

Using insects as potential vectors of *Fusarium tumidum* to control gorse

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ABSTRACT

The adult light brown apple moth *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae), gorse seed weevil *Apion ulicis* Förster, (Coleoptera: Apionidae), gorse pod moth *Cydia ulicetana* Denis and Schiffermüller (Lepidoptera: Tortricidae) and gorse thrips *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae) were assessed for their ability to carry and deposit the conidia of a plant pathogenic fungus *Fusarium tumidum* Sherb. on agar plates in laboratory experiments. *Epiphyas postvittana*, the largest insect species studied, carried and deposited the highest number of *F. tumidum* conidia. The number of colony forming units recovered from all insect species after exposing them to *F. tumidum* sporulating cultures for 24 h, declined with time. In subsequent transmission experiments using only *E. postvittana*, each insect was loaded with 5,000 conidia of *F. tumidum* and caged together with potted healthy gorse (*Ulex europaeus* L.) at eight insects per plant. The insects deposited 310 conidia per plant on average but neither infection nor plant growth effects resulted. Only directly inoculated plants became infected and were significantly shorter with 42% reduction in fresh shoot weight compared with the control treatment. *Epiphyas postvittana* on its own did not cause any significant plant damage and did not enhance *F. tumidum* infection. The insect did not spread the conidia from diseased to healthy plants. The results showed that insects are unlikely to be effective vectors in a 'lure-load-infect' approach for biological control of gorse using *F. tumidum*.

Keywords. *Ulex europaeus*, Light brown apple moth, 'lure-load-infect', biocontrol.

INTRODUCTION

Gorse is a serious pasture and forest plantation weed in New Zealand. It is now considered as New Zealand's worst weed and one of the world's 100 worst invasive weed species (ISSG 2006). Classical biological control using seed and foliage feeding insects such as *Apion ulicis*, *Cydia ulicetana* and *Sericothrips staphylinus* is being used to control this weed in New Zealand. However, to date, insects alone have not produced the high level of control required (Hill *et al.* 2000). Another insect species, *Epiphyas postvittana* is naturally abundant on gorse and the larvae feed on the foliage (Robertson *et al.* 1990). This insect has never been considered as a biocontrol agent outside New Zealand as it is highly polyphagous with over 265 hosts recorded (Thomas 1989).

Studies of foliar pathogens naturally occurring on gorse in New

Zealand identified *Fusarium tumidum* as capable of infecting gorse (Morin *et al.* 1998, 2000). The potential of delivering this pathogen using insects as vectors of inoculum is being contemplated as a novel strategy to control the weed. To date, the combined effect of insects and pathogens against gorse has not been investigated. Synergistic interactions due to insect feeding damage increasing a weed's susceptibility to pathogen infection have been reported for the thistle *Cirsium arvense* L. Scop. (Kluth *et al.* 2001) and leafy spurge *Euphorbia esula* L. (Caesar 2003). Friedli & Bacher (2001) reported that insects may also vector *Puccinia punctiformis* (Str.) Röhl, a plant pathogen of *C. arvense*, although Cripps *et al.* (2009) showed that the level of systemic disease is the same in Europe (disease vectors present) and New Zealand (disease vectors absent) casting doubt on the idea that insects transmit *P. punctiformis*. Reports on transmission of fungal spores to plants by insects include the spread of *Fusarium oxysporum* f. sp. *radicis-lycopersici* by adult fungus gnat (*Bradysia* spp.) and sorghum ergot *Claviceps africana* by the corn earworm moth, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), from diseased to healthy plants (Gillespie & Menzies 1993; Prom *et al.* 2003). In contrast, Connor *et al.* (2000) reported no significant combined effects of *Platypreria virginialis* (Lepidoptera: Arctiidae) and the fungus *Phoma pomorum* on houndstongue *Cynoglossum officinale*, and that the insect appeared to avoid diseased leaves. Similarly, Hill *et al.* (2004) reported that the insect herbivore, *Phytomyza vitalbae* failed to vector or have synergistic interactions with the plant pathogen, *Phoma clematidina*.

A new concept for biological control of weeds referred to as 'lure-load-infect', which is a complementary strategy to be used in combination with mycoherbicides, has been proposed (Hee *et al.* 2004). In this approach, insects are used as vectors to transmit plant pathogens to target weeds. The approach is made feasible by using auto-inoculation systems baited with attractants (e.g., pheromones) to lure insects to the inocula, as has been field tested for insect pathogens of diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Furlong *et al.* 1995). The insects will carry some of the pathogen inocula on their cuticle and deposit them on the target weed upon visit (Fig. 1). This strategy has several potential advantages over the use of broadcast mycoherbicide spray application, being more feasible for use to control gorse on inaccessible land and more cost effective for use on marginal agricultural land. This concept requires feasibility to be demonstrated by assembly of the essential components as

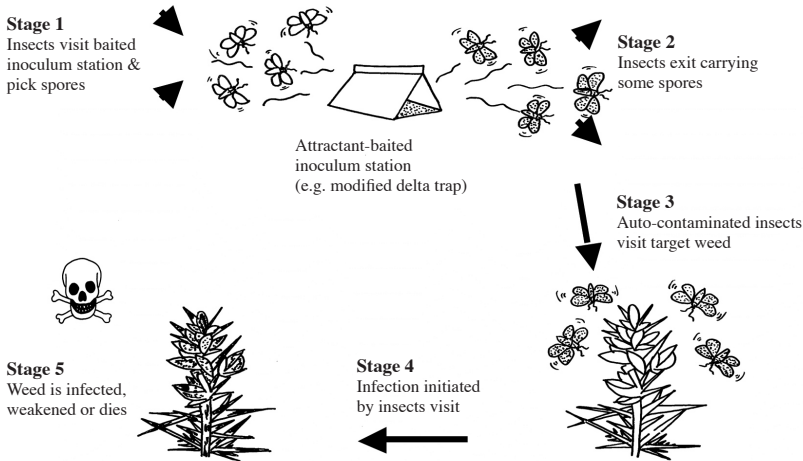


Figure 1. Concept: 'Lure-load-infect' (Diagram courtesy of Alvin Hee).

functional units into a system. This study tackles an early subset of that putative chain of events. The objectives of this study were to (i) select among four gorse-inhabiting insect species (*A. ulicis*, *C. ulicetana*, *E. postvittana* and *S. staphylinus*) the species with the greatest potential to carry and deposit *F. tumidum* conidia (ii) determine the degree of infection of gorse caused by insect-carried *F. tumidum* conidia compared with direct application of the inoculum (iii) determine whether the most potential species can spread the conidia from diseased to healthy plants.

MATERIALS AND METHODS

Fusarium tumidum inoculum production and maintenance

Fusarium tumidum isolate G34-34V, originally isolated from gorse plants in the Bay of Plenty, New Zealand (Morin *et al.* 1998) and supplied by Landcare Research, was used in all experiments. The conidia were stored in 30% sterile glycerol at -80°C and routinely cultured on glucose cornmeal agar (GCMA) or oatmeal agar (OMA) plates under natural light supplemented with white and blue light at 23:18 ± 2°C day: night and a 16-h photoperiod for 14 days for spore production (Yamoah *et al.* 2008a). GCMA contains 2 g of glucose and 17 g of cornmeal agar (Difco) per litre while OMA contains 30 g Fleming's oatmeal and 20 g agar per litre.

Rearing of insects

Male *E. postvittana* were obtained as pupae from a laboratory colony maintained at Plant and Food Research, Mount Albert Research Centre, Auckland, New Zealand. The colony was reared on a semi-synthetic diet and kept at 20°C under a photoperiod of 16:8 h (light: dark). Newly emerged adult moths were kept in humidified polycarbonate boxes (40 cm x 40 cm x 40 cm) at 20°C and 75% relative humidity, under a 16:8 h (light: dark) reverse photoperiod. Moths were provided with 10% sucrose

solution absorbed onto a cotton wick for food. Two to three-day-old virgin males were used in all experiments. All other insect species (*A. ulicis*, *C. ulicetana* and *S. staphylinus*) were collected from the field and none was found to carry *F. tumidum* conidia naturally (Yamoah *et al.* 2008b).

General procedures

The number of *F. tumidum* conidia on the insects was determined by washing the insects in 100 mmol potassium phosphate buffer (PPB; pH 7.0) amended with 0.01% Tween 80 Analar® according to Yamoah *et al.* (2008b). A 50 µL sample of each dilution series (0 and 10⁻¹) was plated onto three replicate Petri dishes containing potato dextrose agar (PDA; Oxoid) amended with 250 µg/mL chloramphenicol and 0.2% of Triton X-100. After 2 days of incubation at 23:18 ± 2°C day: night temperatures, the colony forming units (CFU)/insect were counted. The germination percentage of *F. tumidum* spore suspension inoculum was determined by dilution plating onto PDA and counting the resulting colonies after 2 days incubation. In all experiments, symptomatic tissues from infected gorse plants were plated onto PDA to confirm the cause of infection. Shoot height was measured from the soil level to the tip of the plant. Aluminium foil was spread over the soil surface in each pot to prevent contact of the insects with the soil to minimise contamination. All experiments were designed as a randomised complete block (RCBD).

Carrying of *F. tumidum* conidia from sporulating cultures

Sixty live insects of each species were placed separately on sporulating GCMA cultures of *F. tumidum* for 24 h at five insects per culture plate. Each species was then transferred to sterile plastic containers (measuring 15 x 10 x 6 cm³) using sterile tweezers after being anaesthetised with carbon dioxide (CO₂) according to Gillespie & Menzies (1993) and kept on the laboratory bench at room temperature. *Fusarium tumidum* CFU/

insect was determined at 0, 24, 48 and 72 h after transfer. Five insects of each species were randomly selected and washed in each of three replicate Universal bottles containing 1 mL of 100 mmol PPB as described in the general procedures.

In the next experiment, only *E. postvittana* was used as it picked the largest number of *F. tumidum* conidia in the previous experiments. One hundred and twenty *E. postvittana* were exposed to sporulating cultures of *F. tumidum* for 24 h. The CFU/insect was determined at 0, 24, 48, 72 and 96 h using four replicates as previously described.

Deposition of *F. tumidum* conidia on agar

Sericothrips staphylinus generally picked no *F. tumidum* conidia in the previous experiments so it was excluded in this experiment. The remaining three insect species were used. The number of conidia deposited by each insect species was determined by placing 24 insects of each species on sporulating cultures of *F. tumidum* in a Petri dish for 24 h. Twelve insects were washed immediately after removing them from the cultures and the rest were transferred to new GCMA plates at one insect per plate. The plates were kept on the laboratory bench at room temperature for 24 h and then the insects were removed. The plates were incubated under conditions stated in the general procedures. The number of CFU/insect was counted to determine the number of *F. tumidum* conidia deposited. Based on the results of these experiments, *E. postvittana* was selected and used for all subsequent experiments.

Transmission by inoculated *E. postvittana*

This experiment consisted of four treatments: (i) gorse plant caged together with eight *E. postvittana*; each inoculated with 5,000 conidia of *F. tumidum*, (ii) gorse plant caged together with eight uninoculated *E. postvittana*, (iii) gorse plant directly inoculated with *F. tumidum* and (iv) untreated gorse plants (negative control). The plants were grown in pots at one plant per pot in a glasshouse and used when they were 7 weeks old. Each plant was caged separately using a well ventilated plastic bottle.

To standardise the number of conidia carried by the insects at the start of the experiment, each insect was inoculated with a known count (5,000) of conidia. A 10^7 conidia/mL suspension of *F. tumidum* was prepared according to Yamoah *et al.* (2008a) and amended with 5% Triton X-100. Aliquots (0.5 μ L) were applied to the abdomen of all inoculated *E. postvittana* after being anaesthetised with CO₂. Aliquots of 1 μ L of the suspension (containing 10,000 conidia) were applied to three leaflets and the apical meristem of the inoculated plants. An additional eight *E. postvittana* were inoculated as previously described and washed 1 h later to determine the number of viable *F. tumidum* conidia available for deposition as outlined in the general procedures.

Each treatment consisted of 12 replicate plants, and was placed under 23:18 \pm 2°C day: night temperatures for 12 days. On day four, four insects were randomly selected from each plant (receiving *E. postvittana*), washed, plated and *F. tumidum* CFU/insect determined as stated in the general procedures. The *F. tumidum* CFU/insect of the remaining four insects was

determined at day 7. The plants were incubated for a further 5 days to enable infection to develop. The number of lesions that developed on each plant was recorded and the shoot height was measured. The fresh weight of each plant was also determined to provide information on plant growth. To determine the number of *F. tumidum* conidia deposited by the insects on the gorse, the shoots were cut just above the soil level and washed separately in 5 mL PPB solution. A 100 μ L of each dilution series (0 and 10⁻¹) was plated and *F. tumidum* CFU count determined as outlined in the general procedures. This experiment was modified slightly to determine if *E. postvittana* can spread the conidia from diseased to healthy plants.

Transmission from diseased to healthy gorse

Gorse plants were planted into 1.4 L pots at two plants per pot. When the plants were 10 weeks old, one of each pair was spray-inoculated to run off with a *F. tumidum* suspension of 1.6 x 10⁶ conidia/mL. To prevent the other pair from getting contaminated with *F. tumidum*, it was covered with a transparent plastic bag. The inoculated plants were also bagged separately after inoculation using transparent plastic bags for 24 h after which all plants were placed in a glasshouse. At 12 days after inoculation (DAI), the shoot height and number of plants with dieback were recorded.

Each pair of plants was then covered with a well ventilated 1.0 L plastic container. Twenty *E. postvittana* were introduced to each of 12 cages at 14 DAI while 12 other cages had no *E. postvittana*. Another 12 cages, each containing a pot with a pair of uninoculated gorse plants was set up. Six of them had 20 *E. postvittana* introduced per cage and the remaining were without insects (negative control). All treatments were placed in a glasshouse for a further 7 days and then all the insects were anaesthetised, removed and washed to determine *F. tumidum* CFU/insect picked by the insects from the inoculated gorse. All plants were grown for a further 6 days to allow for infection to develop.

The number of insects present on inoculated and uninoculated plants was counted daily at about 4 pm for 7 days to provide information on the insect's preference for healthy or diseased plants. The shoot height and fresh weight were determined at 27 DAI. Each uninoculated plant was cut and washed separately to determine the number of *F. tumidum* CFU/plant transferred by the insects from the inoculated plants. All inoculated plants were also washed and plated to determine the number of CFU/plant of *F. tumidum* as a source of inoculum. The experiment consisted of 12 replicate plants per treatment.

Statistical analyses

Counts of CFU were log transformed to satisfy the assumption of normality for analysis of variance (ANOVA) and to stabilise the variance. All data were analysed by ANOVA using the GenStat statistical package. To determine the combined effect of *E. postvittana* and *F. tumidum* on gorse, shoot height was recorded before and after the introduction of *E. postvittana*. Mean separation was based on Fisher's protected least significant difference (LSD) tests at $P < 0.05$.

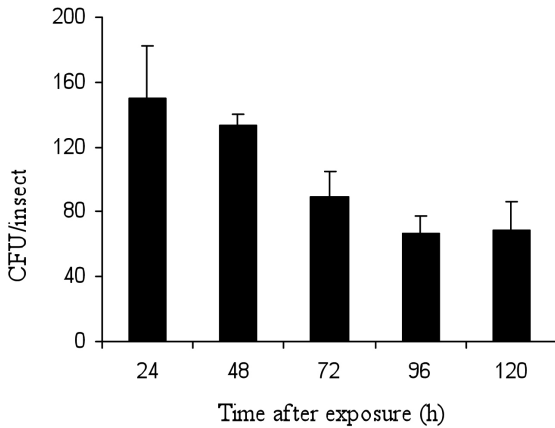


Figure 2. The number of colony forming units (CFU)/insect of *F. tumidum* recovered from *E. postvittana* within 96 h after exposure to sporulating cultures of *F. tumidum* on agar plates for 24h. Bars represent standard error.

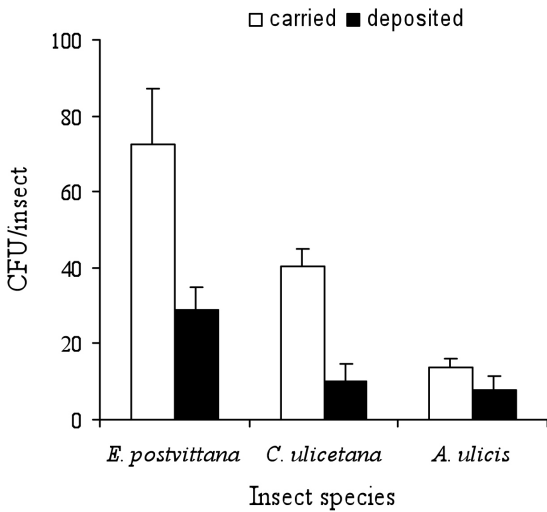


Figure 3. The number of colony forming units (CFU)/insect of *F. tumidum* carried and deposited on agar plates by three insect species. The insects were exposed to sporulating cultures of *F. tumidum* for 24 h. Bars represent standard error.

RESULTS

Carrying and deposition of *F. tumidum* conidia on agar

Following exposure to the sporulating cultures for 24 h, the highest number of conidia (64 CFU/insect) were recovered from *E. postvittana* followed by *C. ulicetana* (17), *A. ulicis* (7) and *S. staphylinus* (0) at 0 h after the exposure ($P = 0.004$). Recovery

of *F. tumidum* generally declined with time after exposure. The conidia viability at the start of the experiment was 60%.

In the experiment using only *E. postvittana*, a similar trend of conidia recovery was observed. The highest number of 150 CFU/insect was recovered immediately after removing them from the cultures or 24 h after exposure to the conidia and the lowest recovered at 96 h after exposure to the conidia (Fig. 2). The rate of linear decline in the number of CFU/insect was significant ($P = 0.002$). In the deposition experiment, *E. postvittana* picked and deposited more conidia than *A. ulicis* and *C. ulicetana* ($P = 0.032$) (Fig. 3). *Cydia ulicetana* picked more conidia than *A. ulicis* but both species deposited similar amount.

Transmission by inoculated *E. postvittana*

The germination percentage of the conidial suspension applied on the insects was 76% representing 3,800 viable conidia in the 5,000 applied. About 3,600 *F. tumidum* CFU/insect were recovered 1 h after inoculation. This represented about 95% of the viable conidia. Insects washed at 4 and 7 DAI had 1,380 and 950 CFU/insect, respectively. The number of *F. tumidum* conidia recovered from the insects dropped significantly ($P < 0.001$) between day 0 and day 4 but remained relatively constant between day 4 and day 7.

The eight inoculated *E. postvittana* deposited a total of 2,480 conidia on each gorse plant, each insect depositing approximately 310 conidia. The number of conidia recovered from directly inoculated plants (9,990) was about four times more than that deposited by the inoculated *E. postvittana* ($P < 0.05$). No *F. tumidum* was recovered from the control treatments. None of the plants caged with inoculated *E. postvittana* developed lesions while all leaflets and apical meristems of directly inoculated plants developed lesions.

Combined effect of *F. tumidum* and *E. postvittana* on growth of gorse

Growth (i.e., shoot height and shoot fresh weight) was not significantly affected by the number of conidia deposited on the plants by the inoculated insects. The uninoculated insects did not affect plant growth compared with the negative control plants. Only the directly inoculated plants were significantly shorter with shoot fresh weight reduced by 42% compared with the negative control treatment (Table 1). Inoculum applied to the leaflets caused lesions at the site of application only and did not spread to adjacent leaflets. However, inoculum applied to the apical meristems caused stunting and dieback. There was a linear and negative correlation between the fresh weight of directly inoculated plants and the number of CFU of *F. tumidum* recovered from the plants ($R^2 = 60\%$). *Epiphyas postvittana* on its own did not cause any visible damage to the plants. Consequently, neither the shoot fresh weight nor height of plants receiving only *E. postvittana* was significantly different from those which had no insects.

Transmission from diseased to healthy gorse

Ninety two percent of the inoculated plants developed dieback and 17% died before *E. postvittana* were introduced. None of the uninoculated plants with/without *E. postvittana* developed dieback or died. The introduction of the insects did not increase

the number of dieback or dead plants. The inoculated plants were significantly shorter (before and after the introduction of the insects) and weighed about half the weight of the uninoculated plants (Table 2). The presence of the insects did not influence the height or the fresh weight of the plants. While inoculated plants with or without insects gained only 0.6 or 0.7 cm, respectively in shoot height in 15 days, the uninoculated plants with or without the insects increased by 4.5 or 4.9 cm, respectively over the same period. The effect of *E. postvittana* on shoot height and fresh weight was not significant. The difference in shoot height (before and after the introduction of the insects) was not significant for either inoculated plants with/without insects or uninoculated plants with/without insects.

Conidia recovery from gorse and disease development

The number of *F. tumidum* CFU recovered from each inoculated gorse without *E. postvittana* (25,880) was approximately two and a half times more than that recovered from inoculated gorse which had *E. postvittana* (10,130), however this was not significantly different ($P = 0.135$). No *F. tumidum* was recovered from the uninoculated gorse pair with/without *E. postvittana*. Consequently, none of the uninoculated gorse was infected or developed dieback. No *F. tumidum* conidia were recovered from the insects introduced to the inoculated plants and none of the control insects naturally carried any *F. tumidum* conidia. More insects were counted on the uninoculated gorse than the inoculated plants at each of the 7 days (Fig. 4).

DISCUSSION

To develop a novel strategy for delivering the biocontrol agent *F. tumidum* to infect gorse using insects as vectors, *E. postvittana* was selected among other potential gorse-inhabiting insect species (i.e., *A. ulicis*, *C. ulicetana* and *S. staphylinus*). *Epiphyas postvittana* was found to carry and deposit most *F. tumidum* conidia on agar and was found in previous study to have the greatest capacity to carry this pathogen (Yamoah *et al.* 2008b).

Table 1. Shoot fresh weight and shoot height of gorse plants after introducing inoculated *E. postvittana* or directly inoculating the plants with *F. tumidum* at 12 days after inoculation (* Values within a column followed by different letters are significantly different based on Fisher's protected LSD tests at $P < 0.05$)

Treatments	Fresh weight (mg)	Height (cm)
Gorse only	387 b [#]	69.0 b
Gorse with inoculated <i>E. postvittana</i>	365 b	74.8 b
Gorse with uninoculated <i>E. postvittana</i>	322 b	71.8 b
Gorse inoculated directly	226 a	54.4 a

Table 2. Shoot fresh weight and shoot height of 10 weeks old gorse at the time of inoculation with *F. tumidum* and assessed before and 13 days after introducing 20 *E. postvittana* († Gorse shoot height before *E. postvittana* were introduced; †† gorse shoot height at 13 days after the introduction of 20 *E. postvittana*/plant. # Values as described in Table 1)

Treatment	Fresh weight (g)	Height (cm)	
		Before [†]	After ^{††}
Inoculated gorse with <i>E. postvittana</i>	0.70 a [#]	8.5 a	9.1 a
Inoculated gorse without <i>E. postvittana</i>	0.63 a	8.2 a	8.9 a
Uninoculated gorse with <i>E. postvittana</i>	1.47 b	15.2 b	19.7 b
Uninoculated gorse without <i>E. postvittana</i>	1.71 b	14.9 b	19.8 b

Moreover, the availability of pheromone (Bellas *et al.* 1983) for attracting the male moths suggested that *E. postvittana* would fit well in the proposed 'lure-load-infect' strategy for gorse control.

When the insects were loaded with the conidia, they deposited significant numbers on gorse plants. However, they did not spread the conidia from diseased to healthy plants as only a few visited the diseased plants. Over the duration of this study, *E. postvittana* alone caused no significant damage to the gorse as the adults do not feed on the plant (which was expected). It is the larvae that feed on gorse foliage and have been shown to transmit grey mold (*Botrytis*) on grapes (Bailey *et al.* 1997). *Epiphyas postvittana* did not enhance the effect of the pathogen on plant growth relative to inoculated plants without the insects. The interaction between *E. postvittana* and *F. tumidum* appeared to be equivalent (i.e., the damage caused by the combination of both agents was equivalent to that caused by the pathogen alone). Equivalent interaction has been reported between the weevil *Perapion antiquum* (Coleoptera: Curculionidae) and the stem blight pathogen *Phomopsis emicis* on the annual weed *Emex australis* in growth room experiments (Shivas & Scott 1993).

The significant effect on growth of gorse observed from direct application of the pathogen to gorse confirms results of earlier pathogenicity tests (Yamoah *et al.* 2008a). This indicates that the isolate of *F. tumidum* used in this study can cause significant levels of disease on gorse. The significant reduction in shoot fresh weight and shoot height was mainly due to inoculum applied to the apical meristem. While application to the apical meristem caused shoot dieback and stunted growth, inoculum applied to the leaflets created lesions at the spot of application only. This implies that the site on gorse plant where the proposed insects would deposit the pathogen is critical in determining the severity of infection and the extent of damage caused. Gorse flowers have been shown to be more susceptible to *F. tumidum* infection than the spines, stem and pods (Yamoah *et al.* 2008a). Therefore, insects that preferentially visit the flowers may be more effective in controlling seed production if they can deposit enough conidia

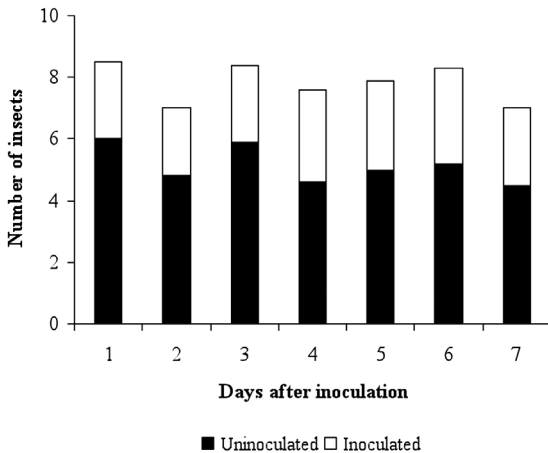


Figure 4. The number of *E. postvittana* on *F. tumidum*-inoculated and uninoculated gorse over 7 days. There were 20 insects per cage each containing an inoculated and an uninoculated gorse plant.

to infect the flowers. *Cydia ulicetana* might therefore be a more effective transmitter even though it carried less inoculum.

Previous pathogenicity trials have shown that about 900 viable conidia are required to form lesions on gorse leaves (Yamoah *et al.* 2008a). In the present study, the average deposition of conidia per insect on gorse was approximately a third of the required number for infection. Moreover, the conidia were probably not deposited at one site, as would be required to cause infection, but more likely were evenly deposited over the entire shoot. It is unrealistic to expect insects to deposit approximately 900 conidia at a close proximity to create infection. Hence, a more virulent pathogen requiring fewer spores to cause infection would be ideal for the 'lure-load-infect' strategy for gorse control. To date, there is no known gorse pathogen which is more virulent than *F. tumidum* (Johnston & Parkes 1994). The ideal pathogen for this novel strategy for weed biocontrol should not only be capable of causing infection with few spores but must be systemic. The use of such pathogen will minimise the number of spores required for infection. For example, a few spores of the fungus *Phaeoisariopsis personata* can infect groundnut (Wadia & Butler 1994).

The insects did not spread the pathogen from diseased to healthy plants. Broadhurst & Johnston (1994) reported that *F. tumidum* conidia are dispersed naturally between gorse plants by water-splash and this probably explains why the conidia were not easily picked by the insects, as opposed to wind dispersed spores like *Botrytis*. Loss of conidia viability is unlikely to account for the sharp decline in the number of conidia recovered from the insects although it cannot be ruled out completely. Reports indicate that a high proportion of dried conidia of *F. tumidum* can remain viable and pathogenic for up to 3 months at room temperature (Fröhlich *et al.* 1998).

The number of *F. tumidum* CFU recovered from the inoculated plants caged with *E. postvittana* was less than half the number recovered from inoculated plants with no insects, although the

difference was not statistically different due to wide variation among the replicates. This low number of conidia recovered from the inoculated plants in the presence of *E. postvittana* is probably due to dislodgment of conidia from the plants by the insects. It is less likely that this low number of conidia was due to transmission by the insects since *F. tumidum* colonies were not recovered from the insects or from the healthy plants. The fact that *F. tumidum* conidia were not recovered from the insects, even though some visited the infected plants is likely due to the low numbers of the insects which visited the inoculated plants. Insects that visited the inoculated plants might have picked only a few conidia of which the washing technique was not sensitive enough to detect.

Although using *E. postvittana* to vector *F. tumidum* did not result in infection of gorse, the results show that the success of this strategy of mycoherbicide delivery would depend on the pathogenicity and mode of infection of the pathogen. A highly virulent and systemic pathogen will ensure that the few spores carried and subsequently deposited by the insects will have a high chance of causing severe disease of the targeted weed. Moreover, a pathogen which is able to spread easily from the initial infection site by producing secondary spores would also be beneficial, whether these are spread by the insects or locally spread by water splash or wind. A classic example is the spread of *Botrytis* (which produces secondary spores) by *Thrips obscuratus* Crawford (Thysanoptera: Thripidae) to infect kiwifruit (Fermaud *et al.* 1994). *Fusarium tumidum* produces only a few secondary spores on gorse (Yamoah *et al.* 2008a) and therefore may not be amenable to spread by insects to cause secondary infection. A model system that could be used to investigate this concept further would be using classical biocontrol insects, *Neochetina* spp. (Coleoptera: Curculionidae) to transmit spores of the pathogen *Alternaria alternata* (Fr.) Keissler to control water hyacinth (*Eichhornia crassipes* (Mart.) Solms. The 'lure-load-infect' concept is laudable but remains to be demonstrated as a feasible concept for weed biocontrol, and will have a chance only if the right combination of weed, pathogen and insects is selected.

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