Fungi Associated with the Quince Borer, *Coryphodema tristis* (Drury) (Lepidoptera: Cossidae), in Grapevines

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Fungal discolouration of wood was found associated with galleries of *Coryphodema tristis* in grapevines. The mean percentage damage caused by larvae and fungal discolouration was 14,8% and 13,5% respectively. Twelve fungal species were isolated from the discoloured region. None of these fungi were transmitted from one generation of the insect to the next.

The quince borer, *Coryphodema tristis* (Drury) is a wood borer found on various plants (Myburgh & Basson, 1960). It is a destructive pest of grapevines in the South-Western Cape, boring in the trunks and arms (G.F.J. Höppner, unpublished data). Damage is caused by larvae whose activity is restricted to live wood older than one year. Larvae initially feed for a period of up to three months on the surface of the wood below the loose bark before boring into the deeper wood layers. This damage is accompanied by the discolouration of tissue surrounding the galleries, similar to that caused by fungi associated with dieback in grapevines (Ferreira 1988). Fungi responsible for rot and stain in wood were found to be associated with galleries of the cossid *Prionoxystus robiniae* (Peck) in oaks (Berry, 1978).

This study was undertaken to determine whether fungi are responsible for the discolouration associated with the galleries of *C. tristis* in grapevines. Furthermore, a possible association between these fungi and *C. tristis* was investigated.

MATERIALS AND METHODS

Determination of damage: Twelve to fifteen year old Chenin blanc vines, infested with *C. tristis*, were cut off approximately 200 mm above soil level in various vineyards in the Bottelary area near Stellenbosch in the South-Western Cape. To determine the damage done by larvae and fungi, five randomly chosen vines were cut into cross sections 30 mm in length. The respective damage due to larval excavation and fungal infestation (represented by discoloured wood) was visually estimated as a percentage of the surface area for each cross section.

The isolation and identification of fungi: To isolate the fungi associated with *C. tristis*, ten infested vines were cut longitudinally to display the larval galleries. From the visually decayed wood surrounding the galleries, 12 wood chips, ca 10 x 10 x 5 mm in size, were removed from each vine. These chips were surface-sterilised in 0.5% sodium hypochlorite, rinsed in sterile water and placed on potato dextrose agar (PDA) in petri dishes. Previous experience indicated that PDA was effective in isolating fungal species from wood with dieback symptoms (Ferreira, 1988).

All plates were incubated at 25°C under a combination of cool white fluorescent and black light with a 12 h photoperiod. After 7 days incubation, all fungi present were identified microscopically and stored on PDA slants in McCartney bottles. These were sent to the Mycological Unit, Plant Protection Research Institute, Pretoria, for the confirmation of identity.

Association between quince borer and fungi: To determine whether fungi are transmitted by any of the life stages of *C. tristis*, isolations were made from the interior and exterior of second and final instar larvae, adults and eggs. Fifteen second and 15 final instar larvae were collected from five vines by careful splitting of the wood. Each larva was removed with a sterile forceps and rolled on PDA in a petri dish. The same larvae were then surface-sterilised in 0,5% sodium hypochlorite and dissected to remove the digestive tract contents. These were placed on PDA in petri dishes. Isolations from the surface of 15 field-collected adults that had emerged from infested grapevines, and their ovaries, and from the surface and contents of 30 eggs, laid on grapevines in a natural infestation, were made as described for larvae. The incubation and identification of fungi were carried out as described previously.

RESULTS AND DISCUSSION

Determination of damage: The damage caused by larvae plus fungi per cross section (n=576) ranged from 0% to 95%, with a mean of 28,3% (SD=19,7%) for all grapevines. The mean damage done by larvae was 14,8% (SD=10,85%), whereas fungal discolouration contributed 13,5% (SD=13,6%). This great variation can be ascribed to the fact that the most damage occurred on the higher part of the trunk and the adaxial parts of the arms.

It appeared that *C. tristis* larvae, after entering the wood, first attack the pith before expanding the galleries to the adjoining wood. In fresh galleries the discoloured area around galleries was narrow (± 2mm), but this expanded as galleries aged. In some cases the discoloured area extended outwards in a wedge shape.

The isolation and identification of fungi: Twelve fungal species were isolated from discoloured areas surrounding border galleries (Fig 1). *Trichoderma harzianum*, *Penicillium purpurascens*, *Cephalosporium* spp., *Fusarium oxyporum* and *Pestalotia quepini* occurred most frequently. Of the isolated

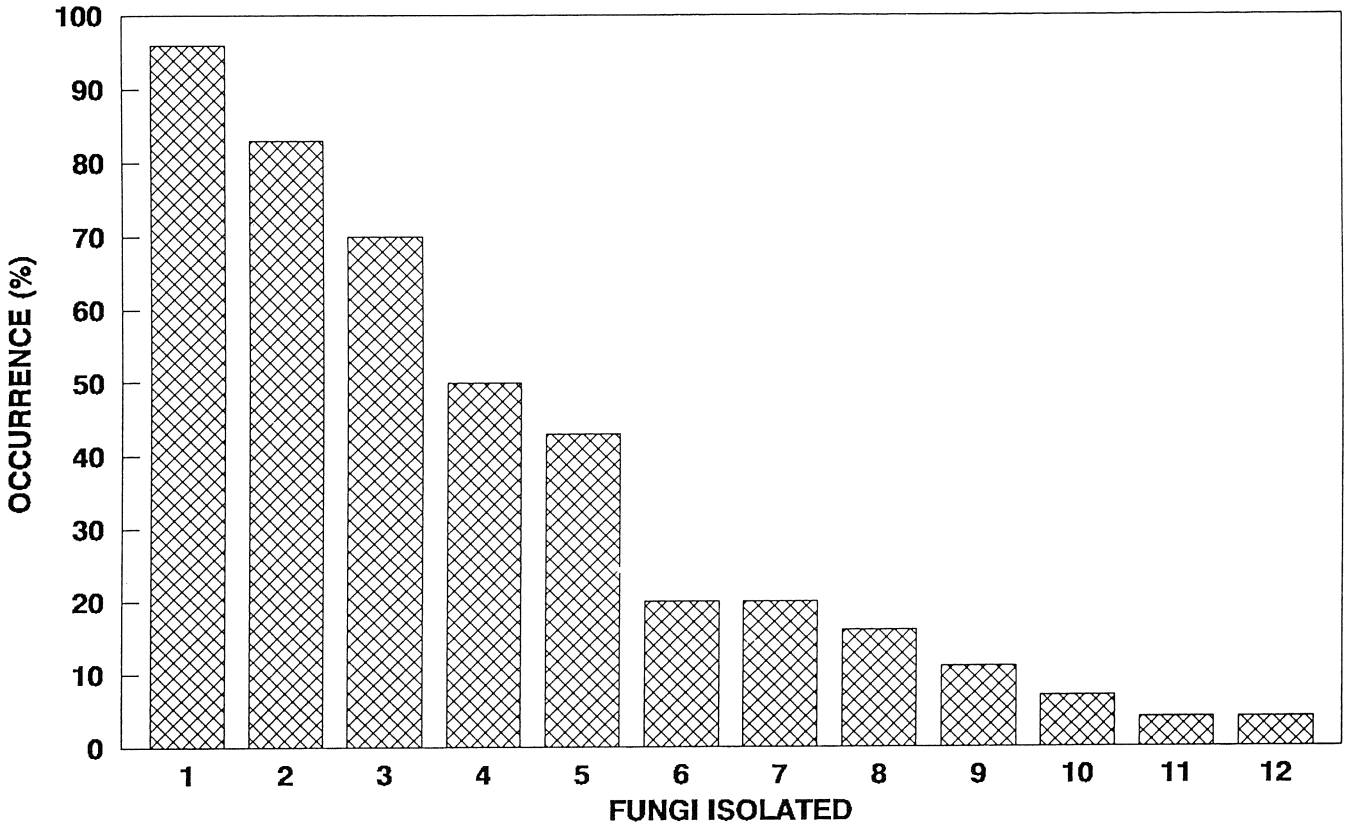

FIGURE 1
Percentage occurrence of fungi isolated from decayed grapevine wood surrounding larval galleries of *Coryphodema tristis*.
1 = *Trichoderma harzianum*, 2 = *Penicillium purpurascens*, 3 = *Cephalosporium* sp., 4 = *Fusarium oxysporum*, 5 = *Pestalotia quepini*, 6 = *Alternaria alternata*, 7 = *Rhizopus* sp., 8 = *Gliocladium* sp., 9 = *Aspergillus* sp., 10 = *Epicoccum purpurascens*, 11 = *Botrytis cinerea*, 12 = *Sphaeropsis* sp.

TABLE 1
Fungi isolated from different stages of *Coryphodema tristis* and frequency of occurrence.

Fungi isolated	Occurrence (%)							
	External				Internal			
	second instar larvae	final instar larvae	adults	eggs	second instar larvae	final instar larvae	ovaries	eggs
Penicillium purpurascens	80,0	100,0	100,0	100,0	13,3	6,7	0	0
Cephalosporium sp.	46,7	33,3	0	0	53,3	33,3	0	0
Epicoccum purpurascens	46,7	20,0	0	0	0	0	0	0
Trichoderma harzianum	46,7	13,3	0	0	0	0	0	0
Alternaria alternata	33,3	53,3	0	0	0	6,7	0	0
Aspergillus sp.	13,3	46,7	0	0	0	46,6	0	0
Rhizopus sp.	6,7	0	0	0	0	0	0	0
Fusarium oxysporum	0	86,6	0	0	0	0	0	0

fungi, however, only *Cephalosporium* spp., *F. oxysporum, P. quepini, Botrytis cinerea* and *Sphaeropsis* spp. can act parasitically, whereas the others are saprophytes. The occurrence of *B. cinerea* and *Sphaeropsis* spp. was very low however, indicating that their role in colonising the wood was negligible. Ferreira (1988) found nine of these fungi to be associated with dieback and pruning wounds, and Hansen (1985) named *Cephalosporium* spp. as some of several fungi responsible for wood decay in grapevines.

Association between quince borer and fungi: During regular sampling of *C. tristis* infested grapevines it was found that larvae drowned in phloem exudates that filled galleries where discoloured wood was absent. Wood-colonising fungi may be responsible for the lowring of moisture in the wood surrounding the galleries, creating a favourable habitat for the development of the larvae. The loss of moisture not only facilitates wood-border invasion but also results in the increased aeration of the microhabitat from which the insects must obtain oxygen for respiration (Graham, 1967). It, therefore, appeared that the fungi may play an important role in the survival of *C. tristis* larvae.

Fungi isolated and their frequency of occurrence on the exterior of larvae, adults and eggs, in the digestive tracts of larvae and in ovaries and eggs are listed in Table 1. The presence of fungi on the surface and in the digestive tract of larvae indicated that the fungi can be transmitted to the deeper layers of the wood. However, no fungi were present in eggs and ovaries which indicates that there was no anatomical adaptation in the insect for the transfer of the fungi from one generation to the next. *Penicillium purpurascens* was the only fungus present on adults and eggs. This fungus may have been transmitted from the adults to the eggs by scales shed during oviposition. Therefore, there was no behavioural or anatomical adaptation in the insect for the transfer of the fungi from one generation to the next. It is assumed that the fungal infection of the wood occurred after wounding during the initial feeding of neonate larvae on the surface and under the loose bark. The lengthy initial period of feeding would allow the fungi to penetrate the wood and create a favourable environment for the survival of the larvae, thus enabling larvae to penetrate the deeper layers of the wood.

It appears that the association between *C. tristis* and the fungi can best be described as protoco-operation: the fungi create a favourable environment for larval survival in the wood but can colonise and develop in vinewood in the absence of *C. tristis* (Ferreira, 1988), while, on the other hand, they benefit in that the larval wounding of wood supplies entrance holes and larvae aid in their dispersal.

CONCLUSIONS

Appreciable fungal decay was associated with borer galleries in grapevines. The isolated fungi apparently played an important role in the ecology of the larvae in that they created a favourable environment for their survival. However, none of the fungi isolated from discoloured wood were transmitted from one generation of the insect to the next since they were not present in or on the adults or eggs. Also, they are not dependent on borers for infection of the wood. It thus seems that protoco-operation exists between *C. tristis* and the fungi isolated.

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