Nectria galligena as the cause of a collar rot disease in organically grown Topaz apple trees

Nectria galligena als Ursache einer Kragenfäule-ähnlichen Krankheit bei der Apfelsorte Topaz im ökologischen Obstbau

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Abstract

Symptoms resembling collar rot were detected in organically managed Topaz trees aged 3-10 years, occurring one to several years after planting of the orchard. Trees were killed within the same growing season in which symptoms were first observed. The disease commonly progressed as a complete covered canker at the base of the tree trunk. Isolation attempts were negative for *Phytophthora* and other Oomycetes, but consistently yielded *Nectria galligena*. The possibility of latent (endophytic) infections of *N. galligena* as the cause of delayed collar rot symptoms is briefly discussed.

Keywords: apple, canker, collar rot, endophyte, *Nectria galligena*, Topaz

Introduction

Seemingly typical collar rot symptoms were observed on apple trees (3-10 years old) grown in an organically managed apple orchard in Northern Germany's most important fruit-growing area, the 'Altes Land' south-west of Hamburg. The trees were of the 'Topaz' variety grafted onto M9 rootstocks, with relatively low graft unions (10-20 cm above soil level). The disease commonly progressed from the graft union upwards as a necrosis of the bark and underlying cambium tissue; upon removal of the bark, the advancing disease lesion was visible as a sharply delimited chocolate-brown zone on the underlying wood. No obvious root or crown rot symptoms were observed on the rootstock of the affected trees. Typical infections quickly enveloped the entire circumference of a tree, causing its death within one growing season. There was no clear-cut correlation with edaphic factors such as soil moisture, and infection foci were not observed. Instead, trees appeared to be affected randomly throughout the plantation.
The summary given here is based on a full account of our results provided by Weber *et al.* (2006).

Material and Methods

With most of the above features pointing towards a *Phytophthora* collar rot, several attempts were made to isolate Oomycetes from the surface-sterilised bark of healthy and diseased trees, as well as their surrounding soil. A medium selective for *Phytophthora* and *Pythium* spp. was used, consisting of cornmeal agar (Difco) augmented with 50 mg nystatin (=mycostatin), 100 mg pentachloronitrobenzene and 200 mg vancomycin litre^{-1} (modified from Erwin & Ribeiro, 1996). Our reference strain of *Phytophthora cactorum* as well as other *Phytophthora* and *Pythium* spp. showed good growth on this medium. Additionally, soil, root and bark samples were incubated in sterile distilled water (Weber *et al.*, 2004). Thirdly, baits (cut apple and potato halves) were buried in the rhizosphere of affected and healthy trees, recovered after 3 days, and plated out onto the selective medium as described above.

Results

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Apart from the sporadic baiting of *Pythium sylvaticum* from orchard soil, no Oomycetes were found in soil or plant tissues. Instead, one morphologically recognisable colony type belonging to a true (*i.e.* non-Oomycete) fungus dominated in our isolation experiments. This grew slowly on the selective agar medium, but more rapidly on standard media without antibiotics. It was ubiquitous in bark samples taken from the advancing disease front, less frequent in dead tissue, and rare in tissue ahead of the advancing infection or in healthy trees (Fig. 1; Weber *et al.*, 2006). Microscopic examination and ITS/5.8 S rDNA sequence analysis permitted the unambiguous identification of this fungus as *Nectria galligena* (conidial state *Cylindrocarpon heteronema*).

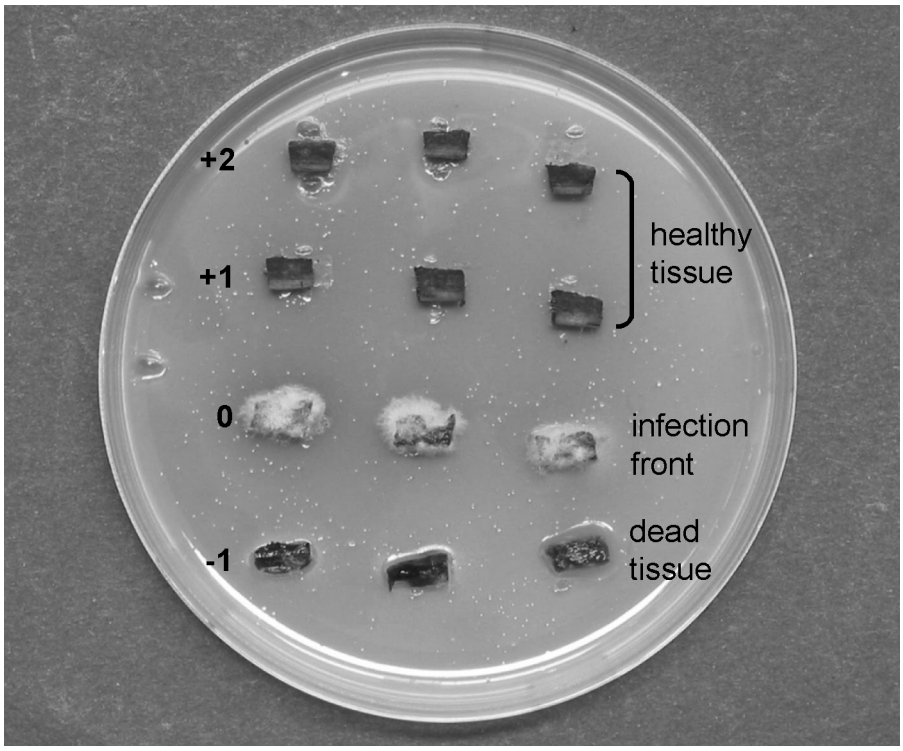

Fig. 1. Agar plate with an Oomycete-selective medium showing the isolation of *Nectria galligena* as a slow-growing white colony from the advancing infection front (0) but not, in this case, from the healthy tissue 1 and 2 cm ahead of it (+1 and +2, respectively) or 1 cm behind it (-1).

Discussion

Nectria galligena is the well-known cause of apple and pear canker. In its typical form the disease is initiated when conidia or ascospores germinate by emitting hyphae which penetrate through wounds or cracks into the bark of above-ground plant organs. Such infections result in open (partial) cankers in which callus-like outgrowths of bark tissue surround a central area of dead bark which often strips off to reveal the underlying wood (Fig. 2). Once such a canker has surrounded the entire circumference of a twig, all portions distal to it die back. Such a disease progression is usually very slow, taking several years to kill the trunk of an established tree.

In the present case, disease symptoms were very different, with lesions frequently at the base of a tree, and disease progression being so rapid that the resulting canker was strikingly similar to a collar rot in entirely surrounding the tree trunk, in the dead bark remaining attached to the wood, and in lacking any callus-like growth. Infection with *N. galligena* therefore appeared to be systemic and endogenous, possibly arising from latent (endophytic) mycelium.

Endophytic fungi are capable of colonising living plant tissue for prolonged periods without causing disease symptoms. Fungi showing this form of association with terrestrial plants are known from

diverse taxonomic groups. Among those related to *Nectria*, the genus *Fusarium* is noteworthy in containing fully pathogenic species (e.g. *F. oxysporum*) as well as closely related strains which colonise their host tissue without overt disease symptoms (Kuldau & Yates, 2000). Other fungi may show a latent endophytic phase followed by an aggressive colonisation of the living plant (as pathogens) or dead plant tissue (as saprotrophs). Could it be that *N. galligena* is capable, under certain circumstances, of establishing endophytic infections in its host, switching to its aggressively pathogenic phase later upon receiving appropriate environmental triggers? McCracken *et al.* (2003) have obtained some evidence for such a scenario; their study of genetic fingerprints of *N. galligena* isolates in apple orchards and nurseries showed that the inoculum of apple canker in young orchards was more closely related to that of the nurseries supplying the trees than to the natural inoculum on trees surrounding the orchard.

In another recent study on *N. galligena* in organic management situations, Jansonius *et al.* (2004) have shown that there can be a very high incidence of apple canker in young trees especially during their first year in the orchard. This was explained by infection during the preceding autumn, *i.e.* after leaf fall but immediately prior to explanting into the orchard. The incidence of such delayed cankers was especially high in trees reared in organic nurseries. Although it is difficult to distinguish such a route of entry from latent endophytic infections, our observations of the rapid onset of complete and covered cankers at the base of trees several years after explantation into the orchard would point towards the existence of an endophytic phase, possibly after infection in the nursery as suggested by McCracken *et al.* (2003) and Jansonius *et al.* (2004).

We are currently examining the possibility of endophytism in *N. galligena*. If this feature turns out to be common, there will be obvious implications for the control of endophytic *N. galligena* infections in nurseries, especially in organic management practices where systemic fungicides cannot be used.

Fig. 2. Typical apple canker caused by *Nectria galligena*. The canker is partial and open, *i.e.* an area of bare wood is surrounded by callus-like host tissue.

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