (C. Bazzi, unpublished). For this experiment, certified cuttings of cv. Malvasia Istriana and the rootstock 420 A were infiltrated with aqueous suspensions of the biological control candidates (10^8 to 10^9 CFU/ml). After 24 h, the cuttings were infiltrated with the tumorigenic strain, CG49, at a similar concentration. Few visible galls developed even after 8 months, but CG49 killed 48.8% of infiltrated plants. The least lethal strain was CG1077, giving 83.8% survival, which was not significantly lower than the water-infiltrated control (89.0%). Strains HLB-2, CG523, and CG1077 gave 69.4, 63.0, and 60.3% marketable vines, respectively, compared with 32.9% for vines infiltrated

with CG49 alone. Interestingly, the vines infiltrated with F2/5 had a high mortality (63.2%), which may be related to the phenomenon of *A. vitis*-induced necrosis (F2/5 appears to cause more necrosis than most other strains).

Cultural control. Preventive control measures for crown gall on grape in France were reported by Boubals (7). For existing vineyards, these include the application of K_2O instead of nitrogen fertilizers to improve resistance of the vines to cold. Also, avoiding wounding of vines and removing and destroying all dead and infected plants is recommended. Where there is a risk of freeze injury, it is advisable to earth-up the foot of the vines (about 0.25 to 0.50 m) in late fall and then remove this coverage in the spring to prevent the formation of adventitious roots. During the growing season, active copper fungicides that may be applied to control downy mildew may also reduce *Agrobacterium* populations. For new plantings, avoid wet, heavy soils in frost-prone areas and exposure to the north, and plant cold-resistant and crown gall-resistant cultivars. It is also recommended to fertilize with K_2O .

Positive results were also obtained in France by treating trunks and canes with Bordeaux mixture plus DNOC (4,6-dinitro-*o*-cresol) or with 5% copper sulfate in the winter (24). In Swiss nurseries, crown gall was significantly reduced with pregraft treatments of oxyquinoline sulfate (21).

In the nursery and in the vineyard, it is advisable to control soilborne nematodes. Studies carried out in Hungary (60) have demonstrated significant increases in the incidence of crown gall on vines that were affected by *Meloidogyne hapla*.

Summary and Outlook

The prospects for controlling grape crown gall in commercial vineyards look very promising. It is now possible to index vines using selective media, antisera, and more recently, by molecular-based methods. *A. vitis*-free vines can be propagated and have thus far remained free of crown gall when planted in soils where grapes were not previously cultivated. Biological control of grape crown gall appears to be very effective and may prove to be a reliable treatment for vines that are to be planted on sites that are contaminated with the pathogen. Selecting planting sites that are not conducive to freeze injury and using rootstocks and scion cultivars that are relatively resistant to crown gall remain important management considerations. However, in spite of many significant research accomplishments, several important questions related to pathogen biology and disease control remain.

Although methods for detection of *A. vitis* are reliable, their sensitivities are largely unknown. Therefore, grapevines and soil that test free of *A. vitis* may be

Table 2. Bacterial strains that have been evaluated as biological controls of grape crown gall

Bacterium	Strain	Origin	Reference
<i>Agrobacterium vitis</i> (nontumorigenic)	F2/5	Grape, South Africa	56
<i>A. vitis</i> (nontumorigenic)	E26	Grape, China	70
<i>A. tumefaciens</i> bv. 2	J73	Plum, South Africa	67
<i>A. radiobacter</i> bv. 1	HLB2	Hop, China	68
<i>A. radiobacter</i> bv. 1	MI 15	Grape, China	69
<i>Agrobacterium</i> spp. (nontumorigenic)		Various, Romania	37
<i>A. tumefaciens</i> (nontumorigenic derivatives)		Soil, Russia	36
Endophytes from grape including <i>Pseudomonas corrugata</i>		Grape, Canada	5

carrying undetectable levels of the pathogen. The recent finding that *A. vitis* is frequently isolated from *V. riparia* vines growing in the wild raises the question of whether the bacterium is a common soil inhabitant that multiplies to detectable levels in association with grape. Also in question is whether all strains from *V. riparia* are in fact nontumorigenic and if they are able to acquire Ti plasmids. The continued development of highly sensitive PCR-based detection methods will provide tools that are essential to help answer these questions.

Another challenge will be to determine how to optimally use biological controls under commercial conditions and to determine mechanisms by which they inhibit crown gall. Although plants are currently being treated with suspensions of the biocontrols when planted, it is unknown how long they will be protected from infection by the pathogen. It is also not known if individual biocontrols will prevent a diverse group of *A. vitis* strains from causing galls or if their activity will be restricted to certain groups of the pathogen. Further research is needed to answer these questions.

A very interesting discovery was that strain F2/5 inhibits galls when coinoculated with pathogenic strains on grape, but not on other plants, suggesting that the biocontrol may be inducing a crown gall resistance reaction that is specific to grape. It will be interesting to study the mechanism of control further and to identify genetic determinants that are associated with control.

Studies on how *A. vitis* has evolved as a grape-specific bacterium will also be of particular interest. The quest for genes that affect *A. vitis* host specificity will be in-

Thomas J. Burr

Dr. Burr received his Ph.D. in plant pathology from the University of California at Berkeley in 1977 in the laboratory of Milt Schroth. Since then, he has been at Cornell in the Department of Plant Pathology at Geneva, where he was promoted to associate professor in 1983 and professor in 1991. In 1987, he spent a sabbatical at the Waite Institute in South Australia with Alan Kerr. His research on *Agrobacterium vitis* has included aspects related to pathogen biology and the development of strategies for crown gall disease control on grape. Dr. Burr is a senior editor for APS Press and Councilor for the NE Division. He was honored by APS in 1986 with the Ciba Geigy Award, in 1990 with the Lee Hutchins Award, and in 1997 as an APS Fellow.

Carlo Bazzi

Dr. Bazzi is an associate professor in the Institute of Plant Pathology at the Faculty of Agriculture, Bologna University, Italy. After graduation in Agricultural Sciences, he received a 3-year fellowship (1971 to 1974) from the National Research Council (CNR) and received his postgraduate degree in phytopathology at the School of Specialization of the Bologna University in 1975. In this period, he carried out studies on fungal diseases of cultivated plants. He joined the Phytobacteriology Laboratory of the Institute of Plant Pathology as a CNR researcher in 1976, and since 1992, he has been doing research and didactic activity in phytopathological bacteriology. His research interests cover several aspects of bacterial plant diseases—and in particular, those caused by quarantine organisms—including detection, epidemiology, biological control, and molecular plant-microbe interactions.

Sandor Süle

Dr. Süle is a research plant pathologist at the Plant Protection Institute, Budapest, Hungary. He received his Ph.D. in 1974 from the Hungarian Academy of Sciences, Budapest, and in the same year he joined the staff of the Plant Protection Institute. Since then, he has worked principally on crown gall and biology of *Agrobacterium tumefaciens*. In 1979, Dr. Süle spent 6 months in Dr. Kado's laboratory at U.C. Davis working on agrocin resistance. In 1984-85, as a Humboldt Fellow, he worked in Professor Seemüller's laboratory in Germany studying the role of ice formation and infection by *Pseudomonas syringae*. Since 1990, he has had a close cooperation with Dr. Burr on grape crown gall research.

Leon Otten

Dr. Otten earned his Ph.D. at the University of Leiden, The Netherlands (1975 to 1979), where he worked on the isolation of the opine synthesis enzymes of crown gall tissues in the group of Rob Schilperoort. He then joined the group of Jeff Schell at the Max Planck Institute of Plant Breeding in Cologne, Germany. His main interests first as a postdoctoral fellow and then as a Max Planck Fellow were the analysis of transgenic plants and their descendence, and the analysis of the *virE* and *virF* genes of *Agrobacterium*. In 1985, he was elected to occupy the chair of molecular phytopathology at the Louis Pasteur University at Strasbourg, France, and moved to the CNRS Institute for Plant Molecular Biology, where he worked on the molecular characterization of the *Agrobacterium vitis* group and its evolution. Since 1996, the isolation of new T-DNA oncogenes and the influence of these genes on the plant cell cycle are the main topics in his group.

triguing. Models predicting the evolution of T-DNAs have provided great insight into *A. vitis* tumorigenesis; however, little is known about the rest of that bacterial genome. It appears from DNA fingerprinting and sequencing of a few chromosomal genes that the *A. vitis* chromosome may be quite divergent from that of other *Agrobacterium* spp. Presently, it is speculated that the ability to produce polygalacturonase, to induce a grape-specific necrosis, and to utilize tartrate may all be related to the specificity of *A. vitis* on grape. It will now be possible to identify genes that are associated with *A. vitis*–grape interactions and to specifically determine their roles. Such information will not only be of academic interest but is also likely to provide information useful for developing novel disease control strategies.

Acknowledgments

We thank the following for research support: NY Wine and Grape Foundation; UST, Inc.; Cornell Center for Advanced Technology-Biotechnology; CNR (Rome, Italy) under Italy-U.S.A. bilateral research project; and the U.S.–Hungarian Joint Board. We also thank M. Borgo and M. Pecile (Istituto Sperimentale per la Viticoltura, Conegliano, Italy) for useful information, E. Sartori and F. Anacleto (Vivai Cooperativi di Rauscedo, Italy) for technical help in biological control experiments, and M. Brisset (INRA, Angers, France) for artwork on Figure 1.

Literature Cited

- Bauer, C., Schulz, T. F., Lorenz, D., Eichhorn, K. W., and Plapp, R. 1994. Population dynamics of *Agrobacterium vitis* in two grapevine varieties during the vegetation period. *Vitis* 33:25-29.
- Bazzi, C., Minardi, P., Burr, T. J., Katz, B. H., Bishop, A. L., and Blanchard, L. M. 1988. Monoclonal and polyclonal antibodies in a comparative serological study of *Agrobacterium* Conn. biovars. *Phytopathol. Mediterr.* 27:51-56.
- Bazzi, C., Piazza, C., and Burr, T. J. 1987. Detection of *Agrobacterium tumefaciens* in grapevine cuttings. *Bull. OEPP/EPPO* 17:105-112.
- Bazzi, C., Stefani, E., Gozzi, R., and Burr, T. J. 1991. Hot water treatment of grape propagation material: Its effects on *Agrobacterium* and on vine growth. *Vitis* 30:177-187.
- Bell, C. R., Dickey, G. A., and Chan, J. W. Y. 1995. Variable response of bacteria isolated from grapevine xylem to control grape crown gall disease in planta. *Am. J. Enol. Vitic.* 46:499-508.
- Bishop, A. L., Burr, T. J., Mittak, V. L., and Katz, B. H. 1989. A monoclonal antibody specific to *Agrobacterium tumefaciens* biovar 3 and its utilization for indexing grapevine propagation material. *Phytopathology* 79:995-998.
- Boubals, D. 1987. Conduite a ténir dans les vignes présentant des broussins. *Progrès Agric. Vitic.* 104:367-368.
- Bouzar, H., and Moore, L. W. 1987. Isolation of different *Agrobacterium* biovars from a natural oak savanna and tallgrass prairie. *Appl. Environ. Microbiol.* 53:717-721.
- Bouzar, H., Quadah, D., Krimi, Z., Jones, J., Trovaoto, M., Petit, A., and Dessauz, T. 1993. Correlative association between resident plasmids and the host chromosome in a diverse *Agrobacterium* soil population. *Appl. Environ. Microbiol.* 59:1310-1317.
- Buchholz, W. B., and Thomashow, M. F. 1984. Comparison of T-DNA complements of *Agrobacterium tumefaciens* tumor-inducing plasmids with limited and wide host ranges. *J. Bacteriol.* 160:319-326.
- Burr, T. J., Bishop, A. L., Katz, B. H., Blanchard, L. M., and Bazzi, C. 1987. A root-specific decay of grapevine caused by *Agrobacterium tumefaciens* and *A. radiobacter* biovar 3. *Phytopathology* 77:1424-1427.
- Burr, T. J., and Katz, B. H. 1984. Grapevine cuttings as potential sites of survival and means of dissemination of *Agrobacterium tumefaciens*. *Plant Dis.* 68:976-978.
- Burr, T. J., Katz, B. H., Bishop, A. L., Meyers, C. A., and Mittak, V. L. 1988. Effect of shoot age and tip culture propagation on grapes on systemic infestation by *Agrobacterium tumefaciens* biovar 3. *Am. J. Enol. Vitic.* 39:67-70.
- Burr, T. J., Norelli, J. L., Katz, B. H., and Bishop, A. L. 1990. Use of Ti-plasmid DNA probes for determining tumorigenicity of *Agrobacterium* strains. *Appl. Environ. Microbiol.* 56:1782-1785.
- Burr, T. J., and Reid, C. L. 1993. Biological control of grape crown gall with nontumorigenic *Agrobacterium vitis* strain F2/5. *Am. J. Enol. Vitic.* 45:213-219.
- Burr, T. J., Reid, C. L., Spittstoesser, D. F., and Yoshimura, M. 1996. Effect of heat treatments on grape bud mortality and survival of *Agrobacterium vitis* in vitro and in dormant grape cuttings. *Am. J. Enol. Vitic.* 47:119-123.
- Burr, T. J., Reid, C. L., Tagliati, E., Bazzi, C., and Süle, S. 1997. Biological control of grape crown gall by strain F2/5 is not associated with agrocinn production or competition for attachment sites on grape cells. *Phytopathology* 87:706-711.
- Burr, T. J., Reid, C. L., Yoshimura, M., Momol, E. A., and Bazzi, C. 1995. Survival and tumorigenicity of *Agrobacterium vitis* in living and decaying grape roots and canes in soil. *Plant Dis.* 79:677-682.
- Canaday, J., Gerard, J.-C., Crouzet, P., and Otten, L. 1992. Organization and functional analysis of three T-DNAs from the vitopine Ti plasmid pTiS4. *Mol. Gen. Genet.* 235:292-303.
- Cavara, F. 1897. Tuberculosis della vite. In: *Torno alla eziologia de alcune malattie di piante coltivate*. Stazioni Sperimentali Agrarie Italiane 30:483-487.
- Cazelles, O., Espard, S., and Simon, J. L. 1991. Influence de la desinfection au sulfate d'oxyquinoleine du potegreffe Berl. X Rip. 5C sur l'expression du broussin. lors de la multiplication de la vigne. *Rev. Suisse Vitic. Arbor. Hortic.* 23:285-288.
- Dong, L.-C., Sun, C.-W., Thies, K. L., Luthe, D. S., and Graves, C. H., Jr. 1992. Use of polymerase chain reaction to detect pathogenic strains of *Agrobacterium*. *Phytopathology* 82:434-439.
- Eastwell, K. C., Willis, L. G., and Cavaleir, T. D. 1995. A rapid and sensitive method to detect *Agrobacterium vitis* in grapevine cuttings using the polymerase chain reaction. *Plant Dis.* 79:822-827.
- Faivre-Amiot, A. 1984. Les tumeurs a *Agrobacterium*. *Phytoma* No. 362:27-31.
- Ferreira, J. H. S., and van Zyl, F. G. H. 1986. Susceptibility of grape-vine rootstocks to strains of *Agrobacterium tumefaciens* biovar 3. *S. Afr. J. Enol. Vitic.* 7:101-104.
- Gerard, J. C., Canaday, J., Szegedi, E., de la Salle, H., and Otten, L. 1992. Physical map of the vitopine Ti plasmid pTiS4. *Plasmid* 28:146-156.
- Goodman, R. N., Grimm, R., and Frank, M. 1993. The influence of grape rootstocks on the crown gall infection process and tumor development. *Am. J. Enol. Vitic.* 44:22-26.
- Haas, J. H., Moore, L. W., Ream, W., and Manulis, S. 1995. Universal PCR primers for detection of phytopathogenic *Agrobacterium* strains. *Appl. Environ. Microbiol.* 61:2869-2884.
- Hedgecock, C. G. 1910. Field studies of the crown-gall of the grape. U.S. Dep. Agric. Bur. Plant Ind. Bull. 183.
- Herlache, T. C., Hotchkiss, A. T., Burr, T. J., and Collmer, A. 1997. Characterization of the *Agrobacterium vitis* *pehA* gene and comparison of the encoded polygalacturonase with the homologous enzymes from *Erwinia carotovora* and *Pseudomonas (Burkholderia) solanacearum*. *Appl. Environ. Microbiol.* 63:338-346.
- Huss, B., Tinland, B., Paulus, F., Walter, B., and Otten, L. 1990. Functional analysis of a complex oncogene arrangement in biotype III *Agrobacterium tumefaciens* strains. *Plant Mol. Biol.* 14:173-186.
- Jager, J. 1990. Untersuchungen zum latentem Vorkommen von *Agrobacterium tumefaciens* Biovar 3 in der Weinrebe (*Vitis vinifera* L.). *Vitic. Enol. Sci.* 45:14-20.
- Kauffman, M., Kassemeyer, H. H., and Otten, L. 1996. Isolation of *Agrobacterium vitis* from grapevine propagating material by means of PCR after immunocapture cultivation. *Vitis* 35:151-153.
- Kerr, A. 1972. Biological control of crown gall: Seed inoculation. *J. Appl. Bacteriol.* 35:493-497.
- Kerr, A., and Panagopoulos, C. G. 1977. Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. *Phytopathol. Z.* 90:172-179.
- Khmel, I. A., Lemanova, N. B., Avdienko, M. I., Ovadis, M. I., Sorokina, T. A., Ismailov, Z. F., and Kernin, L. S. 1991. Potential application of new isolates of soil bacteria for biological control of grapevine against crown gall disease. (Abstr.) *Int. Conf. Pathol. Mol. Biol. Crown Gall. Gif sur Yvette, France*, 25-27 September 1991.
- Kupferberg, S., Zinca, N., Severin, V., and Zamfir, M. 1991. New data concerning the biological control of grapevine crown gall disease. (Abstr.) *Int. Conf. Pathol. Mol. Biol. Crown Gall Disease. Gif sur Yvette, France*, 25-27 September 1991.
- Lehoczy, J. 1968. Spread of *Agrobacterium tumefaciens* in the vessels of the grapevine after natural infection. *Phytopathol. Z.* 63:239-246.
- Lehoczy, J. 1971. Further evidences concerning the systemic spreading of *Agrobacterium tumefaciens* in the vascular system of grapevines. *Vitis* 10:215-221.
- Lehoczy, J. 1978. Root-system of the grapevine as a reservoir of *Agrobacterium tumefaciens* cells. *Proc. 4th Int. Conf. Plant Pathol. Bact. Angers* 239-243.
- Lehoczy, J. 1989. Inoculation experiment on trellised grapevines with *Agrobacterium tumefaciens* for study of the process of crown gall disease. *Acta Phytopathol. Entomol. Hung.* 24:283-291.
- Malenin, L. 1970. Etude de la propagation del' *Agrobacterium tumefaciens* le long des vaisseaux de bois de la vigne et la formation de tumeurs secondaires. *Gradinarika I Lozarska Nauka (Sofia)* 7:127-135.
- Ophel, K., and Kerr, A. 1990. *Agrobacterium vitis* sp. nov. for strains of *Agrobacterium* biovar 3 from grapevines. *Int. J. System. Bacteriol.* 40:236-241.
- Otten, L., Canaday, J., Gerard, J.-C., Fournier, P., Crouzet, P., and Paulus, P. 1992. Evolution of agrobacteria and their Ti plasmids—a review. *Mol. Plant-Microbe Interact.* 5:279-287.
- Otten, L., Crouzet, P., Salomone, J.-Y., de Ruffray, P., and Szegedi, E. 1995. *Agrobacte-*

- rium vitis* strain AB3 harbors two independent tartrate utilization systems, one of which is encoded by the Ti plasmid. *Mol. Plant Microbe Interact.* 8:138-146.
46. Otten, L., de Ruffray, P., Momol, E. A., Momol, M. T., and Burr, T. J. 1996. Phylogenetic relationships between *Agrobacterium vitis* isolates and their Ti plasmids. *Mol. Plant-Microbe Interact.* 9:782-786.
 47. Pansegrau, W., and Lanka, E. 1996. Enzymology of DNA transfer by conjugative mechanisms. Pages 197-251 in: *Progress in Nucleic Acid Research and Molecular Biology*, Vol 4. W. E. Cohn and K. Moldave, eds. Academic Press, San Diego, CA.
 48. Pu, X.-A., and Goodman, R. N. 1993. Tumor formation by *Agrobacterium tumefaciens* is suppressed by *Agrobacterium radiobacter* HLB-2 on grape plants. *Am. J. Enol. Vitic.* 44:249-254.
 49. Reid, C. L., and Burr, T. J. 1994. Wild grape (*Vitis riparia*) as a source of *Agrobacterium vitis*, the pathogen causing grape crown gall. (Abstr.) *Phytopathology* 84:547.
 50. Rodriguez-Palenzuela, P., Burr, T. J., and Collmer, A. 1991. Polygalacturonase is a virulence factor in *Agrobacterium tumefaciens* biovar 3. *J. Bacteriol.* 173:6547-6552.
 51. Sawada, H., Ieki, H., and Matsuda, I. 1995. PCR detection of Ti and Ri Plasmids from phytopathogenic *Agrobacterium* strains. *Appl. Environ. Microbiol.* 61:828-831.
 52. Schroth, M. N., McCain, A. H., Foott, J. H., and Huisman, O. C. 1988. Reduction in yield and vigor of grapevine caused by crown gall disease. *Plant Dis.* 72:241-246.
 53. Schulz, T. F., Lorenz, D., Eichorn, K. W., and Otten, L. 1993. Amplification of different marker sequences for identification of *Agrobacterium vitis* strains. *Vitis* 32:179-182.
 54. Smith, E. F., and Townsend, C. O. 1907. A plant tumor of bacterial origin. *Science* 25:671-673.
 55. Spencer, P. A., Tanaka, A., and Towers, G. H. N. 1990. An *Agrobacterium* signal compound from grapevine cultivars. *Phytochemistry* 29:3785-3788.
 56. Staphorst, J. L., van Zyl, F. G. H., Strijdom, B. W., and Groenewold, Z. E. 1985. Agrocinn-producing pathogenic and nonpathogenic biotype-3 strains of *Agrobacterium tumefaciens* active against biotype-3 pathogens. *Curr. Microbiol.* 12:45-52.
 57. Stover, E. W., Swarz, H. J., and Burr, T. J. 1997. Endophytic *Agrobacterium* in crown gall-resistant and susceptible *Vitis* genotypes. *Vitis* 36:21-26.
 58. Stover, E. W., Swarz, H. J., and Burr, T. J. 1997. Susceptibility of a diverse collection of *Vitis* genotypes to crown gall caused by *Agrobacterium vitis*. *J. Am. Soc. Enol. Vitic.* 48:26-32.
 59. Süle, S., and Burr, T. J. 1998. The influence of rootstock resistance to crown gall (*Agrobacterium* spp.) on the susceptibility of scions in grape vine cultivars. *Plant Pathol.* 47:84-88.
 60. Süle, S., Lehoczy, J., Jenser, G., Nagy, P., and Burr, T. J. 1995. Infection of grapevine roots by *Agrobacterium vitis* and *Meloidogyne hapla*. *J. Phytopathol.* 143:169-171.
 61. Süle, S., Mozsar, J., and Burr, T. J. 1994. Crown gall resistance of *Vitis* spp. and grapevine rootstocks. *Phytopathology* 84:607-611.
 62. Szegedi, E., and Kozma, J. R. 1984. Studies on the inheritance of resistance to crown gall disease of grapevine. *Vitis* 23:121-126.
 63. Szegedi, E., and Nemeth, J. 1996. Investigation of grape shoots for *Agrobacterium vitis*. *Novenyvedelem* 32:605-609.
 64. Tarbah, F., and Goodman, R. N. 1987. Systemic spread of *Agrobacterium tumefaciens* biovar 3 in the vascular system of grapes. *Phytopathology* 77:915-920.
 65. Tarbah, F. A., and Goodman, R. N. 1986. Rapid detection of *Agrobacterium tumefaciens* in grapevine propagating material and the basis for an efficient indexing system. *Plant Dis.* 70:566-568.
 66. Wample, R. L. 1993. Influence of pre- and post-treatment storage on budbreak of hot water treated cuttings of Cabernet Sauvignon. *Am. J. Enol. Vitic.* 44:153-158.
 67. Webster, J., Dos Santos, M., and Thomson, J. A. 1986. Agrocinn-producing *Agrobacterium tumefaciens* strains active against grapevine isolates. *Appl. Environ. Microbiol.* 52:217-219.
 68. Xiaoying, C., and Wangnian, X. 1986. A strain of *Agrobacterium radiobacter* inhibits growth and gall formation by biotype III strain of *A. tumefaciens* from grapevine. *Acta Microbiol. Sin.* 26:193-199.
 69. Xie, X. M., You, J. F., Chen, P. M., and Guo, J. M. 1993. On a strain MI 15 of *Agrobacterium radiobacter* for the biological control of grapevine crown gall. *Acta Phytopathol. Sin.* 23:137-141.
 70. Yajie, L., Jaiying, Z., and Dequin, M. 1990. A biotype 3 strain of *Agrobacterium radiobacter* inhibits crown gall formation on grapevine. *Acta Phytopathol. Sin.* 30:165-171.
 71. Zupan, J. R., and Zambryski, P. 1995. Transfer of T-DNA from *Agrobacterium* to the plant cell. *Plant Physiol.* 107:1041-1047.
 72. Zupan, J. R., and Zambryski, P. 1997. The *Agrobacterium* DNA transfer complex. *Crit. Rev. Plant Sci.* 16:279-295.