

Assessing the Pathogenic Effect of *Fusarium*, *Geosmithia* and *Ophiostoma* Fungi from Broad-Leaved Trees

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ABSTRACT. Phytopathogenic effect of *Geosmithia pallida*, *G. langdonii*, *Ophiostoma grandicarpum*, *O. querci*, two isolates of *O. piceae*, and two isolates of *Fusarium solani* was compared using plant growth test (stem and root length of garden cress plants seeded on mycelium-covered potato carrot agar); *Ophiostoma* spp. and *F. solani* were isolated from oak, *Geosmithia* spp. from galleries of *Scolytus intricatus* on beech. All fungi inhibited more the root elongation than that of stems. *F. solani* led to plant collapse after briefly stimulating the growth of stem and in one case also root. *G. langdonii* inhibited stem and root growth to 20 % and led to plant collapse. *G. pallida* inhibited root growth to 25 % whereas stem growth was almost unimpaired. *Ophiostoma* spp. reduced stem growth to ≈60–80 % and root growth to 25–60 %. *O. piceae* and *O. querci* caused plant collapse after 15–20 d.

Combination of various stressors such as severe seasonal droughts and sudden freezing temperatures in winter together with water stress, defoliating insects, other pest insects and fungal pathogens are thought to be the most important predisposing and concomitantly acting factors in oak decline (for review see Marcu 1987; Führer 1998).

One of the hypotheses for explanation of the *Quercus petraea* dieback in Central Europe was based on an epidemic concept analogous to the North American oak wilt disease or elm disease (Čapek 1987; Leontovyč and Čapek 1987; Igmandy 1987). A ‘tracheomycosis’, caused by fungi of the genus *Ceratocystis/Ophiostoma* and transmitted by insects (*Scolytus intricatus* RATZ., *Agrilus* spp., etc.), was considered to be the main causative factor responsible for tree mortality (Oszako 1990, 1992).

Another factor in oak decline was the infection with *Phytophthora* spp. (Jung *et al.* 1996). Other fungi were found associated with bark beetles and oak decline; e.g., *Fusarium solani*, *F. eumartii* and *Verticillium dahliae* were associated with *S. intricatus* in Italy (Ragazzi *et al.* 1993; Riziero *et al.* 2002).

Phloemophagous bark beetles (*Coleoptera:Scolytidae*) live in symbiosis with various fungal species. The most researched association is that of scolytids and phytopathogenic ophiostomatal fungi, influencing their mutual evolution (Farrell *et al.* 2001).

Recently, we found a novel and widespread association of scolytids with fungi of the genus *Geosmithia* (Kolařík *et al.* 2004; Kubátová *et al.* 2004), first observed on declining oaks (Kubátová 2000). The mycoflora of these species of bark beetles was almost unknown because of their limited phytopathological importance.

Geosmithia is a polyphyletic taxon with affinities to *Hypocreales* and *Eurotiales* (Ogawa *et al.* 1997; Peterson 2000; Iwamoto *et al.* 2002). As shown by rDNA comparison, all new species of *Geosmithia* that we found associated with bark beetles belong to *Hypocreales* (Kolařík *et al.* 2004).

The aim of this study was to evaluate the putative plant inhibitory effect of two species of *Geosmithia* in comparison with known pathogens *Ophiostoma* spp. and *F. solani*, using our method of assay influencing the whole plant.

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MATERIAL AND METHODS

Isolates. Putative pathogenic effects were studied on 2 isolates of genus *Fusarium*, 2 representatives of *Geosmithia*, and 4 isolates of *Ophiostoma* (Table I). The isolates were chosen for their rapid growth and, in the case of *Ophiostoma*, also for fruiting body formation.

Table I. Used isolates and their origin

Species	Isolate	Year	Source	Host	Location
<i>Fusarium solani</i>	Fus-06	2000	branch cut	<i>Q. robur</i>	Dubovce, South Bohemia
	AK 109/93	1993	root	<i>ditto</i>	Dešov, Znojmo, South Moravia
<i>Geosmithia langdonii</i>	MK 373	2003	adult of <i>S. intricatus</i> ^a	<i>F. sylvatica</i>	Hříškov, Louny, North Bohemia
	<i>pallida</i> MK 386	2003	<i>ditto</i>	<i>ditto</i>	<i>ditto</i>
<i>Ophiostoma grandicarpum</i> <i>piceae sensu lato</i> <i>querci</i>	Oph-24	2000	branch cut ^b	<i>Q. robur</i>	Koclířov, South Bohemia
	Oph-02	2000	<i>ditto</i>	<i>ditto</i>	<i>ditto</i>
	Oph-12	2000	<i>ditto</i>	<i>ditto</i>	Dubovce, South Bohemia
	Oph-10	2000	<i>ditto</i>	<i>ditto</i>	Velký Tisý, South Bohemia

^aFrom beech (Kolařík *et al.* 2004); in some *S. intricatus* galleries, growth of the *Geosmithia* fungi was abundant to such an extent that it was directly visible.

^bNovotný and Šrůtka (2004).

Pathogenicity test. The surface of potato–carrot agar (100 mL) in a 250-mL Erlenmeyer flask was inoculated in 5 spots by the isolate tested. After 15 d of growth, ca. 200 seeds of *Lepidium sativum* L. var. *capitatum* (surface-sterilized by 20 min in 1 % sodium hypochlorite) were placed on the mycelial mat. The seeds started germinating in 1 d. During the test, the length of the available stems and roots was measured from outside at 2–5-d intervals without opening the flask; the test was done at 22–28 °C. Each culture was run in 6 parallels, the length value for each parallel flask was the average length of the plants or roots measured.

RESULTS AND DISCUSSION

The presence of both isolates of *F. solani* (Fig. 1A) initially stimulated stem growth of the plantlets but, later, the plants were smaller than the controls, and collapsed after 10 d. The effect of both isolates on stem growth was similar. A larger difference was observed in their influence on roots. Isolate AK 109/93 stimulated root growth, which then slowed down to 80 % of the control level, whereas the isolate Fus-06 depressed root growth from the beginning by 25 to 60 %. As *F. solani* is a well-known plant pathogen, the initial stem growth stimulation preceding plant collapse was unexpected.

The two *Geosmithia* species differed in their effect (Fig. 1B). *G. pallida* depressed stem growth very slightly, root growth was suppressed to 35–25 %. On the other hand, *G. langdonii* slowed down stem growth to ≈25 % of control and almost completely inhibited root formation, exceeding the detrimental effects of *Fusarium*.

Ophiostoma fungi (Fig. 1C) influenced more markedly root than stem length. The strongest inhibitory effect was observed with *O. grandicarpum*, where the root length reached only ≈25 % and maximum stem length was 82 % of control. However, the plants remained alive until the end of the experiment. Isolates of *O. piceae*, although less inhibitory for elongation growth than *O. grandicarpum*, destroyed the plants after 18 and 25 d. *O. querci* reduced the maximum length of roots to 50 % and the maximum stem length was ≈88 % of the control; the plants collapsed after 25 d.

In our experimental setting, *Geosmithia* spp. showed plant inhibition potential comparable to that of *Ophiostoma* spp. and *F. solani*, especially on roots. The direct destructive effect of mycelial growth on plants may be increased by toxin production. In *Ophiostoma* spp., production of toxic metabolites was shown (Salemink *et al.* 1965; Takai and Richards 1978).

The decline of oaks is a complex process in which climatic changes, water stress, subcortical insects and fungi introduced by these vectors cooperate. The main inciting cause was found to be drought. The infestation of weakened trees with scolytids and introduction and often intensive growth of *Geosmithia* fungi that have phytopathogenic potential might worsen their condition.

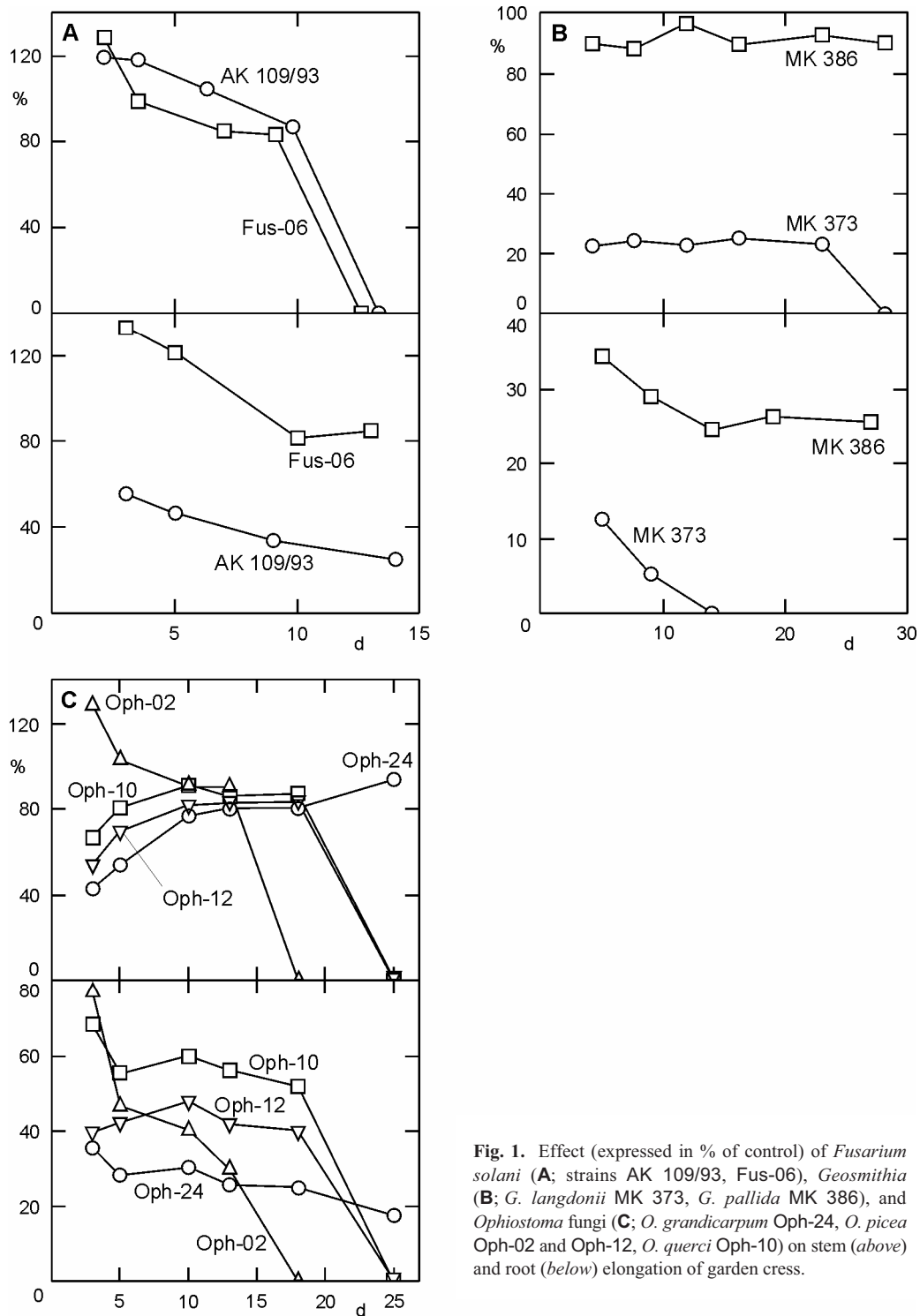


Fig. 1. Effect (expressed in % of control) of *Fusarium solani* (A; strains AK 109/93, Fus-06), *Geosmithia* (B; *G. langdonii* MK 373, *G. pallida* MK 386), and *Ophiostoma* fungi (C; *O. grandicarpum* Oph-24, *O. picea* Oph-02 and Oph-12, *O. querci* Oph-10) on stem (above) and root (below) elongation of garden cress.

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