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Fungal Disease of Grapevines

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ABSTRACT

Grapes have the longest history of all the cultivated fruits and are among the most widely grown. Cooler temperatures and high relative humidity and/or moisture favour the development of fungal diseases of grapes. These diseases affect the leaves, shoots, stems and fruit. Fungal diseases can render fruit unusable and can very easily cause severe losses in yield. Grapevines are fairly adaptable plants, growing in a wide variety of soil types, from light sand to packed clay, and flourishing around the globe in the temperate bands between 20° and 50° latitude, north or south of the Equator. Black rot (*Guignardia bidwellii* (ELL.) V. *et* R. (anamorph: *Phyllosticta ampelicida* (Engelm.) van der Aa), white rot (*Metasphaeria diplodiella* (Viala *et* Ravaz) Berl. anamorph: *Coniella diplodiella* (Speg.) Pet. *et* Syd.), powdery mildew (*Uncinula necator* (Schein.) Burr. anamorph: *Oidium tuckeri* Berk.), downy mildew (*Plasmopara viticola (*Berk. & Curt. *ex*. de Bary) and grey mould (*Botrytis bunch rot – B. fuckeliana* (de Bary) Whetzel) are the most common fungal diseases of grapevine. Recently, decline symptoms (*Phaeomoniella chlamydospora* (W. Gams, Crous, M. J. Wingf. & L. Mugnai) in young grapevines have increased in areas with new planted vineyards resulting in poor vineyard establishment. In this review we describe symptoms of disease and their management, and the effect of climatic factors.

Keywords: climatic factor, management of diseases, *Vitis*, yield losses
Abbreviations: BR, black root; **DM**, downy mildew; **DS**, decline symptoms; **GM**, grey mould; **PA**, phenylamides; **PM**, powdery mildew; **WR**, white rot

CONTENTS

INTRODUCTION

The main conditions for cultivating grape are: level of soil fertility, weather-related advances and delays in development, and the ratio of exposed leaf area to fruit all influence the quantity and quality of grapes produced. For cultivating, it is generally best to purchase varieties that in most years perform well. The performance of any one variety is greatly influenced by local growing conditions and climate (Pearson and Goheen 1988). Grapes require full sunlight, – if possible against a sunny wall (Tonietto and Carbonneau 2004).

Grapes can be grown from two types of cuttings, dormant or hardwood, and green cuttings. The presence of diseases from cuttings could be removed by treatment with hot water (over 50°C) or 5% chlorine bleach solution, because under favourable environmental conditions, disease can develop (Sommer *et al*. 1970; Ophel *et al*. 1990). Grapes prefer deep, well-drained, sandy, or gravel loam soils. Recommended chemical herbicides may also be used for weed control in grapes, but mulching is the preferred management practice. Using mulching with grass clippings, straw, or other suitable material will help keep young vines free from competing weeds and will also help to conserve soil moisture (Scopel *et al*. 1994). To maintain a balance between vegetative growth and fruit production the vine needs to be pruned (Reynolds and Wardle 2001). Pruning of vines received a great deal of attention over the centuries resulting in the development of the curtain trellis system (Read and Gu 2003).

These are the most important cultural practices for maintaining vine productivity. By altering any one of these factors under favourable conditions disease will spread. The aim of our review is to describe the most favourable ecological conditions for the development of fungi and their spread as a pathogen and their subsequent management.

PREDISPOSION FACTOR OF DISEASE

Climatic factor, yield losses

Cool, wet spring conditions allow the disease-causing organism black rot (BR) of grape, caused by the ascomycete *Guignardia bidwellii* (Ellis) Viala & Ravaz (anamorph:

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Invited Mini-Review

Phyllosticta ampelicida (Englem.) van der Aa), to develop and produce both ascospores and conidia in infected fruit that have mummified and overwintered in the vineyard.

The optimum temperature for fungal development during the crop's growing season is 10-25°C. As the disease progresses, the fruit will turn bluish-black in colour and berries shrivel (turn into raisins). Crop losses can be devastating, ranging from 5 to 80% depending on the amount of disease in the vineyard, weather, and cultivar susceptibility. The fungus can infect all green parts of the vine including the fruit, shoots, leaves, and tendrils (Hoffman and Wilcox 2002). Most damaging is the effect on fruit. The fruit-infection phase of the disease can result in serious economic loss. Berries are susceptible to the infection from bloom until shortly after bloom. Protection should be maintained until the berries begin their final ripening stage, at about 5% sugar (Pearson and Goheen 1988).

White rot (WR) is caused by (*Metasphaeria diplodiella* (Viala *et* Ravaz) Berl. anamorph: *Coniella diplodiella* (Speg.) Pet. *et* Syd, the causal organism of white rot of vine. For the activation phase of spore germination tartaric acid was necessary (Aragno 1974). It has been reported from most countries in Europe, from North and South America, Australasia, Oceania, Asia and Africa. In the latter continent it has been reported from South Africa (Matthee and Thomas 1981), Tanzania and Zambia (IMI distribution map No. 335, 3rd Edn, issued 1992). It was first identified in Slovakia in 1995 (Kakalíková and Šrobárová 1996), and is now presently regarded as an international organism of importance on all grapevine propagating material (Crous and Carstens 2000).

The most important environmental factor in the development of downy mildew (DM) is moisture. The most damaging fungal disease of grapevine (*Vitis* spp.) worldwide, DM, is caused by the heterothallic diploid *Plasmopara viticola* (Berk. & Curt. *ex*. de Bary), a biotrofic Oomycete (Stramenopiles) native of North America but in the late 1870s was accidentally introduced to Europe, Australia, and Africa, and in 1990 in New Zealand (Beresford *et al*. 1999).

Wet winters and springs are particularly important for oospore survival and germination. In addition, these spores must be subjected to a prolonged cold spell before they will germinate. Wet summers and suitable temperature (25°C) favour the survival and germination of sporangia and zoospores. Maximum and minimum temperatures for disease development are 8 and 32°C, respectively. Frequent periods with favourable weather conditions and optimal temperature favour disease (i.e. 20-28°C)

Optimum temperatures for Powdery mildew (PM) – *Uncinula necator* (Schein.) Burr. anamorph: *Oidium tuckeri* Berk.) are 20-25°C with a RH anywhere from 40-100%; free water actually inhibits germination (Gadoury *et al*. 1997). Fungal infection on leaves appears as a whitish, powdery covering the upper and lower leaf surfaces (**Fig. 1**). Infected green shoots have brown-black blotches; these are apparent on dormant canes as well. On fruit, the whitish powdery sporulation also appears. Infected berries ripen unevenly and will often split.

Grey mould (GM) *Botryotinia fuckeliana* (anamorph *Botrytis cinerea)* causes a very destructive grey mould rot of the ripening berries of grapevine. The crop is particularly susceptible to infection, which is favoured by rainy conditions from flowering onwards.

GM is favoured by temperatures of 15-20ºC and free water or at least 90% RH. The disease spreads rapidly during moist periods, especially close to harvest. In certain cultivars, slow-developing, late-season infections are termed "noble rot" because they contribute to the production of exceptionally sweet wines (Rogiers *et al*. 2005). Invertase (β-fructofuranosidase) activity was shown to be stimulated in grape berries after infection with GM (Ruiz and Ruffner 2002). The fungus overwinters as mycelium or sclerotia (small black structures) in mummified fruit and other infected plant parts.

Grapevine decline symptoms (DS) - *Phaeomoniella*

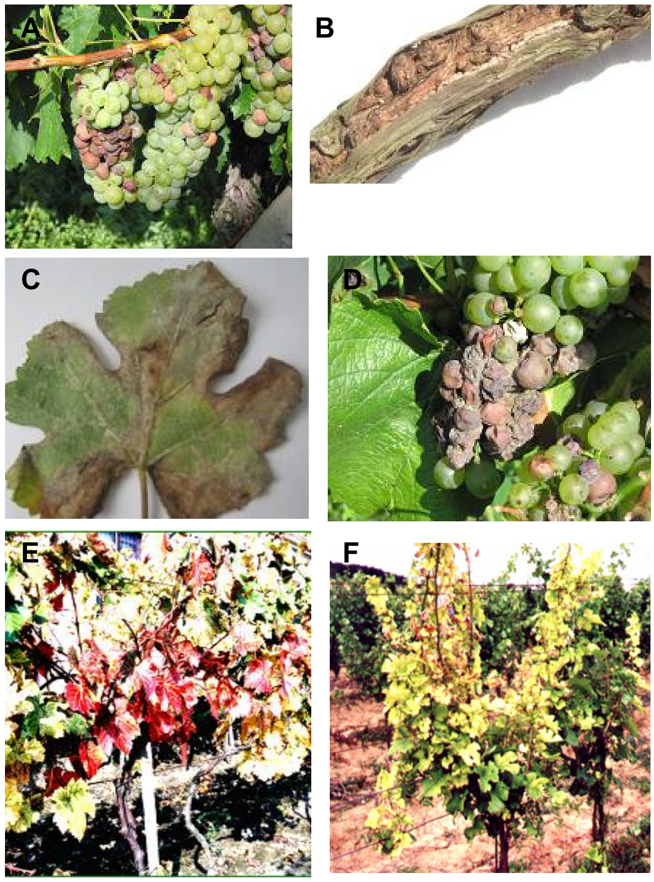

Fig. 1 Symptoms of diseases. White mould on (**A**) grape berries and (**B**) rootstocks, (**C**) powdery mildew, (**D**) grey root. Decline symptoms (**E**) on a leaves of red-berries cultivars and white cultivars (**F**).

chlamydospora (W. Gams, Crous, M. J. Wingf. & L. Mugnai) has been recognized throughout the world in California, Europe, Australia, New Zealand and South Africa (Stewart *et al*. 2003). Young DS caused by the pathogen develops slowly in the first few seasons of vineyard establishment and production. Young vines generally appear normal at planting, but differences in vigour become marked by reduced calliper size of the trunk, shortened internodes, reduced foliage, and reduced leaf size. The first 3 to 5 years after planting, onset of foliar symptoms may appear as intervened chlorosis, followed by necrosis and early defoliation. Viewing trunks of DS in cross-section, dark-brown to black streaking is evident in the vascular elements due to plugging of individual or aggregates of xylem vessels with amber to black gum (gummosis) and formation of tyloses (Chialarappa 2000). A few to most vascular elements may be discoloured.

Below ground, symptoms include reduced total root biomass, reduced numbers of feeder roots, and sunken, necrotic root lesions.

DISEASE

Black rot (BR) is the main fungal disease that infects grapevines in spring, and has the greatest potential of infecting vines during the first month of vegetative growth. Black rot is caused by *Guignardia bidwellii* (ELL.) V. *et* R. (anamorph: *Phyllosticta ampelicida* (Engelm.) van der Aa), and can infect immature leaves, clusters in bloom and green berries (<5% sugar content). Infected fruit generally develops necrotic spots that turn into concentric lesions on the fruit (Pscheidt and Pearson 1989). Whitish dots indicate the infection of berries, later surrounded by a reddish brown ring. The berries then develop to blue-black mummies. The fungus produces ascocarps (pseudothecia) on overwintering mummies. Discharge of the ascospores begins in spring after bud-burst. Young leaves, flowers and young fruits are infected by ascospores. Free water is required for germination. Conidia are formed in black pycnidia on both berries and leaves and cause secondary infection under conditions similar to those for primary infection. Leaf spots appear one to two weeks after infection. The spots are small, tan and circular, enlarging to 0.25 to 0. 50 cm in diameter (Hoffman and Wilcox 2002). The colour of the leaf spots deepens to reddish brown, and the spots are bordered by a dark brown margin. Numerous tiny black structures (pycnidia) of the fungus can be seen within the spot centres. Pycnidia are the fruiting bodies of the fungus, and bear spores, which infect new tissue.

White rot (WR) is caused by (*Metasphaeria diplodiella* (Viala *et* Ravaz) Berl. anamorph: *Coniella diplodiella* (Speg.) Pet. *et* Syd. It is widely distributed internationally, and is closely associated with its host (Matthee and Thomas 1981). Infected berries smell like vinegar.

Shoots of rootstocks infected by WR were seen to have local, grey-green spots with a blackish, slightly ulcerous edge, whose centre later dropped and acquired a fibre-like structure. Mass transport can be largely disrupted by subsequent phloem damage. Leaf infection symptoms were intercostal dark spots which later took on the appearance of shot-hole disease (Brendel 1975). Experimental infection of Mueller-Thurgau confirmed these symptoms. *Coniella diplodiella* (Speg.) Pet. *et* Syd., which has a growth and germination optimum of 23-27ºC, was isolated as the pathogenic organism. Rootstock with latent infections caused damage which led to a high incidence of preplanting deaths. It is therefore proposed that white rot of grapevines be retained as a disease of quarantine significance until local grapevine isolates have been compared on a molecular basis with the established international gene pool of these fungi (Crous and Carstens 2000).

Downy mildew (DM) *Plasmopara viticola* (Berk. & Curt. *ex*. de Bary) earned its fame as part of a mixed blessing sent from America to Europe. American *Vitis* (grape) species were resistant to the aphid so rootstocks of these species were sent to Europe to help control the problem. Unfortunately, DM is endemic in America and the causal fungus was also sent overseas (Tran Manh Sung *et al*. 1990). The fungus is an obligate parasite. DM is found worldwide wherever grapes are grown, occurring primarily where warm, humid conditions exist during the growing season. All common cultivated and wild species of grape as well as a few hosts outside the *Vitis* species are susceptible to this disease. The fungus over-seasons as oospores in the soil or contained in shed or attached leaves. It can also survive as mycelium in infected twigs. *Plasmopara viticola* becomes active in the spring when oospores germinate to form a sporangium. Sporangia are formed in the dark and are disseminated by wind or rain splash. They germinate on host tissue when free moisture is present releasing zoospores. These spores swim to stomata on leaves, twigs or berries and encyst. Primary infection occurs when these spores germinate and penetrate the host through stomata. The fungus becomes established as an intercellular mycelium and then produces sporangia that exit host tissue through stoma on the undersides of leaves or lenticels on fruit. Zoospores (Santili 1957) released from these structures cause secondary infections, entering the host through stomata or lenticels. As the season progresses, oospores are formed from mycelia within host tissue completing the life cycle. In contrast, the presence of downy mildew did not significantly affect the assimilation rate of the healthy tissue portion of infected leaves (Moriondo *et al*. 2005), the reduction in intercellular concentration of CO_2 and photosynthetic pigment may explain the lower assimilation rates in the healthy tissues and DM leaves respectively.

Anomalous spots of the fungus (i.e. atypical coloration of leaves or mosaic) on leaf tissues of a sensitive *V. vinifera* grapevine were observed. The anomalous symptoms were often associated with the typical 'oil spots' – were absent in leaf tissues without DM spots, but showing severe ultrastructural modifications (Musetti *et al*. 2005). Several plant virus infections were found in these grapevines. The development and sporulation of some phytopathogenic fungi inside their hosts can be limited by virus infections. Further experimental approaches are required to determine if resistance to alterations in *P. viticola* mycelium as endophytes are present.

Powdery mildew (PM; *Uncinula necator* (Schein.) Burr. anamorph: *Oidium tuckeri* Berk.) is perhaps the most important fungal disease of grapevines worldwide. It was previously thought that the PM fungus overwintered inside dormant buds of the grapevine. Spores released by spring rains are the primary inoculums. Ascospore release always began after bud burst and generally ended before blossoming. The release periods of ascospores were always associated with a rainfall higher than 2 mm, a wetting duration greater than 2.5 h, an average temperature generally above 11°C and a daily mean temperature sum from November 1 to the first ascospore release above 1100°C (Jailloux *et al*. 1999). There was no relation between earliness, number of ascospores released, and disease severity on grapes (*Vitis vinifera* L. cv. 'Merlot'). The primary infection did not appear to be important for the increase of the powdery mildew population; on the other hand, the weather conditions of April (rainfall and temperature) seemed to strongly influence disease severity on berries by enabling good growth of the pathogen on leaves. Wind carries the fungus where it grows on any green surface of the vine. The infected area has a dusty or powdery appearance. Spores produced in infected areas provide secondary inoculums for infections throughout the growing season. Esterase activity is associated with conidia and infection structures. Adhesion assays showed that the presence of esterase-cutinase inhibitors on the cuticle did not significantly affect adhesion (Rumbolz *et al*. 2000). This disease is native to eastern North America, but gained notoriety when it was introduced into European vineyards in 1845, causing extensive losses as it spread rapidly throughout the continent.

Grey mould (GM; *Botrytis bunch rot – B. fuckeliana* (de Bary) Whetzel exists in all vine growing areas throughout the world (Nair and Allen 1993), where it is given the right climatic conditions and insufficient control measures and can quickly colonise the berries, macerate them enzymatically and cover them with a thick conidial layer. The fungus can infect all green parts of the vine, though bunch rot tends to be the biggest problem. In early spring, buds and young shoots may be infected and turn brown. In late spring, V-shaped or irregular brown patches may appear on leaves. Inflorescences may also be blighted and wither away. Some flower infections can remain latent until version (Cole *et al*. 1996). From version onward, the fungus can infect grape berries directly through the epidermis or through wounds and may continue to invade the entire cluster. During dry weather, infected berries dry out; in wet weather, they tend to burst and become covered with a greyish mould, which contains millions of spores. These spores are spread by wind to new infection sites. The fungus is spread by anything that moves soil or plant debris, or transports sclerotia. The fungus requires free moisture (wet surfaces) for germination and cool (15-25ºC), damp weather with little wind for optimal infection, growth, sporulation, and spore release. *Botrytis* is also active at low temperatures, and can cause problems on vegetables stored for weeks or months at temperatures ranging from 0-10ºC (Broome *et al*. 1995). Infection rarely occurs at temperatures above 25ºC. Once infection occurs, the fungus grows over a range of 0-35ºC.

Decline symptoms (DS) - *Phaeomoniella chlamydospora* (W. Gams, Crous, M. J. Wingf. & L. Mugnai) in young grapevines have increased in areas with new planted vineyards resulting in poor vineyards establishment. Chlorosis of lamina, reddening and necrotic areas on the margins (**Fig. 1**), although not consistent, develop on the leaves of inoculated plants. Very seldom leaf rolls occur there. Dark streaks are found extending from the inoculated site along the trunk. Symptoms of disease were described by several authors, but pathogenicity of associated fungi was confirmed by Crous and Gams (2000). Epidemiology studies by researchers in Spain (Armengol *et al*. 2001), showed that *Phaeomoniella chlamydospora* colonises parenchyma cells, and actively penetrates the cells. The defence response of the plant reacts by the production of tyllae and accumulation of phenols in the tissue, whereby browning occurs around the infection site. This response could have an influence on the rate of spread of the fungi through the host.

Observed field, foliar and internal wood symptoms like those in vines with decline associated with *P. chlamydospora* and *Phaeoacremonium* spp. were described by many authors in different countries (Chialarappa 2000). Various names were used for this disease, ranging from black goo to slow dieback, slow decline and *Phaeoacremonium* grapevine decline. During the International Workshop on Grapevine Trunk Diseases held in Portugal (September, 2001), consensus was reached that the disease will henceforth be called Petri disease. Esca is regarded as the most important disease of older grapevines in Europe (Surico 2002), and *P. chlamydospora* fungus may be considered as the main pathogen causing the decline of young or older grapevines. The presence of pathogenic fungi probably depends on vineyard vigour, age and environmental conditions (Oliveira *et al*. 2004) and expression of decline symptoms is deeply influenced by the environment. Another explanation exists: fungi are present as endophytes of wood without coloration, and they produce some metabolites which may be involved in symptom expression of Petri disease.

APPLIED CONTROLS

Biological methods

Considerable effort was put into basic research involving the identification of the new organisms and their antagonists, and the development of techniques for effectively estimating pathogen populations. It was at this time that the movement towards biological control was initiated. Histochemical analyses of such HR lesions revealed the accumulation of lignin-like deposits in the host cells after infection by DM (Cohen *et al*. 1999).

Virtually all agricultural crop cultivars in use today have some form of genetic resistance incorporated, generally against a number of diseases on which the selection of genes to genetically engineer into plants to protect against fungal diseases has been based. However, following expression of different types of chitinases in a range of transgenic plant species, the rate of lesion development and the overall size and number of lesions were reduced upon challenge with many fungal pathogens, including those with a broad host range, such as *Botrytis cinerea*. Reduction of this pathogen could be developed in preliminary tests (Kikkert *et al*. 2000) by the expression of the *Trichoderma harzianum* endochitinase gene. Rice chitinase reduced development of *Uncinula necator* and fewer lesions due to *Elisinoe ampelina* (Yamamoto *et al*. 2000).

The oomycete grape DM (*Plasmopara viticola* Berk. & Curt. *ex*. de Bary) is a serious pathogen of grapevine and spreads by extremely efficient cycles of asexual propagation.

The high efficiency must involve efficient sensing of the host. Experiments (Kortecamp 2006) were conducted to elucidate whether or not grapevine plants susceptible to DM exhibit an identical defence response after inoculation with the non-host pathogen. Expression analysis of defence-related genes revealed marked differences between the susceptible cultivar ‘Riesling’ (*Vitis vinifera*) and the resistant cultivar ‘Gloire de Montpellier’ (*Vitis riparia*). Whereas some genes seem to be expressed constitutively in ‘Gloire’ or induced after an inoculation with both pathogens, expression of defence-related genes in ‘Riesling’ was influenced mainly after inoculation with the non-host pathogen: PR-2, PR-3, PR-4, a PGIP gene, and especially genes encoding enzymes involved in anthocyanin biosynthesis (DFR, F3H, LDOX) were affected (Kortecamp 2006). Catechins and related phenolic compounds were produced within the first 48 hai (hours after inoculation) with *P. viticola* in the susceptible cultivar, whereas in resistant ‘Gloire de Montpellier’ no further production was seen (Kortecamp 2006), which may be due to the high content of polyphenolics as observed in control leaves. Therefore, the occurrence of the respective products (flavans and other phenolics) in inoculated leaves was investigated (Downey *et al*. 2006). Phytochemicals are used intensively in vineyards to limit pathogen infections.

The β-1,3-glucan laminarin derived from the brown algae *Laminaria digitata* was shown both to be an efficient elicitor of defense responses in grapevine cells and plants (Aziz *et al*. 2003). Laminarin did not induce cell death, but rather reduced infection by *B. cinerea* and *P. viticola* by approximately 55 and 75%, respectively. The activation of defense responses using elicitors could be a valuable strategy to protect plants against pathogens.

Recently Laquitaine *et al*. (2006) used ergosterol, a non-specific fungal elicitor, to trigger lipid transfer protein 1 (VvLTP1) and 1,420-bp of its promoter and stilbene synthase gene expression in grape plantlets and enhanced protection against *Botrytis cinerea*.

Biological preventive treatment to control decline symptoms have been used: hot water treatment of cuttings gave excellent control in two trials, but the biological control treatment Trichoflow gave poor results (Fourie and Halleen 2006).

There are several non-chemical control options: plant resistant cultivars; leaf removal around clusters to reduce humidity and wetness; training systems that help expose the fruit.

Chemical control

Managing fungal diseases in grapevines is an annual event. Control currently depends heavily on the use of elemental sulphur (both for organic plantings and for non-organic production plantings). Other options available include sterol inhibitors (currently used to some extent), strobilurins, oils, lime-sulphur + sulphur (used somewhat), and a biocontrol fungal parasite, *Ampelomyces quisquallis* (AQ10). Assessment of control efficacies for these options and a new biocontrol option Serenade (Serenade 10W) is needed to help the wine grape industry knowledge to ably select the best options for their vineyard situations (Larsen and Caspari 2005). In addition, assessment is needed to validate the use of the grape PM disease model.

Protection of the vine against BR is to use an applicant from the group of ditiocarbamates, which are also used against DM, namly with active group of mancozeb (Dithane M 45, Dithane DG; Novozir MN 80, Polyram WG, Mancosan 80WP) as well as applicants, which operate against PM (Rubigan 12 EC, Topas 100 EC, Punch 10 EW, Bayleton 25 WP). Very proper application is the use of Cabrio Top (Metirame-zinc 55% + Pyraclostrobine 5%) with good effects against DM and PM (Kakalíková and Šrobárová 2006). Applications used to protect vines against BR are presented in **Table 1**. Attendance realize preventative in years above standard rainfall - permanent rainy and dumpy weather, by humidity up to 100% RH and optimalization temperature 20-27°C, as it was in August 2005 in Slovakia. A fungicide with good eradicative activity, such as Nova, Elite, or Bayleton, should be applied as soon as possible, if black rot infection is expected to have occurred, the sterol inhibitors would be good choices.

The strobilurins (Abound flowable 2.08F, Flint - 50 WDG - trifloxystrobin Sovran- Sovran 50WDG) have some eradicative activity, but should be used primarily as protectants. Both Sovran and Abound will provide only fair control of BR, and both have at least fair activity against GM. When the efficacy of Rovral (Rovral 75 WG) for GM control appears to become reduced (Kakalikova and Šrobárová 2006), new materials Vangard fungicide (Cyprodonil) and Elevate fungicide (Fenhexamid) should be introduced into

Table 1 Applicants to control black rot of grape, fungicides with an active substance, appropriate concentration and days for prevention.

Fungicide	Common name of active substance	Concentration (%)	Days to prevention
Dithane M45	mancozeb	0.30	42
Dithane DG	mancozeb	0.30	42
Novozir MN 80	mancozeb	0.30	42
Cabrio Top	strobilurin+metiram	0.20	42
Polyram WG	metiram	0.30	42
Mancosan 80WP	mancozeb	0.30	42
Bayleton 25 WP	triadimefon	0.02	28
Rubigan 12 EC	fenarimol	0.02	28
Topas 100 EC	penconazol	0.03	35
Punch 10 EW	flusilazol	0.03	35

Table 2 Applicants to control white rot of grape, in concentration from 0.3 to 0.30, with 21 to 35 days of prevention and 7 days for table grape.

Fungicides	Concentration (%)	Common name of active substance	Days to prevention
Hattrick	0.30	tebuconazole + tolylfluanid	35
Hattrick WG	0.20-0.30	tebuconazole + tolylfluanid	35
Shavit F 71,5 WP	0.20	triadimenol + folpet	35
Folpan 50 WP	0.30	folpet	35
Folpan 80 WDG	0.20		35
Quadris	0.10	azoxystrobin	21
Discus	0.03	kresoxim - methyl	35
Forum FP	0.20	folpet + dimetomorph	35
Melody Combi WP	0.25	folpet + iprovalicarb	35
Euparen multi	0.15-0.3	tolylfluanid	35

the fungicide program for GM control.

Beltanol-50 (Quinosol) treatment was not possible with WR material, but wood with a surface infection could successfully be disinfected (Brendel 1975). In a very high pressure of infection in 2005 we used different fungicides with a different active substances to protect grape (**Table 2**) with a very high efficacy (Kakalíková and Šrobárová 2006).

Fungicides inhibiting the mitochondrial respiration of plant pathogens by binding to the cytochrome bc1 enzyme complex (complex III) at the Qo site (Qo inhibitors, QoIs) were first introduced to the market in 1996 (Wellmann *et al.* 1996); later Gisi *et al.* (2002) described them for control of DM populations. The main fungicides used for DM control belong to just four groups of chemicals, those with phenylamides (mostly metalaxyl) being the most important because of their systemic and curative activity. Phenylamides are single site inhibitors, however, and bear a high intrinsic risk for selection of pathogen resistance, which is now prevalent. Also there are already reports of DM resistance to new groups of fungicides such as the strobilurins. The ultimate goal towards prolonging effectiveness of fungicides is optimal application, with early and reliable diagnosis as a key component of integrated crop management programmes. The sensitivity of populations fluctuates from year to year and within a season. Sensitive, intermediate and resistant isolates can be detected in fields that have or have not been treated with PAs and are in a "dynamic equilibrium" with each other. The dynamics of resistance evolution are driven not only by selection through PA fungicides; equally important are the inheritance and genetic background of resistance, as well as fitness and migration of isolates (Downy *et al.* 2006).

In high pressure of infection, a mixture of experiments indicated that selection pressure was affected most by the dose of the QoI fungicide (Genet *et al.* 2006) and the nature of the partner fungicide. Folpet 80 WDG delayed selection pressure most effectively when it was associated with famoxadone or azoxystrobin. Mancozeb is marketed by the trade names Dithane, Manzeb, Nemispot, Manzane and was least effective at reducing the rate of selection compared with the QoI (QoI – Quinone outside inhibitor) alone, and fosetyl-aluminium (Fosetyl-Al) was intermediate.

Following the discovery and use of Bordeaux mixture, several relatively insoluble copper compounds or fixed coppers were developed. Cupric products at a low dose and alternative compounds have been tested to control DM in an organic vineyard (La Torre *et al*. 2005). It was found that copper compounds (commercial formulations of fixed copper include C-O-C-S, Kocide 101, Tribasic Copper sulphate, Champ, and Tenn-Copp 5E) control DM in a satisfactory way, reducing, at the same time, the dose of copper metal.

Copper fungicides - Benalaxyl, oxadixyl and ofurace at 10 mg/L and Fosetyl-Al and phosphorous acid at 100 mg/L also completely inhibited sporulation of each of the isolates in Australia tested for each fungicide (Wick e*t al.* 1987). Timorex is a new organic bio-pesticide. The 66 EC formulation of Timorex (containing 66% tea tree oil) was used in all experiments. Timorex (1%) inhibited spore germination and had a prophylactic activity against powdery and downy mildews on young plants (Reuveni *et al* 2005).

Recently chemicals together with biological control to decline symptoms (DS) have been publishing by Fourie and Halleen (2006). Products containing *T. harzianum* (Trichoflow-T), hydrogen peroxide (Bio-Sterilizer) and 8-hydroxyquinoline sulphate (Chinosol) gave inconsistent results, whereas Bronocide (a blend of halogenated alcohols and water) proved to be a good sterilising agent, but reduced certifiable plant yield significantly. Benomyl at 100 g/100 L, Sporekill (a patented didecyldimethylammonium chloride formulation at 150 ml/100 L) and captan (at 1,000 ml/100 L) were consistently the best treatments as growth parameters were not negatively influenced and pathogen incidences in basal ends and graft unions of uprooted plants were reduced. Results obtained in our laboratory indicate that fungicides Falcon 460 EC have an effect *in vitro* against DS - *P. chlamydospora*, in particular one of its components, spiroxamine. Falcon 460 in a mixture with other components had a synergic effect (Jankura *et al*. 2007). The *in planta* (in laboratory) experiments suggest that preventive treatment of vines with Falcon 460 EC can help to avoid the development and spread of DS.

Most fungicides are registered for control of BR, DM, PM, and leaf spot. Some recent research (Laquitaine *et al.* 2006) suggests that some fungicides have fair to good activity in the control of *Botrytis*. For example, the sterol-inhibiting fungicides such as Nova provide excellent control of BR and PD, but are not effective against DM. Thus, a tankmix of Nova 40 W 4 plus Penncozeb 75 DF (mancozeb) has been recommended to control all these diseases simultaneously (Leavitt and Duvall 2005): Nova 40 W 4 (myclobutanil) for BR and PM and Penncozeb 75 DF (mancozeb) for DM and *Phomopsis*. They have good residual activity and provide good control in 10-14 day spray schedules. They are also "locally" systemic and provide some level of "after-infection" activity. One problem with the strobilurins is that they are a high risk for fungicide resistance development.

Fungicide resistance development is a serious problem being facing with these new fungicides, as well as previously registered materials such as the sterol-inhibitors (Bayleton, Nova 40 W 4, Rubigan (fenarimol), Procure and Elite) Leavitt and Duvall (2005). When Triadimefon (Bayleton 50% DF) was first registered in the world, it could be used at 57 g product per acre on a 21-day schedule and provide excellent PM control. After years of continual use, the PM fungus developed a high level (Ypema and Gubler 1997a, 1997b) of resistance to Bayleton.

CONCLUSION

Fungi are responsible for a range of serious plant diseases such as blight, grey mould, bunts, powdery mildew, and downy mildew. Fungal plant diseases are usually managed with applications of chemical fungicides or heavy metals. In some cases, conventional breeding has provided fungus resistant cultivars.

Genetic engineering enables new ways of managing fungal infections. Several approaches have been taken: 1) introducing genes from other plants or bacteria encoding enzymes like chitinase or glucanase (Kortecamp 2006). A gene genetically controlling fungal infection- protein 1 (VvLTP1) and 1,420-bp has been introgressed into *V. vinifera* using a pseudo-backcross strategy, and genetic markers have previously been identified that are linked to the resistance locus (Laquitaine *et al*. 2006) enhanced protection against *Botrytis cinerea*; 2) Introducing (Kikkert *et al*. 2000) plant genes to enhance innate plant defence mechanisms (e.g. activing phytoalexins, proteinase inhibitors, or toxic proteins); 3) Invoking the hypersensitive reaction (Cohen *et al*. 1999). Plants varieties that are naturally resistant to specific types of fungal diseases are often programmed to have individual cells quickly die at the site of fungal infection. Despite such research having begun many years ago, no commercial cultivars of vine are available today that use these approaches.

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