

Slow Dieback of Grapevine: Association of *Phialophora parasitica* with Slow Dieback of Grapevines

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Phialophora parasitica was consistently isolated from vines showing symptoms of slow dieback and decline. Pathogenicity tests were conducted on *in vitro* plantlets, grafted plants in a glasshouse and on graft unions. The fungus caused discolouration of wood as well as extensive plugging of xylem tissue. Plugging material seemed to be of gum as well as parenchyma origin. Callusing of graft unions was negatively affected.

Unequal and stunted growth of grapevines in the Cape Province of South Africa has been known to occur sporadically since 1940 (A.S. Zeeman, personal communication 1992). Several fungi have been isolated from grapevines with stunted growth and dieback symptoms in the past (unpublished data). Recently, Loubser & Van Huyssteen (1992) suggested fungi, nematodes and certain soil conditions as possible causes of stunted growth and dieback of vines. The aetiology of the disease, however, remains unresolved.

The association *P. parasitica* with plants showing symptoms of slow dieback was investigated.

MATERIALS AND METHODS

Isolation: Diseased plants were obtained from different viticultural districts (Table 1). Isolations were made from diseased vines by placing small fragments of affected xylem tissue on tea leaf agar (TLA) in petri dishes. This medium proved to sustain good growth and sporulation of the fungus and consisted of 25 g dry used tea leaves, 15 g agar, and 1 ℓ distilled water. Petri dishes were incubated at 24°C under a combination of fluorescent and ultraviolet light with a 12 h photoperiod. Cultures were examined macro- and microscopically after seven days and identified.

Scanning electron microscopy (SEM): Root and inter-node samples were collected from vines (Pinotage grafted onto rootstock 101-14 Mgt) showing dieback symptoms. The tissue was fixed in 5% glutaraldehyde in NaH₂-PO₄.H₂O phosphate buffer (pH 7.0), dehydrated in an acetone series (30-95% acetone in distilled water), critical point dried and sputter coated with gold. The vascular tissue was studied in a scanning electron microscope (ISI-100-A).

Inoculum: Inoculum was prepared from 14-d-old cultures of the isolate on TLA. Twenty millilitres sterile, distilled water was poured into each petri dish and the fungal colonies agitated with a bent glassrod. The resulting spore suspension was filtered through a double layer of cheesecloth and adjusted to 1 x 10⁶ spores/ml.

Pathogenicity tests: Pathogenicity tests were performed on tissue-cultured plantlets in the laboratory, grafted plants in a glasshouse and on graft unions during grafting.

1. **Laboratory:** Tissue-culture plantlets (rootstock 101-14 Mgt) in petri dishes were inoculated by placing a small piece of agar (2 x 2 mm) from a TLA culture of the isolate onto the roots and stems of 10 individual plantlets. These plantlets were incubated at 24°C for 12 days, whereafter hand sections of shoots were made and stained in lactophenol cotton blue and examined microscopically.

2. **Glasshouse:** Forty one-year-old plants, 20 each of the cultivars Cabernet Sauvignon and Pinotage, grafted onto rootstock 101-14 Mgt, were planted in polyethylene bags and grown in a glasshouse at 25°C.

Plants were inoculated by drilling a hole (Ø 4 mm) 3 mm deep into the rootstock near the soil surface. Each plant was inoculated by forcing a few microlitres of the spore suspension into the drilled hole with a syringe. Holes were then sealed with petroleum jelly and covered with masking tape. Control plants were injected with sterile distilled water. After nine months, plants were taken to the laboratory where the bark was removed with a sharp knife to expose the lesions formed by the fungus. Lesion length was measured on each plant and small pieces of xylem tissue underneath the lesion removed and placed on TLA in petri dishes after surface sterilisation in 0.5% sodium hypochlorite.

3. **Graft unions:** Graft unions of 200 Chenin blanc cuttings, grafted onto 99 Richter, were dipped into a spore suspension of the isolate before being packed into vermiculite in a callus tray. Control plants were dipped into sterile water only. Cuttings were left to callus at 26°C for four weeks whereafter percentage callus formation was recorded before plants were planted in polyethylene bags in a glasshouse. The number of surviving plants was determined after three months.

RESULTS

Isolation: The fungus grew very slowly on TLA and had a colony diameter of only 30 mm after two weeks. It had a fluffy greyish appearance, sporulated abundantly, and was identified as *Phialophora parasitica* Ajello, Georg & Wang. The fungus was isolated from 22 grapevines, with poor growth or decline (Fig. 1). The cultivar/rootstock combinations and their origin are given in Table 1.

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TABLE 1

Cultivar, rootstock and district where grapevines showing symptoms of slow dieback and decline were sampled.

Cultivar/Rootstock	District
Raisin blanc/Ramsey	Stellenbosch
Chenin blanc/Ramsey	Rawsonville
Sauvignon blanc/99 Richter	Paarl
Merlot/99 Richter	Paarl
Chenin blanc/101-14 Mgt	Robertson
La Rochelle/99 Richter	Porterville
Chenin blanc/99 Richter	Paarl
Bien Donne/Jacquez	Wellington
Chenin blanc/99 Richter	Bonnievale
Chenin blanc/101-14 Mgt	Worcester
Sauvignon blanc/143-B	Robertson
Chenin blanc/101-14 Mgt	Robertson
Riesling/101-14 Mgt	Robertson
Chardonnay/99 Richter	Wellington
Ruby cabernet/99 Richter	Wellington
Muscat d'Alexandrie/99 Richter	Wellington
Chenin blanc/99 Richter	Worcester
Colombar/99 Richter	Worcester
Chenin blanc/99 Richter	Robertson
Colombar/99 Richter.	Ladismith
Riesling/99 Richter	Worcester
Pinotage/101-14 Mgt	Paarl



FIGURE 1

Vines showing symptoms of slow dieback.

Scanning Electron Microscopy: Extensive plugging occurred in xylem tissue in the roots and stems of the rootstocks (Fig. 2). Occlusions seemed to be gummoses as well as parenchymatous in nature (Fig. 3).



FIGURE 2

Plugging of infected xylem tissue (arrowed).

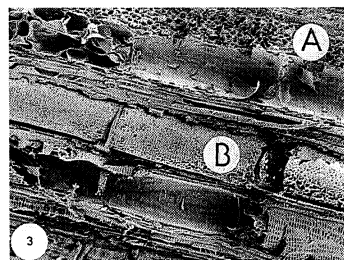


FIGURE 3

Infected xylem tissue with (A) parenchymatous and (B) gummoses-like material.

Pathogenicity tests

1. *Laboratory:* Mycelia of the fungus penetrated the young rootlets and stems of the plants, causing a brown colouration of the surrounding tissue. Mycelia grew intercellularly in the tissue (Fig. 4) and pathogenicity was confirmed by completion of Koch's postulates.

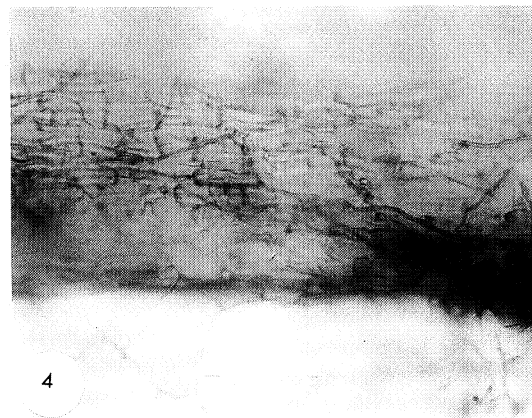


FIGURE 4

Mycelia of *Phialophora parasitica* developing intercellularly in plant tissue.

2. *Glasshouse:* Dark coloured, oval lesions extending downward and upward from the point of inoculation occurred (Fig. 5). The largest mean lesion length was recorded on Pinotage (Table 2). The mean length of lesions on control plants was approximately five times less.

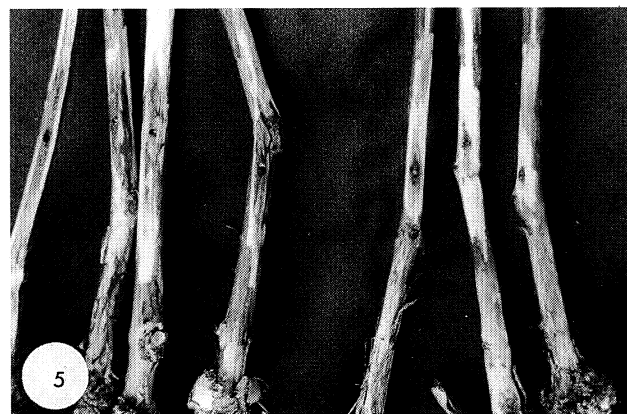


FIGURE 5

Dark coloured lesions on *Phialophora parasitica* inoculated cuttings (Control cuttings are shown on the right).

TABLE 2

Mean lesion length caused by *Phialophora parasitica* on 101-14 Mgt with Cabernet Sauvignon and Pinotage as scion cultivars.

Cultivar	Lesion Length (mm)	N	Range (mm)
Cabernet Sauvignon	65,3	40	48-85
Pinotage	80,0	40	50-90
Control	14,7	40	8-25

3. *Graft unions*: The mean percentage callus formation (percentage of graft union covered by callus tissue) of inoculated cuttings was 29% compared to 76% in control cuttings. Inoculated cuttings exhibited a brown colouration of the wood tissue in the graft union, whereas control graft unions had a healthy, yellowish colour (Fig. 6). Only 32% of the inoculated plants survived compared to 84% of the uninoculated control plants (data not shown).



FIGURE 6

Brown colouration of *Phialophora parasitica* inoculated graft unions after four weeks (Healthy graft unions are shown on the left).

DISCUSSION

Phialophora parasitica was first isolated from a subcutaneous infection in a kidney transplant (the patient was on immunosuppressive maintenance therapy) and described as a new species by Ajello *et al.* (1974). Following its description as a human pathogen, a number of earlier isolates from disease conditions in several tree species proved to be conspecific (Hawksworth, Gibson & Gams, 1976). Recently, *P. parasitica* was also suggested to be the cause of cherry dieback (Rumbos, 1986). *Phialophora parasitica* has thus been associated with disease symptoms ranging from twig dieback to dieback and wilting in several tree species. In all these cases infection was suggested to have occurred on aboveground parts of plants.

The partial plugging of xylem vessels found in the roots and stems of inoculated rootstocks may well coincide with the slow development of dieback under field conditions. It is suspected that as more vessels become plugged, less growth can be sustained beyond the occlusion and the plants evidently starve to death. Halliwell (1966) suggested that disease symptoms associated with decline of oak resulted from the pathogen exerting some mechanical interference with the passage of water and (or) to the presence of a toxin.

In the present study the plugs within the xylem vessels were similar to pseudooparenchyma of *Phialophora zeicola* in cortical cells of maize (Deacon & Scott, 1983) and are therefore believed to consist of parenchyma of *P. parasitica*.

The pathogen has been isolated from a variety of culti-

var/rootstock combinations. Of particular interest is the absence of Shiraz from the list of affected cultivars. To our knowledge slow dieback has also not been recorded on Shiraz.

The majority of diseased vines received at Nietvoorbij were grafted to 99 Richter rootstock, which is a popular rootstock among South African farmers, but it may be susceptible to *P. parasitica*. This rootstock is known to be highly susceptible to the root pathogen *Phytophthora cinnamomi* Rands (Marais, 1983).

It is possible that in the case of slow dieback of grapevine that we report on infection probably takes place through the root system.

Symptoms of slow dieback on grapevines are only recognised four to five years after planting. Complying with Koch's postulates will therefore be a long-term project. However, for the time being, circumstantial evidence strengthens the hypothesis that *P. parasitica* is the cause of slow dieback. Not only is the organism involved in similar dieback diseases in other hosts, but the type of fungal growth produced in the vessels by this organism is also typical for closely related species.

At this stage the isolates from infected grapevine are best classed under *P. parasitica*. However, they are morphologically slightly different from *P. parasitica*, and may finally be classed in a new species. Furthermore, the isolates currently referred to as *P. parasitica* strongly deviated from the accepted generic concept within *Phialophora* (Gams & McGinnis, 1983) and may therefore more likely be accommodated in a new genus.

Further research will be conducted on the mechanism of plugging as well as a possible toxin production by the fungus.

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