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Fungal Trunk Pathogens in the Grapevine Propagation Process: Potential Inoculum Sources, Detection, Identification, and Management Strategies

Symptoms of young grapevine decline and failure of planting material have plagued the raisin, table, and wine grape industry since the 1990s. Evaluation of declining young vineyards has revealed that many factors are involved in the poor performance of vines. Fungal trunk pathogens have been extensively investigated and are now considered one of the major causes of this syndrome. Black-foot and Petri diseases, and species of Botryosphaeriaceae, all contribute to young grapevine decline, reducing productivity and longevity, thereby causing considerable economic loss to the industry. Subsequent investigations have led to the conclusion that planting material used in young vineyards is already infected, either systemically from infected mother vines or by contamination during the propagation process. In this review, the causal organisms and their associated symptoms are discussed. Specific attention is given to the grapevine propagating process, the potential inoculum sources, and the detection tools being developed to facilitate rapid identification of these pathogens. The review also evaluates the currently known management strategies applied in nurseries. Lastly, an overview is given of how to minimize the economic impact of these pathogens and to improve the quality of planting material.

Importance and Impact of Fungal Trunk Pathogens in Young Vineyards

Decline symptoms in young vineyards have dramatically increased all over the world since the early 1990s, when the wine industry entered a period of rapid expansion that was sustained for a decade. As a consequence, growers have been forced to replant sizeable vineyard areas, causing substantial economic losses and compromising the stable production in viticulture and the wine industry (218).

Evaluation of declining young vineyards has revealed that many factors are involved in the poor performance of vines (128,183,192,193,218). These factors have been divided into three major groups: (i) nursery induced stress (structural vine defects, extended cold storage, limited vine carbohydrates and pot-bound root systems), (ii) vineyard establishment and management stresses (inadequate ground preparation, incorrect planting, inappropriate irrigation and nutritional deficiencies or excesses), and (iii) biological stresses (nematodes, root-colonizing fungal pathogens, viruses, rootstock/scion incompatibilities, or fungal trunk pathogens) (192,193). In the course of investigations, it has become evident that the causes of young vine decline and failures are numerous and complex; however, fungal trunk pathogens have been extensively investigated and are now considered one of the major causes of this syndrome.

The main fungal trunk diseases associated with young grapevine decline are black-foot disease, caused by *Cylindrocarpon* spp. (*Cylindrocarpon destructans* (Zinnsm.) Scholten, *C. liriodendri* J.D. MacDon. & E.E. Butler, *C. macrodidymum* Schroers, Halleen & Crous, and *C. pauciseptatum* Schroers & Crous) and *Campylocarpon* spp. (*Campylocarpon fasciculare* Schroers, Halleen & Crous and *Campyl. pseudofasciculare* Halleen, Schroers & Crous) (81,85,86,184), and Petri disease, caused by *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams, as well as numerous species of the genus *Phaeoacremonium* (30,77,134).

Field symptoms of black-foot or Petri disease affected vines are frequently indistinguishable. External symptoms include stunted growth, reduced vigor, retarded or absent sprouting (Fig. 1A), shortened internodes, sparse and chlorotic foliage with necrotic margins, wilting, and dieback (Fig. 1B) (57,161,183).

In addition, characteristic symptoms of vines affected by *Cylindrocarpon* spp. and *Campylocarpon* spp. are sunken necrotic root lesions with a reduction in root biomass and root hairs (8,81). To compensate for the loss in root mass, a second crown of horizontally growing roots is sometimes formed close to the soil surface (103). Removal of rootstock bark reveals black discoloration and necrosis of wood tissue which develops from the base of the rootstock (Fig. 1C). A cross-section through these lesions reveals the development of necrosis extending from the bark to the pith (57,103). Characteristic blackened sectors occurring at the base of the rootstock have given the disease the name of black-foot (75,116).

By contrast, dissected vines affected by Petri disease show a typical black discoloration of the xylem vessels, which is a result of tyloses, gums, and phenolic compounds formed inside these vessels by the host in response to the fungus growing in and around the xylem vessels (Fig. 1D and E) (134).

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It is believed that the host is predisposed to the pathogenic phase of these fungi by stress, in particular water stress (54). Blocked xylem vessels accentuate the water stress and lead to insufficient water and nutrient supply to the vegetative plant parts. This leads to symptom expression, which usually occurs during periods of high water demand (103,134).

Environmental factors and host stress such as malnutrition, poor drainage, soil compaction, heavy crop loads on young plants, planting of vines in poorly prepared soil and improper plant holes also play an important part in the development of black-foot and Petri diseases (57,103).

Several phialophora-like and *Acremonium* species have also been involved in the decline of young vines, mainly species of the genus *Cadophora* Lagerb. & Melin (84,144). Of those, *Ca. luteo-olivacea* (J.F.H. Beyma) T.C. Harr. & McNew has been recently shown to be quite common on grapevines affected by esca and Petri disease in California (174), South Africa (84), Uruguay (2), New Zealand (117), Northeastern America (169), and Spain (73). *Pleurostomophora richardsiae* (Nannf.) L. Mostert, W. Gams & Crous (basionym: *Ca. richardsiae* Nannf.) has also been associated with esca and Petri disease in California (168), and caused vascular discoloration after field and glasshouse inoculations similar to that seen in Petri diseased grapevines in South Africa (84).

Additionally, species of the family Botryosphaeriaceae have been frequently isolated from declining young vineyards in different grapevine-growing areas worldwide (66,70,80,119,140, 151,202). To date, 17 different members of the Botryosphaeriaceae, placed in the anamorphic genera *Diplodia*, *Dothiorella*,

Fig. 1. A, Growth of good and poor quality young vines from the same nursery. Affected plants show stunted growth, reduced vigor, and retarded sprouting. **B,** Symptoms of severe leaf wilting and dieback (plant indicated by arrow). **C,** Black discoloration and necrosis of wood tissue which develops from the base of the rootstock characteristic of black-foot disease. **D,** Rootstock cross section showing a ring of necrotic xylem vessels surrounding the pith, characteristic of Petri disease. **E,** rootstock longitudinal section showing dark brown to black streaking of the xylem tissues. **F,** Dead arm affected by Botryosphaeriaceae species. **G,** Cross section showing a wedge-shaped necrosis caused by Botryosphaeriaceae species.

Fusicoccum, *Lasiodiplodia*, and *Neofusicoccum*, have been reported to be pathogenic on grapevines (16,197,200,203–205,209).

Symptom expression caused by Botryosphaeriaceae species has been shown to differ from region to region and among different grapevine cultivars (110,209). Typical disease symptoms include cankers and other dieback symptoms such as bud mortality, leaf chlorosis, fruit rot, sectorial wood necrosis, shoot dieback, cane bleaching, and graft union failure (Fig. 1F and G) (102,109,110,150). Symptoms caused by species of Botryosphaeriaceae are also especially severe in cases where the host plant has been subjected to stress (149,156), and this is probably the reason why the most severe losses due to this disease occur in grapevines that are 8 years and older (109).

Cases of decline of young grapevines attributed to black-foot disease and Petri disease pathogens, and other related fungi such as *Ca. luteo-olivacea*, as well as species of Botryosphaeriaceae have been reported from most grape-growing regions in the world, including South Africa (55), the United States of America (126,183), Italy (133,134), France (107), Chile (18), Australia (145), Turkey (10,99), Portugal (28,161), Austria (162), Spain (11), Argentina (65), Greece (180), New Zealand (166), Germany (56), Uruguay (2), Iran (125), Brazil (181), and Argelia (19).

The capacity of fungal grapevine trunk pathogens to have a very severe impact on the sustainability of the industry's expansion focused the attention of researchers during the late 1990s. The need for closer coordination between scientists working on these diseases was realized, and the idea of an International Council on Grapevine Trunk Disease (ICGTD) was conceived. Since then, there have been seven International Workshops on Grapevine Trunk Diseases (IWGTD) (1999, 2001, 2003, 2005, 2006, 2008, and 2010) organized by the ICGTD. These workshops have provided an excellent forum for the exchange of information on developments in grapevine trunk disease research around the world, and much work has been published on epidemiological, management, and diagnostic aspects of trunk diseases. Detection of fungi involved in young vine decline and their spread through propagation material has been of particular concern for researchers. The subsequent increase in studies on this aspect has led to the conclusion that planting material is already infected in young vineyards, either systemically from infected mother vines (13,40,53,58,59,80,146, 152,161,164,166,186,227) or by contamination during the propagation process (12,13,20,22,66,73,106,154,164,210,212,225, 230,232).

The presence of endogenous pathogens in planting material in newly established vineyards has been recognized as a cause of poor vine vigor, lower than acceptable yields, and commensurate reductions in income and return on capital (218). Infected parent material used for vine propagation means that a very high proportion of new vineyards will be affected. Even if these vineyards do not fail to establish, it is likely that vines will begin to decline as they mature (214,216). This has created significant problems for growers and resulted in a number of cases of accusation, litigation, and extensive replanting. Thus researchers, in an effort to minimize the economic impact in the grapevine industry, focused their attention on the detection, identification, and control of fungal grapevine trunk pathogens during the propagation process.

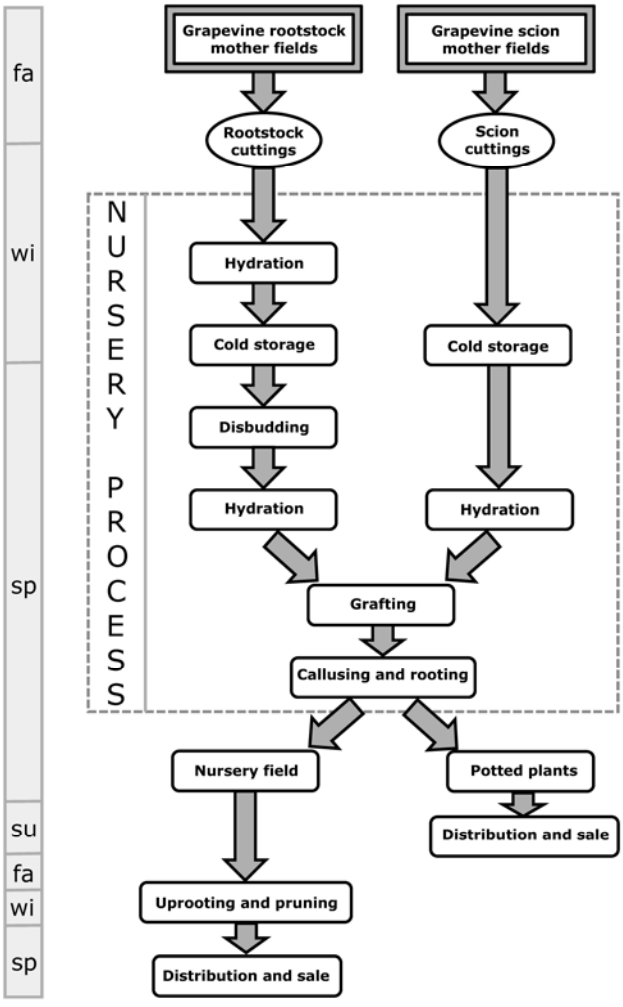

Fig. 2. Diagram representing the propagation process of grafted plants in grapevine nurseries. Abbreviations: fa, fall; wi, winter; sp, spring; su, summer. In this figure, the seasons correspond to the Northern Hemisphere.

Production of Vines

In general, the fundamentals of the grapevine propagation process are quite similar in all grapevine production areas of the world. A diagram representing the production of grafted plants is shown in Figure 2.

Traditional grapevine propagation techniques use rootstock or scion mother vines, from which dormant cuttings are taken for bench grafting, rooting, or field budding. However, some practices, such as hydration, cold storage conditions, and general standards of nursery hygiene, that can have a significant effect on the quality of the vines produced, may vary considerably within and between nurseries. Mother vines are generally planted from dormant rootings or potted plants and require about 3 years to produce generous amounts of cuttings (138).

Once the cuttings have been collected from rootstock and scion mother plants (Fig. 3A and B) in autumn and early winter, they are usually immersed in water for periods between 1 and 12 h (62,232). Fungicides (Chinosol or Rovral) and general biocides (Vibrex) are sometimes added to the soaking water or applied in a separate soaking bath (138). However, practices vary depending on the country and the individual nursery. For instance, the practice of soaking rootstock cuttings in water and fungicides before cold storage is not common in Chilean (3), French (9), and Spanish nurseries (13,92). Following the initial soaking, the cuttings are held in cold storage at 2 to 3°C (62,232) or 5 to 6°C (13) with 90% humidity until late winter or early spring (Fig. 3C) (62).

Following cold storage, rootstock and scion cuttings are usually soaked again in water for periods of 2 to 4 h to 4 days (Fig. 3D) (3,62,92,229,232) and then either bench-grafted and callused or, if they are to be grafted in the field, simply callused. Bench-grafting is the most commonly used method for grafting vines throughout the world, as it is the most suited for mass production of grafted vines (138). Nurseries in most of the vine producing countries usually join rootstock and scion cutting using 'omega-cut' or 'V-cut' grafting machines (Fig. 3E) (3,9,138,192). In South African nurseries, grapevines are grafted mainly by means of long whip and tongue hand-grafting, and to a lesser extent by 'omega-cut' grafting machines (88,111). Following grafting, the graft unions are dipped in a melted (70 to 75°C) wax formulation that may contain plant growth regulators or fungicides that encourage graft

union callus development while inhibiting fungal contamination (Fig. 3F) (92,138).

For callusing, cuttings and grafted vines are held under growth-stimulating, warm and humid callusing conditions until callus forms at the base of the cutting and around the graft union (Fig. 3G) (88,229). This method of callusing is preferred because cuttings can be removed from the cool room, grafted, and callused in batches to facilitate efficient use of labor. The older practice of callusing cuttings in sand or sawdust pits in the open is largely redundant because it requires a high labor input over a very short period.

Cuttings and grafted vines for callusing are generally packed in crates or boxes with coarse vermiculite, perlite, moss, or sawdust moistened to prevent dehydration of the vines and held in humidified chambers for 2 to 3 weeks depending on the temperature and the variety (92,138,229). The temperature, humidity, and callusing medium may vary between nurseries. For example, in Italian nurseries, plants are put in forcing boxes filled with wood shavings and maintained at 30 to 32°C with 75 to 90% relative humidity (RH) for about 2 weeks (230). In South Africa, hand-grafted cuttings are cold-callused at circa 18°C for a period up to 5 weeks, while machine-grafted cuttings are hot-callused at 26 to 28°C and 70% RH for a period of up to 3 weeks followed by a hardening off period of 1 to 2 weeks under shade netting (62). The callusing medium used in South Africa consists of fresh pine sawdust drenched in a broad-spectrum fungicide (164). In Spain, grafted plants are placed in plastic boxes with a 10-cm peat bed and stored at 24 to 26°C and 80% RH for 16 to 20 days (13,92). Australian

Fig. 3. A, Grapevine rootstock mother plants in early stages of development. **B,** Grapevine scion mother plants. **C,** Grapevine cuttings in cold storage. **D,** Cuttings being soaked in water tanks. **E,** Omega-cut grafting machine. **F,** Graft unions being dipped in a melted wax formulation. **G,** Grafted vines under growth-stimulating, warm and humid callusing conditions. **H,** Vines planted in an open-root field nursery. **I,** Vines planted into pots. **J,** Cuttings pushed into the soil by hand leaving at least two nodes exposed. **K,** Control treatments of weeds using herbicides at pre-emergence.

nurseries mainly use callusing boxes containing coarse vermiculite, and the resulting grafted cuttings are held at 28 to 29°C for 2 weeks (138). In Chile, grafts are placed in a suitable packing medium and stored at 20 to 25°C and 85 to 90% RH for 25 to 30 days (3). North American nurseries usually pack callusing grafts into boxes containing peat moss, coarse sawdust, or wood shaving at 24 to 29°C from 10 days to 2 weeks (221,229).

Following successful callusing, grafts are removed from the callusing boxes, and the shoots are trimmed if too long. They are dipped again in a suitable grafting wax to give a thin coating over the scion and graft union and are usually dipped into cold water immediately after waxing to cool them down. The bottom of the grafts can also be dipped in a fungicide as a protection against pathogen attack (3,92,138), although this is not a universal practice. Vines are then transported and planted in an open-root field nursery (Fig. 3H) or planted into biodegradable pots filled with a standard commercial potting mixture, sandy loam soil, or peat/perlite pots and grown in a glasshouse before hardening off in shade (Fig. 3I) (3,92,138,221).

If the soil is infected with parasitic nematodes, it should be fumigated by injecting a suitable nematicide at a depth of 15 cm with soil moisture at seedbed condition, and when the soil reaches the temperature recommended for each specific fumigant (138). The planting distances for cuttings in a nursery vary according to the equipment used, but are normally in the range of 5 to 7 cm (92,138,229). This can range from a hand plow pulled by a horse to a medium size tractor with big plows and discs. A row spacing of 1.2 to 1.5 m is suitable to allow access by a normal size vineyard tractor. A furrow is ripped along each row, water is applied along the full length of the furrow, and the cuttings are pushed into the soil, leaving at least two nodes exposed (Fig. 3J). A planting machine can also be used which allows a person sitting on the machine to push cuttings into a furrow opened and closed as the machine progresses (138). There is little root development at this stage, as roots may break off during planting with consequent loss of reserves and more susceptibility to soilborne pathogen attack (94). In South African nurseries, graft unions are covered with soil, which is later removed following successful bud burst (62). This practice is used to prevent drying of the callus tissue, but could increase the occurrence of soilborne pathogens such as *Cylindrocarpon* spp. in this plant zone, and is rarely used in other countries as the grafting wax provides sufficient protection from dehydration. In cool climate areas, rooting may be improved by planting the cuttings through polyethylene sheeting. This gives a higher soil temperature, reduces water loss, and eliminates competition from weeds (92,94,138). However, plastic covering of the soil had no apparent effect on the performance of the nursery vines in South Africa (93).

The care of a nursery established in an open site is quite similar to that devoted to any field crop. The supply of adequate water of good quality is probably the most crucial requirement during the growing season. Adequate soil moisture and aeration is also important since overwatering favors most soilborne pathogenic fungi and reduces aeration in the root zone (199). During the early part of plant growth, vines are watered until the root system has become established, especially if the weather is hot and dry (3,92). Overhead sprinklers are frequently used in South African nurseries (62) and other warm climates (H. Waite, *personal communication*) and are often considered a good method of irrigation provided the sprinklers have a uniform distribution pattern and are mounted high enough to clear the foliage (138); however, this method could enhance pathogen survival and dispersal and disease development (98), and drip irrigation is often used as the main source of irrigation once the vine root systems are established. A recent study demonstrated that overhead sprinkler irrigation can trigger Botryophaeriaeae spore release in some vineyard sites in California (201).

In a vine nursery, weed control is essential, as weeds compete for water, nutrients, and sunlight and impede the lifting operation (3). Additionally, weeds have recently shown to be potential hosts for black-foot and Petri disease pathogens (4). Herbicides are normally used to control weeds in field nurseries. Herbicides including Devrinol, Surflan, or Trifluraline can be used in nurseries at preemergence (Fig. 3K) (138). Once the vine canopies have grown enough to shade the soil, weed growth is much reduced. Grapevine nurseries require little fertilizer if the soil is reasonably fertile. On less fertile soils, small quantities of urea and ammonium nitrate can be applied regularly during the growing season, but applications are usually stopped at the end of the summer to allow shoots to harden (3,92,229). Nurseries are subject to the same pests and diseases as are vineyards. In fact, downy mildew and oidium infections are favored by the crowded conditions of the nursery. Protective copper-sulfur-based sprays are applied soon after budburst and regularly thereafter, depending on weather conditions, to control downy mildew, oidium, erinose, and budmite (138).

Dormant field finished plants are usually lifted during winter. This work can be done by hand or by mechanical implements (92,229). A U-shaped or L-shaped digger fitted to a tractor can be used to cut the roots and lift the vines. The grafts are then carefully graded, pruned, and once more dipped in paraffin at 70 to 75°C to prevent dehydration of the buds (138). They are then kept in cold storage and sold to growers as dormant bench-grafts the following spring (9,229). Alternatively, callused cuttings and grafted vines that have been planted into biodegradable pots and grown in a glasshouse and shade house are ready for spring/summer delivery and planting by early summer in the year of propagation. Although potted grapevine plants do perform well, their utility is more restricted than dormant field-finished product. Potted products are more difficult to check for defects and can rapidly become stressed by remaining too long in the pot before planting (138,193), or by exposure to harsh field conditions before they are properly acclimated. Some nurseries also offer rootstock rootings for field graftings. These nongrafted vines are callused and field grown prior to sale as dormant rootings the following spring in the same manner as grafted plants (138).

Potential Inoculum Sources in the Grapevine Propagation Process

Rootstock and scion mother fields. The role of rootstock mother vines as a primary source of grapevine trunk pathogens has been well-documented by several authors. However, the role of scion mother vines is less defined, with conflicting data both affirming (38,146) and negating (14,20,80,187,232) its contribution to disease in young vines. Australian researchers have reported more common isolation of grapevine trunk pathogens from scion material, leading them to conclude that the source of infection may be either rootstocks or scion mother vines (38,40,146). This may be related to the Australian practice of growing predominately own-rooted scions, hence providing a greater amount of older scion material for the pathogen to be isolated from.

Most nurseries allow mother vines to sprawl on the ground, which in combination with flood irrigation can result in water-soaked cuttings. Some nurseries in California and South Africa, however, cultivate rootstock on a trellis, which is more labor intensive but can eliminate potential soil-surface pathogen contamination and thereby produce better quality cuttings (93,193).

Surveys of rootstock mother plants as a source of Petri disease pathogens have been focused mainly on the detection of *Pa. chlamydospora* (1,58,59,104,152,154,163,164,186,227). Species of *Phaeoacremonium*, mainly *Pm. aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai, have also been isolated from rootstock mother plants (13,59,161) and from scion cuttings (230).

Species of *Cylindrocarpon* have also been detected in rootstock mother plants; however, the incidence of black-foot disease and Petri disease pathogens was extremely low in all cases (13,58,59,63,80,104,105,140,158,161,210,230). Infections caused by several species of Botryosphaeriaceae have been frequently observed in rootstock mother fields (13,22,58,59,80,140,153,158).

In many cases, the presence of black-foot and Petri disease pathogens as well as Botryosphaeriaceae species in the grapevine rootstock mother plants was not related to the observed external

symptoms (13,40,59,80,84). The demonstrated presence of these pathogens in symptomless grapevines with internal black wood streaking could be explained by reports (54) that the fungi can behave as an endophyte or latent pathogen until the grapevines are stressed, for example by water deficit (54) or nematode populations (192), after which these diseases become evident. Thus, this demonstrates the high potential risk of mother vines as an inoculum source in the vegetative propagation process.

Given this incidence of infection in mother vines, it was important to establish whether infection could be passed systemically into current season's growth. In this sense, conidial distribution of *Pm. aleophilum* and *Pm. inflatipes* W. Gams, Crous & M.J. Wingf. in xylem vessels (52) and active growth of *Pa. chlamydospora* mycelium within shoots (42,146) has been observed. It has therefore been hypothesized that spores are carried in sap flow of infected mother plants, which causes the subsequent contamination of canes (42,170). The ability of *Pa. chlamydospora* to move from the retained rootstock mother-vine trunk or head into current season's growth was investigated by determining disease levels in own-rooted rootstock vines propagated from cuttings collected at different distances from the head (227). Vines from dormant cuttings collected nearest the head had a higher level of infection at the 8-month assessment (42%) than those collected from further away (6 to 8%). At the 20-month assessment, infection in vines from close to the head and vines from actively growing cuttings collected furthest from the head were similar (53 and 55%, respectively).

It is clear that infected mother plants act as reservoirs of inoculum from which trunk disease pathogens infect the rootstock or scion canes; but how do mother vines become infected and where is the original source of inoculum in the field?

First, it has been hypothesized that soil can act as a source of the pathogens (20,127,196). Species of *Cylindrocarpon* are commonly known to be saprobes in soil, occur on dead plant substrata, or act as weak pathogens of plants infecting wounds on roots and stems of various hosts (81,184). The production of chlamydospores would allow *Cylindrocarpon* spp. to survive for extended periods in soil (85).

It has been suggested that *Pa. chlamydospora* is a soilborne pathogen due to its ability to produce chlamydospores in culture (20,134,187). Chlamydospores are thought to form conidia that can penetrate uninjured roots of vines in nurseries or vineyards (20,52). The presence of *Pm. aleophilum* in the soil (47,171) and standing water under grapevines (171) was confirmed by molecular methods. *Pa. chlamydospora* was detected in vineyard soil using different polymerase chain reaction (PCR) techniques in New Zealand (226) and in South Africa (32,164). Retief et al. (164) suggested that *Pa. chlamydospora* might be present in these infected soils as mycelium, conidia, chlamydospores, and/or other fruiting structures originating from infected mother plants. Ridgway et al. (167) detected *Pa. chlamydospora* DNA from soil inoculated with viable spores at 17 weeks, indicating that spores could persist in soil and the inoculum could build up over time. Pathogenicity studies have shown that *Pa. chlamydospora*, *Pm. aleophilum,* and *Pm. inflatipes* can infect dormant grape wood through cut wounds exposed to infested sand, although *Pa. chlamydospora* was only minimally successful in colonizing wood from the soil (96). Whiting et al. (228) demonstrated that *Pa. chlamydospora* and *Phaeoacremonium* spp. were able to adapt to a wide range of water potentials, possibly as a survival strategy in soil.

Pruning wounds are also a possible point of entry into grapevine mother plants for these pathogens. *Pa. chlamydospora* produces a phoma-like synanamorph on canes and litter (31,38,39,108), resulting in inoculum dispersal via rain splash and irrigation, leading to pruning wound infection (45,107,146,157). Some species belonging to the genus *Phaeoacremonium* produce perithecia (*Togninia* as a sexual stage) in old, rotted, vascular tissue of pruning wounds and in deep cracks in cordons, trunks, and spurs (48,49,172,175–178). Ascospores are released from these overwintering structures with rainfall and infect the grapevine through pruning wounds (45,48,49). Spores of *Pa. chlamydospora* and *Phaeoacremonium* spp. were collected on jelly-coated microscope slides placed close to vine trunks and cordons (45,108). However, recent studies failed to trap spores of *Pa. chlamydospora* and *Phaeoacremonium* spp. using a volumetric spore trap (124,208). In the case of *Pm. aleophilum*, the presence of both mating types (129) and the formation of *T. minima* perithecia on incubated grapevine wood (147) have indicated that the teleomorph could easily form in the field under the right environmental conditions. Several studies have shown that *Pa. chlamydospora* and *Pm. aleophilum* could readily infect pruning wounds inoculated with conidia (44,96,108) and that pruning wounds were particularly vulnerable to colonization when pruning was done early in the season (44,96,103,107,185). Insect transmission of sexual spores may also occur (45), although no study has been conducted within vineyards to prove this. Evidence of this type of dispersal has been found in the isolation of *Pm. scolyti* L. Mostert, Summerb. & Crous (101) from insect larvae as well as in the isolation of *Pm. parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf. (90,179) and *Pm. mortoniae* Crous & W. Gams (89) from larval galleries inside tree bark.

There is also considerable evidence to support the hypothesis that infection by Botryosphaeriaceae occurs via wounds (112,188,208,213). A number of studies have, however, also shown that these fungi can infect directly through lenticels, stomata, or other openings on healthy plants (26,97,123). Species of Botryosphaeriaceae are able to produce pycnidia and pseudothecia on diseased wood or pruning debris from which propagules are released during wet, rainy weather (91,112,149,201,208). These propagules are then wind or splash dispersed by rain or sprinkler irrigation to infect wounds (91).

Grafting and callusing. Much work has been published on the detection and identification of fungal trunk pathogens at various stages of the propagation process. One of the earliest stages where infection can occur is during the postharvest soaking in water (hydration) prior to cold storage. The water itself may be a source of waterborne microorganisms, but even if the soaking water is clean it will be contaminated by field-acquired microorganisms and abiotic contaminants on the bark of the bud sections dispersing into the soaking water (219).

In New Zealand nurseries, Whiteman et al. (225) found that infection rates of *Pa. chlamydopora* in cuttings increased from 39% prior to nursery processing to 70% after processing and identified prestorage and pregrafting hydration and fungicide tanks as potential sources of inoculum. Retief et al. (164) also found a very high percentage of positive water samples of *Pa. chlamydospora* collected after prestorage hydration (40%) and during grafting (67%) in South African commercial nurseries. These authors suggested that mycelium and conidia present on the surface of cuttings might wash off into the water during hydration, or they might even ooze from xylem vessels into the water. *Pa. chlamydospora* (37) and *Phaeoacremonium* spp. (13) were detected in poststorage hydration tanks by molecular techniques in Australian and Spanish nurseries, respectively. Species of Botryosphaeriaceae and Petri disease pathogens (*Pa. chlamydospora* and *Phaeoacremonium* spp.) were detected by PCR on the surface of grafted varieties and rootstocks and in hydration and callusing baths in French nurseries (212). In Italy, Pollastro et al. (154) detected *Pa. chlamydospora* DNA in 28% of pregrafting and 23% of precallusing hydration tanks. Water used in commercial cool-down tanks after hot-water treatment (HWT) is usually chlorinated, but it is not sterile and has also been demonstrated as a potential source of *Pa. chlamydospora* and other microbial contaminants (29,37,164,225).

Many wounds are produced during cutting and graft preparation, such as those resulting from disbudding, grafting, improperly matched or healed graft unions, or the rooting process. The large number of cuts and wounds make the propagation material very susceptible to infection by fungal trunk pathogens (20,183). This is further accentuated by the fact that *Pa. chlamydospora*, *Pm. aleophilum,* and species of Botryosphaeriaceae have been shown to be potential pruning wound colonizers (108,188,190).

Rego et al. (161) isolated *Cylindrocarpon* spp., *Pa. chlamydospora,* and *Pm. aleophilum* from symptomatic rootstock cuttings prior to grafting in Portuguese nurseries. In Italy, Zanzotto et al. (232) investigated the occurrence of fungal pathogens on plants' rootstock and scions before grafting and plants after grafting, and found that *Phaeoacremonium* spp. occurred in many of the grafted plants and were frequently isolated from both the rootstocks and the graft union, whereas *Pa. chlamydospora* isolation rates from grafted plants were much lower.

In 2003, New Zealand researchers reported detecting *Pa. chlamydospora* contamination during the grafting process using a nested-PCR approach (225). Subsequently, Retief et al. (164) found in South African nurseries that a percentage of the rootstock (42%) and scion (16%) cuttings that were sampled during grafting tested positive for the presence of *Pa. chlamydospora*. The PCR assays were able to detect *Pa. chlamydospora* DNA from washings of grafting tools in Spain (13) and in cuttings postgrafting in Australia (37). In France, Vigues et al. (210) reported that the presence of Botryosphaeriaceae and *Pa. chlamydospora* in the propgating material increased as the nursery process progressed. Conamination by Botryosphaeriaceae increased after cutting rehydraion and by *Pa. chlamydospora,* during the callusing stage. *Pa. chlaydospora* was also detected in grapevine sap flow (7%) and on grafting machines (29%) by nested-PCR in Italian nurseries (154).

High temperatures (26 to 28°C) and humidity in callusing boxes and callusing rooms favor the growth of pathogens. Halleen et al. (80) isolated high percentages of several *Phaeoacremonium* spp. and *Pa. chlamydospora* from callused cuttings prior to planting in South African nurseries. However the incidence of *Cylindrocarpon* spp. was much lower. Retief et al. (164) found that a very small number (8%) of the callusing medium samples tested positive for *Pa. chlamydospora*. These results correlate with those from Whiteman et al. (225), who found a very low percentage of positive *Pa. chlamydospora* samples from washings of callusing media in New Zealand nurseries. In Australian nurseries, Wallace et al. (220) reported reduced percentage of certifiable vines due to callus inhibition by *Pa. chlamydospora* infections. Edwards et al. (37) also detected *Pa. chlamydospora* in water from callusing media using PCR. Larignon et al. (104,106) demonstrated that *Pa. chlamydospora* contamination is possible during the callusing stage in French nurseries by bringing inoculated plants into contact with healthy plants. In Italy, Pollastro et al. (154) found *Pa. chlamydospora* DNA in rootstock cuttings and graftings before and after callusing (2 to 6%), and grafted rootstocks (57%).

The detection of *Pa. chlamydospora* in most of these studies was based on the presence of pathogen genomic DNA. However, it is important to consider that the mere presence of DNA does not indicate whether viable pathogen propagules are present. However, Aroca et al. (13) were able to detect viable propagules of *Phaeoacremonium* spp. and *Pa. chlamydospora* from washing pruning shears and callusing media by filtering the water samples and culturing the filtrate on appropriate media. Viable propagules of *Ca. luteo-olivacea* were also obtained from hydration tanks, pruning shears, and grafting machines using the same technique (73).

Finished vines. In general, vines propagated from infected cuttings are slow to establish, or may never make satisfactory growth. Grafts may fail in severe cases. This unsatisfactory establishment of young vines has been consistently associated with fungal trunk pathogen infections in field nurseries.

Isolation studies conducted in South African grapevine nurseries demonstrated that black-foot pathogens (*Cylindrocarpon* spp. and *Campylocarpon* spp.) from soils infected grafted grapevines once planted in field nurseries (80). These authors also isolated *Cylindrocarpon* spp., *Pa. chlamydospora*, *Phaeoacremonium* spp., and Botryosphaeriaceae species from rootstocks and graft unions and suggested that the occurrence of *Cylindrocarpon* spp. in graft unions might be explained by the nursery practice of covering this plant zone with soil for a period of approximately 5 weeks to prevent drying of the callus tissue.

Several researchers have indicated that black-foot pathogens rarely occurred in rootstock propagation material prior to planting (58,78,140,161). Nevertheless, at the time of planting, the susceptible basal ends (especially the pith area) of most of the nursery cuttings are partly or even fully exposed. Callus roots also break during the planting process, resulting in small wounds susceptible to infection by soilborne pathogens (80,82,161).

Fourie and Halleen (59) isolated *Pa. chlamydospora* from the stained wood of failed rooted cuttings and grafted grapevines, indicating that contamination with untreated water, soil, or dust occurred during the propagation process. Giménez-Jaime et al. (66) surveyed cuttings at different stages of the grafting process, and *Pm. aleophilum* and *Pa. chlamydospora* were exclusively isolated from grafted plants 2 months after they were planted in a nursery field. In Australia, Edwards and Pascoe (40) also isolated *Pa. chlamydospora* and *Cylindrocarpon* spp. from 1-year-old grafted plants with symptoms of poor or distorted growth, stunting, and graft failure. Surveys in nurseries and agricultural cooperatives in Spain showed that grafted plants and rooted rootstocks ready to be planted in the field were infected by species of Botryosphaeriaceae and *Cylindrocarpon*, as well as *Pa. chlamydospora* and *Phaeoacremonium* spp. (12). Gramaje et al. (74) isolated several species of *Phaeoacremonium* and *Cylindrocarpon*, as well as *Pa. chlamydospora* and *Ca. luteo-olivacea* from grafted grapevines associated with Syrah decline in nursery fields in Spain. More recently, Spagnolo et al. (189) screened several lots of dormant grafted rooted cuttings ready to be commercialized by national nurseries in Italy and France, and found several species of Botryosphaeriaceae and *Pa. chlamydospora*. Species of Botryosphaeriaceae have also been frequently isolated from failed graft unions of young grapevines in field nurseries (21,22,70,151).

Despite the large body of work indicating the presence of grapevine trunk pathogens in nursery plants at the end of the propagation process, other authors question the role of these fungi in the symptom failure or poor performance of plants in the field nursery and vineyard. Rumbos and Rumbou (180) examined nursery plants ready for planting in the vineyard and found very low incidences of *Cylindrocarpon* spp., Petri disease pathogens, and Botryosphaeriaceae species in vines with failed graft unions and concluded that these pathogens could not by themselves be the cause of young grapevine decline. Abiotic causes, such as lesions from improperly healed rootstock disbudding sites and graft unions made in the nursery, as well as improper storage and transportation conditions of the propagated material, must also have played a role and made the decline more acute. Zanzotto et al. (232) also reported that after 1 year in the vineyard, the isolation percentages for *Phaeoacremonium* spp. and *Pa. chlamydospora* were substantially lower than in the original stock of grafted vines. These authors hypothesized that, in the first year of cultivation in the vineyard, the percentage of fungi involved in Petri disease could be masked or reduced by competition with other microorganisms in the complex natural environment.

Tools for Detection and Identification of Fungal Trunk Pathogens

The detection and identification of fungal trunk pathogens involves taking samples of grapevine tissue and placing them on nutrient-rich agar plates. Over a period of several days (or weeks), the fungi present in the sample will grow and thus be examined under a microscope for a number of phenotypical features. This particular identification process is not only very slow, but also requires expert knowledge because: (i) not all fungi will grow out of the wood sample onto the agar, and these are never detected; (ii) several fungal species may be present in a single vine wood sample; (iii) different fungi grow at different rates, and therefore the faster growing fungi could outgrow the slower species, which means that if the pathogenic fungi are slow growing, as are *Pa. chlamydospora* and *Phaeoacremonium* spp., they can be easily missed; and (iv) the identification of these pathogens through phenotypical characteristics is not straightforward. Colony colors and

textures, conidial dimensions, and amounts of sporulation are heterogeneous. Within a given species, isolates have variable phenotypes, and the range of these phenotypic characters overlap between species.

For these reasons, there is a growing demand for molecular methods for the detection of these pathogens in plant disease diagnostics. Molecular detection by means of DNA extraction and species-specific PCR offers a fast alternative, which avoids the above problems. These analyses are much less time-consuming, and results are generally available after 1 or 2 days. Conventional PCR, nested-PCR, and quantitative PCR have been developed for the detection and identification of the main causal agents of black-foot, Petri disease, and Botryosphaeriaceae and for their detection directly in wood, water, and soil samples from nurseries and vineyards.

For species of *Cylindrocarpon*, Nascimento et al. (136) used the universal primer located in the internal transcribed spacer region (ITS), ITS4, and the fungus-specific primer ITS1F in a first-stage fungus specific amplification, followed by a second-stage amplification with the primers Dest 1 and Dest 4 designed by Hamelin et al. (87) using the PCR product from stage one. This was a simple and reliable method for detection of *Cylindrocarpon* spp. directly from infected grapevines, although they amplified indistinctly *C. destructans* and *C. obtusisporum* (Cooke & Harkn.) Wollenw. (136). Damm and Fourie (32) developed a method for the extraction of fungal DNA from soil to study the epidemiology of grapevine trunk disease pathogens in South African grapevine nurseries and vineyards. The extracted DNA was tested for *Cylindrocarpon* spp. by using the primers Dest 1 and Dest 4. *Cylindrocarpon* spp. were detected in 66% of the samples investigated. Dubrosky and Fabritius (35) later designed a pair of genus-specific primers to detect *Cylindrocarpon* from infected grapevines in Californian nurseries. The main weakness of these techniques is the fact that the genus-specific primers cannot distinguish among *Cylindrocarpon* species. This was recently improved by Alaniz et al. (6) and Mostert et al. (132). The set of three primers pairs designed by Alaniz et al. (6) (Lir1/Lir2, Mac1/MaPa2, and Pau1/MaPa2) clearly distinguished *C. liriodendri*, *C. macrodidymum,* and *C. pauciseptatum* in a multiplex PCR assay. Additionally, a nested multiplex PCR developed to detect these species from artificially inoculated grapevine rootstock cuttings reached a selective amplification of the target pathogen. Mostert et al. (132) developed species-specific primers from the beta-tubulin (BT) nuclear gene area to identify *C. liriodendri* (CyliF and CyliR), *C. macrodidymum* (CymaF and CymaR), *Campyl. fasciculare* (CafaF and CafaR), and *Campyl. pseudofasciculare* (CapsF and CapsR) from soil and grapevine root material with promising results. Recently, a quantitative PCR (qPCR) using species-specific primers from the BT nuclear gene was developed to test large soil samples for the presence of *C. liriodendri* and *C. macrodidymum* (155). Results showed that this method was able to detect small quantities of the specific DNA in soil, and also demonstrated the apparent loss of fungal DNA over time.

The development of molecular techniques to detect and identify Petri disease pathogens has been intensively studied in recent years, mainly because of the difficulty of isolating these slow-growing fungi on culture media. Firstly, the sequence of the ITS regions was used by several research groups for designing species-specific PCR primers to facilitate accurate detection of *Pa. chlamydospora* (76,198). However, because of the presence of PCR inhibitors, these methods were not suitable for the detection of DNA from lignified wood. Ridgway et al. (166) therefore devised an extraction protocol and species-specific PCR method to detect *Pa. chlamydospora* in grapevine wood. The PCR procedure was performed using the species-specific primers developed by Tegli et al. (198) (Pch1 and Pch2), and results showed the high sensitivity of the PCR, detecting <1 pg of *Pa. chlamydospora* genomic DNA. Later, a conventional PCR method was also developed for detection of *Pa. chlamydospora* in grapevine wood, detecting up to 1 pg of fungus DNA (163). Whiteman et al. (224,226) published a sensitive nested-PCR using the primers developed by Tegli et al. (198) for detecting as little as 50 fg of *Pa. chlamydospora* genomic DNA from artificially infested soil. The identity of PCR products obtained with the species-specific primers had to be differentiated from other closely related fungi using restriction enzyme digestion (224,226). When traditional plating methods were compared with PCR detection, Retief et al. (163) found on average four times less positive detection with traditional plating methods than with PCR detection in naturally infected grapevine material.

The development of a technique for extracting DNA from water and callusing media, along with other techniques previously published for extraction from soil (32) and wood (163), led to the optimization of a one-tube nested-PCR technique to detect *Pa. chlamydospora* in all these grapevine nursery stages (164). In this study, as well as in the study of Whiteman et al. (224), the published *Pa. chlamydospora* species-specific primers Pch1 and Pch2 (198) were not found to be species-specific, since the ITS regions of a few unknown fungi were also amplified. Diagnostic protocols based on single or nested-PCR for the detection of *Phaeoacremonium* spp. and *Pa. chlamydospora* at different stages of the grapevine nursery process have been frequently used by different research groups in Italy (1,25), New Zealand (225), and Spain (13). Recently, Martos et al. (120) adapted the cooperational PCR coupled with dot-blot hybridization for the detection of *Pa. chlamydospora* in infected grapevine wood. A specific probe (Pch2D) targeting the ITS2 region in the rDNA only hybridized with DNA amplicons of *Pa. chlamydospora* isolates proving the specific detection of this fungus. This technique was further validated using artificially inoculated grapevine cuttings with *Pa. chlamydospora* in two independent blind tests, and the efficacy of detection was established at 75 and 85%, respectively.

Interest in the genus *Phaeoacremonium* has increased in the last decade because of the continuous identification of new species and their involvement in plant as well as human diseases (130,131). Therefore, researchers have also focused their attention on the development of molecular tools to detect and identify species of *Phaeoacremonium* associated with Petri disease and esca. Species-specific primers have been developed from the internal transcribed spacers ITS1 and ITS2 of the rRNA gene and used for the detection of *Pm. aleophilum* (Pal1N + Pal2) (198). Restriction fragment length polymorphism (RFLP) patterns of the ITS region were used to distinguish *Pm. aleophilum*, *Pm. inflatipes,* and *Pm. rubrigenum* W. Gams, Crous & M.J. Wingf. (198). Dupont et al. (36) distinguished five species of *Phaeoacremonium*, namely *Pm. aleophilum*, *Pm. inflatipes*, *Pm. parasiticum*, *Pm. rubrigenum,* and *Pm. viticola* J. Dupont, using PCR-RFLP markers from the ITS regions and the partial BT gene. A rapid identification method was developed for 22 species of *Phaeoacremonium* (130,131). It involved the use of 23 species-specific primers, including 20 primers targeting the BT gene and three targeting the actin gene. The information generated in these works provided a polyphasic identification tool including morphological and cultural characters as well as BT sequences. This *Phaeoacremonium* database including all of the known *Phaeoacremonium* species can be accessed from the website of the Centraalbureau voor Schimmelcultures (www.cbs.knaw.nl/phaeoacremonium.htm).

A pair of genus-specific primers located in the ITS region has been successfully used in a nested-PCR for detecting any species of *Phaeoacremonium* in plants (15). The PCR product obtained was subsequently digested by restriction enzymes, and the band pattern was used to identify nine *Phaeoacremonium* species.

Few works have been published on the development of molecular tools for the detection and identification of Botryosphaeriaceae species in grapevine nurseries and vineyards. Martos et al. (121) developed a sensitive tool for rapid detection and subsequent identification of Botryosphaeriaceae species occurring on grapevines based on the cooperational PCR technique coupled with dot-blot hybridization. This technique was able to recognize the eight Botryosphaeriaceae species tested with a similar sensitive level to nested-PCR methods. Spagnolo et al. (189) recently developed two

ITS-rDNA-based nested PCR assays, one with a narrow target range to detect *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips and the closely related species complex, and another, with a wider range, to detect all 17 species of Botryosphaeriaceae previously reported as potential wood pathogens of grapevines. These two protocols were found to be efficient, sensitive, and reliable when used with naturally infected grapevine tissues. A set of two multispecies primers (BOT100F and BOT427R) able to detect DNA of six Botryosphaeriaceae species commonly found in New Zealand vineyards was successfully developed by Ridgway et al. (165). Additionally, using a single-stranded conformational polymorphism (SSCP) analysis, the single amplicon produced for all six species tested were resolved into four individual species and a duo of either *N. parvum* or *N. ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips (165).

Recent advances in DNA-based techniques like real-time PCR (rtPCR) have provided new tools for accurately detecting fungal trunk pathogens in grapevine tissue. Overton et al. (142,143) designed primer pairs (Pmo1f/Pmo2r and Pac1f/Pac2r) for species and genus-specific amplification of *Pa. chlamydospora* and *Phaeoacremonium* spp., respectively, using a quantitative rtPCR assay with SYBR Green technology. *Pa. chlamydospora* was detected in roots, shoots, and young trunks of drill-inoculated vines while *Phaeoacremonium* spp. were detected in trunk cross-sections of naturally infected vines. Edwards et al. (37) compared different molecular tests (single PCR, nested-PCR, and quantitative PCR with SYBR Green and TaqMan technology) for detection of *Pa. chlamydospora* during grapevine nursery propagation, and concluded that all techniques were able to detect the pathogen in water samples, although nested-PCR and rtPCR were the most sensitive, TaqMan being more reliable than SYBR Green. A comparison between rtPCR with TaqMan technology and a nested-PCR was carried out by Aroca et al. (17) for detecting *Phaeoacremonium* spp. in naturally infected grapevine cuttings. These authors concluded that rtPCR assay was much more sensitive and reproducible than nested-PCR or isolation on culturing media. Luchi et al. (113) successfully developed an rtPCR assay with TaqMan technology, designing taxon-specific primers for the specific detection of Botryosphaeriaceae species in grapevine propagation material.

Simultaneous identification techniques of multiple fungal pathogens have also been recently developed. Weir and Graham (222,223) adapted the terminal-RFLP to monitor endophytic and pathogenic fungal populations in the xylem of grapevines during nursery propagation. This method was able to detect *Cylindrocarpon* spp., Botryosphaeriaceae species, *Phaeacremonium* spp., and *Pa. chlamydospora* to species level. Lummerzheim et al. (114) tested a multiplex PCR method for the simultaneous identification of *Botryosphaeria dothidea* (Moug.) Ces. & De Not., *Diplodia seriata* De Not., *Pm. aleophilum,* and *Pa. chlamydospora* on pure fungal DNA, crude mycelium, inoculated vine-stocks, and naturally infected cordons and wood, with no conclusive results.

Management Strategies

A healthy vine is fundamental to the successful beginning and sustainability of all grape vineyards. Growers depend on commercial grapevine nurseries for vine stock that is free of known pathogens and serious viruses and true to type. This is not an easy task. At present, it is not possible for nurseries to ensure a fungal trunk pathogen–free stock.

Control of endogenous pathogens in grapevines is problematic. In vineyards, management strategies recommended for prevention and disease management mainly involve the prevention and/or correction of predisposing stress situations (57).

There are many opportunities for infection by trunk disease pathogens during propagation processes. Wounds are made in the tissue at every stage of production from collection and disbudding of cuttings to bench grafting and lifting and trimming of finished vines. Improperly healed graft unions are also vulnerable to infection in the nursery, and if the vines survive, after planting in the vineyard. Poor cold storage and transport conditions can also result in stress and cross-contamination. In recent years, there have been advances in the development of procedures and products to prevent or reduce infection of woody tissue by fungal trunk pathogens during propagation. Consequently, good hygiene and wound protection are of the utmost importance.

In this section, we review research on the management of black-foot and Petri diseases as well as Botryosphaeriaceae species in mother fields, nurseries, and open root field nurseries. Chemical, physical, and biological control, host resistance, and other management strategies will be discussed.

Chemical control. The prevention of pruning wound infections, as well as sanitation to reduce inoculum sources, have been recommended in mother blocks (59). In recent years, pruning wound protection with fungicide applications has been intensively studied. Halleen et al. (83) demonstrated that wound protection under vineyard conditions with benomyl and flusilazole reduced natural *Pa. chlamydospora* infections of pruning wounds by circa 80%. Recently, Rolshausen et al. (168) evaluated the efficacy of selected fungicides to control Botryosphaeriaceae species and Petri disease pathogens. Thiophanate-methyl was, overall, the most efficacious fungicide. However, the protection of pruning wounds in mother blocks remains problematic. The range of registered products is limited, they generally do not provide long-term wound protection or broad spectrum control and application can be difficult and expensive (168).

The application of chemical strategies to control fungal trunk pathogens in the nursery process is also difficult. Traditional techniques such as chemical sprays and dips used for the control of surface pathogens do not penetrate dormant grapevine cuttings sufficiently to control organisms inhabiting the phloem and xylem tissue (27,141).

Hydration tanks containing drench water (pre-storage, pre- and post-grafting) are an important focal point for management strategies. Soaking propagation material prior to cold storage or grafting in benomyl, carbendazim, didecyldimethylammonium chloride, or captan resulted in significantly reduced *Pa. chlamydospora* and *Phaeoacremonium* infection levels in basal ends and graft unions of nursery plants (60,62,68). However, these treatments did not have an effect on infection by black-foot pathogens, most likely due to the fact that infection by these pathogens was shown to occur from nursery soils (80). Eskalen et al. (50) tested several fungicides on naturally infested dormant propagation materials and demonstrated that vines soaked with ziram, thiram, thiophanate-methyl, or lime sulfur prior to grafting showed significant reductions in *Pm. aleophilum* after 1 year in the field nursery. In similar experiments, Rego et al. (160) and Vigues et al. (211) concluded that soaking naturally infested grapevines prior to grafting in the mixture cyprodinil + fludioxonil or pyraclostrobin + metiram reduced the presence of *Cylindrocarpon, Pa. chlamydospora,* and Botryosphaeriaceae species.

The effect of fungicides during the callusing stage and before planting in nurseries has also been investigated. Results from in vivo studies conducted on preinoculated potted grapevines in Portugal proved that benomyl, tebuconazole, carbendazim + flusilazole, and cyprodinil + fludioxonil significantly improved plant growth and decreased disease incidence of *Cylindrocarpon* spp. (159). Nascimento et al. (137) later confirmed that cyprodinil + fludioxonil reduced the incidence of *C. liriodendri* and *Pa. chlamydospora* in greenhouse trials with potted grapevines. Halleen et al. (82) evaluated various chemical preplanting treatments for prevention of infection by black-foot and Petri disease pathogens, and the results were inconsistent, perhaps because of generally low and varying infection levels in the roots and rootstocks, respectively. Recently, Alaniz et al. (5) conducted a pot assay with several fungicides in order to determine their potential to prevent infections caused by *C. liriodendri* and *C. macrodidymum* during the rooting phase in the grapevine propagation process. Results showed that captan, carbendazim, copper oxychloride, didecyldimethylammonium chloride, hydroxyquinoline sulfate, and prochloraz decreased the root disease severity values in both species compared with

control treatment; but only captan, carbendazim, and didecyldimethylammonium chloride presented a percentage of reisolation values significantly different from control treatment in the case of the cuttings inoculated with *C. liriodendri*, and prochloraz in the case of those inoculated with *C. macrodidymum*.

Hot-water treatment. The use of HWT has been reported as a promising method for the control of black-foot and Petri disease pathogens in grapevine propagating material. However, there have been, and continue to be, irregular reports of unacceptably high losses when long duration HWT (50°C for 30 min) is applied to commercial batches of cuttings and rootlings.

HWT of rootstock cuttings prior to grafting (43,50,60) or HWT of dormant nursery plants after uprooting (58,60,68,82) has been strongly recommended for their effectiveness in reducing infection levels in nursery plants. Although HWT can be applied to young rooted vines just prior to dispatch, Australian nurseries prefer to use it as a precallusing treatment, before or after cold storage, to avoid the possibility of litigation from clients arising from the occasional unpredictable failure of HWT vines in the vineyard (215,218).

The optimal temperature-time combination of HWT has been intensively investigated and discussed among researchers, but no clear consensus has emerged. Until now, the regular protocol has been standardized at 50°C for 30 min. However, recent research has demonstrated that 50°C for 30 min may not always be an effective control for the target pathogens (79,173,186,228), or may result in unacceptable damage to young vines (23,79). Waite and Morton (219) suggested that tolerance of plants and their accompanying pathogens to HWT is affected by the climate in which the cuttings are grown. In this regard, Graham (67) and Bleach et al. (23) found that both cuttings grown in cool climate in New Zealand and their pathogens were more susceptible to HWT, and the use of temperatures below 50°C reduced the incidence of *Pa. chlamydospora* and *Cylindrocarpon* spp., respectively. Conversely, Gramaje et al. (68,69,71) fixed 53°C for 30 min as the most effective treatment to reduce conidial germination and mycelial growth of black-foot and Petri disease pathogens in Spain without detrimental effects to grapevine cuttings.

Another option would be to increase the time of treatment. This has been recently tested by different researchers with promising results. Habib et al. (79) evaluated the effect of HWT at 50°C for 45 min on naturally infected planting material, and although treatments did not affect the intensity of wood discoloration, the frequency of *Pa. chlamydospora* detection was strongly reduced as compared to the untreated material. Using the same temperature and time combination, Vigues et al. (211) concluded that HWT was the only practice among different control methods tested (chemical, biological, and technological methods) that showed promising results by reducing *B. dothidea*, *D. seriata*, and *Pa. chlamydospora* infections for several years in French nurseries.

Biological control. The potential use of biocontrol agents as a wound protectant and growth stimulant in grapevine nurseries have also been reported (60,64,122). In a semi-commercial nursery trial, Fourie et al. (64) demonstrated the growth stimulating attributes of commercial products of *Trichoderma*, as well as the positive effect on natural infection by *Cylindrocarpon* spp., *Phaeoacremonium* spp., and *Pa. chlamydospora*. Low levels of these pathogens were recorded, but the *Trichoderma* treatments notably reduced their incidence in roots of nursery grapevines. *Trichoderma* also significantly improved root development, which could make plants more tolerant to black-foot disease when subjected to stress.

Pruning wound protection by *T. harzianum* Rifai and *T. longibrachiatum* Rifai against artificial infection by *Pa. chlamydospora* was demonstrated by Di Marco et al. (34). Kotze et al. (100) reported that *T. atroviride* P. Karst and *T. harzianum* strongly reduced artificial infection by *Pa. chlamydospora* and species of Botryosphaeriaceae. The ability of *Trichoderma* spp. to colonize pruning wounds and reduce infection by pruning wound pathogens was also demonstrated (61,100,135). Soaking rootstock cuttings prior to cold storage or grafting in *Trichoderma* formulations also reduced the incidence of *Pa. chlamydospora* and *Phaeoacremonium* in basal ends and graft unions of nursery plants (60). The mycorrhizal fungus *Glomus intraradices* N.C. Schenck & G.S. Sm. also provided excellent control against black-foot disease when applied preventively (148).

Host resistance. Given the difficulty of controlling grapevine trunk pathogens using the measures previously described, an obvious solution would be the use of resistant rootstock or scion cultivars. However, this has limited potential because surveys of naturally infected material have demonstrated that all major rootstock cultivars and scions are susceptible (11,12,146,157,200,202, 206,230).

However, the results of artificial inoculation experiments have demonstrated differences in susceptibility of grapevine rootstock and scion cultivars, mainly to Petri disease pathogens. There were no resistant cultivars observed among 20 grapevine rootstocks inoculated with *Pa. chlamydospora*, *Pm. aleophilum,* or *Pm. inflatipes* (46). Recently, Gramaje et al. (72) found that 161-49 Couderc was the least susceptible among five grapevine rootstocks previously vacuum inoculated with *Ca. luteo-olivacea*, five species of *Phaeoacremonium,* or *Pa. chlamydospora* under field conditions in Spain. In contrast, rootstocks 110 R and 140 Ru (both crosses of *V. berlandieri* × *V. rupestris*) were greatly affected by these fungi. On the north coast of California, large-scale replanting of grapevine rootstock crosses of *V. berlandieri* × *V. riparia* by new rootstock crosses of *V. riparia* × *V. rupestris* and *V. berlandieri* × *V. rupestris* resulted in increased signs of plant decline and subsequent death from the early 1990s (78). Species of *Phaeoacremonium* and *Pa. chlamydospora* were later isolated from these affected vines. This information and the results published by Gramaje et al. (72) suggest that grapevine rootstock crosses of *V. riparia* × *V. berlandieri* could be the least susceptible to Petri disease pathogens.

Santos et al. (182) also reported that Baga and Maria Gomes cultivars were more susceptible to *Pa. chlamydospora* and *Pm. angustius* than 3309 Couderc rootstock, and also noted differences between Baga and Maria Gomes. Artificial inoculation of rootstock cuttings (1103 Paulsen and 110 Richter) and *V. vinifera* cultivars (Chardonnay and Anglianico) with *Pa. chlamydospora* showed that rootstock cuttings had a higher susceptibility than *V. vinifera* cultivars to infection by this pathogen (231). Similar results were observed in Australia, where seven grapevine rootstocks (Ramsey, 99 Richter, Schwarzmann, Kober 5BB, 1103 P, 101-14 Millardet, and SO4) were also reported to be more susceptible to *Pa. chlamydospora* than the *V. vinifera* cultivars (Merlot, Cabernet Sauvignon, Pinot Noir, Shiraz PT10, and Shiraz PT23) (220).

Feliciano et al. (51) demonstrated that Thompson Seedless was significantly more susceptible to *Pa. chlamydospora* and *Pm. aleophilum* than Grenache and Cabernet Sauvignon cultivars. In a 3-year field trial where *Pa. chlamydospora* and *Pm. aleophilum* were inoculated on spurs of Italia and Matilda cultivars, the latter cultivar was more resistant (191). In Australia, Edwards and Pascoe (40) only diagnosed Petri disease and esca in a few Riesling or Sultana cultivars, and no Colombard or Ruby Cabernet cultivars. Marchi (188) studied the disease incidence and progression of esca in a mixed cultivar vineyard in Italy and found four susceptibility groups among the 17 cultivars evaluated, with Semillon the most, and Roussanne the least susceptible. Two-year visual inspections of 10 different cultivars in Italy demonstrated that the incidence of esca was higher in cultivars Cabernet Sauvignon, Sangiovese, and Trebbiano toscano, and lower in Montepulciano and Merlot (157).

Regarding black-foot disease, Gubler et al. (78) indicated that the rootstocks *Vitis riparia* 039-16 and Freedom had a good degree of resistance to this disease. Jaspers et al. (95) evaluated the susceptibility of the more commonly planted grapevine rootstocks in New Zealand under greenhouse conditions. The results showed that all rootstock varieties included in the study were susceptible to *Cylindrocarpon* spp. to some degree. These findings were in agreement with those obtained recently by Alaniz et al. (7), who evaluated the susceptibility of the grapevine rootstocks most commonly

used in Spain to *C. liriodendri* and *C. macrodidymum* and found that all rootstocks inoculated were affected by the disease.

Miscellaneous. The use of ameliorative treatments to limit symptom expression and disease progress has been investigated. Edwards and Pascoe (41) demonstrated that applications of compost, nutrient fertilizers, extra water, phosphonates, and Brotomax over periods of 2 to 5 years were ineffective in reducing Petri disease occurrence.

In grapevine nursery fields, Halleen et al. (80) indicated that standard nursery practice of a 2-year rotation system, whereby cuttings are planted every second year, alternated with a cover crop, might have led to a build-up of soilborne pathogens such as species of *Cylindrocarpon*.

Di Marco and Osti (33) evaluated the potential use of electrolyzed acid water in cutting hydration after the cold-stored period to control *Pm. aleophilum* and *Pa. chlamydospora.* Results of the laboratory assays demonstrated that this disinfectant was effective in reducing conidial germination of both pathogens without affecting plant growth and development in the nursery field. The impact of ozonation on grapevine scion decontamination was evaluated by Mailhac et al. (115) and Vigues et al. (211), and while the former showed inconclusive results, the latter concluded that this oxidative agent did not control Botryosphaeriaceae species and *Pa. chlamydospora* in French nurseries.

The potential of the biofumigant crop Indian mustard (*Brassica juncea*) was evaluated in nursery fields as a possible alternative for methyl bromide and metham sodium for the control of *C. destructans* (195). These authors concluded that this biofumigant did not reduce the percentage of root or stem tissue containing this pathogen at harvest. In New Zealand, biofumigation using mustard gave useful reductions in disease when callused rootstock cuttings were planted into artificially inoculated soil (24). It appeared that mustard meal incorporated into infested soil was as good as growing the plants and incorporating the plant into the soil. This may give a valuable control tool for growers who replant into a pathogen-contaminated site after the removal of infected plants in an established vineyard.

On the basis of the previous research, an integrated management program that includes HWT, chemical, biological, or other control measures has been suggested to be the most interesting procedure to reduce infections by fungal trunk pathogens during the nursery stages (57,59–62,64,69).

Improving the Quality of Planting Material in Nurseries

The identification of nursery vines as a significant source of trunk diseases in vineyards around the world highlights the important role of nurseries in the management and control of serious grapevine pests and diseases and the urgent need for significant improvement in the quality of planting material offered to grape growers. The results of a survey of grapevine nurseries and the regular detection of trunk disease pathogens in nursery vines in every vine points to an urgent need for a set of standard operating procedures that can be incorporated into nursery accreditation and quality assurance schemes for both cuttings and 1-year-old vines (215). For instance, in the case of the EU countries, there is a certification scheme elaborated by the European and Mediterranean Plant Protection Organization (EPPO), which provides detailed guidance on the production of pathogen-tested material of grafted grapevine varieties and rootstocks (139). Planting material produced according to this certification scheme is derived from nuclear-stock plants that have been tested and found free from some viruses and phytoplasmas, and checked for the presence of other pathogens such as *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora*.

Stamp (192) screened over one million grapevine plants including dormant bench-grafts, dormant rootstock rootings, and current season and dormant (year-old) potted grapevine plants in California and Oregon. Inspection of vines for physical quality criteria including condition of root system, rootstock shaft, and graft union, and vascular symptoms of Petri disease resulted in the detection of significant defects in 35% of dormant rootstock rootings and 39% of dormant bench-grafted vines in a two-season study on nursery stock (192). It has been demonstrated that nursery derived imperfections in grapevine stock are a critical source of stress for new and established vineyards, and it is clear that planting vines without significant defects would result in healthier vineyards that are less likely to succumb to stress-induced disease (194).

Waite (216) recently provided a general guideline for producing and procuring quality grapevine planting material for nurseries and grape growers. This work, together with others published by Stamp (192), Hunter et al. (93), and Waite and Morton (219), describes the characteristics of sound, healthy nursery vines and the critical factors upon which successful grapevine propagation depends and discusses the steps that can be taken to improve the quality of planting material available to growers.

To this end, propagating material should be obtained from registered source blocks of known disease status. The most reliable sources of superior grapevine cuttings are those that are established and managed specially to supply registered disease-free cuttings to propagators. Cutting sources from unregistered vineyards are frequently inferior and of unknown disease status and type. Purchase of vines propagated from unregistered source areas not only carries a serious risk of introducing diseases, but also carries a risk of establishing a vineyard that is not of the desired variety and clone (219). Irrigation and fertilization practices in mother vine source blocks should be designed to support production of the highest quality cuttings with maximum carbohydrate reserves and physical attributes most suited to grafting (93). Nurseries should also have comprehensive quality assurance programs, and/or participate in accreditation schemes managed by appropriate organizations such as government regulatory bodies or nursery industry organizations that set standards and oversee nursery practices.

Correct treatment and handling of harvested cuttings would minimize contamination and spread of grapevine pathogens. Nurseries should also have a high standard of general hygiene. Hydration should be minimized, and tanks should be sterilized after every hydration period and the water treated with chemical and/or biological control agents, since unprotected wounds on cuttings provide ideal infection openings for species of Botryosphaeriaceae and Petri disease pathogens (44,59,64,91,106) or other waterborne contaminants (219). Immediately after grafting, nurseries may dip vines into specialized waxes containing plant growth regulators or fungicide-impregnated formulations such as Graftseal which encourage graft union callus development while inhibiting fungal contamination (62,93,192).

Hot-water treatment equipment should be calibrated by an independent authority, and the nursery should employ a trained operator. It is important to note that HWT is a significant stress and can result in the loss of treated material if not applied correctly (93). Hot-water treated cuttings should be stored in perforated bags to ensure the material has adequate oxygen (217). The undesirable but common practice of packaging hot-water treated cuttings and vines in sealed plastic bags within a few hours of treatment may prolong the anaerobiosis in the vine tissue brought about by HWT and cause the accumulation of ethanol and acetylaldehyde in the bags, further exacerbating the damage already caused to the tissue.

Given the wide range of biotic and abiotic stresses that new vines can be subjected to before and after planting, ranging from nursery-derived effects and extended cold storage to improper planting, insufficient irrigation, and microbial contamination, it is obvious that any measures that result in the planting of vines with superior physical quality and reduced pathogen contamination represent a worthwhile investment.

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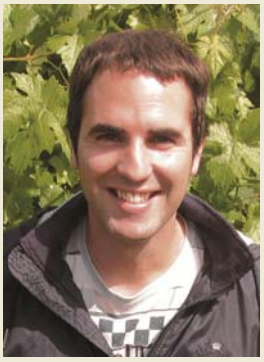

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