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Nature and physiological effects of grapevine diseases

by G.P. Martelli, A. Graniti and G.L. Ercolani*

*Dipartimento di Patologia vegetale and *Istituto di Microbiologia agraria, University of Bari, Via G. Amendola 165/A, I-70126 Bari (Italy)*

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Several factors are known to have detrimental effects to the productivity and appearance of grapevines, through interference with various basic physiological processes. The causes of some adverse effects can be identified as abiotic factors such as climatic adversities, air pollution and nutritional imbalance. Others are infections by pathogens; fungi, bacteria and viruses are most often involved. The effects of infection by the most important of such pathogens will be outlined below.

1. Fungal diseases

A considerable number of fungi are known to cause diseases of grapevines, including species that in many areas are the most important pathogens for this crop. Apart from control costs, the economic importance of most fungal diseases of the grapevines is mainly related to a reduction in the quantity and quality of the yield, particularly of table grapes. For the wine industry, additional losses may arise from the undesirable effect on fermentation of substances produced during the plant-pathogen interaction processes. Only a limited number of fungi cause destructive diseases which may shorten the lifetime of the vines or cause them to die.

Because of the vastness of the pertinent literature, only some physiological aspects of two major diseases, downy mildew and grey mold, will be discussed in detail here.

1.1 Downy mildew

Spring symptoms of downy mildew appear on leaves, green shoots, inflorescences and young fruit clusters. Leaf spots are at first pale green and lightly translucent ('oily spots'), then yellowish – or reddish in some red-berried cultivars – and eventually turn brown and wither. On older leaves, the summer or autumn lesions of mildew are restricted to smaller, polyhedrical, interveinal areas ('mosaic spots'), the tissues of which usually harbor gametangia and oospores of the pathogen, *Plasmopara viticola* (Berk et Curt.) Berl. et de Toni. The asexual sporulation occurs on the lower surface of mildew spots, appearing as a whitish mold. Heavy infections may cause severe leaf fall. Attacks on flowers and developing clusters cause discoloration and distortion ('S'-shaped bending) of the rachis, 'grey-rot' and loss of young fruits, withering and dropping of part or all of the inflorescence. Later, mildew infections through the fruit peduncles and bunch axes may cause 'brown rot' of grapes. Shoots, green nodes of older canes, and buds are also subject to attack and may be severely damaged.

Infection occurs by stomatal penetration of germ tubes developed from encysted zoospores. Before encysting, the zoospores released from a zoosporangium head for open stomata by swimming in a thin film of rain or dew water on the plant surface. Attraction of zoospores by stomata is probably chemotactic with reference to concentration gradients of oxygen or exudates. Several zoospores (4–5) often congregate around a single stoma which is penetrated by as many thin germ tubes. These enlarge in the substomatal cavity to form vesicles from which the invading hyphae of the pathogen originate.

Infection of the grapevine by *P. viticola* is relatively rapid, the entire process, from the deposition of the sporangial inoculum on the leaf surface to the formation of the first haustorium being completed in about 3 h[55]. Colonization of the host tissue is due to the growth of branched coenocytic hyphae of the pathogen in the intercellular spaces. The biotrophic habit of *P. viticola* is shown by a profuse development of pyriform haustoria within the host cells. The bud-shaped hyphal outgrowths, from which the haustoria originate, pierce the plant cell wall mostly mechanically, although enzymic degradation of cell-wall components is supposed to be involved as well. In the early phase of haustorial penetration, the host plasmalemma is invaginated by the growing haustorium. Later, the continuity of plasmalemma around the fully developed haustorium is no longer detected in electron micrographs, possibly because the plasmalemma is not distinguishable from the electron-dense layer surrounding the

haustorial cell wall, whereas it clearly covers the collar formed around the haustorial neck[55, 34]. This collar is presumably a wound response of the host cell and consists mainly of β-glucans (callose). Cells newly penetrated by haustoria show a proliferation of the cytoplasm in the form of a continuous layer around the haustorium, a reduction of the vacuolar system, and some alteration of the chloroplasts[55, 34]. Little is known about the exchange of metabolites and other substances between host and pathogen at the haustorium-cytoplasm interface during the time in which *P. viticola* actively grows as a parasite. At a later stage, either the host cytoplasm degenerates while the haustorium apparently remains intact, or the haustorium collapses while the host cytoplasm shows no sign of incipient necrosis[55]. This, however, is ultimately the fate of the infected tissues of *Vitis vinifera* L. *cult.* and other susceptible hosts.

Vitis species or cultivars which are resistant to *P. viticola* may show hypersensitive reactions leading to a necrotic browning of host cells at the site of infection. In *V. riparia* Michx., brown areas develop around infected stomata within 2–3 days after infection. During the first day, the pathogen is allowed to penetrate the stomatal pore and to form substomatal vesicles, invading hyphae and even the first haustoria; thereafter its further progress is hindered or greatly reduced. Sometimes, however, the hyphal development is affected before the hypersensitive response of the host becomes evident[55]. In some *Vitis* hybrids used as rootstocks, the development of infection hyphae and haustoria is variously affected, being either delayed, reduced or even completely prevented[36]. More than to preformed factors, the resistance of *Vitis* species to *P. viticola* is thought to be due to active defence mechanisms, which are triggered by the pathogen. Several antifungal substances which accumulate in grapevines as stress metabolites, in response to infections by potential pathogens or to various forms of injury, have been associated with the resistance of vines to mildew and other diseases. Such responses include the production of a range of biosynthetically related di- and oligomers of a simple stilbene, *trans*-resveratrol (3,5,4′-trihydrostilbene), collectively called viniferins, and of *trans*-pterostilbene (3,5-dimetoxy-4′-hydroxystilbene)[54, 56, 86]. In compatible interactions of *P. viticola* with susceptible cultivars of *V. vinifera,* the relative concentration of all these secondary metabolites is low in comparison with resistant species *(V. riparia),* where ε-viniferin, α-viniferin and resveratrol, but not pterostilbene, are accumulated more rapidly and in greater amounts[54].

Unfortunately, no comparable advances have been made in the knowledge of the nature and production by *P. viticola* of elicitors of the hypersensitive reactions in grapevine. The elicitation of phytoalexins or other fungitoxic substances may represent an effective method for the selection of vines resistant to diseases[71]. The dual culture of *P. viticola* and grapevine in vitro has also been suggested as a screening test for mildew resistant grape cultivars[57].

1.2 Grey mold and bunch rot

Infections of *Botryotinia fuckeliana* Wetz. may occur throughout the vegetative growth of grapevines. Under favorable conditions, particularly when mild and humid conditions prevail, shoots, leaves, inflorescences, flowers and fruits are an easy prey for this plurivorous fungus. The typical mold which develops on infected plant tissues is due to the abundant sporulation of the conidial anamorph (*Botrytis cinerea* Pers.). Woody organs of the grapevine are also subject to attacks both in the vineyard and during the storage of propagative material, and often show a profuse formation of sclerotia.

Although grapes may be infected at different stages of fruit development, it is most often at or near maturity (bunch rot). Under certain circumstances, a mild attack on white grapes results in a favorable effect on wine ('noble rot'). However, if severe attacks of grey mold have occurred on the grapes before harvesting, both the taste and color of wine may be spoiled ('casse')[73, 76]. A number of substances excreted by *B. fuckeliana* in the infected berries, or during fermentation, produce changes in wine components with either beneficial or undesirable consequences. For example, glycerol and pectolytic enzymes substantially contribute to wine clarification and aging processes, whereas laccases may cause an excessive oxidation and deposition of anthocyanins and other phenolics; moreover, β-glucans and other high molecular weight substances are responsible for wine filtration problems. For table grapes, losses are not proportional to the extent of rot, because the whole bunch is commercially devalued or becomes unmarketable even if only a few berries are affected. Finally, post-harvest decay and rot may develop during transportation and storage, even if the grapes are kept at relatively low temperature.

Colonization of aborted flowers, senescent or necrotic flower parts or other decayed tissues trapped in the developing bunch when it tightens up, as well as infection of the berry stylar-end contribute to the over-summering of the fungus and provide the inoculum for its subsequent spread[24, 84]. Infection of berries can arise either from conidia or hyphae growing on their surface, or from mycelial strands or cushions formed on adjacent infected fruits. The pathogen may remain with ungerminated conidia or other structures on fruits and other plant surfaces, where it successfully competes with epithytic fungi and bacteria, probably because of its ability to produce several organic acids (oxalic, citric, gluconic, etc.) and antibiotics (botrydial, related sesquiterpenes and botrylactone)[10, 91]. Fractures at the pedicel, and wounds and lesions on berries caused by any adverse factor, favor the entry and the establishment of the pathogen in the inner tissues. Even on apparently uninjured berries, minute openings and cracks usually occur in the peristomatal areas, which represent preferential sites of infection[5]. Conidia germinate on plant surfaces provided that periods of wetness or high relative humidity are assured, free water probably being needed by dry conidia for imbibition[10]. After germination, appressoria are formed on the host surface, then a thin infection hypha pierces the cuticle and enters the subcuticular layers of the outer periclinal wall of the epidermis. Alternative mechanisms may enable a direct penetration of the intact epicarp; controversial findings support the view that either mechanical or chemical processes may take place[94].

The cuticle of grape berries is mainly composed by cutin and waxes; the latter also form an epicuticular layer on the berry surface. Thin (0.5–2 μm) pores, left through the

otherwise continuous cuticular texture, may help the pathogen to penetrate. Their number varies with the age and variety of grapes, and is higher in highly susceptible cultivars, and very low in those resistant to the pathogen[9]. A correlation also exists between epidermal thickness and the resistance of grapes to *B. fuckeliana*[94], especially when the grapes are not in compact bunches[7].

Grape isolates of the pathogen actually produce cutinolytic enzymes when their colonies are grown in vitro on media containing cutin preparations from various sources, including grape skin. At least ten C_{12} to C_{22} cutin acids have been detected in culture filtrates, and these include mono- and dicarboxy, mono- and dihydroxy fatty acids, the main component which accumulates in culture brews being a C_{16} unsaturated acid, 10, 16-dihydroxy-hexadecanoic acid[83]. However, there is still a lack of evidence that the infection structures of germinating conidia actually produce such enzymes on grapes, as apparently occurs on tomatoes and other hosts[77, 94]. Apart from specificity of the fungal strain[59], it is likely that the pathogen's ability to break down the grape cuticle is influenced by several factors, such as leakage of nutrients from the host and microbial contamination of the carpoplane.

Once the cuticle is pierced, the fungal hyphae proceed through the epidermis and reach the underlying hypodermal tissues, where they extend mostly parallel to the berry surface and cause marked physiological and structural changes. These include maceration, cell death and browning, associated with cell-wall breakdown, loss of membrane permeability and oxidation of phenolic substances.

The main components of the host cell-walls and middle lamellae, (i.e. pectic substances, hemicelluloses and cellulose) may undergo enzymic degradation, a soft rot being the result of the fungal growth within the outer layers of the mesocarp, whereas the overlying cuticle remains undegraded for a long time. Among the wide array of extracellular cell-wall degrading enzymes produced by *B. fuckeliana,* pectolytic enzymes are most effective. They include exo- and endopolygalacturonases, polygalacturonate *trans*-eliminases and pectin methylesterases[62, 94]. Together with some poorly known hemicellulases, cellulolytic enzymes, especially C_X, are also produced by the pathogen, which is able to carry out a slow degradation of cellulose[95].

It has been supposed that the death of host cells which follows the action of purified pectolytic enzymes is due to an inherent toxicity of the enzymes themselves of their *iso*-forms, but this is still debated[42, 23]. The killing of plant protoplasts may simply be an osmotic effect which follows the disruption of the integrity of cell walls and middle lamellae. However, protoplasts might be killed if their plasmalemma contained a substrate for pectolytic enzymes, which could alter membrane permeability in this way[23]. Finally, it has been suggested that toxins are produced by the pathogen and have a function in pathogenesis, but there is little still known about their chemical nature, occurrence and effect on plant cells[31, 94].

A rapid colonization of plant tissues by the pathogen, and the killing of host cells before they can respond, may prevent or suppress active defense mechanisms taking place in susceptible grapevine cultivars. Production of enzymes or toxic metabolites by infecting structures soon after penetration or diffusion of these substances from adjacent infected tissues may likewise reduce the host response to a minimum.

Among the several mechanisms which enable plants to resist colonization by species of *Botryotinia*[61], preformed plant constituents are supposed to play a role in the natural defence of grapevines against *B. fuckeliana.* For example, the resistance of several cultivars and species of *Vitis* shows a relationship to the amounts of condensed tannins present in the leaves[8]. These substances could inhibit the cell-wall degrading enzymes produced by conidial germ tubes and other infection structures of the pathogen. Incidentally, even fungal metabolites such as glycerol, released by the pathogen itself in older lesions, might have a similar effect on pectic lyases[74].

All these reactions are able to retard the growth of *B. fuckeliana,* allowing accumulation of phytoalexins or other antifungal substances in diseased tissues. This hypersensitive reaction which probably occurs in resistant *Vitis* genotypes is considered to be the most efficient way to restrict the pathogen in limited lesions.

It is known that a number of substances, mostly carbohydrates and glycoproteins, extracted from germinating conidia or mycelium of *B. fuckeliana,* may act as elicitors of phytoalexins in different host species[61]. In the grapevine, substances capable of eliciting host resistance include mucic acid and are thought to trigger a defence reaction leading to accumulation of the stress metabolites already mentioned for the *P. viticola* – grapevine interaction[8]. In tissues surrounding non-spreading lesions of *B. fuckeliana* on mature leaves of *V. vinifera,* α-viniferin predominates compared with both resveratrol and ε-viniferin, whereas in similar leaf tissues of the resistant *V. riparia,* all these stilbenes are produced in larger amounts, ε-viniferin being the main component[54]. In many cases, the resistance of *V. vinifera* cultivars and of *V. riparia* and hybrids to *B. fuckeliana* appears to be related to the concentration of the stress metabolites produced in response to infection[54, 60].

2. *Bacterial diseases*

Several bacteria have been associated with grapevine diseases at one time or another[37]. However, only two of them, i.e., *Agrobacterium tumefaciens* (Smith et Townsend) Conn and *Xanthomonas ampelina* Panagopoulos, have been characterized sufficiently, and only the physiology of the crown gall disease, induced by the former, has been studied in any detail.

2.1 Crown gall

A hyperplastic syndrome known as crown gall has been noted on several hundred plant species belonging to over 300 genera, mostly gymnosperms and dicotyledonous angiosperms[29]. Typical symptoms are most often observed as excrescences on the roots and around the collar of affected plants. On grapevines, rather convoluted outgrowths, frequently in the form of elongated ridges, can be seen emerging through fissured bark on the trunk, arms and canes of plants which may or may not show evidence of the disease on the roots. These outgrowths are called tumors because they result from unlimited host

plant cell proliferation. Galled plants or plant parts usually grow poorly but are not necessarily killed.

Bacteria that cause the crown gall disease are conventionally referred by phytopathological criteria to the species *Agrobacterium tumefaciens*. This, however, is not a satisfactory arrangement because it overweights a plasmid-borne character (see below) and fails to recognize the true taxonomic subdivisions within the genus *Agrobacterium*. Contemporary views tend to regard as the real taxa those entities that are currently called biovars (formerly biotypes), at least three of which can be circumscribed by standard bacteriological techniques[50,58]. Biovar 3 agrobacteria are typically found in association with crown gall on grapevines and have been isolated also from the sap of galled as well as of apparently healthy 'bleeding' vines, and from vineyard and other soils[18,32,49].

All virulent agrobacteria carry one of a diverse array of large (circa 90 to over 160 Mdal) so-called Ti (tumor-inducing) plasmids which may co-reside in the bacterial cell with one or more additional plasmids of various size[68,69]. Each plasmid consists of a circular molecule of double-stranded DNA independent of chromosomal DNA. In addition to ancillary bacteriological traits, Ti-plasmids encode several functions essential for pathogenic interaction of the agrobacteria with the host plant[45]. Diversity in the expression of some of these functions allows classification of Ti-plasmids into groups that are usually based either on the host plant range they largely contribute to determining in the bacterial strain where they reside, or on their ability to determine the synthesis of a given family of tumor-specific amino acid derivatives called opines. Agrobacteria associated with crown gall on grapevines typically harbor limited host range Ti-plasmids and induce tumors that synthesize opines of the octopine family[68,70,90].

Crown gall pathogenesis has been an area of intensive research in recent years[68,69]. Following inoculation into wounded plant sites, agrobacteria bind to exposed plant cells through an interaction that apparently involves plant cell wall components and the lipopolysaccharide of the bacterial outer membrane. There is no evidence that the bacteria subsequently become intracellular but it is firmly established that part of the Ti-plasmid DNA (transfer or T-DNA) is transferred to the plant cells where it becomes covalently integrated into the nuclear DNA. Transcription of T-DNA and expression of the oncogenes in the cell environment results in autonomous cell proliferation and local tumor formation. In contrast to normal plant cells which do not usually grow in vitro unless two plant hormones, an auxin and a cytokinin, are exogenously added, tumor cells are phytohormone-independent. It thus appears that uncontrolled tumor cell proliferation results from a persistent alteration in phytohormone metabolism. This is in line with the presence of high levels of an auxin, indoleacetic acid (IAA), and an extraordinary variety of cytokinins in sterile cultured tumor tissue. The relative endogenous levels of auxin and cytokinin determine the morphology of the tumor, according to the same patterns that are established for healthy tissue. Thus, Ti-plasmids control not only tumor growth, but also tumor morphology. Tumors of the octopine type usually produce such high levels of auxin that differentiation is inhibited, therefore they are usually unorganized[17,68].

The synthesis of opines, products never found in normal plant tissue, is another striking feature of tumor physiology besides phytohormone autotrophy[68]. Opines are not necessary for tumor maintenance but are selectively used as carbon and nitrogen sources by inducing bacteria. Each opine is now known to be produced through the activity of specific opine synthase enzymes which are coded for by genes located in the T-DNA. The genes encoding opine uptake and utilization by the inducing bacterium are also present on the Ti-plasmid, where they

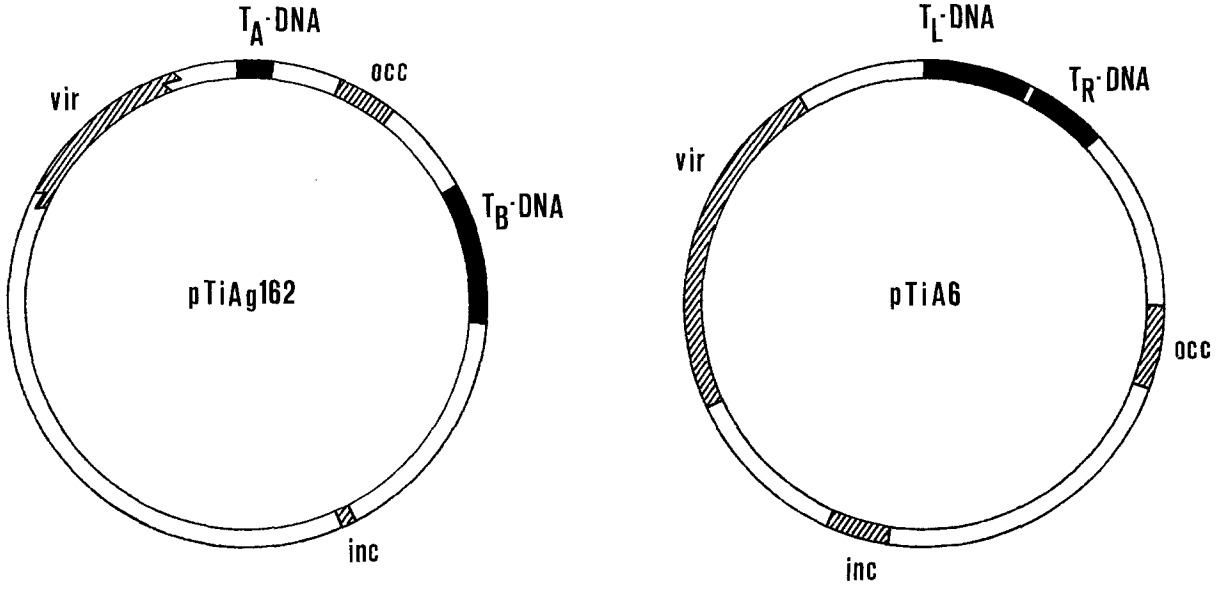

Figure 1. Simplified maps of two agrobacterial Ti-plasmids with limited (pTiAg162) and wide (pTiA6) host range. The location of the *vir* (virulence) genes and of the genes encoding octopine catabolism *(occ)* and incompatibility with other plasmids *(inc)* are shown relative to the portions of DNA (T-DNA) that are transferred and integrated into the plant cell nuclear genome during pathogenesis. Based on Knauf et al.[52].

map outside the T-DNA, and are more or less specific for the same opine that is synthesized under the control of the T-DNA on the same plasmid. Therefore each bacterial strain can normally metabolize only the opines whose synthesis it induces.

Comparative analysis of several Ti-plasmids has revealed analogies as well as differences in organization[68]. Thus, it is a rule that the T-DNA can be resolved into two segments both in wide host range (T_L-DNA and T_R-DNA) and in limited host range Ti-plasmids (T_A-DNA and T_B-DNA), but these lie close together in the former and far apart in the latter[17, 52]. A region containing the so-called *vir* genes which are essential at an early stage of pathogenesis, when they apparently play a role in mobilizing the T-DNA[45], is also invariably found outside the T-DNA, but differs by extension and location in wide and in limited host range Ti-plasmids (fig. 1).

Present knowledge seems to justify the view that crown gall is an example of natural genetic engineering by which a parasitic bacterium re-programs host plant cells to manufacture a novel metabolite it can selectively utilize, and also stimulates the transformed cells to multiply in order that the nutrient supply be increased. Efforts are currently being made to turn some of these bacterial properties to man's advantage. Since the T-DNA can be modified by insertion of foreign DNA without apparently affecting its transferability, the rationale is to introduce plant genes into T-DNA and to remove oncogenes from it. The resulting modified Ti-plasmid can then be used as a vector for conferring alien genes to host plant cells without disturbing normal growth and differentiation of the latter[19].

Crown gall resistance has recently been incorporated into several *Vitis vinifera* × *V. amuriensis* hybrids, one of which has been certified as variety Kunbarat[87, 88]. After a period of skepticism[49], it has now become apparent also that crown gall of grapevines may be amenable to biological control by using strains of non-pathogenic, agrocin-producing biovar 3 agrobacteria with a selective 'killer' action against the strains of the same biovar that induce the disease[85].

2.2 Bacterial blight and canker

The disease is known to be induced by *Xanthomonas ampelina* in several European countries and in South Africa. Old records of a disease supposedly caused by a bacterium called *Erwinia vitivora* should also probably be re-interpreted as misidentified outbreaks of *X. ampelina*[16].

Early symptoms during the growing season include bud failure and stunted growth of the spurs, that may show cankers and underlying hyperplasia of cambial tissue. Cankers may follow on shoots and canes. One-sided necrosis of leaf blades and of flower and fruit clusters can be seen in summer, resulting from cankers on the sides of leaf petioles and of flower and fruit stalks. Spots and marginal necrosis may also affect the leaves.

Combined evidence from symptomatology and from the observed association of causal bacteria with the vascular as well as with the parenchymatous tissues of diseased plants suggests that phytohormone metabolism and vascular transport are affected, but the underlying mechanisms have not been elucidated.

2.3 Pierce's disease

The causative agent of the disease has long been referred to as a rickettsia-like (rippled-walled) bacterium. Data obtained after isolation in pure culture, however, indicate that its identification as a eubacterium of uncertain taxonomic affiliation is probably more correct[28]. The bacterium has a very wide host range but is presently known to occur only in the Americas. The acronym PD-ALS bacterium under which it is sometimes described refers to the organism's ability to induce almond leaf scorch (ALS) in addition to Pierce's disease (PD).

Delayed sprouting followed by marginal leaf necrosis and possibly by separation of the leaf blade from the petiole is typical of the disease. Other symptoms include withering of fruit clusters before harvesting and patchy maturation of the canes.

The overall symptomatology is strongly suggestive of a disrupted water supply and can probably be explained by the finding that the bacteria selectively invade xylem vessels where they form aggregates of cells held together by extracellular material of bacterial origin[28, 46]. In fact there is evidence that the aggregates, together with gum and tylose which may also form, can physically block a sufficient number of vessels to disrupt the regular ascending flow of the sap[46].

2.4 Flavescence dorée and black wood

It has been postulated[37] but not confirmed that mycoplasma-like (wall-free) bacteria are involved in two partly overlapping syndromes in Europe. Both are characterized by reduced plant growth and by downward rolling and brittling of leaf blades, which show yellow areas on white cultivars and reddening of the entire lamina on red cultivars. The symptomatology is completed ('black wood') or not ('flavescence dorée' proper) by blackening of immature portions of the canes in winter. Disease physiology is largely unknown.

3. Virus and virus-like diseases

Among the pathogens of the grapevine, viruses are still the least known. Their elusive nature and a lack of adequate technology have impaired for many years substantial progresses in the understanding of their relationships with the host plant. In the last couple of decades, however, advances have been made especially in the definition of the etiology of some diseases and in certain aspects of virus-host interaction. Some of this information, relating to the main virus and virus-like diseases currently known, will be briefly reviewed.

3.1 Virus diseases

Nature and symptomatology. Of the 24 sap-transmissible viruses isolated from *Vitis* species in different grape-growing areas of the world[15, 93], only those transmitted in nature through the soil by longidorid nematodes (i.e. Nepoviruses) have been identified as causal agents of widespread and economically important disorders. Aphid-transmitted viruses such as alfalfa mosaic (AMV) and broad bean wilt (BBWV), and a score of additional viruses without any known vector, can infect grapevines and induce disease, but these are of negligible importance because of their low incidence and limited geographical distribution[15].

According to the inducing viral agent and field symptomatology, soil-borne diseases can broadly be grouped into two major types of syndromes denoted 'grapevine degeneration' and 'grapevine decline'[63]. Degenerative diseases include fanleaf, caused by grapevine fanleaf virus (GFV) and comparable disorders induced by other European Nepoviruses: arabis mosaic (ArMV), tomato black ring (TBRV), raspberry ringspot (RRV), strawberry latent ringspot (SLRV) and grapevine chrome mosaic (GCMV). Fanleaf proper is ubiquitous, whereas the other diseases are mostly restricted to continental Europe. The field syndromes are often the same irrespective of whether they are caused by a single virus or an association of different viruses, so that it is virtually impossible to identify specific infections on a symptomatological basis in the vineyard.

Degenerative diseases are characterized by a wide range of symptoms depending on the strain of the infective agent. For instance, GFV, ArMV and CGMV possess distorting and chromogenous strains that cannot be distinguished serologically, or morphologically (i.e. host range responses, transmissibility by vectors) from one another, but cause strikingly different field syndromes known by different names[10]. Distorting strains cause malformations of leaves and canes, sometimes accompanied by chlorotic mottling (e.g. fanleaf), whereas chromogenous strains elict bright chrome-yellow discolorations of the foliage without much deformation (e.g. yellow mosaic, vein banding, chrome-yellow mosaic). Infections by all viruses are accompanied by reduced vigor and yield, stunting and shortening of the productive life of the vineyard.

Decline is more typical of diseases induced by tomato ringspot (TomRSV), tobacco ringspot (TRSV) and peach rosette mosaic (PRMV), three American Nepoviruses especially injurious to grapes (*Vitis labrusca* and *V. vinifera* hybrids) grown in the north-eastern part of the United States and Canada. Symptoms consist of delayed sprouting, malformation and mottling of the leaves, loss of crop, generalized and progressive decline.

Virus-host interactions. Symptomatological responses to plant virus infections are a consequence of diverse interacting factors, primarily physiological and structural disturbances. However, all the efforts spent in analyzing metabolic changes in virus-infected plants have failed to provide unequivocal biochemical explanations for the majority of symptoms[11, 30, 35]. From this point of view, grapevine viruses are no exception, even though occasional indications have been obtained of possible ways whereby modification of outward appearance of the host plant could be traced back to a specific physiological change. For instance, altered growth hormone production because of deranged nitrogen metabolism has been held responsible for induction of stunting in GFV- and GCMV-diseased vines, which was apparently due to a reduced synthesis of β-indolacetic acid subsequent to a significant accumulation of tryptophan in infected plants[13, 14, 47].

Further evidence that GCMV interferes with nitrogen metabolism was obtained by Jakò and co-workers[47], who found a substantially higher content of alcohol-soluble free amino acids but lower levels of protein-bound amino acids, total nitrogen and protein nitrogen in symptomatic grapevine leaves. However, whether these changes had a bearing on symptom expression is unclear. Schaefer and Brückbauer[82], in fact, found decreased concentrations of a major protein, possibly identical with protein fraction I (i.e. ribulose-bisphosphate carboxylase), in leaves of vines infected with distorting and chromogenous strains of Nepoviruses, but could not correlate this with visible symptoms. Conversely, as with other virus-host combinations, GFV-induced foliar mottling could perhaps be linked with increased peroxidase activity[4].

The intensity of chromatic alterations was directly related to decreased photosynthesis in vines infected with strains of GFV and GCMV that induce yellowing of tissues[72]. Distorting strains of GFV were also reported to interfere with CO_2 fixation, causing a 20% average reduction of carbohydrate assimilation in infected grapevine leaves[2].

Modifications of the photosynthetic process brought about by the above viruses seem to be consequential to a dramatic fall of chlorophyll 'a' and 'b' content[48] and to derangement of the fine structure of chloroplasts[38]. As expected, the outcome of such metabolic and ultrastructural changes is a reduced total sugar content which, in tissues of severely affected vines, was lowered by 3.5%[48]. Carbohydrate metabolism was also modified in that a remarkable accumulation of reducing sugars, linked with a diminished acid phosphatase acitivty, was detected in GFV-diseased leaves[13, 14].

GFV was reported to affect negatively the transpiration rate of infected vines, possibly because of their smaller green mass and reduced root system, as compared with healthy controls[1].

The results of comparative studies on phenol metabolism of vines of several German cultivars infected with distorting and chromogenous strains of Nepoviruses were inconclusive, because no significant differences were detected in the total amount and seasonal variation of phenolic substances in healthy and diseased tissues[81]. Yet, in GFV-infected grape leaves, Bosc[12] had observed the virtual disappearance of phenols from vacuoles of epidermal cells.

Nepoviurses spread systemically in diseased hosts invading all tissues, including apical meristems[75]. As reviewed by Russo[80], modifications of the fine structure can be observed in the cell walls (finger-like protrusions centered on plasmodesmata), cell wall-cytoplasm interface (paramural bodies, callose depositions) and cytoplasm. The latter contains vacuolate-vesiculate inclusion bodies (fig. 2c), regarded as likely sites of viral RNA synthesis. Empty viral capsids of GFV and ArMV accumulate intranuclearly, whereas virus particles of all Nepoviruses are in the ground cytoplasm, in single rows within tubular structures or in crystalline or paracrystalline aggregates (fig. 2b).

3.2 Virus-like diseases

There are some 13 graft-transmissible disorders of grapevines whose etiology, albeit suspected to be viral, has not yet been experimentally ascertained[15, 63]. Of these only leafroll, stem pitting (legno riccio) and corky bark are geographically widespread and economically relevant.

Leafroll. Several viruses are regarded as possible elicitors of leafroll. As recently reviewed[64], besides a potyvirus

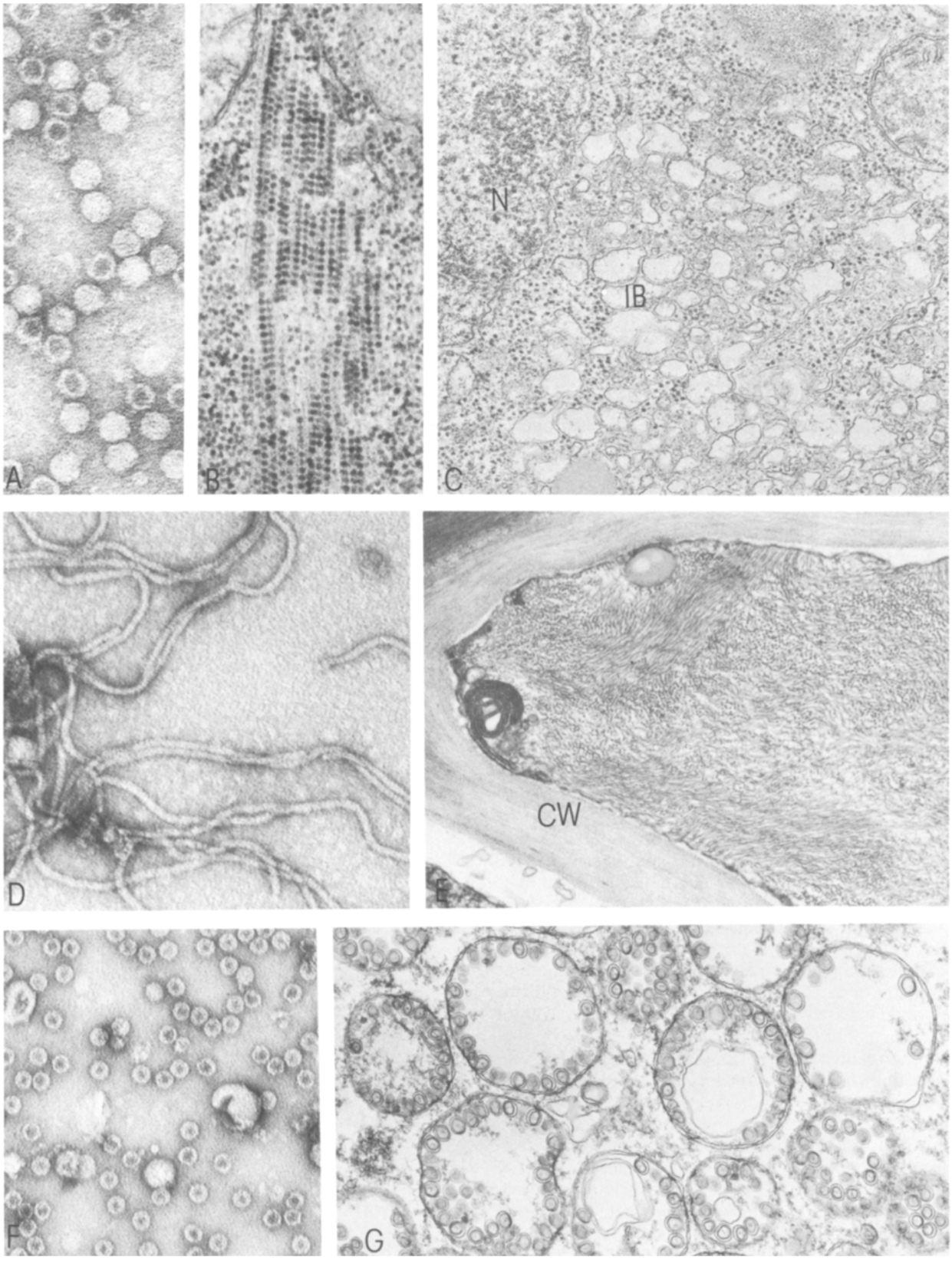

Figure 2. *A* Negatively stained particles of the Nepoviurs tomato black ring, × 170000. *B* Intracellular paracrystalline aggregate of grapevine fanleaf virus particles, × 60000. *C* Nepovirus-induced vesiculate-vacuolate inclusion body (IB), a possible site of viral RNA replication. N = nucleus, × 30000. *D* Negatively stained particles of grapevine virus A, × 115000. *E* Intracellular accumulation of grapevine virus A particles. CW = cell wall, × 45000. *F* Negatively stained isometric virus-like particles isolated from a leafroll-infected grapevine, × 100000. *G* A group of vesiculated bodies derived from deranged mitochondria, associated with the above particles, × 35000.

reported from Israel, candidate etiological agents of this disease are closteroviruses with particle length ranging from 800 to over 2000 nm[40]. One of these viruses, known as grapevine virus A[25], is transmitted by different mealybug species[78,79] (fig. 2d). A non mechanically transmissible isometric virus (fig. 2f) has also been observed in the phloem of some diseased vines[21,22].

Leafroll-affected plants exhibit thick, brittle and downward rolled leaves which turn prematurely reddish or yellowish, in red- and white-berried varieties, respectively. Bunches mature late and irregularly, and are often pale colored, low in sugar and inferior in quality and quantity. In certain cultivars, the extent of these undesirable characteristics is directly related to the severity of symptoms[3].

Leafroll infection has been shown to interfere with several physiological processes. Nitrogen metabolism is affected in that significantly lower levels of nitrogen compounds were detected in fruits[51] and leaves[89] of diseased vines. Millikan and co-workers[65], however, found a higher nitrogen content in diseased leaves.

Modifications of protein content comparable to those observed in Nepovirus-infected plants (i.e. reduced protein fraction I, changes in the pattern of isoenzymes of peroxidase and polyphenoloxidase) were also recorded in vines with leafroll, but no correlation could be established with symptom expression[40,41]. Conversely, a deranged distribution of mineral nutrients, especially potassium, has a dramatic impact on symptoms and on the quality of the crop. Cook and Goheen[26] discovered that potassium accumulates in very large quantity in petioles of leafroll-diseased vines. This blockage has a 2-fold effect: (1) reduces K^+ content in leaf laminae which, therefore, develop symptoms akin those of K^+ deficiency (i.e. reddening, rolling, marginal necrosis); (2) favors K^+ translocation to bunches, an excess of which evokes increased level of malate and tartrate, hence of titratable acidity[41,51].

Potassium content, regardless of its level, does not influence grape anatomy which, instead, is severely affected by other causes. The primary anatomical effect of infection is phloem degeneration in the vascular bundles of leaves, stems and fruit pedicels. Sieve elements are obliterated and crushed, thus impairing carbohydrate translocation from foliar parenchymas[44,96]. Hence, starch accumulates in degenerated chloroplasts[96], causing brittleness and increased thickness of leaf blades, as well as low sugar content of fruits.

Although callose abounds in phloem tissues of leaf veins and petioles of symptomatic leaves[96], no increased β-1.3.-glucan hydrolase activity was detected in leafroll-infected plants[43], as could have been expected by analogy with other virus-host combinations[66].

The ultrastructure of leafroll-diseased vines is characterized by the presence of inclusion bodies in phloem cells. According to the infecting agent, these are made up of: (1) accumulations of closterovirus particles intermingled[33,67,92] or not[21] (fig. 2e) with membranous vesicles; (2) globose to ovoid multivesiculate structures derived from modified mitochondria, which are possibly involved in nucleic acid synthesis[20] (fig. 2g).

Stem pitting (legno riccio) and corky bark. Whereas there is non final proof that stem pitting (legno riccio)[15] and corky bark[15] are related or identical diseases, there is no doubt that they both induce xylem alterations having a comparable ontogeny. The woody cylinder of infected vines is more or less severely indented with pits (stem pitting) and/or grooves (stem grooving) that correspond to pegs and ridges protruding from the cambial face of the bark. These anatomical abnormalities originate from the altered behavior of the vascular cambium which gives rise to hypertrophy, hyperplasia, hypoplasia and parenchymatosis in the secondary xylem and phloem[6,39,53].

The cause of neither of these diseases is yet known, although closteroviruses are more and more frequently found associated with both[27,92]. Since these viruses may be similar or the same (e.g. grapevine virus A[25]) as those found also in leafroll-infected vines, it is clear that further studies are needed for the etiological definition of these virus-like diseases as a whole.

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Full Papers

Cimetidine induces hepatic heme oxygenase activity without altering hepatic heme catabolism

J. Reichen, C. Hoilien and G. R. Kirshenbaum

Divisions of Gastroenterology and Clinical Pharmacology, Department of Medicine, Colorado University School of Medicine, Denver (Colorado, USA), and Division of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Berne (Switzerland), 30 August 1985

Summary. Cimetidine inhibits oxidative drug metabolism; it is not known whether this drug alters the catabolic fate of hepatic heme. We therefore investigated hepatic heme turnover both by a ^{14}CO breath test and directly by labeling the heme pool. Neither acute (150 mg/kg i.p.) nor chronic (150 mg/kg i.p. bid for 3 days) cimetidine administration significantly affected hepatic heme turnover. Chronic, but not acute, cimetidine significantly ($p < 0.025$) increased heme oxygenase activity. Cimetidine inhibited heme oxygenase activity in vitro at concentrations achieved in vivo.
Key words. Cimetidine; heme; heme oxygenase; cytochromes; breath test.

Introduction

Cimetidine has been shown to inhibit the microsomal monooxygenase system both in vivo and in vitro[1–3]. This has been ascribed to a reversible high-affinity binding of the drug to microsomal cytochrome P-450[3,4]. It is not known whether this binding leads to destruction of the cytochrome and whether turnover of heme is altered by cimetidine.

Materials

Male Sprague-Dawley rats, b.wt 175–225 g, were obtained from Charles River Breeding Laboratories, Wilmington, MA. 5-^{14}C-delta-amino-levulinic acid (ALA, sp.A. 48.9 mCi/mmole) was obtained from Research Products International, Elk Grove, Ill. Methanol HPLC grade and D-4 (dibutylamine-phosphate) were from Waters, Milford, Mass. Cimetidine and cimetidine sulfoxide