

***Diplodia seriata*, cause of black fruit rot in organically grown apples in Holland, Belgium and Northern Germany**

Marc Trapman¹, Peter Maxin², Roland W. S. Weber^{3,*}

Abstract

A fruit rot resembling Gloeosporium infections but appearing on fruits prior to harvest was noticed in organic apple orchards in Holland, Belgium and Northern Germany in 2007. Infections were most commonly observed on 'Elstar', but other cultivars were also affected. Fruit colonisation progressed in two steps, whereby a latent stage of sunken black lesions in immature fruits gave rise to a rapidly spreading firm brown rot upon fruit ripening. Isolation experiments from both stages consistently yielded a single species of fungus identified as Diplodia seriata, formerly known under the teleomorph name Botryosphaeria obtusa. Lesions of D. seriata were also seen on leaves as necrotic light brown spots surrounded by a purple halo, and occasionally on small twigs as cankers. Fruit mummies on apple twigs were heavily colonised by D. seriata and are thus likely to carry inoculum for fruit infections during late summer or in the following growing season.

Keywords: *Botryosphaeria obtusa*, *Diplodia seriata*, apple, black rot, fruit mummies

Introduction

In August and September 2007 an unusual fruit rot was noticed on ripening apples in Belgium, the Netherlands and in the Lower Elbe Valley of Northern Germany. Whilst 'Elstar' was most commonly affected, the rot was also seen on several other apple varieties including 'Ingrid Marie', 'Dalinco', 'Dalinel', 'Jonagold' and 'Gerlinde'. The disease was observed only in organically managed orchards where it appeared to progress in two stages. The first visible symptoms were small black lesions (2-4 mm diam. and in depth) which were slightly sunken and had a somewhat corky texture upon cutting. These black lesions did not enlarge further and were regarded as a dormant stage of infection because they gave rise to a rapidly progressing pale brown rot only during the 2-3 weeks preceding harvest (Fig. 1). The active fruit rot showed a concentric zonation of lighter and darker brown colours (Figs. 1 and 6). Fruits affected by this kind of brown rot were entirely colonised by it within 3-5 d. In contrast, no brown rot arose from the black lesions when infected apples were harvested and stored at low temperatures (2-4 °C) for up to 6 weeks, although the rot appeared within a few days of incubating these stored apples at room temperature.

Leaves of infected trees showed a purple halo surrounding necrotic tan-coloured spots, some of which carried pycnidial primordia (Fig. 2). Severe leaf infections led to premature defoliation soon after harvest and were seen predominantly on trees also carrying infected fruits. Less severe leaf infections were observed on trees up to 100 m away from the infection focus. In severely affected orchards, up to 25% of the harvest was lost. Since we had not previously observed this disease, we isolated and identified the causal agent.

¹ Bio Fruit Advies, Dorpsstraat 32, 4111 KT Zoelmond, Netherlands

² KÖN and ³ OVA, Obstbau Versuchs- und Beratungszentrum (OVB) Jork, Moorende 53, 21635 Jork, Germany

* Corresponding author: roland.weber@lwk-niedersachsen.de



Figures 1-2. Obvious symptoms of *D. seriata* on 'Gerlinde'. Fig. 1: Successive stages of fruit rot from left to right. Fig. 2: Leaf infections of the 'frog-eye' type in which a pale brown necrotic centre is surrounded by a reddish-purple halo.

Material and methods

Fruits showing dormant black lesions and/or spreading brown rot were surface-sterilised by wiping with tissue paper soaked in 70% ethanol. Small tissue pieces (2-4 mm³) were cut from the advancing margin of infections with a sterile scalpel and were transferred to 2% malt extract agar (MEA) augmented with penicillin G and streptomycin sulphate (each at 200 mg l⁻¹). For initiation of pycnidial development and spore production, isolates were subcultured onto tap-water agar (TWA) bearing small (5 mm) lengths of *Pinus* needles (Slippers *et al.*, 2007). Because of its distinctive spores, it was possible subsequently to identify the fungus by direct microscopic analysis of sporulating lesions taken from other infected host organs, especially twig cankers and fruit mummies.

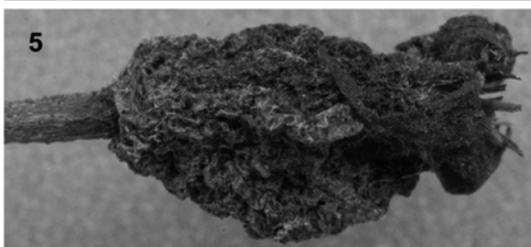
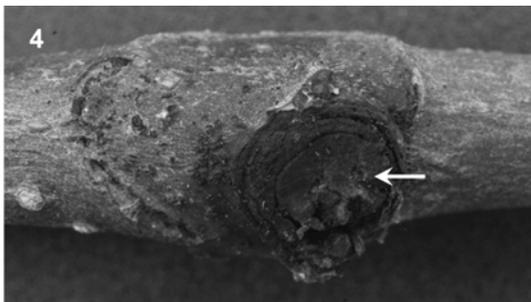
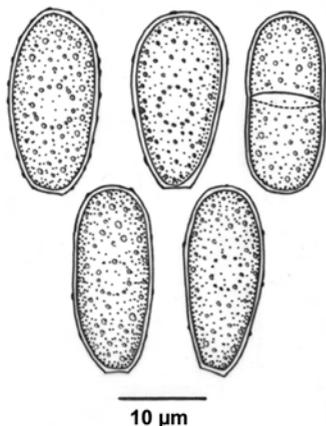
Results

Isolation of *D. seriata*. – On MEA, one and the same species of fungus was consistently isolated from dormant black lesions as well as actively spreading brown rot infections. Whereas rapid growth ensued within 24 h of plating out brown rot samples, with black lesions a lag time of 48-72 h was observed before mycelium became visible. Colonies on MEA were initially hyaline, but produced dark green melanised hyphae and pycnidium initials within 7 d incubation at room temperature (20-22 °C). Mature pycnidia with typical conidia were observed on TWA with pine needles after 14-21 d incubation.

Description of *D. seriata*. – Conidia were ellipsoid to cylindrical ($22\text{-}25 \times 9.5\text{-}11.0 \mu\text{m}$) with a distinct basal scar. They had conspicuously thick ($>0.5 \mu\text{m}$) melanised cell walls and contained dense cytoplasmic deposits of lipid droplets which made it impossible to state with certainty whether the walls were smooth or slightly roughened. Exuded as aseptate and fully pigmented spores, conidia were occasionally observed to form one central transverse septum at a later stage (Fig. 3). Identification as *Diplodia seriata* De Not. was unequivocal on the basis of the descriptions given by Punithalingam & Waller (1973), Jones & Aldwinckle (1990) and Phillips *et al.* (2007). Pale brown leaf spots (Fig. 2) showing the typical ‘frog-eye’ appearance described by Jones & Aldwinckle (1990) were also assigned to infections by *D. seriata*.

Distribution of *D. seriata*. – On the basis of its conspicuous conidia, we were able to examine trees from an orchard heavily infested with fruit rot (‘Gerlinde’; Neuenfelde, Altes Land, Germany) for the presence of *D. seriata* on other host organs. Whereas cankers were only rarely observed on two-year-old twigs (Fig. 4), infections of fruit mummies were extremely abundant (Fig. 5). Of 100 mummies collected from 10 trees with fruit rot symptoms, 97 were heavily colonised by *D. seriata*. Similarly dense colonisation of fruit mummies was observed on material from other Northern German orchards as well as from Belgium and Holland. We were able to confirm *D. seriata* in fruit rots from the following samples provided by organic fruit farmers: (1) Northern Germany, on ‘Dalinbel’, ‘Elstar’, ‘Gerlinde’, ‘Holsteiner Cox’ and ‘Ingrid Marie’ in Hove, Neuenschleuse, Estebürgge, Neuenfelde (Altes Land, Lower Elbe Valley); (2) Netherlands, on ‘Dalinco’, ‘Elstar’ and ‘Jonagold’ in Beemster and ‘s-Hertogenbosch; (3) Belgium, on ‘Elstar’ in Reinrode.

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Figures 3-5. Further diagnostic features of *D. seriata*. Fig. 3: Conidia from colonised fruit mummies (top row) and from an agar culture (bottom). Fig. 4: Canker on a two-year-old ‘Gerlinde’ twig. Infection probably occurred through the pruning wound (arrow). Fig. 5: Infected fruit mummy from a ‘Gerlinde’ tree.

Discussion

Diplodia seriata, better known in the literature as *Botryosphaeria obtusa* (Schwein.) Shoemaker, is readily recognised by its large, ellipsoidal to cylindrical conidia which are aseptate but fully pigmented while still within the pycnidium, and it can be distinguished on that basis from other *Botryosphaeria* spp. Conidia of *B. stevensii* Shoemaker (anamorph *Diplodia mutila* (Fr.) Mont.) become septate early in their development, but melanisation is delayed until they are outside the pycnidium; whilst *B. dothidea* (Moug.:Fr.) Ces. & De Not. (anamorph *Fusicoccum aesculi* Corda) has conidia which remain hyaline and aseptate even at maturity (Jones & Aldwinckle, 1990; Phillips, 2007). Despite these clear-cut differences, the taxonomic placement of *D. seriata* has been in a state of confusion which persists to the present day. Slippers *et al.* (2007) have shown that this species is not closely related to currently recognised *Botryosphaeria* spp. such as *B. stevensii* and *B. dothidea*, and they have proposed that it should no longer be called *B. obtusa*. For the moment, the best available name seems to be *D. seriata* (Phillips *et al.*, 2007). The circumscription of this fungus is further complicated by the likely existence of numerous strains or cryptic species (Phillips, 2007).

Our observation of the existence of a latent phase of *D. seriata* in apple fruits agrees with several reports on *B. dothidea* which can infect apples within 7 weeks of petal fall but shows a delay of brown rot until the defence system weakens during fruit ripening (Parker & Sutton, 1993; Kim *et al.*, 2001). This aspect of the infection biology of *D. seriata* and *B. dothidea* is very similar also to *Gloeosporium* spp. causing storage rots of apples. Such similarities extend to the colonisation of fruit mummies and the bark of trees as niches for overwintering. We were astonished to find that nearly all mummies on trees with fruit rot symptoms were infected by *D. seriata*. These mummies (1-2 cm diam.) partly originated from aborted fruits of the current growing season, and partly represented overwintered structures from the previous year. We regard them as the key to the success of *D. seriata* as an apple pathogen (Fig. 6), and it is probably no coincidence that varieties with a strong tendency to retain their mummies (e.g. 'Elstar' or 'Gerlinde') were more frequently infected than others. Extended warm and wet periods in Northern Europe in May, June and July 2007 would have been favourable for fruit infections from colonised mummies, rainsplash being the main dispersal mechanism for *D. seriata* conidia (Arauz & Sutton, 1989).

We have not (yet) found *D. seriata* in orchards under integrated pest management (IPM) in Northern Germany. This could, of course, be coincidental, *i.e.* due to chance observations by organic farmers but not their conventional colleagues. More likely, the latter may have achieved collateral control of *D. seriata* by fungicide treatments against *Gloeosporium* spp. or other pathogenic fungi during the growing season. The near-absence of sooty blotch disease from IPM orchards in Northern Germany probably has a similar explanation.

In organic fruit production, the removal of fruit mummies from the trees, although labour-intensive, may be a good way to control *D. seriata* and to retard its further spread. In the same instance, this would contribute to the control of several other fungal pathogens, notably *Gloeosporium* and *Fusarium* spp. which can co-colonise fruit mummies infected by *D. seriata* (Weber *et al.*, 2008). However, such hygiene measures should take into account that *D. seriata* has numerous alternative hosts, including quince, pear, peach, plum, grapevine and many other woody plants (Punithalingam & Waller, 1973; Phillips *et al.*, 2007; Slippers *et al.*, 2007). When considering other plants as alternative hosts, one should also test for possible symptomless (endophytic) colonisation of twigs by *D. seriata* (Slippers & Wingfield, 2007). Further work on the spread, infection biology and control of *D. seriata* in Northern European apple orchards is therefore urgently required before more secure control measures can be recommended.



Figure 6: Fruit mummies were the likely source of infection for black lesions and a spreading brown rot (arrow) of *Diplodia seriata*.

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