

Mycotoxin Producing *Fusarium* Species – the Cause of Primary Stem Canker of Deciduous Forest Plants

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OBJECTIVE

Fusarium species are spread worldwide and causing damages at different stages of tree development as well as are involved in many complex diseases of forest ecosystems. Most impact was assessed in forest nurseries with seedling diseases like root and hypocotyls rot and wilt. Moreover, afforestations and natural stands of young forest broadleaved trees were affected by a range of species with symptoms of foliage withering and dieback of branches as well as bark necrosis and canker. Because those alarming symptoms appeared more widespread in the last decade, *Fusarium* strains were isolated from affected young trees of black locust, birch, alder, and aspen from nurseries, afforestations and from natural stands in Germany. The isolates were classified in *F. avenaceum*, *F. tricinctum*, *F. sporotrichioides*, and *F. sambucinum* by conidia morphology and ITS sequencing of rDNA. Fifteen isolates were examined regarding their pathogenicity on six broadleaved tree species with artificial inoculation under glasshouse conditions. Furthermore, the mycotoxic properties of these strains were investigated from cell extracts produced in six different culture media by means of on-line couplings LC-PDA-Q-TOF-ESI-MS as well as LC-UV-NMR, and MALDI-TOF-MS.

MATERIAL AND METHODS

Fusarium strains were isolated from bark necroses of black locust, birch, alder, and aspen selected in nurseries and plantations. The isolates were determined by conidia formation and by partial 16S rDNA analysis. Inoculation experiments were carried out with containerized two year-old plants of *Sorbus aucuparia*, *Acer platanoides*, *Tilia cordata*, *Prunus avium* (all tested in 2005 and 2006), *Fraxinus excelsior* (2005), and *Quercus robur* (2006) by artificial inoculation with conidia suspension ($\sim 10^4$ conidia / ml) with six replications. Control plants were grown without conidia treatment. *Fusarium* strains tested in 2005 and 2006 resp. were *F. avenaceum* (9 and 7 strains, resp.), *F. sporotrichioides* (2 and 4, resp.), *F. sambucinum* (2 and 4, resp.), and *F. tricinctum* (1 strain). Plants were estimated regarding formation of necroses and canker symptoms by a gradual score after 9 months: Without

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symptoms =1, spot infection =2, small necrosis not broader than the half of shoot =3; extended necrosis with canker =4, canker with withering and dieback =5.

Mycotoxin analysis of fungal strains: Rapid identification of secondary metabolites from pure culture (PDA) was carried out by combination of MALDI-TOF- and -TOF/ TOF-MS, as well as by on-line couplings LC-PDA-ESI-Q-TOF-MS and LC-UV-NMR. The mutual completion of structure information delivered by the spectroscopic methods UV/VIS, MS and NMR is of special importance for rapid identification of secondary metabolites directly in crude extracts.

RESULTS

Inoculation experiments had shown that all isolates caused shoot necrosis, canker, and dieback symptoms of tree species tested (Figure 1, 2). Differences between *Fusarium* species were visible resulting in different intensity of symptoms. These can be traced back to the secondary metabolite profiles of strains showing the appearance of a range of metabolites known for their phytotoxic properties and of novel metabolites. Strains of *F. sambucinum* and *F. sporotrichioides*, which have caused severe damage, produced mainly mycotoxins from the trichothecene group. *F. tricinctum* inoculation induced only light damage. This species accumulated mainly cyclodepsipeptides such as enniatins. Between the different isolates of *F. avenaceum* a high variation of virulence was determined. The mycotoxin profiles of those strains had shown a large spectrum of compounds ranging from formation of cyclodepsipeptides alone up to cyclodepsipeptides and trichothecene mycotoxins.

Experiment 2005

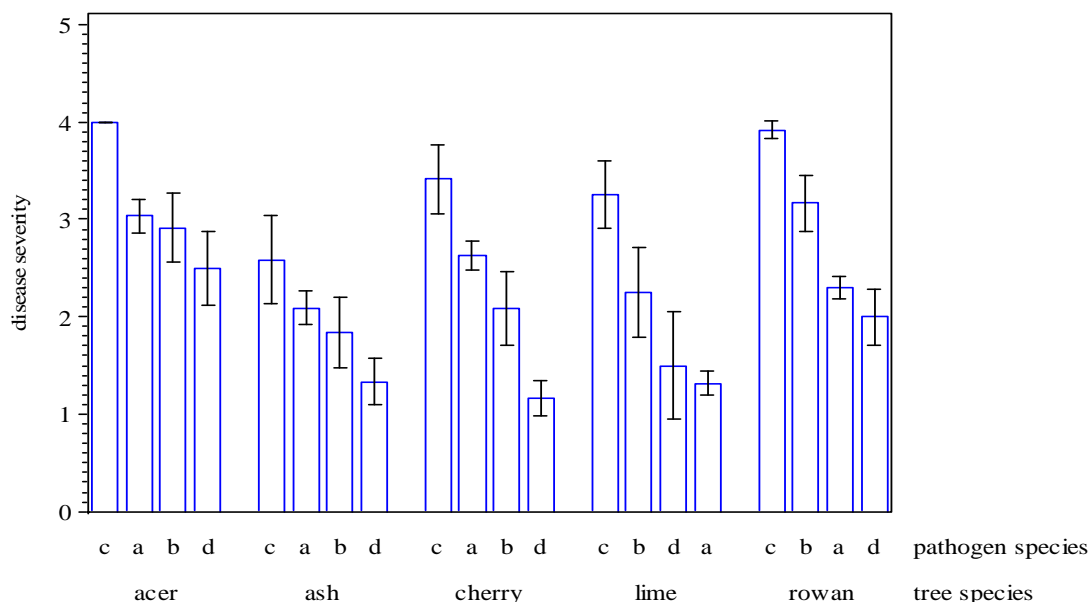


Figure 1. Mean of disease severity of deciduous tree species caused by four different *Fusarium* species 9 months after artificial inoculation from experiment 2005; the letters are indicating the fungal species: a *F. avenaceum* (n=54), b *F. sporotrichioides* (n=12), c *F. sambucinum* (n=12), d *F. tricinctum* (n=6).

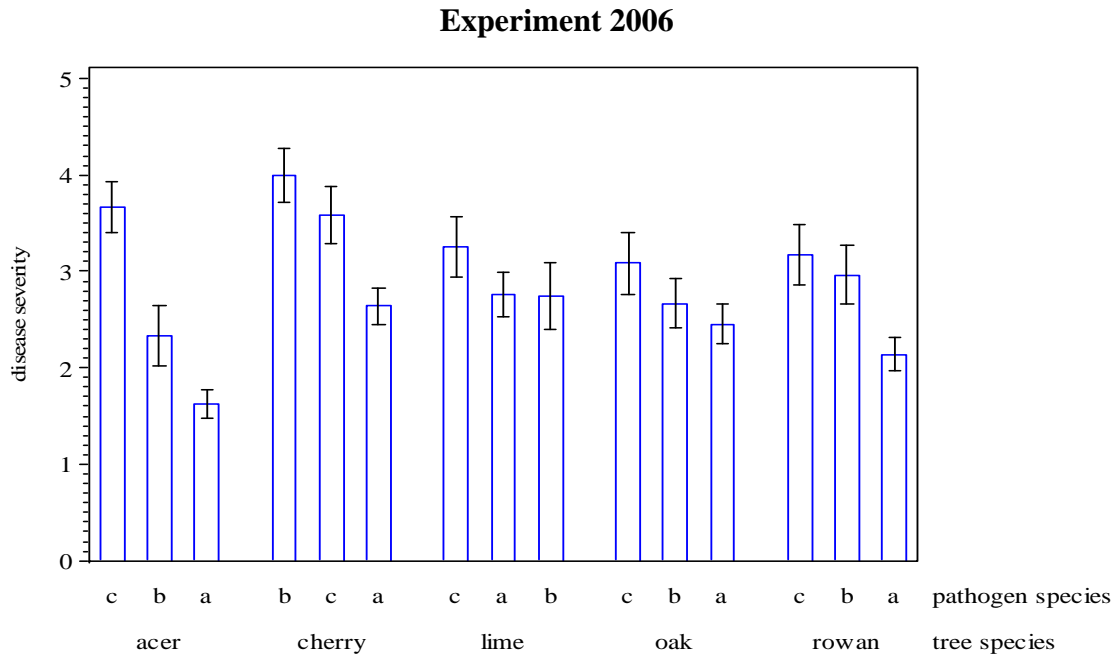


Figure 2. Mean of disease severity of deciduous tree species caused by three different *Fusarium* species 9 months after artificial inoculation from experiment 2006; the letters are indicating the following fungal species: a *F. avenaceum* (n=42), b *F. sporotrichioides* (n=24), c *F. sambucinum* (n=24).

DISCUSSION

Our results had shown the phytotoxic effect of a range of mycotoxin producing *Fusarium* strains on six different forest tree species was not specific. *F. sambucinum* had proven as a species with the highest pathogenicity to the young broadleaves tested followed by *F. sporotrichioides* and *F. avenaceum*.

Reports about damage in terms of bark necrosis, canker, wilt and dieback caused by *Fusarium* species are more common in the last years. Particularly the neophytic tree species *Robinia pseudoacacia* was investigated regarding these pathogens because it plays an increasing role in woody biomass production (Szabó 2000, Halász 2002).

Pathogenic *Fusarium* species are characterized by the formation of a large variety of toxic metabolites. More than 100 toxigenic secondary metabolites have been described (DeNijs et al. 1996). Enniatins were long known as phytotoxins from *Fusarium* species and associated with plant diseases characterized by wilt and necrosis formation. Furthermore, beauvericin, moniliformin, as well as toxins from the trichothecene group are produced by members of the genus (Logrieco et al. 2002). The interaction between the trait of mycotoxin-production of a strain and their virulence could be proven at *F. graminearum* and *F. avenaceum* (Desjardins et al. 1996), where trichothecene-nonproducing and enniatin-nonproducing mutants resp. showed a reduced virulence at their hosts.

The recent study showed the possible role of members of this genus to evolve into serious pathogens for different broadleaved tree species in forested landscapes. This may become important under the aspect of transformation of arable land to areas for production of woody biomass.

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