
CHAPTER THREE

Factors influencing pathogenicity of *Fusarium tumidum* on gorse

3.1 SUMMARY

Factors promoting pathogenicity of *Fusarium tumidum* Sherb., were determined as part of an overall objective of using insects as vectors of *F. tumidum* conidia for biological control of gorse (*Ulex europaeus* L.). Maximum infection was caused with inoculum concentration of 10^6 conidia/mL. A minimum number of approximately 900 viable conidia/infection site were required to form lesions in the leaves, indicative of low pathogenicity against gorse. The flowers and leaves were more susceptible to infection than the pods, spines and stem.

To determine if wounding enhances infection, gorse plants aged 4, 8, 16 and 32 wk were wounded and inoculated with a 10^6 conidia/mL suspension of *F. tumidum*. All the 4 wk old wounded plants died while 83% of the non wounded plants died from *F. tumidum* infection ($P > 0.05$). Eight and 16 wk old plants which were wounded and inoculated had significantly greater infection, were shorter in height, produced less biomass ($P < 0.05$) and developed more tip dieback compared with non wounded plants treated with the fungus. Eighty per cent of wounded inoculated 32 wk old plants developed tip dieback compared with only 32% of non wounded 32 wk old plants from two experiments. Wounding of gorse tissues may release nutrients that enhance conidia germination, germ tube growth and provide easier access for penetration by the pathogen as it normally enters only through the stomata as indicated by scanning electron micrographs. Conidial germination (93%) and germ tube length (407 μm) were significantly greater when incubated in 0.2% gorse extract solution for 24 h than in water (62% germination, germ tube length 42 μm). Inoculation of gorse plants with *F. tumidum* conidial suspension supplemented with 0.2% gorse extract resulted in similar shoot dry weight reduction ($P =$

0.012) as wounded and inoculated plants. To determine the number of infection sites required to cause plant mortality, 10 wk old plants were sprayed to cover 0, 25, 50, 75 and 100% of the plant's surface with a *F. tumidum* suspension of 10^6 conidia/mL. Only the 100% spray cover caused plant mortality. The percentage of plant surface inoculated correlated strongly ($R^2 = 99.3\%$) with severity of infection. At least 50% of the plant was required to be inoculated for a significant reduction in shoot dry weight. The results indicate that wounding of gorse plants facilitates significant infection. However, supplementing *F. tumidum* suspension with gorse extract at the appropriate concentration could provide a similar effect on gorse as wounding.

3.2 INTRODUCTION

Gorse (*Ulex europaeus* L.) is a major weed adversely affecting native vegetation, biodiversity and productivity of forest plantations in New Zealand. It is estimated to occupy about 1 million ha with seed production of over 36,000 seeds/m²/year (Rees and Hill, 2001). It is found on a wide variety of terrain including hill slopes, agricultural areas, coastland, disturbed areas and forests. In pastures and forest plantations, it can reduce establishment and production of desirable plant species by competing for light, nutrients and water. Due to its thorny and impenetrable characteristics, useful pasture plants growing under or beside gorse are often avoided by grazing livestock, thus reducing the utilisation of pasture plants (Matthews *et al.*, 1999). In 1984, the loss in production to farming was estimated at about \$22 million per annum and the cost of controlling this weed was estimated at approximately \$27 million per annum to the forestry and farming industries (Monsanto, 1984).

In plantation forestry, herbicides and fire are the main tools used against this weed (Rees and Hill, 2001). Seed and foliage feeding insect species such as *Apion ulicis*, *Cydia ulicetana* and *Sericothrips staphylinus* have also been introduced for classical biological control (Hill *et al.*, 2000; Syrett *et al.*, 1999). These insects acting alone, to date, have not significantly reduced the population of gorse (Hill *et al.*, 2000; Syrett *et al.*, 1999).

Studies of foliar pathogens naturally found on gorse in New Zealand have shown *Fusarium tumidum* Sherb., has potential to be developed as a mycoherbicide for gorse (Johnston *et al.*, 1995; Morin *et al.*, 1998; 2000). To date, no study has investigated the combined effect of insects and pathogens against gorse, using the insects as vectors of the pathogens.

Control of gorse using insect-vectored *F. tumidum* requires information on the conditions favouring *F. tumidum* pathogenicity especially, against mature gorse plants. The seedlings however, are very susceptible to *F. tumidum* infection (Morin *et al.*, 1998). Since gorse can grow for up to 30 years (Lee *et al.*, 1986), it is important to find conditions under which mature plants could succumb to *F. tumidum* infection. One of the methods to achieve this may be through wounding of the plant tissues. Wounding provides easier access for fungi and provides the nutrients for germ tube growth (Hale and Griffin, 1976; Klein and Auld, 1996). The feeding or oviposition of some gorse-associated insect species such as *A. ulicis*, *C. ulicetana* *S. staphylinus* and *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) may provide wound sites for *F. tumidum*. Insect feeding (Lepidoptera larvae), oviposition and artificial wounding of *Cirsium arvense* (Californian thistle) provided wound sites for the rust fungus *Puccinia punctiformis* (Kluth *et al.*, 2001) and enhanced infection caused by the fungal pathogen *Sclerotinia sclerotiorum* (Dillard and Cobb, 1995). There is no report on susceptibility of injured gorse tissues to *F. tumidum* infection.

Potential limiting factors to the success of this strategy of mycoherbicide delivery may be the number of *F. tumidum* conidia required to infect and kill the weed. In addition, the *F. tumidum* conidia are large (Broadhurst and Johnston, 1994) and this may limit the number the insects will carry. Reports indicate that *Fusarium* blight of gorse generally increases with pathogen inoculum density and the volume of application (Fröhlich *et al.*, 2000; Morin *et al.*, 1998). In order to use insects as vectors of *F. tumidum*, it is essential to determine the minimum number of conidia required for infection. This will provide information about the number of insects required to facilitate successful *Fusarium* infection.

In this chapter, 12 experiments are reported that were carried out to determine the factors that would enhance pathogenicity of *F. tumidum* on gorse. The optimum inoculum concentration, minimum number of *F. tumidum* conidia required for infection and the number of infection sites required for plant mortality were determined. The effect of wounding of gorse plants and the susceptibility of the various morphological structures of gorse to infection were also determined. The effect of exogenous nutrients on *F. tumidum* conidia germination, germ tube elongation and pathogenicity against mature gorse plants were also evaluated.

3.3 MATERIALS AND METHODS

3.3.1 Seed germination and growth

Gorse seeds were collected from Auckland (Latitude 36° 51' S, Longitude 174° 46' E) and three sites (Gebbies Pass, Springston and Tai Tapu) in Canterbury (Latitude 43° 00' S, Longitude 172° 42' E) in New Zealand. The seeds were germinated according to Morin *et al.* (1998) with slight modifications. Seeds were surface sterilised in 5% sodium hypochlorite (NaOCl) for 5 min, rinsed in tap water, scarified between two layers of sand paper and soaked in distilled water for 24 h. They were then placed on moistened paper towels in trays, covered with plastic bags to maintain high humidity and incubated at 16°C, 8 h photoperiod for about 1 wk until germination has occurred. During this period, the paper towels were kept moist using sterile distilled water to prevent desiccation of the seeds. Germinated seeds were sown in a standard potting mix and kept under a controlled environment cabinet at 25:16°C day: night, 16-h photoperiod. Three weeks after germination, the seedlings were planted into larger plastic pots (1.0 or 1.4 L) at one seedling per pot and kept in a greenhouse for all subsequent experiments. Watering was done on a regular basis and weeds were uprooted from the pots by hand.

3.3.2 *F. tumidum* source and maintenance

F. tumidum isolates G34-34M, G34-34T and G34-34V, provided by Landcare Research, Auckland were used for Experiment 1. For all other experiments in this chapter, G34-34V was used. These isolates were recovered from gorse growing near the highway between Katikati and Tauranga, Bay of Plenty, New Zealand. Inoculum for the experiments was prepared by transferring pieces of freeze-dried conidia and mycelium onto glucose cornmeal agar (GCMA) (Appendix 3.1) or Fleming's oatmeal agar (FOMA) (Appendix 3.2) in Petri plates. The plates were incubated under natural light supplemented with white and blue light at $23:18 \pm 2^\circ\text{C}$ day: night, 16-h photoperiod for 14 days. Conidia produced were stored in 30% sterilised glycerol at -80°C (Appendix 3.3) and routinely used for mass inoculum production by transferring a few conidia onto FOMA plates and incubating the plates under similar conditions as stated previously.

Spore production on FOMA plates was about 10 times that produced on GCMA and was therefore used for spore production in the experiments. Conidia were harvested by flooding each plate with 15 mL of sterile distilled water amended with 0.01% Tween 80 (polyoxyethylene sorbitan monooleate) and dislodging the conidia with a sterile glass rod. The resulting suspensions were then filtered through a layer of sterile Miracloth (Calbiochem[®], EMD Biosciences, Inc., CA, USA) to remove mycelial fragments, quantified using a haemocytometer and adjusted to the required concentration. Respective concentrations of viable conidia were verified by plating 0.1mL of 10^2 and 10^3 conidia/mL onto GCMA and counting the number of *F. tumidum* colonies formed.

3.3.3 General procedures for inoculation of plants and data collection

In each of the 12 experiments in this chapter, all plants were sprayed to run-off with a suspension of *F. tumidum* conidia using a hand-sprayer. Control plants were sprayed with distilled water-Tween 80 solution. All plants were subjected to the following standard post-inoculation conditions (unless stated otherwise): plants were placed in a mist chamber at 20°C , 92% humidity for 24 h (Morin *et al.*, 1998) and then transferred to the glasshouse at $18-21^\circ\text{C}$. Disease severity on each plant was visually assessed by scoring

the amount of necrotic foliage using the following index: 1, no visible symptoms; 2, 1-25% necrosis; 3, 26-50% necrosis; 4, 51-75% necrosis and 5, 76-100% necrosis. In all experiments, lesioned tissue from infected plants were plated onto GCMA to confirm the cause of infection. Plant mortality, shoot height and dry weight (shoot and root) of the surviving plants were assessed at 4 wk after inoculation (WAI) (unless stated otherwise). Shoot height was measured from the soil level to the tip of the plant. The shoot dry weight was determined by cutting each stem just above the soil surface (discarding all necrotic portions unless stated otherwise) and oven drying it in a separate paper bags to a constant weight at 70°C. The roots were washed under running tap water to remove all soil particles before drying to a constant weight. All experiments were set up as a randomised complete block (RCBD).

3.3.4 Pathogenicity experiments

3.3.4.1 Experiment 1: *F. tumidum* isolate comparison

To determine the pathogenicity of three *F. tumidum* isolates against gorse, 8 wk-old plants were sprayed with a 10^5 conidia/mL suspension of *F. tumidum* isolates G34-34M, G34-34T, G34-34V or 10^6 conidia/mL of G34-34V and exposed to the standard post-inoculation conditions. The higher inoculum concentration was included for comparison with the 10^5 conidia/mL concentration. There were eight replicate plants per treatment. Disease severity was assessed twice a week over 4 wk using the disease severity index outlined in section 3.3.3.

3.3.4.2 Experiment 2: Susceptibility of gorse from four different sites

This experiment was conducted to assess the pathogenicity of *F. tumidum* against gorse plants sourced from four sites in New Zealand (Auckland, Gebbies Pass, Springston and Tai Tapu). Eight weeks old gorse plants grown from seeds obtained from each of the four locations were sprayed with 10^5 conidia/mL of G34-34V suspension produced as described in section 3.3.2 and exposed to the standard post-inoculation conditions. There were eight replicate plants per treatment. Disease severity was assessed at weekly interval

for 4 wk and the shoot dry weight of all surviving plants was determined. The number of plants with tip dieback symptoms was counted.

3.3.4.3 Experiment 3: Inoculum concentration

This experiment was conducted to determine the inoculum concentration of *F. tumidum* conidial suspension required to cause maximum infection of gorse plants. A conidial suspension of 10^6 conidia/mL was prepared as described in section 3.3.2. Five serial dilutions made of 10^1 , 10^2 , 10^3 , 10^4 and 10^5 conidia/mL. Respective concentrations of viable conidia were verified by dilution plating onto GCMA plates and counting the number of *F. tumidum* colonies formed after 2 days of incubation as previously described.

Plants aged 16 wk were sprayed with each inoculum concentration and exposed to the standard post-inoculation conditions. Six replicates per treatment were used. Disease severity was assessed weekly for 4 wk. The number of plants showing tip dieback symptoms and plant mortality was counted at 4 WAI. Root and shoot dry weight (DM) were determined at 4 and 6 WAI (three plants harvested at each sampling). Both dead and living plant tissues were included in dry weight determination. The shoot and root growth rate (GR) were 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. 14 days) between two successive sampling periods. This experiment was also conducted in separate experiments using 12 and 8 wk old gorse plants with *F. tumidum* suspensions of 10^4 , 10^5 and 10^6 conidia/mL (12 wk old) and 10^5 and 10^6 conidia/mL (8 wk old) with eight replicate plants.

3.3.4.4 Experiment 4: Infection sites required for plant mortality

Gorse plants (10 wk old) were sprayed to cover 0, 25, 50, 75 or 100% of the plant surface (beginning from the top of the plant) with a *F. tumidum* suspension of 10^6 conidia/mL and exposed to the standard post-inoculation conditions. There were six replicate plants for each treatment. Disease severity was scored every other day for 14 days. Plant mortality,

height and shoot dry weight were determined as outlined in the general procedures (section 3.3.3).

3.3.4.5 Experiments 5, 6 & 7: Minimum number of conidia required for infection

Three experiments were conducted to determine the minimum number of *F. tumidum* conidia required to create a lesion on a gorse leaf. In Experiment 5, 10 µL aliquot of conidial suspensions containing 12,000, 1,200, 120, 60, 12 and 0 *F. tumidum* conidia (prepared as described in section 3.3.2) were deposited on the upper surfaces of ten of the leaflets of each of eight replicate 12 wk old potted plants. The germination percentage of the spores was determined by plating 0.1 mL of the lower dilutions on GCMA plates and counting the number of conidia germinating after 2 days incubation. The plants were exposed to the standard post-inoculation conditions for 48 h and then transferred to a glasshouse. The lesions per plant were counted and their diameters were measured at 1 WAI.

In Experiment 6, *F. tumidum* conidial suspensions containing 1,200, 800, 400, 200 and 0 conidia/10 µL were used for inoculating 12 wk old gorse plants as in Experiment 5. There were 15 replicate plants for each of the five treatments. A 10 µL of each suspension was applied to five randomly selected leaves on each plant including the top leaf (i.e. apical meristem). The plants were exposed to the standard post-inoculation conditions for 48 h and then transferred to a glasshouse. The germination percentage of the inoculum and the number of lesions/plant were assessed as previously described.

In Experiment 7, *F. tumidum* conidial suspensions containing 1,500, 900, 600, 300 and 0 conidia/10 µL were amended with 5% Triton X-100 (Rohm and Haas Co., Philadelphia, USA). There were 10 replicate plants per treatment. Ten microlitres of each suspension was applied to five randomly selected leaves on each plant including the top leaf. The plants were exposed to the standard post-inoculation conditions for 48 h and then transferred to a glasshouse. The number of lesions/plant and the percentage of plants which developed tip dieback were assessed at 1 WAI.

3.3.4.6 Experiment 8: Susceptibility of gorse structures to infection

This experiment assessed the susceptibility of five different morphological structures of gorse to *F. tumidum* infection. Detached portions of gorse leaves, spines, stems (from 16 wk old plants), flowers and pods (from older plants obtained from the field) in three replicates were assessed. The samples were either sprayed with 10^6 conidia/mL of *F. tumidum* suspension or water-Tween 80 as control treatments. Each sample was placed on a Petri plate and incubated inside a plastic container containing moist tissue paper to maintain high humidity. Incubation was at 23:19°C day: night, 16-h photoperiod for 2 wk. Disease severity was assessed one and two weeks after treatment.

3.3.4.7 Experiment 9: Effect of nutrient on conidial germination and germ tube elongation

The objectives of this experiment were to determine whether exogenous nutrients enhance conidial germination and germ tube growth of *F. tumidum*. Additionally, penetration of the leaf by the germ tube and hyphal growth were observed by a scanning electron microscope (SEM).

A conidial suspension of *F. tumidum* was prepared (as described in section 3.3.2), centrifuged at $670 \times g$ for 3 min and the supernatant discarded. Sterile water with 0.01% Tween 80 was added and vortex briefly to re-suspend the conidia. This was repeated twice to wash off traces of nutrients acquired from the agar (FOMA) used for culturing. The resulting pellets were re-suspended in sterile water, vortexed and the concentration was adjusted to 5×10^3 conidia/mL based on haemocytometer counts. Aliquots of 1 mL of the suspension was transferred into sterile 1.5 mL tubes and centrifuged at $8,000 \times g$ for 2 min. The supernatant was discarded and the pellets were suspended in 1 mL of either 0.2% (w/v) of gorse extract solution, sterile water (control), 0.05, 0.1 or 0.2% (w/v) glucose solution. The gorse extract was prepared by grinding 0.2 g of gorse shoot sample (which had been surface sterilised in 1% NaOCl for 90 s, and rinsed twice in sterile distilled water) in a sterile mortar with pestle. The ground sample was mixed with 100 mL of sterile water and filtered through sterile Miracloth. Aliquots (10 μ L) of each treatment were applied to three wells on each of ten microscope slides. A sixth treatment

consisted of placing 10 μL samples of the conidial suspension in sterile water on detached gorse leaves at one sample per leaf. The leaves were then placed on slides, three leaves per slide. Each of the ten slides was placed on double 9 cm Whatman's filter paper saturated with 5 mL sterile water in a Petri dish and incubated at 23:19°C day: night, 12 h photoperiod. Conidial germination was determined after 24 h from 120 conidia from each replicate by counting 40 conidia from each of the three wells or leaves under a light microscope. The length of the germ tubes were measured using a DP12 digital camera system connected to a light microscope (BX51, Olympus New Zealand Ltd, Auckland, New Zealand) connected to the computer driven software AnalySIS[®] (Soft Imaging System GmbH, Münster, Germany). The germ tube lengths of 40 to 45 germinated conidia from each replicate were measured.

To determine *F. tumidum* germ tube penetration of the leaf tissues and whether wounding releases nutrients for hyphal growth, detached gorse leaves were either pierced with a sterile needle (five holes per leaf) or left unpierced and then inoculated with 10 μL of 5×10^3 conidia/mL of *F. tumidum* suspension. Five replicate leaves of each treatment were incubated for 48 h as stated previously; air dried in a laminar flow hood and examined using a scanning electron microscope as described in section 2.3.11.

3.3.4.8 Experiments 10 & 11: Effect of wounding gorse plants

Two experiments were conducted to determine whether wounding of gorse plants would enhance infection caused by *F. tumidum*.

In Experiment 10, the shoots of 4, 8, 16 and 32 wk old gorse plants were either wounded or not. Wounding was done over 40% of the shoot surface using sharp sterile needles. The plants were either sprayed with *F. tumidum* suspension of 10^6 conidia/mL immediately after wounding (to avoid callus formation before inoculum was applied) or with water-Tween 80. The plants were exposed to the standard post-inoculation conditions for 48 h and then transferred to a glasshouse. The experiment was a randomised complete block design with a 2 x 2 factorial treatment structure (wounding/non wounding and inoculated/uninoculated) replicated six times. Thus, each

age group consisted of 24 plants. Disease severity, plant mortality and tip dieback infection were assessed at 2 WAI since maximum infection was observed in the previous experiment to occur at this time. Shoot dry weights of all surviving plants were determined at 5 WAI.

In Experiment 11, plants aged 8, 16 and 32 wk were used to repeat Experiment 10. The 4 wk old plants were excluded because the pathogen did not require wounding to infect the young plants significantly. The experimental design was the same as Experiment 10 with 13 replicate plants per treatment. Wounding and inoculation of the plants were done as described in Experiment 10. Disease severity was assessed every other day for 2 wk for all plant ages and then at weekly intervals for a further 3 wk for the 16 wk old plants. Each age group was treated and assessed separately. Shoot and root dry weights and height of surviving plants were determined as in Experiment 10. Total plant dry weight was determined from the sum of shoot and root dry weights.

3.3.4.9 Experiment 12: Effect of gorse extract on infection

The objective of this experiment was to determine whether any changes in lesion development with wounding are due to nutrients released from the damaged tissues, the physical damage, or both. The treatments were untreated plants (control), plants sprayed to run off with a 1.3×10^6 conidia/mL of *F. tumidum* suspension, plants wounded and sprayed with the suspension of conidia as previously described and plants sprayed with the suspension of conidia supplemented with 0.2% gorse extract. The gorse extract was prepared as described in section 3.3.4.7. Each treatment consisted of 10 replicates of 24 wk old gorse plants. After inoculation, the plants were placed in a growth cabinet at 23°C, 92% humidity for 48 h and then transferred to a glasshouse. Disease severity was assessed using a modified disease-rating system. Plants showing no visible symptoms were rated 1, necrosis of 1-10% was scored as 2; 11-20% = 3, 21-30% = 4, 31-40% = 5, 41-50% = 6, 51-75% = 7, 76-100% = 8. Shoot dry weight of all surviving plants was determined at 4 WAI.

3.3.5 Statistical analyses

The effect of treatment on disease severity, height and dry weight of surviving plants, conidial germination, and germ tube length, were assessed using analysis of variance (ANOVA) (Snedecor and Cochran, 1980). To satisfy the assumptions of normal distribution, logarithmic transformation was applied to the dry weight and height data before analysis. All data for experiments where the treatments caused severe infection or plant mortality, were $\log_{10}(x+1)$ transformed to cope with decimal and zero figures. Conidial germination was angular transformed prior to analysis to stabilise the variance. Disease severity data for all uninoculated (control) plants were excluded from analysis since none of the control plants were infected and therefore were assigned a disease score of 1. Treatment effect on plant mortality or tip dieback was determined by percentages over the total number of plants. Linear regression was used to determine the relationship between root and shoot dry weights and between the surface area of gorse plants inoculated and maximum disease scores. Mean separation was based on Fisher's protected least significant difference (LSD) tests at $P < 0.05$.

3.4 RESULTS

3.4.1 Experiment 1: Pathogenicity of *F. tumidum* isolates

This study showed no significant differences ($P = 0.311$) in pathogenicity among the three isolates of *F. tumidum* applied at 10^5 conidia/mL and assessed at period of maximum infection. Maximum infection for all treatments occurred at about 14 days after inoculation (DAI) (Fig. 3.1). The higher inoculum concentration (10^6 conidia/mL), caused significantly more infection (DSS = 4.9) compared with all strains applied at 10^5 conidia/mL and 50% plant mortality but none of the isolates applied at the sub-optimal concentration (10^5 conidia/mL) caused any plant death. The decline in disease score was due to regeneration of new lateral shoots by the plants. G34-34V was selected for all subsequent experiments because it sporulated more abundantly than the other two isolates (G34-34M and G34-34T). Spore production of this isolate was further enhanced (10 fold increase) by culturing on Fleming's oatmeal agar compared with glucose cornmeal agar which has been used for previous studies (Morin *et al.*, 1998). Sealing the Petri plates with clear polyethylene film (Glad[®] Wrap; Oakland, CA) reduced spore production indicating lack of gaseous exchange may limit spore production.

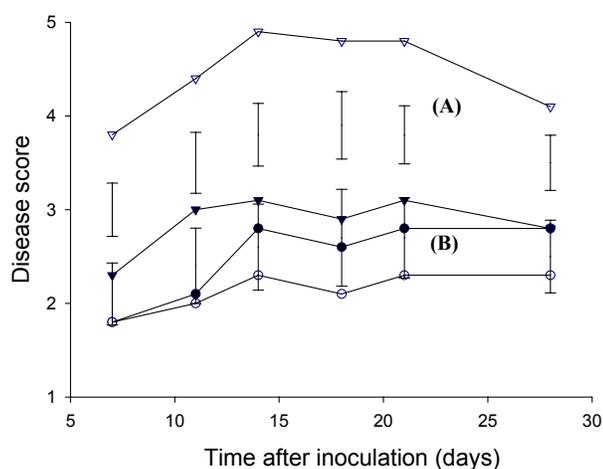


Figure 3.1 Disease score (1-5 scale) of gorse plants in Experiment 1 inoculated at 8 wk old with three isolates of *F. tumidum* [G34-34V (●), G34-34M (○), G34-34T (▼)] applied at 10^5 conidia/mL and G34-34V (▽) applied at 10^6 conidia/mL. Mean of eight plants. Bars show LSD involving (A) all four treatments; (B) only 10^5 conidia/mL treatments.

3.4.2 Experiment 2: Susceptibility of gorse from four different sites

Gorse seedlings from Auckland, Gebbies Pass, Springston and Tai Tapu showed no significant difference in their susceptibility to *F. tumidum* infection and were highly susceptible to the pathogen with a mean disease score of 3.4 (Table 3.1). In this experiment, a sub-optimal inoculum concentration was used to better differentiate any differences in susceptibility between seed sources. The shoot dry weight of the inoculated plants did not vary significantly among the four locations the seeds were sourced from. Seedlings from Springston and Auckland, respectively recorded the lowest (50%) and highest (83%) tip dieback infection.

Table 3.1. Disease score (1-5 scale) at 2 wk after inoculation and shoot dry weight of gorse sourced from four locations in New Zealand and grown in pots after inoculation with 10^5 conidia/L of *F. tumidum* in Experiment 2. Values in parentheses are back-transformed means after \log_{10} transformation, n = 6.

Location	Disease score	Shoot dry weight (g/plant) ^a	
Auckland	3.2	(0.32)	0.119
Gebbies Pass	3.2	(0.25)	0.094
Springston	3.5	(0.30)	0.113
Tai Tapu	3.5	(0.24)	0.093
LSD ($P < 0.05$) ^b	0.86		0.0327

^a Data were $\log_{10}(x + 1)$ transformed prior to analysis by ANOVA.

^b LSD = Least significant difference calculated from ANOVA.

3.4.3 Experiment 3: Inoculum concentration

Disease severity

Disease severity increased with increasing inoculum concentration for all plant ages assessed but decreased with increasing plant age. Uninoculated control plants were not infected and had disease score of 1. Disease severity of 16 wk old plants inoculated at 10^6 conidia/mL peaked at 3.3 at 14 DAI with a predicted concentration for maximum infection at $10^{7.4}$ conidia/mL (Fig. 3.2). The younger plants (8 and 12 wk old) attained maximum disease score of approximately 5 with 10^6 conidia/mL (Fig. 3.3A). At 10^5 conidia/mL, disease levels peaked at 2.8 for the 8 wk old plants (Fig. 3.3B) and at 2.5 for the 16 wk old plants. Infected plants showed symptoms 2-3 DAI which progressed up to

14 DAI and declined thereafter as a result of the plants regenerating new lateral shoots (except when the infection resulted in plant mortality).

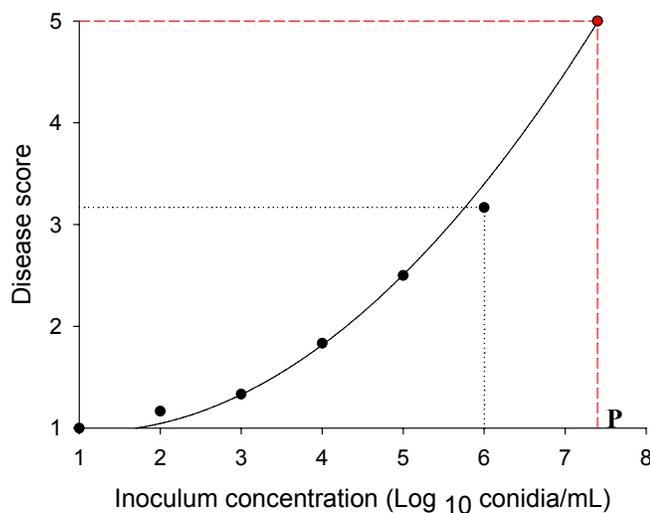


Figure 3.2. Relationship between *F. tumidum* inoculum concentration and disease score (1-5 scale) of 16 wk old gorse plants assessed at 14 days after inoculation in Experiment 3. The data points represent the means of six replicate plants. Regression equation: $Y=1.14-0.21x+0.10x^2$ ($R^2=99.8\%$), where Y = disease score and x = (\log_{10} of inoculum concentration). P: predicted concentration ($10^{7.4}$ conidia/mL) for maximum disease.

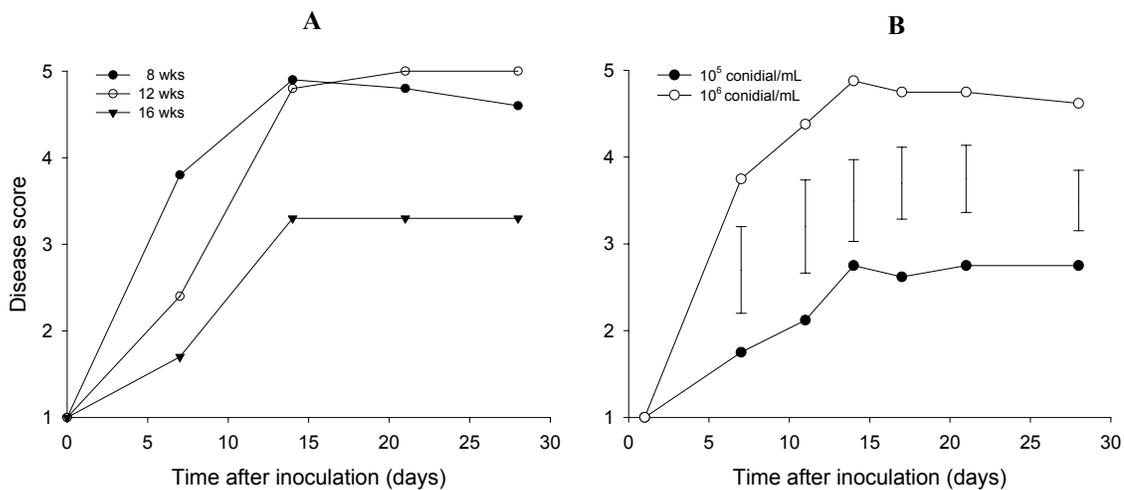


Figure 3.3. Disease score (1-5 scale) of gorse plants aged 8, 12 and 16 wk inoculated with 10^6 conidia/mL of *F. tumidum* (A) and 8 wk old plants inoculated with 10^5 or 10^6 conidia/mL (B) in Experiment 3. Bars indicate LSD at $P < 0.05$ at each assessment time.

Tip dieback and plant mortality

At the highest inoculum concentration (10^6 conidia/mL), mortality was greatest (50%) in the 8 wk old gorse (Fig. 3.4) followed by 40% mortality of the 12 wk old plant. Although none of the plants older than 12 wk were killed, 83% of the 16 wk old plants developed tip dieback (Fig. 3.5). All the 8 and 12 wk old plants treated with 10^6 conidia/mL developed tip dieback. Inoculum concentration of 10^5 conidia/mL caused a relatively low mortality (13%) of only the youngest plants (8 wk old). However, even at this lower inoculum concentration, 100% (8 wk), 60% (12 wk) and 67% (16 wk) of treated plants developed tip dieback. At concentration of 10^4 conidia/mL, no plant death occurred.



Figure 3.4. Gorse plants inoculated at 8 wk old, killed by *F. tumidum* compared with the untreated control plants at 14 days after inoculation.



Figure 3.5. Gorse plants inoculated at 16 wk old, showing tip dieback (arrowed) at 14 days after inoculation with *F. tumidum*.

Dry weights and growth rates

There were no significant differences between untreated and treated plants in the shoot and root dry weight of the 16 wk old plants harvested at 4 or 6 WAI. Consequently, the shoot growth rates of the 16 wk old plants inoculated with 10^6 conidia/mL (20.3 mgDM/d) and the untreated plants (55.7 mgDM/d) did not vary significantly ($P = 0.695$). The inoculum concentration that caused maximum reduction in shoot and root dry weights of the 12 and 8 wk old gorse plants was 10^6 conidia/mL (Tables 3.2 & 3.3, respectively). Twelve weeks old plants inoculated at 10^6 conidia/mL, produced only 5.9 mg above ground dry matter per day compared with 29.1 mgDM/d produced by the uninoculated plants (Table 3.2). This represented a reduction of 79.7% ($P = 0.071$). The shoot dry weights of 12 and 8 wk old plants were reduced by 56 and 69%, respectively compared with the control treatment at 6 WAI with the 10^6 conidia/mL. The shoot and root dry weights correlated positively for the 12 wk ($R^2 = 77\%$) and the 16 wk ($R^2 = 81\%$) old plants. The root growth rate of the 8 and 12 wk old gorse plants were not affected by any of the inoculum concentrations applied.

Table 3.2. Shoot and root dry weight (g/plant) of gorse plants treated at 12 wk old at 4 and 6 wk after inoculation with different concentrations of *F. tumidum* conidial suspension in Experiment 3. Values in parentheses are back-transformed means after \log_{10} transformation.

Inoculum concentration (conidia/mL)	Dry weight (g/plant) ^a				Growth rate (mgDM/d)
	4 wk		6 wk		
Shoot					
Control	(0.45)	0.160 b ^z	(0.85)	0.267 c	29.1 b
10^4	(0.41)	0.150 b	(0.60)	0.203 b	13.3 ab
10^5	(0.40)	0.145 b	(0.50)	0.175 ab	7.3 a
10^6	(0.29)	0.109 a	(0.37)	0.136 a	5.9 a
LSD ($P < 0.05$)		0.0333		0.0595	18.50
Root					
Control	(0.16)	0.063 b	0.25 c	0.096 b	6.6
10^4	(0.13)	0.054 ab	0.19 b	0.075 a	3.9
10^5	(0.15)	0.060 b	0.18 b	0.071 a	2.0
10^6	(0.08)	0.035 a	0.13 a	0.055 a	3.6
LSD ($P < 0.05$)		0.0199		0.0202	6.35

^a Data were $\log_{10}(x + 1)$ transformed prior to analysis by ANOVA.

^z Treatment means within columns followed by the same letter are not significantly different based on LSD tests at $P < 0.05$.

Table 3.3. Shoot and root dry weight (g/plant) of gorse plants treated at 8 wk old at 4 and 6 wk after inoculation with different concentrations of *F. tumidum* conidial suspension in Experiment 3. Values in parentheses are back-transformed means after \log_{10} transformation.

Inoculum concentration (conidia/mL)	Dry weight (g/plant) ^a				Growth rate (mgDM/d)
	4 wk		6 wk		
Shoot					
Control	(0.22)	0.087 c ^z	(0.26)	0.101 b	2.8
10 ⁵	(0.12)	0.048 b	(0.15)	0.061 a	2.4
10 ⁶	(0.07)	0.029 a	(0.08)	0.032 a	0.4
LSD ($P < 0.05$)		0.0180		0.0361	6.44
Root					
Control	(0.07)	0.030 b	(0.09)	0.036 c	1.1
10 ⁵	(0.04)	0.016 a	(0.04)	0.018 b	0.5
10 ⁶	(0.02)	0.009 a	(0.02)	0.008 a	-0.2
LSD ($P < 0.05$)		0.0085		0.0096	1.49

^{a, z} footnotes same as Table 3.2.

3.4.4 Experiment 4: Infection sites required for plant mortality

Disease severity

Application of *F. tumidum* inoculum to the entire plant surface (i.e. 100% coverage) caused the highest infection DSI of 4.6 (Fig. 3.6) while plants with only 25% coverage had the least infection (DSI = 2.2) ($P < 0.001$). The plants receiving 75 and 50% coverage had disease scores of 3.8 and 2.8, respectively. None of the untreated control plants were infected. There was a positive and linear relationship between the surface area of plant inoculated and the maximum disease score ($R^2 = 99.3\%$).

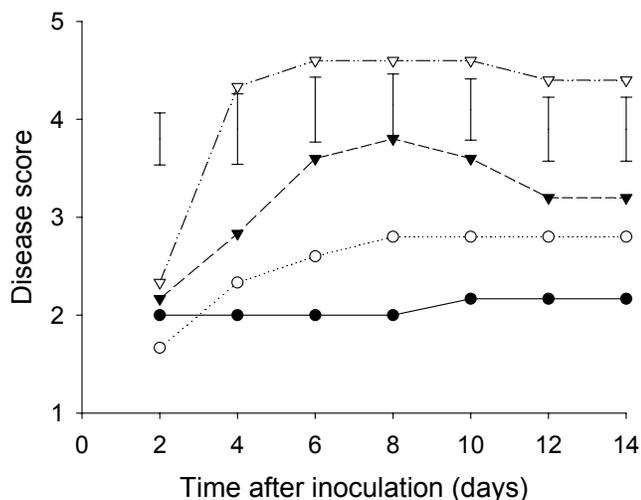


Figure 3.6. Disease score (1-5 scale) of 10 wk old gorse plants inoculated with 10^6 conidia/mL of *F. tumidum* to cover 25 (●), 50 (○), 75 (▼) and 100% (▽) of the plant's surface area in Experiment 4. Control treatments were sprayed with water-Tween 80 and were not infected. Mean of six replicate plants. Bars represent LSDs at $P < 0.05$.

Shoot height and dry weight

Applying *F. tumidum* inoculum to only 25% of the surface area of 10 wk old gorse significantly reduced the shoot height by 61% compared with the control treatment (Table 3.4). This reduction was comparable to the 73% reduction in shoot height obtained by 100% coverage. Inoculating the entire plant caused the most reduction in shoot dry weight (58%) compared with the control treatment. Fifty per cent coverage reduced the shoot dry weight by 45% and this was not significantly different from shoot dry weight of 100% coverage plants. Only the 100% coverage caused plant death (17%).

Table 3.4. Shoot height and dry weight of 10 wk old gorse sprayed with 10^6 conidia/mL of *F. tumidum* conidial suspension to cover 0, 25, 50, 75 and 100% of the plant surface area in Experiment 4. Values in parentheses are back-transformed means after \log_{10} transformation. Mean of six replicate plants.

Treatment	Number of <i>F. tumidum</i> conidia applied/plant (10^6) ^b	Shoot height (cm) ^a	Dry weight (g/plant) ^a
Control (0%)	0.0	(30.1) 1.504 c ^z	(1.30) 0.356 c
25 % coverage	0.5	(11.6) 1.099 ab	(0.91) 0.278 bc
50 % coverage	1.0	(15.4) 1.164 b	(0.71) 0.223 ab
75 % coverage	1.5	(11.1) 1.080 ab	(0.71) 0.230 ab
100 % coverage	2.0	(8.2) 0.849 a	(0.54) 0.174 a
LSD ($P < 0.05$)		0.2528	0.0986

^{a, z} footnotes same as Table 3.2.

^b The number of conidia applied/plant was estimated from the volume of 2.5 mL used to spray the 100 % coverage plants with the assumption that 0.5 mL was lost during spraying.

3.4.5 Experiment 5-7: Minimum number of conidia required for infection

In Experiment 5, the minimum number of *F. tumidum* conidia per infection site that created a lesion on gorse leaf (Fig. 3.7) was 1,200 (Table 3.5). The germination percentage of the conidia on agar was 75.8% after 2 days incubation. Thus, the minimum number of viable conidia required for infection was approximately 900. This number of conidia created an average of 6.6 lesions out of 10 leaves inoculated per plant. Twelve thousand conidia created lesions in about 90% of the leaves inoculated which was significantly ($P = 0.01$) more than that caused by 1,200 conidia.

In Experiment 6, lesions were produced on 65% of leaves (about 3.3 leaves/plant) inoculated with 1,200 conidia. The 800 conidia treatment only caused lesions on the apical meristems (youngest) and only on leaves in 3 out of 15 plants. No lesions were produced with any other treatment (data not shown). The germination percentage of the inoculum on agar was comparable to the previous experiment (79.2%).

In Experiment 7, when the inoculum suspension was amended with 5% Triton X-100, lesions were formed with lower number of conidia (i.e. 300 conidia) in two out of five inoculated leaves per plant (Table 3.6). Some of the control treatments (1.7 leaves/plant) also developed lesions probably caused by the Triton X-100. Consequently, the number

of lesions formed with 300 conidia was not significantly different from that of the control treatment. A significant number of lesions were formed with 600 conidia or 475 viable conidia ($P = 0.022$). Infection was localised and did not spread to uninoculated leaflets as shown in Figure 3.7.



Figure 3.7. Lesions (LE) caused by *F. tumidum* on gorse leaves in Experiment 5. A: control treatment; B; inoculated plant.

Table 3.5. The number of lesions/plant and lesion diameter at 1 wk after inoculation with *F. tumidum* in Experiment 5. Mean of eight replicate plants with 10 leaves/plant inoculated.

Number of Conidia	Number of viable conidia	Number of lesions/plant (10 drops/plant) ^a	Lesion diameter (mm)
12,000	9,100	9.1 b ^z	5.2 b
1,200	900	6.6 a	4.3 a
120	90	0	-
60	45	0	-
12	9	0	-
0	0	0	-
LSD ($P < 0.05$)		1.67	0.13

^a ANOVA was carried out on data from 12,000 and 1,200 conidia only since the lower conidia numbers failed to create lesions.

^z footnote same as Table 3.2.

Table 3.6. The number of lesions/plant and tip dieback (%) at 1 week after inoculation with *F. tumidum* suspension amended with 5% Triton X-100 in Experiment 7. Mean of 10 replicate plants with 5 leaves/plant inoculated.

Number of Conidia	Number of viable conidia	Number of lesions/plant (5 drops/plant)	Tip dieback (%)
1,500	1,188	3.6 c ^z	100
900	713	3.0 bc	70
600	475	3.1 bc	40
300	238	2.0 ab	0
0	0	1.7 a	10
LSD ($P < 0.05$)	-	1.27	-

^z footnote same as Table 3.2.

3.4.6 Experiment 8: Susceptibility of gorse structures

The flowers and leaves were the most susceptible morphological structures to *F. tumidum* infection (Table 3.7). Disease severity was highest with inoculations of flowers and leaves with a mean disease score of 5 at 7 DAI ($P < 0.001$) and lowest with inoculations of spine and pod. None of the control treatments were infected. The flowers were all senesced by the second week of the experiment.

Table 3.7. Effect of *F. tumidum* on different morphological structures of gorse assessed at 7 days after inoculation in Experiment 8.

Morphological structure	Disease score
Flowers	5.0 b ^z
Leaves	5.0 b
Stems	2.7 a
Spines	2.3 a
Pods	2.3 a
LSD ($P < 0.05$)	0.91

^z footnote same as Table 3.2.

3.4.7 Experiment 9: Effect of nutrient on conidial germination and germ tube elongation

Conidium was considered germinated when its germ tube measured at least half the spore length. Conidial germination and germ tube length increased significantly with 0.2% glucose or 0.2% gorse extract supplement when incubated for 24 h (Fig. 3.8). A similar result was obtained with *F. tumidum* conidia incubated on gorse leaves. Conidial germination was highest (93%) in the 0.2% gorse extract solution and lowest (62%) in water after 24 h of incubation ($P < 0.001$). *F. tumidum* suspension amended with glucose at 0.1 or 0.05% did not have any significant effect on the percentage of germinated conidia or the length of germ tube compared with those in water (Fig. 3.8B). Germ tubes were longest (407 μm) in 0.2% gorse extract solution and shortest (42 μm) in water ($P < 0.001$) as illustrated in Figure 3.9.

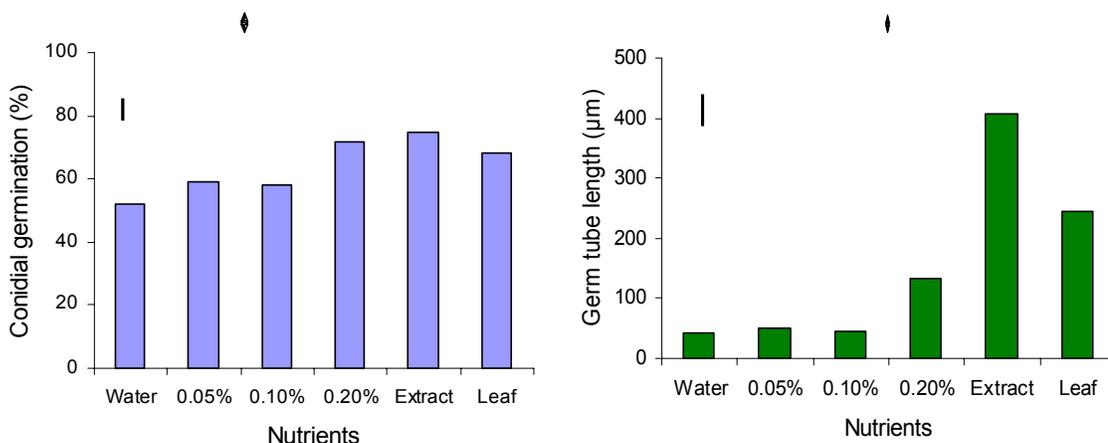


Figure 3.8. Per cent germination (A) and germ tube length (B) of *F. tumidum* conidia amended with water, glucose (0.05, 0.1, 0.2%), gorse extract or incubated on gorse leaves for 24 h in Experiment 9. Germination percentages are angular transformed means. Bars indicate LSDs at $P < 0.05$.



Figure 3.9. Germ tubes (GT) and germ tube branches (GTb) arising from germinating conidia (GC) of *F. tumidum* conidia in water and in 0.2% gorse extract solution after 24 h of incubation. NC: non-germinated conidia.

SEM studies

F. tumidum germ tube penetration of gorse leaf tissue was through the stomata as shown in Figure 3.10. No infection cushions or appressoria were observed. Hyphal growth was more extensive on wounded leaves than on non wounded leaves (Fig. 3.11).

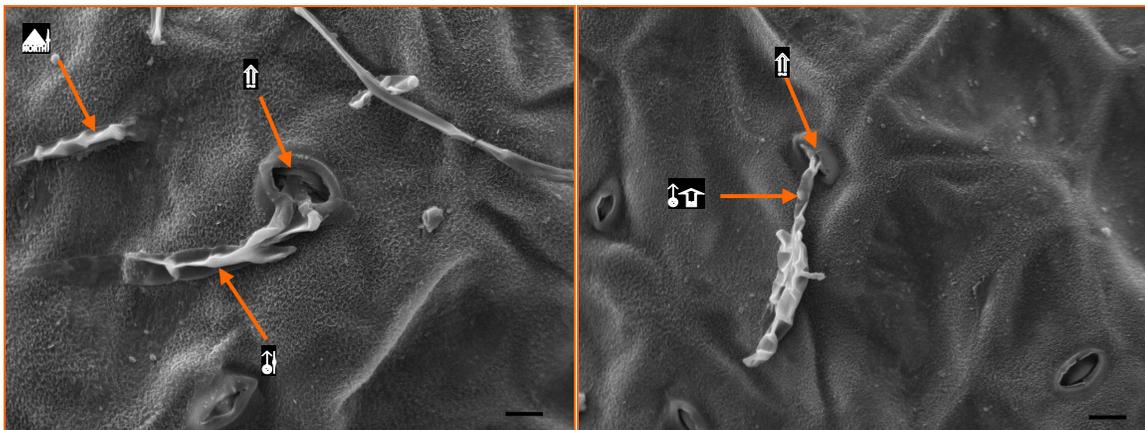


Figure 3.10. Scanning electron micrographs showing penetration of gorse leaf tissues by *F. tumidum* germ tubes (GT) through the stomata (S). Picture was taken at 48 hours after inoculation. GC: germinating conidia; NC: non-germinated conidia; Bar indicates 10 µm.

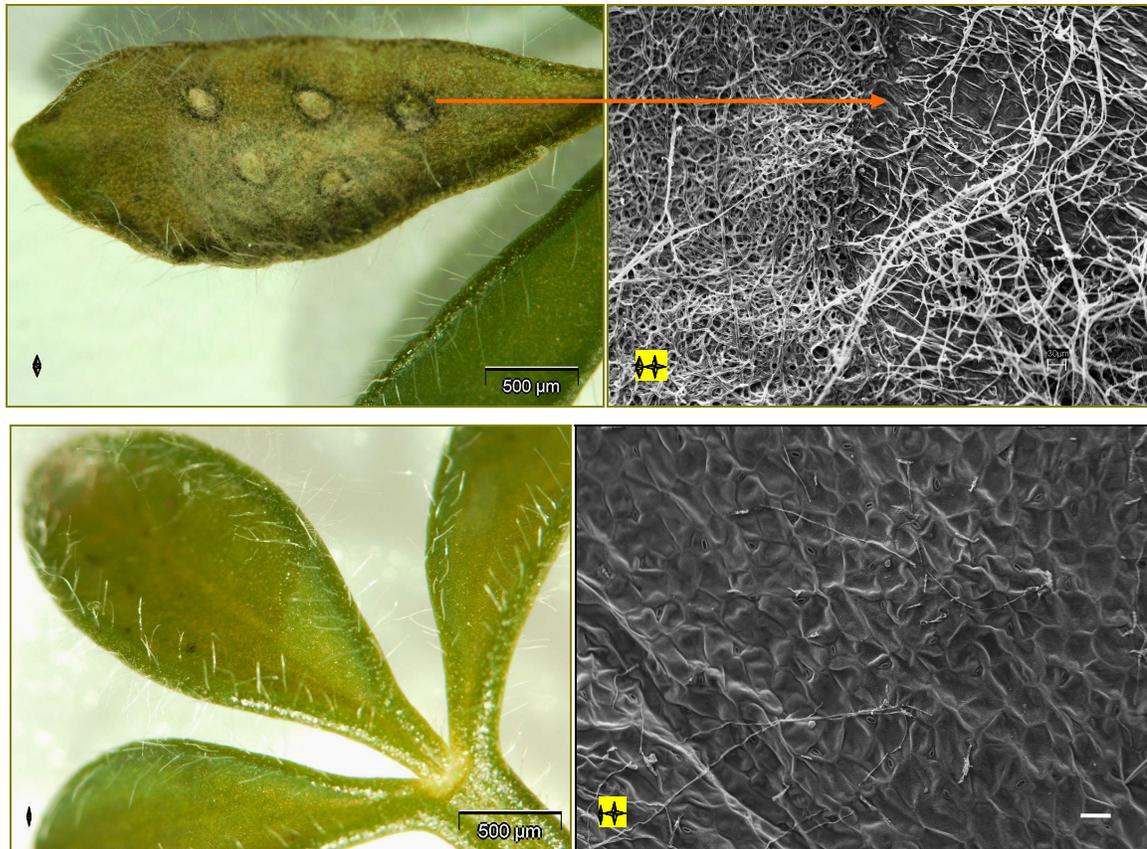


Figure 3.11. Hyphal growth of *F. tumidum* on wounded (A) and non wounded (B) gorse leaves. Above: arrow indicates wound crevice on leaf. A2 and B2: scanning electron micrographs, Bar = 30 μm .

3.4.8 Experiment 10: Effect of wounding gorse plants

Disease severity

The effect of wounding on disease severity was significant for the 8 wk ($P = 0.003$) and 16 wk ($P = 0.046$) old plants (Fig. 3.12). All inoculated 4 wk old plants were severely infected and had a similar disease severity score of 5 for wounded and 4.8 for non wounded plants and there was no significant effect of wounding ($P = 0.333$). The 32 wk old plants which were inoculated were the least infected of all ages assessed ($P = 0.188$) and had disease scores of 2.3 and 1.8, respectively for the wounded and non wounded plants. None of the uninoculated plants (wounded or non wounded) were infected.

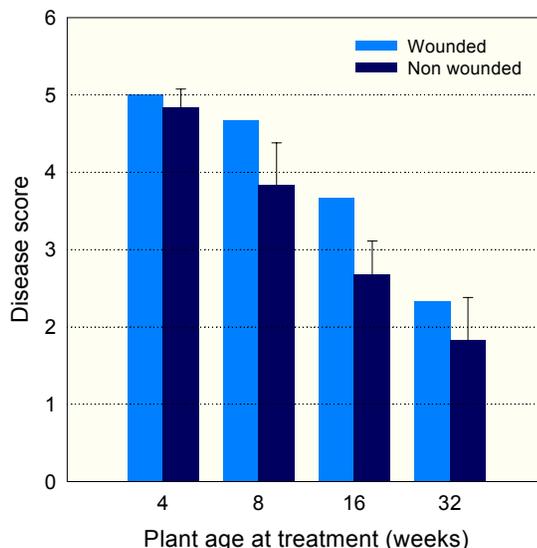


Figure 3.12. Disease score (1-5 scale) of wounded and non wounded gorse (4, 8, 16 and 32 wk old) at 2 wk after inoculating with *F. tumidum* in Experiment 10. Mean of six replicate plants. Bars represent LSD at $P < 0.05$ for comparison between wounded and non wounded for each plant age.

Shoot dry weight

The effect of wounding on the shoot dry weight was significant in the 8 and 16 wk plants (Table 3.8). *F. tumidum* significantly reduced by 90% the shoot dry weight of the 8 wk old plants which were wounded and that of the non wounded plants by 66% compared with the control treatment. Similarly, the pathogen significantly reduced shoot dry weight of wounded and non wounded 16 wk old gorse by 44 and 22%, respectively.

Shoot height

Eight week old plants which were wounded and inoculated were shorter ($P = 0.005$) than those which were only inoculated (Table 3.9). *F. tumidum* on its own reduced the shoot height of the 32 wk old plants by 28% ($P = 0.008$) compared with the control treatment. Wounding of 32 wk old plants had a similar effect on plant shoot height as applying *F. tumidum* alone and was not significantly different to the combined effect of both wounding and *F. tumidum*. Similar effect was seen with 16 wk old plants with no

significant difference between effect of *F. tumidum* alone and combined effect of both wounding and *F. tumidum*.

Table 3.8. Shoot dry weight of gorse aged 4, 8 16 and 32 wk assessed 5 wk after inoculating with 10^6 conidia/mL of *F. tumidum* suspension in Experiment 10. Mean of six replicate plants. Values in parentheses are back-transformed means after \log_{10} transformation.

Treatment	Shoot dry weight (g/plant)							
	4 wk ^a		8 wk ^a		16 wk		32 wk	
Control	(0.19)	0.075 b ^z	(1.21)	0.341 d	(5.94)	0.769 c	(15.38)	1.161
Wounding only	(0.16)	0.064 b	(0.83)	0.261 c	(6.26)	0.790 c	(14.93)	1.165
<i>Fusarium</i> only	(0.02)	0.010 a	(0.41)	0.146 b	(4.62)	0.663 b	(15.01)	1.161
W & <i>Fusarium</i> [†]	(0.00)	0.000 a	(0.12)	0.044 a	(3.30)	0.509 a	(12.28)	1.068
LSD ($P < 0.05$)	-	0.0231	-	0.0624	-	0.1013	-	0.1665
Interaction (P)	-	0.905	-	0.627	-	0.019	-	0.390
Main effect								
No wounding	(0.11)	0.042	(0.81)	0.244	(5.28)	0.716	(15.19)	1.161
Wounding	(0.08)	0.032	(0.47)	0.152	(4.78)	0.650	(13.60)	1.117
No <i>Fusarium</i>	(0.17)	0.069	(1.02)	0.301	(6.10)	0.780	(15.15)	1.163
<i>Fusarium</i>	(0.01)	0.005	(0.27)	0.095	(3.96)	0.586	(13.64)	1.115
LSD ($P < 0.05$)	-	0.0164	-	0.0441	-	0.0716	-	0.1177

^a Data were $\log_{10}(x + 1)$ transformed prior to analysis by ANOVA.

^z Treatment means within columns followed by the same letter are not significantly different based on LSD tests at $P < 0.05$.

[†] Wounding and *Fusarium* treatment.

Table 3.9. Height of gorse plants aged 4, 8 16 and 32 wk assessed 5 wk after inoculating with 10^6 conidia/mL of *F. tumidum* suspension in Experiment 10. Mean of six replicate plants. Values in parentheses are back-transformed means after \log_{10} transformation.

Treatment	Plant height (cm)							
	4 wk ^a		8 wk ^a		16 wk		32 wk	
Control	(12.0)	1.092 b ^z	(27.0)	1.445 b	(57.0)	1.752 b	(88.3)	1.943 b
Wounding only	(12.8)	1.138 b	(22.8)	1.375 b	(52.3)	1.715 b	(77.0)	1.879ab
<i>Fusarium</i> only	(1.4)	0.163 a	(15.1)	1.188 b	(35.6)	1.534 a	(64.0)	1.795 a
W & <i>Fusarium</i> [†]	(0.0)	0.000 a	(3.4)	0.401 a	(27.8)	1.440 a	(65.0)	1.805 a
LSD ($P < 0.05$)	-	0.2751	-	0.3254	-	0.1212	-	0.1086
Interaction (P)	-	0.271	-	< 0.005	-	0.489	-	0.325
Main effect								
No wounding	(6.7)	0.628	(21.0)	1.317	(46.3)	1.643	(76.1)	1.869
Wounding	(6.4)	0.569	(13.1)	0.888	(40.1)	1.578	(71.0)	1.842
No <i>Fusarium</i>	(12.4)	1.115	(24.9)	1.410	(54.7)	1.733	(82.6)	1.911
<i>Fusarium</i>	(0.7)	0.081	(9.3)	0.795	(31.7)	1.487	(64.5)	1.800
LSD ($P < 0.05$)	-	0.1945	-	0.2300	-	0.0857	-	0.0768

^{a, z, †} footnotes same as Table 3.8.

Plant mortality and tip dieback

F. tumidum infected and killed almost all the 4 wk old plants, irrespective of the wounding treatment. Plant mortality was 100 and 83%, respectively for the wounded and non wounded 4 wk old plants. *F. tumidum* killed 67% of 8 wk old plants which were wounded and 50% of those that were not wounded. None of the older plants (16 or 32 wk) died in response to the treatments although a high percentage (83%) of the 32 wk old which were wounded developed tip dieback after inoculation compared with only 33% of those plants that were not wounded. All 4 and 8 wk old plants developed tip dieback in response to inoculation with *F. tumidum* irrespective of the wounding treatment.

3.4.9 Experiment 11: Effect of wounding gorse plants*Disease severity*

In Experiment 11, disease severity followed a similar pattern to that in Experiment 10 for all ages of gorse assessed (8, 16 and 32 wk old) (Fig. 3.13). In all instances, disease score increased to a peak at about 10 DAI and then declined gradually as a result of regrowth. The overall disease severity was comparable to that of Experiment 10 for all plant ages apart from the 16 wk old plants which had higher disease scores compared to Experiment 10.

The 16 wk old plants which were wounded and inoculated attained maximum disease score of 5 while disease score of plants which were only inoculated peaked at 3.8. Differences in severity of infection between the wounded and non wounded plants in response to inoculation with *F. tumidum* were highly significant ($P < 0.001$) throughout the experiment. None of the uninoculated plants either wounded or non wounded were infected.

Shoot dry weight

Application of *F. tumidum* alone significantly reduced the shoot dry weight of the 8 and 16 wk old plants (Table 3.10). However, shoot dry weight of 8 and 16 wk old plants which were wounded and inoculated with *F. tumidum* were significantly lower than those

which were only inoculated. Inoculation alone reduced the shoot dry weight of non wounded 8 wk old plants by 41% and that of wounded plants by 57% compared with the control treatment. Similarly, the shoot dry weight of 16 wk old plants which were only inoculated were reduced by 59% while plants which were wounded and inoculated were reduced by 76% compared with the control treatment. These results were comparable to that of Experiment 10. Wounding 32 wk old plants had a similar effect on plant shoot dry weight as applying *F. tumidum* only and was not significantly different to the combined effect of both wounding and *F. tumidum*. The wounding treatment alone caused no significant reduction in shoot weight of all ages of plants assessed.

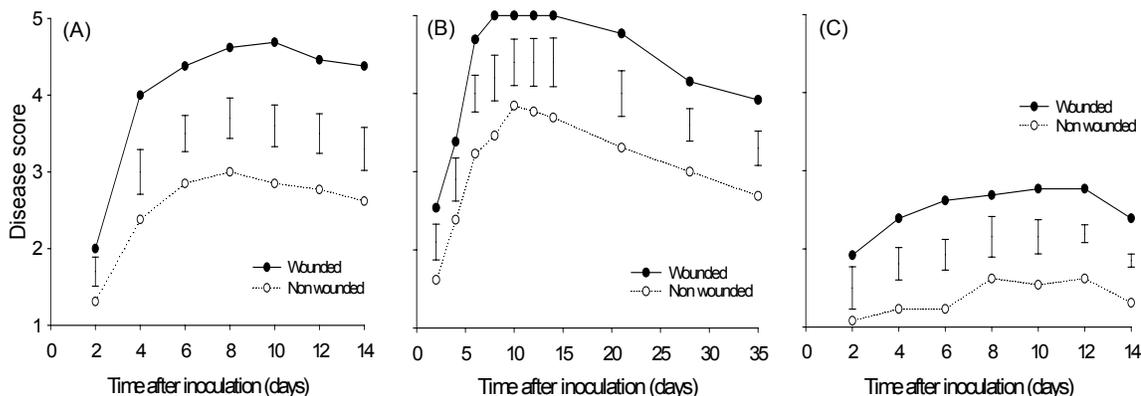


Figure 3.13. Disease score (1-5 scale) of wounded and non wounded 8 (A) 16 (B) and 32 (C) wk-old gorse inoculated with 10^6 conidia/mL of *F. tumidum* suspension in Experiment 11. The data points represent the mean of 13 replicate plants. Bars represent LSD at $P < 0.05$ at each assessment time.

Table 3.10. Shoot dry weight of 8, 16 and 32 wk old gorse assessed 5 wk after inoculating with 10^6 conidia/mL of *F. tumidum* suspension in Experiment 11. Mean of 13 replicate plants. Values in parentheses are back-transformed means after \log_{10} transformation.

Treatment	Shoot dry weight (g/plant)					
	8 wk ^a		16 wk ^a		32 wk	
Control	(0.51)	0.176 c ^z	(2.90)	0.584 c	(19.59)	1.259
Wounding only	(0.51)	0.178 c	(2.81)	0.570 c	(19.04)	1.250
<i>Fusarium</i> only	(0.30)	0.111 b	(1.19)	0.328 b	(19.38)	1.274
W & <i>Fusarium</i> [†]	(0.22)	0.082 a	(0.70)	0.223 a	(17.17)	1.209
LSD ($P < 0.05$)	-	0.0290	-	0.0742	-	0.0719
Interaction (P)	-	0.134	-	0.086	-	0.271
Main effect						
No wounding	(0.41)	0.144	(2.05)	0.456	(19.49)	1.267
Wounding	(0.37)	0.130	(1.75)	0.396	(18.10)	1.229
No <i>Fusarium</i>	(0.51)	0.177	(2.86)	0.577	(19.32)	1.255
<i>Fusarium</i>	(0.26)	0.096	(0.94)	0.276	(18.28)	1.242
LSD ($P < 0.05$)	-	0.0205	-	0.0525	-	0.0508

^{a, z, †} footnotes same as Table 3.8.

Root dry weight

F. tumidum reduced significantly the root dry weights of the 8 and 16 wk old plants which were wounded (Table 3.11). Unlike the shoot dry weight, there was no significant difference between root dry weights of plants which were wounded and those which were not wounded in their response to inoculation with *F. tumidum*. Root dry weight of 8 wk old plants, wounded and inoculated were 33% lower than the control treatments while that of 16 wk old plants with similar treatment were 76% lower than the control treatments. The treatments did not have any significant effect on the root dry weight of the 32 wk old plants. Total plant dry weights of 8 and 16 wk old plants which were wounded and inoculated were significantly lower than the same age plants which were not wounded in response to *F. tumidum* inoculation (Appendix 3.4).

Shoot height

The 8, 16 and 32 wk old plants which were wounded and then inoculated, were significantly shorter than those that were only inoculated and the untreated control plants (Table 3.12). *F. tumidum* on its own reduced the shoot height of the 8 and 16 wk old plants significantly by 34 and 48%, respectively compared with the control treatment. However, the wounding with inoculation treatment reduced the shoot height by 69% (8 wk old plants) and by 60% (16 wk old plants; Fig. 3.14) compared with the control treatment. For the 32 wk old plants, the wounding with inoculation treatment reduced the shoot height by 23% ($P < 0.05$) while the shoot height of only inoculated plants were not significantly different from the control treatment. Height of plants which were only wounded did not differ significantly from the untreated control plants for any of the plant ages.

Plant mortality and tip dieback

Seventy seven per cent of the 32 wk plants wounded and inoculated, developed tip dieback while only 31% of those plants which were only inoculated developed tip dieback. Similar results were obtained for wounded and non wounded 32 wk old plants in Experiment 10. All 16 wk old plants which were wounded and inoculated developed tip dieback while only 69% of the plants that were only inoculated had. All 8 wk old plants developed tip dieback but only 15% of the wounded and inoculated plants died. None of the older plants (16 or 32 wk old) were killed by the treatments.

Table 3.11. Root dry weight of 8, 16 and 32 wk old gorse assessed 5 wk after inoculating with 10^6 conidia/mL of *F. tumidum* suspension in Experiment 11. Mean of 13 replicate plants. Values in parentheses are back-transformed means after \log_{10} transformation.

Treatment	Root dry weight (g/plant)					
	8 wk ^a		16 wk ^a		32 wk	
Control	(0.12)	0.048 b ^z	(0.92)	0.281 b	(9.33)	0.929
Wounding only	(0.12)	0.051 b	(0.77)	0.245 b	(7.34)	0.855
<i>Fusarium</i> only	(0.10)	0.041 ab	(0.31)	0.115 a	(7.64)	0.878
W & <i>Fusarium</i> [†]	(0.08)	0.032 a	(0.22)	0.085 a	(7.76)	0.870
LSD ($P < 0.05$)	-	0.0102	-	0.0382	-	0.0983
Interaction (P)	-	0.119	-	0.821	-	0.339
Main effect						
No wounding	(0.11)	0.044	(0.50)	0.198	(8.48)	0.903
Wounding	(0.10)	0.042	(0.62)	0.165	(7.55)	0.862
No <i>Fusarium</i>	(0.12)	0.049	(0.85)	0.263	(8.33)	0.892
<i>Fusarium</i>	(0.09)	0.036	(0.26)	0.100	(7.70)	0.874
LSD ($P < 0.05$)	-	0.0072	-	0.0270	-	0.0695

^a, ^z, [†] footnotes same as Table 3.8.

Table 3.12. Height (cm) of 8, 16 and 32 wk old gorse assessed 5 wk after inoculating with 10^6 conidia/mL of *F. tumidum* suspension in Experiment 11. Mean of 13 replicate plants. Values in parentheses are back-transformed means after \log_{10} transformation.

Treatment	Shoot height (cm)					
	8 wk ^a		16 wk		32 wk	
Control	(22.5)	1.368 c ^z	(34.6)	1.533 c	(84.4)	1.920 b
Wounding only	(19.9)	1.312 bc	(32.1)	1.498 c	(81.2)	1.904 b
<i>Fusarium</i> only	(14.9)	1.172 b	(18.0)	1.228 b	(80.6)	1.900 b
W & <i>Fusarium</i> [†]	(7.0)	0.818 a	(13.7)	1.124 a	(65.3)	1.809 a
LSD ($P < 0.05$)	-	0.1443	-	0.0896	-	0.0460
Interaction (P)	-	0.134	-	0.272	-	0.025
Main effect						
No wounding	(18.7)	1.270	(26.3)	1.381	(82.5)	1.911
Wounding	(13.5)	1.065	(22.9)	1.311	(73.2)	1.857
No <i>Fusarium</i>	(21.2)	1.340	(33.3)	1.515	(82.8)	1.912
<i>Fusarium</i>	(11.0)	0.995	(15.9)	1.176	(72.9)	1.855
LSD ($P < 0.05$)	-	0.1020	-	0.0634	-	0.0325

^a, ^z, [†] footnotes same as Table 3.8.



Figure 3.14. Gorse plants inoculated at 16 wk old with *F. tumidum* (A), untreated control plants (B) and wounded and inoculated plants (C) at 2 wk after inoculation in Experiment 11.

3.4.10 Experiment 12: Effect of gorse extract on infection

Disease severity assessment at 8 and 14 DAI with *F. tumidum* showed that plants which were wounded and then inoculated were the most infected (Table 3.13). Inoculation of plants with *F. tumidum* conidial suspension supplemented with 0.2% gorse extract resulted in significantly higher disease than inoculation with *F. tumidum* only at 8 DAI but not at 14 DAI and resulted in similar shoot dry weight reduction ($P = 0.012$) as the wounded and inoculated plants. Plants which were wounded and then inoculated had the lowest shoot dry weight. Only *F. tumidum* treatment in combination with wounding or the 0.2% gorse extract supplement treatments significantly reduced the mean shoot dry weights of the plants compared with the control treatment.

Table 3.13. Disease score (1-8 scale) at 8 and 14 days after inoculation (DAI) and shoot dry weight of 24 wk old gorse inoculated with *F. tumidum* suspension amended with gorse extract and assessed at 28 DAI. Mean of 10 replicate plants. Values in parentheses are back-transformed means after \log_{10} transformation.

Treatment	Disease score		Shoot dry weight at 28 DAI (g)	
	8 DAI	14 DAI		
Control	1.0 *	1.0	(7.26)	0.833 b
<i>Fusarium</i> only	3.0 a ^z	3.0 a	(5.81)	0.747 ab
Wounding & <i>Fusarium</i>	5.5 c	5.2 b	(4.76)	0.667 a
Gorse extract & <i>Fusarium</i>	3.9 b	3.6 a	(5.20)	0.701 a
LSD ($P < 0.05$)	0.88	1.09		0.0993

* The untreated control plants were not infected and were excluded from ANOVA.

^z footnote same as Table 3.8.

3.5 DISCUSSION

To develop a novel strategy for delivering *F. tumidum* to infect gorse using insects as vectors, factors that would enhance pathogenicity of *F. tumidum* were determined. *F. tumidum* consistently infected and reduced shoot and root dry weight of gorse plants aged 16 wk or younger when applied at 10^6 conidia/mL, confirming previous reports (Morin *et al.*, 1998). In all pathogenicity experiments, symptoms appeared 2-3 DAI and disease severity increased to a maximum at about 10-14 days and declined thereafter. In most instances, inoculated plants developed tip dieback symptom. Gorse plants, in drought condition or under moisture stress, can also develop tip dieback. Hence, in all experiments, *F. tumidum* was isolated from samples of lesions to confirm the cause of infection. Tip dieback infection usually causes the growing tip to wilt. It is probably due to restriction in water and nutrient flow to tissues above the point of infection. Infected plants compensated by the development of more lateral shoots which accounted for the decline in disease scores often recorded after 14 days following inoculation. It must be emphasised that the decline in disease score was not due to the recovery of already infected tissues. Disease levels of inoculated plants were still high at 35 DAI (Experiment 11). Significant disease levels have been reported even at 7 months after inoculation (Fröhlich *et al.*, 2000).

Under severe infection (as observed in plants 16 wk old or younger), large portions of the shoot wither. Wilting of the shoot damages chloroplasts (which intercept light) and reduces the number and the surface area of healthy green leaves available for photosynthesis. This decreases the photosynthetic rate and dry matter accumulation (Kwapata *et al.*, 1990). Thus, the shoot dry weights of severely infected plants were significantly lower than that of the uninoculated plants. The shoot dry weight was correlated with the root dry weight which indicates that severe shoot infection could restrict root development and growth as less photosynthate are translocated to the roots.

In Experiment 3, disease severity of all plant ages assessed increased with inoculum concentration. A suspension of 10^6 conidia/mL was found to be the best concentration

required to cause plant mortality. However, at this concentration, none of the 16 wk or older plants was killed, confirming earlier reports by Morin *et al.* (1998). Using the model: $y = 1.14 - 0.21x + 0.10x^2$ generated from the relationship between disease severity score and inoculum concentration, a suspension of about $10^{7.4}$ conidia/mL would be required to cause maximum infection and probably plant mortality of the 16 wk old plants. Disease severity though is not expected to increase indefinitely with increase in inoculum concentration as was observed by Kurt and Tok (2006). They found that lesion development of *Septoria petroselini* increased with increasing inoculum concentration from 10^4 to 2×10^6 conidia/mL and declined at higher concentrations (10^7 conidia/mL).

As infected gorse plants generate new lateral shoots (at about 10-14 DAI), a second application of the inoculum would be required to prolong infection of plants which are at least 16 wk old. The second application should be done when regrowth are young, tender and therefore are most susceptible to infection. Results from this study showed that almost all inoculated 4 wk old plants were killed by the pathogen. Fröhlich *et al.* (2000) have pointed out that due to the large size of *F. tumidum* conidia, suspensions containing more than 10^6 conidia/mL may be difficult to apply with commonly used agricultural spray nozzles due to high viscosity.

In Experiment 4, strong correlation between the number of infection sites and disease score ($R^2=99.3\%$) implies that the entire plant would have to be inoculated in order to cause plant mortality. Only 100% spray coverage caused any plant mortality. *F. tumidum* infection did not spread from the inoculated sites to other parts of the plant. Conidia were produced on disease lesions but no secondary lesions were observed. A similar mode of infection has been reported in *Chondrostereum purpureum* which is another fungal pathogen of gorse (Bourdôt *et al.*, 2006). As *F. tumidum* is more aggressive towards soft tissues of the weed, spraying methods targeting the apical meristem may restrict plant growth as much as spraying the entire plant. For instance, shoot height was significantly reduced when only 25% of the plant surface area (from the apical meristem) was inoculated. However, significant reduction in shoot dry weight was only achieved with at least 50% spray coverage.

In Experiment 5, the minimum of about 900 viable conidia required to create a lesion is high compared with only one spore required by the groundnut pathogen *Phaeoisariopsis personata* or two spores required by *Aspergillus flavus* (peanut pathogen) to form lesions (Griffin and Garren, 1974; Wadia and Butler, 1994). This high number of conidia suggests that *F. tumidum* is a weak pathogen against gorse. Furthermore, since *F. tumidum* penetrates via stomata only and the stomata must be opened, the whole penetration process must be completed before the conidium depletes its nutrient reserves. A high percentage of the conidia may then germinate, develop germ tubes but not penetrate the host tissues if unable to find open stomata. These three stages are critical for successful infection (Gäumann, 1950) and the limiting factor for *F. tumidum* may be the presence of open stomata. Contributing to this is the fact that stomatal frequency on the upper epidermis of the leaf, where the inoculum was applied, is less than the lower epidermis (Martin *et al.*, 1983). All these factors may account for the relatively high number of conidia required for successful lesion development. This high number of conidia required for infection may restrict the use of insects as vectors of *F. tumidum* for successful control of gorse. However, incorporation of amendments in the formulation such as Triton X-100 (which acts as a surfactant to break surface tension) and was found in this study to cause some damage of the host tissues, might decrease the number of conidia required to form lesions.

The spines, stems and pods, as a result of the high proportion of woody tissues, were less susceptible to infection compared with the leaves and flowers. As gorse matures, most of the leaves are reduced to form spines (Roy *et al.*, 2004). The plant thus, becoming more lignified than the seedling. Zabkiewicz and Balneaves (1984) attributed the decline in susceptibility to infection with maturity to changes in the composition of lignin, foliar waxes and thickening of the cuticle and high root: shoot ratio. Since the survival of gorse populations is largely due to its prolific seed production ability (Rees and Hill, 2001), inoculation of high percentage of the flowers with *F. tumidum* may minimise seed production and help reduce the spread of the weed.

In the experiment to determine the effect of exogenous nutrients on germ tube elongation, germ tubes supplemented with 0.2% gorse extract were about 10 times longer and had more branching than those cultured in sterile water and were almost twice as long as those grown on gorse leaf. Glucose, sucrose, amino-N compounds, Mg and Zn are important for conidia germination and germ tube elongation (Beyer *et al.*, 2004; Farach *et al.*, 1979; Hale and Griffin, 1976). Reports by the Integrated Pest Management Information Service, IPM (2000) and Jobson and Thomas (1964) indicates the presence of nutrients such as Ca, Cl, Co, Cu, Fe, K, Mg, Mn, N, Na, P and Zn in gorse plants. Some of these nutrients might have supported the growth of *F. tumidum* germ tubes but such minerals were not determined in this study. Wounding of gorse tissues (caused by insects or by physical damage) can release nutrients from sap exuding from the wounds and also provide direct access for *F. tumidum* to the subcuticular tissues. In this study, wounding of gorse plants enhanced the amount of mycelium on the leaf surface and generally increased disease severity. The cuticle is often the main barrier to fungal infection. Wounding damages the cuticle and provides easier access for fungal penetration. Wounding of mature gorse plants in particular would be necessary to enhance *F. tumidum* infection as stomatal frequency declines with plant age (Willmer, 1983). Additionally, higher disease levels achieved with wounding and inoculation than with 0.2% gorse extract supplement of the inoculum, shows that wounding provides not only nutrients for the pathogen but also access to the plant tissues. Studies have shown that damaged plant tissues are generally more susceptible to infection than healthy ones as they provide easier access for penetration (Bateman, 1997; Bourdôt *et al.*, 2004). Bateman (1997) reported a significant increase in disease severity caused by *Fusarium solani* and *F. avenaceum* in wounded hypocotyls of white lupin (*Lupinus albus*) than non wounded hypocotyls. Pleysier *et al.* (2006) have also shown that wounded leaf tissues of *Paulownia fortunei* were significantly more susceptible to *Alternaria alternata* infection than non wounded leaf tissues. Wounding might decrease the number of conidia required to form lesions and warrants investigation.

This study has shown that dry weight and growth rate of gorse severely infected by *F. tumidum* (at inoculum concentration of 10^6 conidia/mL), were significantly reduced. A

higher inoculum concentration however, would be required to kill or cause severe infection of mature plants. In the field, this decrease in biomass may be sufficient to reduce the competitiveness of gorse against forestry plant species for light, nutrients and water. It has also been demonstrated in this study that wounding of older tissues will be required to facilitate *F. tumidum* infection. The level of wounding required for significant plant response to inoculation should increase with plant age. The feeding by larvae of insect species such as the gorse stem miner (*Anisoplaca ptyoptera* Meyrick), the lemon tree borer (*Oemona hirta* Fabricius) and *Epiphyas postvittana* which are naturally found on gorse causes severe damage to the plant tissues. Other sources of wounding may include mechanical damage, sunburn, abrasion, wind damage and oviposition by insects such as *A. ulicis* and stem boring insects. Small amounts of herbicides can also be used to augment the damage caused by insects. The level of wounding inflicted on the plants in this study was minimal as dry weights of wounded plants generally did not vary from that of the untreated plants. A more severe wounding could impose more stress on the weed (Moellenbeck *et al.*, 1992) and reduce its defence mechanism against infection.

Furthermore, this study has shown that *F. tumidum* is capable of infecting gorse plants sourced from different sites in New Zealand. Report indicates that *F. tumidum* has a host range including related species to gorse in the tribe Genisteae (Barton *et al.*, 2003). It is therefore not surprising that this pathogen was capable of infecting gorse collected as seed from a range of sites in New Zealand. Using insect species as vectors of *F. tumidum* to infect gorse would however demand large numbers of insects because of the large number of conidia required to initiate infection. The number of *F. tumidum* conidia that each of four potential insect vectors can carry is presented in Chapter Five.

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