SUPPRESSION OF EMERGENCE AND GROWTH OF GORSE (*ULEX EUROPAEUS*) SEEDLINGS BY *FUSARIUM TUMIDUM*

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ABSTRACT

The effect of *Fusarium tumidum*, a potential mycoherbicide, on gorse seedling emergence and growth was examined in three experiments. In Experiment 1, *F. tumidum*-inoculated wheat grains (one, two or three) were placed close to pre-germinated gorse seeds at sowing. Shoot and root dry weights of inoculated seedlings were lower than the control treatment at all three inoculum densities but there was no significant difference in percentage emergence. In Experiments 2 and 3, two inoculated wheat grains were placed in contact with the seedlings at sowing. Less than 50% of inoculated seedlings emerged compared with 97% in the control treatments from both experiments. Soon after emergence, about one-third of the inoculated seedlings that had emerged died from damping-off disease caused by *F. tumidum*. Shoot and root dry weights of inoculated seedlings were significantly lower than the control treatment. The results suggest that *F. tumidum* can suppress gorse seedling emergence and growth.

Keywords: *Fusarium tumidum*, *Ulex europaeus*, biological control, seedling emergence, mycotoxins, trichothecenes.

INTRODUCTION

Gorse (*Ulex europaeus* L.) is an invasive weed adversely affecting forest plantations, native vegetation and biodiversity. It is estimated to occupy about 1 million ha of the land area of New Zealand, with seed production of over 36,000 seeds/m^2/year (Rees & Hill 2001). The ability of the seeds to remain viable in the soil for many years greatly accounts for its persistence despite control measures.

Fusarium tumidum Sherb., is a potential mycoherbicide against gorse (Fröhlich et al. 2000). The young and soft tissues have been shown to be more susceptible to infection than the older and woody tissues (Morin et al. 1998, 2000). Hence, the seedlings may be the most susceptible phase of the weed to *Fusarium* infection. A related fungus, *F. oxysporum*, has been shown to suppress emergence of weeds such as sunflower broomrape (*Orobanche cumana*) and striga (*Striga hermonthica*) (Müller-Stöver et al. 2004; Yonli et al. 2004). Research on controlling gorse with *F. tumidum* has focused on aerial application of the inoculum (Fröhlich et al. 2000, Morin et al. 2000). Since the survival of gorse is largely dependent on its prolific seed production, control practices targeting seed proliferation and emergence, could minimise the spread of the weed. The objective of this study was to determine the effect of *F. tumidum* on gorse seedling emergence and growth.

MATERIALS AND METHODS

Wheat grain inoculum preparation

Fusarium tumidum isolate G34-34V, originally isolated from gorse plants (Morin et al. 1998), was used for this study. Wheat grains (100 g) were sterilised by auto-claving according to Sneh et al. (2004), inoculated with 5 ml *F. tumidum* conidial suspension (1 x 10^6 conidia/ml) and incubated for 14 days at 24:19°C day:night temperatures with 12 h photoperiod. During this period, the grains were shaken daily to prevent them from sticking together due to hyphal growth. To ensure that the wheat grains were colonised with the fungus, 60 grains were selected, placed on cornmeal agar (DIFCO laboratories, Detroit, MI) amended with 2 g/litre glucose (CMGA) and assessed for *F. tumidum* growth.

Experiment 1

Pre-germinated gorse seeds (2 days old) were sown in standard potting mix saturated with 4 mg metalaxyl/ml (Ridomil, CIBA-GEIGY Ltd, Basle) suspension (to inhibit *Pythium* spp.). Each seed was sown 2 cm deep without (control) or with one, two or three *F. tumidum* inoculated wheat grains placed about 1 cm from the seed. Each treatment consisted of three replicate pots, containing five gorse seeds per pot. The pots were incubated in a growth cabinet at 20°C, 70% humidity and 660 µmols/m^2/s light at 16 h photoperiod for 17 days, after which the seedlings were moved to a glasshouse (18°C, 55% humidity) for a further 32 days. The numbers of seedlings that emerged were counted daily between day 2 and day 16 and expressed as percentages. Seedlings were considered emerged when the first two true leaves had opened. Time taken for each treatment to attain 80% emergence was recorded. Root and shoot dry weights were determined at 49 days after sowing (DAS). Plant samples were cut just above the soil surface and the roots were washed in water to remove soil particles. The samples were oven dried at 70°C to a constant weight. Dead parts of inoculated plants were not included in dry weight determination. Samples of infected roots were sterilised in 1% sodium hypochlorite for 5 min, rinsed in sterile water and plated onto CMGA to determine *F. tumidum* infection.

Experiment 2

Each pre-germinated gorse seed was sown without or with two *F. tumidum* inoculated wheat grains, placed in contact with the seed. Each treatment consisted of three replicate pots, containing seven seeds per pot. The pots were incubated as described for Experiment 1. Seedling emergence was determined as described previously. Plant height, shoot and root dry weights were determined at day 49 (DM_1) and day 64 (DM_2). The growth rate (GR) was calculated from the DM using the Gardner et al. (1985) equation: $GR=(DM_2–DM_1)/(t_2–t_1)$, where $t_2–t_1$ is the time interval (i.e. 15 days) between two successive sampling periods.

Experiment 3

Each pre-germinated seed was sown without or in contact with two *F. tumidum* inoculated wheat grains, either in the standard potting mix or in 3x3 mm sieved potting mix. Each treatment consisted of six replicate pots, containing nine seeds per pot. Seedling emergence was determined over 28 days. Shoot dry weight was determined at 49 DAS.

Analysis

All experiments were set up as a completely randomised design. Emergence was angular transformed prior to analysis to stabilise the variance. Linear regression (Genstat) was used to determine the relationship between root and shoot dry weights for Experiment 2. All results were analysed by analysis of variance and mean separation was based on least significant difference (LSD) tests at $P<0.05$.

RESULTS

All inoculated wheat grains cultured on CMGA were colonised by *F. tumidum*. In Experiment 1, the number of *F. tumidum* colonised wheat grains (one, two or three) sown with the seedlings did not affect seedling emergence hence, the data were pooled

(Table 1). The control seedlings attained 80% emergence at 7 DAS while only 40% of inoculated seedlings had emerged (data not shown). Inoculated seedlings attained maximum emergence of 80% at 16 DAS while the control seedlings reached a maximum emergence of 93% at 7 days earlier. However, the effect of *F. tumidum* on final seedling emergence when inoculated grains were placed close to the seedlings at sowing was not significant. The root and shoot dry weights of the inoculated plants were 56 and 42% lower compared with the control treatment, respectively (Table 1). No difference was observed in the dry weight of the plants between the three different *F. tumidum* inoculum densities.

TABLE 1: Gorse seedling emergence at 11 days after sowing (%) and root and shoot dry weight (mg/plant) after inoculation with *F. tumidum* in Experiment 1. Values in parentheses are means after angular transformation of data for seedling emergence.

Treatment	Emergence (%)		Root dry weight	Shoot dry weight
Control seedlings	93.3	(81.1)	34.2	88.0
Inoculated seedlings	70.7	(57.3)	14.9	51.1
P value		(0.064)	0.002	0.024

In Experiment 2, the control seedlings attained 80% emergence at 6 DAS, while only 14% of the inoculated seedlings had emerged. The control treatment and inoculated seedlings attained maximum emergence of 100 and 43%, respectively over 16 days. At 11 DAS, seedling emergence of the inoculated treatment was reduced by 65% compared with the control treatment (Table 2). A few days after emergence, one-third of the inoculated seedlings that had emerged died from damping-off disease caused by *F. tumidum*. Thus, only 29% of the total inoculated seedlings survived to the final harvest at 64 DAS. *Fusarium tumidum* was isolated from root samples of inoculated seedlings. Although there was no visible symptom of tip dieback infection (associated with aerial

TABLE 2: Effect of *F. tumidum* inoculation on gorse seedling emergence at 11 days after sowing (%) and weight of roots and shoots (mg dry matter) and growth rate (mg dry matter/day) at 49 and 64 days after sowing in Experiment 2. Values in parentheses are means after angular transformation.

Treatment	Control	Inoculated	LSD (P<0.05)
Emergence (%)	95.2 (82.6)	33.4 (35.2)	(22.03)
Height (cm)			
49 days	6.1	2.4	0.91
64 days	11.7	4.4	3.32
Root dry weight (mg)			
49 days	47.8	13.0	27.19
64 days	66.7	20.0	24.49
Shoot dry weight (mg)			
49 days	140.0	26.7	52.35
64 days	247.0	63.0	85.80
Growth rate (mg DM/d)			
Root	1.3	0.5	2.22
Shoot	7.1	2.4	3.18

application of the fungus), inoculated plants were stunted, and reached only a third of the height of the control plants (Table 2). At 64 days, root and shoot dry weight and shoot growth rate of inoculated plants were reduced significantly by 70, 74 and 66%, respectively compared with the control treatment (Table 2). There was a strong correlation ($R^2 = 0.87$) between the root and shoot dry weights.

In Experiment 3, inoculated seedlings sown in sieved potting mix had lower emergence than those sown in standard potting mix (Fig. 1). Uninoculated seedlings had much greater emergence than inoculated seedlings and had similar emergence rates in sieved and standard potting mix (Fig. 1). At 49 days after sowing, shoot dry weight of inoculated seedlings sown in standard potting mix (52.5 mg/plant) was reduced by 55% compared to uninoculated seedlings (116.0 mg/plant), while the shoot dry weight of inoculated seedlings sown in sieved potting mix (14.2 mg/plant) was reduced by 87% compared to uninoculated seedlings (113.3 mg/plant) (LSD (P<0.05) = 31.07).

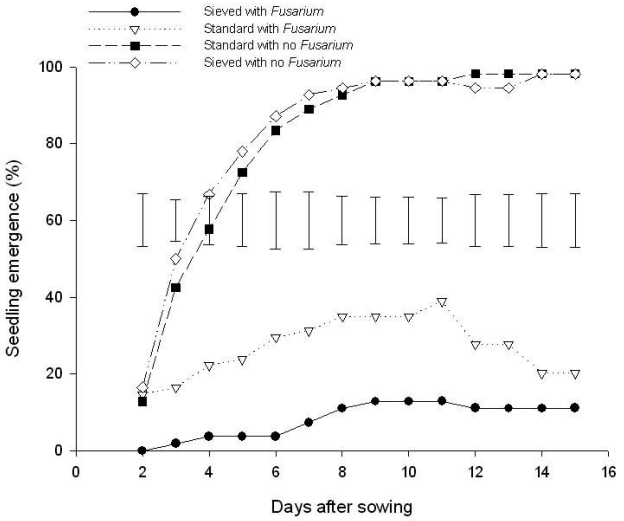

FIGURE 1: Seedling emergence (%) of pre-germinated gorse seeds sown with or without *Fusarium tumidum* inoculated wheat grains in sieved or standard potting mix in Experiment 3. Error bars represent LSD at P<0.05.

DISCUSSION

In all experiments, *F. tumidum* inoculation delayed and significantly reduced emergence of gorse seedlings and reduced both shoot and root dry weights. In Experiment 1, 80% of the inoculated seeds emerged 9 days after the uninoculated seeds had attained similar emergence. This delay of seedling emergence by *F. tumidum* is in accordance with reports by Yonli et al. (2004) and Müller-Stöver et al. (2004) for *F. oxysporum* isolates. Yonli et al. (2004) reported *F. oxysporum* treatment delayed emergence of *S. hermonthica* by the same number of days observed in Experiment 1. However, the reduction in total emergence was only significant when the inoculum was placed in contact with the seedlings. This may indicate poor growth of the pathogen in soil. *Fusarium tumidum* is a foliar pathogen (Broadhurst & Johnston 1994) and therefore, its growth may be restricted when applied as a soil inoculant. However, the fungus was capable of colonising nearby roots of gorse seedlings, as *F. tumidum* was

consistently isolated from root samples from treated seedlings. A better distribution of the pathogen in soil could probably be achieved by using smaller inoculated grain size, as Müller-Stöver et al. (2004) reported better control of *O. cumana* from formulations of *F. oxysporum* on smaller granules than larger ones. In addition, a split application of the inoculum may be more effective than a single application to ensure that the inoculum remains viable in the soil long enough to infect new roots of the weed. The fact that the total emergence and root/shoot biomass of inoculated seedlings were not affected by the inoculum density indicates that a single *F. tumidum* colonised wheat grain provides sufficient inoculum to infect the seedling.

Type A trichothecenes (neosolaniol and T-2 toxin) produced by *F. tumidum* (Mule et al. 1997; Morin et al. 2000) may be involved in delaying and reducing emergence of the seedlings and reducing the root/shoot biomass. Poor drainage (due to fine soil texture) could lead to higher accumulation of these toxins in the sieved potting mix, which resulted in higher seedling mortality than those sown in standard potting mix. Further research is needed to determine whether trichothecenes have a direct bioherbicidal effect on gorse, since Morin et al. (2000) found no correlation between levels of T-2 tetraol produced and pathogenicity of different *F. tumidum* isolates. A recent study has shown that trichothecenes exacerbate the spread of disease after infection has been initiated by a pathogen (Wang et al. 2006). Other workers (Kroschel & Elzein 2004) have reported a reduction in the emergence of *Striga* spp. by the mycotoxin fumonisin B_1 produced by *Fusarium nygamai*.

Fusarium tumidum restricted root and shoot growth rates of inoculated seedlings, which attained only a third of the height of that of the control treatment. A strong correlation between root and shoot dry weight indicates that root infection resulted in poor shoot growth. Applying inoculum to the soil should have a direct impact on the root system and reduce the number of seedlings that emerge and grow from the seed bank annually. Use of this control method would be more beneficial in areas with large number of gorse seeds in the seed bank or after fire disturbance. This is because fire helps to break the dormancy of the seeds, resulting in the emergence of many seedlings. However, more work is needed to assess the effect of this soil application method on roots of mature gorse plants.

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REFERENCES

Broadhurst PG, Johnston PR 1994. *Gibbererella tumida* sp. Nov-teleomorph of *Fusarium tumidum* from gorse in New Zealand. Mycology Research 98: 729-732.

Fröhlich J, Zabkiewicz JA, Gianotti AF, Ray JW, Vanner AL, Liu ZQ, Gous S 2000. Field evaluation of *Fusarium tumidum* as a bioherbicide against gorse and broom. New Zealand Plant Protection 53: 59-65.

Gardner FP, Pearce RB, Mitchell RL 1985. Growth and development. In: Gardner FP, Pearce RB, Mitchell RL ed. Physiology of crop plants. Iowa State University Press, Iowa, USA. Pp. 187-208.

Kroschel J, Elzein A 2004. Bioherbicidal effect of Fumonisin B_1, a phytotoxic metabolite naturally produced by *Fusarium nygamai*, on parasitic weeds of the genus *Striga*. Biocontrol Science and Technology 14(2): 117-128.

Morin L, Gianotti AF, Barker R, Johnston PR 1998. Favourable conditions for the bioherbicide candidate *Fusarium tumidum* to infect and cause severe disease on gorse *Ulex europaeus* in a controlled environment. Biocontrol Science and Technology 8: 301-311.

Morin L, Gianotti AF, Lauren DL 2000. Trichothecene production and pathogenicity of *Fusarium tumidum*, a candidate bioherbicide for gorse and broom in New Zealand. Mycological Research 104: 993-999.

Mule G, Logrieco A, Stea G, Bottalico A 1997. Clustering of Trichothecene-producing *Fusarium* strains determined from 28S Ribosomal DNA sequences. Applied and Environmental Microbiology 63: 1843-1846.

Müller-Stöver D, Thomas H, Sauerborn J, Kroschel J 2004. Two granular formulations of *Fusarium oxysporum* f.sp. *orthoceras* to mitigate sunflower broomrape *Orobanche cumana*. BioControl 49: 595-602.

Rees M, Hill RL 2001. Large-scale disturbances, biological control and the dynamics of gorse populations. Applied Ecology 38: 364-377.

Sneh B, Yamoah E, Stewart A 2004. Hypovirulent *Rhizoctonia* spp. isolates from New Zealand soils protect radish seedlings against damping-off caused by *R. solani*. New Zealand Plant Protection 57: 54-58.

Wang H, Hwang SF, Eudes F, Chang KF, Howard RJ, Turnbull GD 2006. Trichothecenes and aggressiveness of *Fusarium graminearum* causing seedling blight and root rot in cereals. Plant Pathology 55: 224-230.

Yonli D, Traore H, Hess DE, Abbasher AA, Boussim IJ 2004. Effect of growth medium and method of application of *Fusarium oxysporum* on infestation of sorghum by *Striga hermonthica* in Burkina Faso. Biocontrol Science and Technology 14(4): 417-421.