Black foot disease in South African vineyards and grapevine nurseries

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Over the last few years a drastic reduction has been noted in the survival rate of vine cuttings in nurseries, as well as in young vineyards in the Western Cape Province of South Africa. The low average take percentages of young vines can be attributed to several factors, including fungal, bacterial and viral diseases, insect and nematode pests, abiotic factors, as well as nutritional deficiencies and toxicities. *Cylindrocarpon* spp., which cause black foot disease of grapevine were found to be associated with the decline of young vines in South Africa. Results obtained from the diagnostic service at ARC Infruitec-Nietvoorbij (Disease Management Division) showed that *Cylindrocarpon* spp. were isolated from 52%, 22% and 29% of diseased vines during 1999-2002, 2002-2003 and 2003-2004, respectively. However, it was unclear how and where these infections occurred. Very little information is available regarding the aetiology and epidemiology of the fungi believed to be involved in black foot disease. Diseased grapevines delivered for diagnosis were typically less than 5 years old and in some instances symptoms even appeared during the first year of planting. Diseased plants display an array of decline symptoms. In vineyards, the first visible symptoms are usually delayed or absence of budding. Subsequently shoots develop abnormally (shortened internodes and small discoloured leaves) which may dry and even die during summer. When symptomatic plants are removed from the soil, inspection of the roots may reveal the presence of grey to black necrosis which ultimately leads to the underdevelopment of the entire root system. Further root abnormalities include the development of secondary root crowns with roots growing parallel to the soil surface. Internal symptoms include brown-black discoloration of xylem vessels originating from the base of the rootstock, brown-black discoloration in roots, and necrosis from the bark to the centre of roots and rootstocks (Fourie & Halleen, 2001).

The purpose of this communication is to provide a short overview of the results obtained from research conducted during the past 5 years. The primary aims of research have been (1) to conduct nursery surveys in order to determine which fungi are involved in the decline phenomenon, with special reference to the involvement of *Cylindrocarpon* spp., (2) to identify the organisms believed to be the causal organisms of black foot disease, and (3) the development of control and/or management strategies to prevent or eradicate *Cylindrocarpon* infections.

Materials and Methods

Nursery survey: Nursery grapevines that were sampled from 3 commercial nurseries in the Wellington area of the Western Cape province of South Africa were investigated during the 1999/2000 season by means of destructive sampling. The first isolations were made in September from callused cuttings prior to planting in nurseries. After planting, asymptomatic rooted cuttings were selected from nurseries after 3, 6 and 9 months. Isolations were made from the roots, rootstocks, graft unions and scions.

Identification of the causal organism: *Cylindrocarpon*-like isolates, obtained from the nursery survey, as well as from the grapevine diagnostic service at ARC Infruitec-Nietvoorbij, were collected for further investigation. Substantial variation in cultural and morphological characters was observed among these isolates. Morphological and phylogenetic studies were therefore conducted to identify these *Cylindrocarpon* spp. and to establish their association with black foot disease. Sequences of the

partial nuclear large subunit ribosomal DNA (LSU rDNA), internal transcribed spacers 1 and 2 of the rDNA including the 5.8S rDNA gene (ITS), and partial β-tubulin gene introns and exons were used for phylogenetic inference.

Control: Knowledge obtained pertaining to the infection period and site in the nursery survey suggested that any suitable control method will have to focus on preventing or eradicating infection of the basal ends of nursery cuttings. However, at present, no fungicides are registered for control of this disease in South African vineyards or nurseries. *In vitro* studies were conducted to evaluate the effectiveness of various fungicides. Benomyl, flusilazole and prochloraz manganese chloride were the most effective fungicides tested against these species *in vitro*. These fungicides, alone or in combination with a wax formulation or adjuvant, were included with biological and physical treatments in the semi-commercial field trials. These treatments were aimed at protecting the basal ends of rootstocks against infection. After callusing, the basal ends of grafted cuttings were dipped in various treatments prior to planting. Additional treatments involved soil amendments with *Trichoderma* formulations and hot water treatment of uprooted dormant nursery grapevines (30 min at 50°C). Nursery plants were uprooted after eight months. The trials were conducted at two field nurseries in Wellington during the 2002-2003 and 2003-2004 seasons.

Results and Discussion

Nursery survey: Isolation studies conducted in the nurseries clearly demonstrated that different *Cylindrocarpon* spp. infected cuttings from soil once planted in the nurseries. These species rarely occurred in rootstock propagation material prior to planting. 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. The isolation studies revealed that the first infections occurred in the roots, followed by infections of the rootstocks. Furthermore, these infections also increased progressively during the course of the growing season (Halleen *et al*., 2003).

Identification of the causal organism: Phylogenetic analyses confirmed the diversity observed among the isolates and four *Cylindrocarpon*-like species were identified. One of these groups were identified as *Cylindrocarpon destructans*. A second group was newly described in this study as *Cylindrocarpon macrodidymum* (*Neonectria macrodidyma*). The two remaining *Cylindrocarpon*-like species were placed in a new genus, *Campylocarpon*. The two species were named *Campylocarpon fasciculare* and *Campylocarpon pseudofasciculare*. Pathogenicity studies confirmed that all 4 species were able to reduce root and shoot mass significantly (Halleen *et al*., 2004).

Control: No *Cylindrocarpon* or *Campylocarpon* spp. was isolated from any of the control plants before planting in the nurseries. Isolations from uprooted grapevines at the end of the growing season revealed very low levels (4.1%) of *Cylindrocarpon* and *Campylocarpon* spp. in the roots of untreated control plants and no significant differences between treatments. However, infection levels in the basal ends were substantially higher and *Cylindrocarpon* and *Campylocarpon* spp. were isolated from 31.1% of untreated control plants. If all factors are taken into consideration (percentage fungal infection, take percentages, root- and shoot mass), none of the chemical and biological treatments prevented infection. Hot water treatment of uprooted dormant nursery grapevines completely eradicated *Cylindrocarpon* and *Campylocarpon* infection in the plants.

Conclusion

The diversity of species associated with black foot disease has been confirmed by this study. The fact that *Cylindrocarpon* and *Campylocarpon* spp. have the ability to infect grapevine cuttings in nursery soils has clearly placed the emphasis on the importance of suitable control measures to prevent or eradicate these infections. However, none of the chemical and biological treatments evaluated in this study prevented infection of nursery grapevines. The reduction of *Cylindrocarpon* and *Campylocarpon* infection in uprooted dormant nursery grapevines caused by the hot water treatment clearly demonstrated the potential of this control measure. This treatment was also recommended for the eradication of *Phytophthora cinnamomi* (Von Broembsen & Marais, 1978) and *Meloidogyne*

javanica (Barbercheck, 1986) from dormant nursery grapevines and was also found to be effective against *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. that cause Petri disease of grapevine (Fourie & Halleen, 2004). Apart from these pro-active management strategies in grapevine nurseries, no curative strategy is known for declining grapevines in vineyards. Producers are therefore urged to heed general recommendations to prevent and/or correct predisposing stress situations, such as soil compaction and poor drainage.

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