**The Potential of *Miscanthus* to Harbour Known Cereal Pathogens**

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**Abstract**

*Miscanthus* holds great potential as a bioenergy crop and Ireland has ideal conditions for its cultivation, however limited information is available about the interactions between *Miscanthus* and soil fungi which are pathogenic to other crops grown in Ireland and the UK. *Miscanthus* may therefore be susceptible to soil-borne pathogens present in the soil prior to crop establishment or may harbour pathogens and facilitate transmission of disease to other crops. The response of *Miscanthus* to a number of fungal species was recorded to determine the vulnerability of *Miscanthus* to some of the most important cereal pathogens in Ireland. The microbial species were selected based on their presence in soil and their known pathogenicity towards cereal crops currently grown in Ireland. A number of fungi caused a significant level of infection on detached *Miscanthus* leaves: *Rhizoctonia solani*, *Fusarium poae* (*Fusarium sporotrichiella* var. *poae*) and *Sordaria fimicola* caused the greatest level of symptoms while *Fusarium culmorum* caused the greatest visual disease symptoms in living tissue during whole plant tests. The results suggest that *Miscanthus* is susceptible to a number of cereal fungal pathogens, and that of all the species investigated

*Fusarium* species pose the greatest threat to *Miscanthus* plantings in Ireland. *Fusarium* is a known causative agent of blight in cereals, thus its ability to survive both on living and discarded *Miscanthus* tissue is important as it suggests that *Miscanthus* could act as a "disease bridge" for cereal pathogens.

**Keywords:** *Miscanthus*; fungal cereal pathogens; detached leaf test; whole plant test; Ireland.

**1. Introduction**

*Miscanthus* is a perennial rhizomatous grass with the $C_4$ photosynthetic pathway. The adaptability of *Miscanthus* to different environments makes this crop suitable for establishment under a range of European and North American climatic conditions (Lewandowski et al. 2000). Ireland's maritime climate, characterised by high rainfall, low summer temperatures, and relatively high winter temperatures, makes it an ideal location for *Miscanthus* cultivation. *Miscanthus* is planted in spring and once planted can remain *in situ* for at least fifteen years. Under ideal conditions crop yields of 7-10 t $ha^{-1}$ of *Miscanthus* can be achieved in Ireland (Meehan et al. 2012).

Limited information is available about diseases which affect *Miscanthus* (Stewart and Cromey 2011) though it is known that *Miscanthus* is susceptible to diseases in its native Asia (Caslin et al. 2011). A number of diseases have been observed on various *Miscanthus* species in plantations throughout the world including *Miscanthus* blight on *M. sinesis,* barley yellow dwarf luteovirus on *M. sacchariflorus*, and *Fusarium miscanthus* on *M. sinesis* straw (O'Neill and Farr (1996); Christian et al. (1994); Gams et al. (1999)). Research has shown that energy crops such as *Miscanthus* and willow (*Salix* sp) have phytoremediatory potential and may be an avenue for treatment of organic biosolids (Galbally et al. 2013), an additionally beneficial role to their use as alternative solid fuels. The cultivation of *Miscanthus* in Ireland

is a relatively new concept, and what interactions, if any, will arise between non-indigenous bioenergy crops and indigenous microorganisms is something that will only be revealed in time.

Some soil-borne species such as *Rhizoctonia* and *Pythium* are considered a threat to optimum *Miscanthus* yields. These species have a wide host range (Liu et al. 2011) and can affect growth and yield of both broadleaf and grass-type crops. *Rhizoctonia* root rot can be exacerbated by a lack of tillage as cultivation breaks up hyphal networks thereby reducing the size and inoculum potential of infested host residues (Paulitz et al. 2002). This factor could have a large bearing on a *Miscanthus* stand as there is no tillage involved in the lifecycle of the crop once it has been established. Root rot of *Miscanthus* has been reported in central Europe and in America, associated with *Pythium selvaticum* (Ahonsi et al. 2011) and *Pithomyces chartarum* (Ahonsi et al. 2010) as well as with *F. culmorum* and *F. proliferatum* (Goßmann 2000).

Ireland has a long tradition of cereal cultivation; the major crops grown in Ireland are barley, wheat, and oats (CSO 2013). There are a number of fungal pathogens associated with cereals crop grown in Ireland, specifically *Septoria tritici* (synonym: *Mycosphaerella graminicola*), *Tapesia acuformis* (synonym: *Oculimacula acuformis*), and *Rhynchosporium secalis* (O'Sullivan 2004). These pathogens have shown resistance to fungicides so may pose a threat to crops planted subsequently in the soil if the pathogens are not first eliminated. This is particularly relevant for energy crops with lifecycles without tillage, such as *Miscanthus*. *Fusarium* Head Blight (FHB) is a significant disease of commercial cereals which can cause mycotoxin production in the grain which is harmful to human health (HGCA 2008; Brennan et al. 2007). The causative agents of *Fusarium* blight depend on the growing environment: in northern Europe where temperatures are lower the main causative species

are *Microdochium nivale* and *M*. *majus* whereas in warmer climates *F*. *culmorum* and *F*. *graminearum* (synonym: *Gibberella zeae*) are more dominant (Glynn et al. 2007).

Ireland has a very limited supply of indigenous fossil fuels and relies on imports to meet its energy demand (Howley et al. 2012). Domestically-grown bioenergy crops could offer an alternative fuel option for Ireland, particularly as the local climate is ideal for growth of *Miscanthus.* Achieving optimum yields of *Miscanthus* biomass is dependent on a number of cultivation conditions, not least of which is the extent of disease penetration into a crop. If bioenergy crop cultivation were to be expanded significantly in Ireland it may encroach on land which had previously been used for cereal production, which may have an indigenous fungal pathogen population. What effect, if any, a population of microorganisms known to be pathogenic towards cereal crops will have on bioenergy crops is unknown. Furthermore, the ability of *Miscanthus* to act as a host for these cereal pathogens, storing and potentially passing on infections to subsequent or adjacent cereal crops is also currently unknown. The main objective of this study was therefore to isolate and identify important fungal and oomycete cereal pathogens in the soil-seed environment and to assess their ability to infect and survive on *M.* x *giganteus* biomass. Once the ability of these species to grow on *Miscanthus* has been established, conclusions can be drawn in relation to the potential risk to yields both of bioenergy and cereal crops which may result from the presence of fungi and oomycetes in the soil environment; that is, the potential of *Miscanthus* to act as a "disease bridge" for known cereal pathogens, facilitating transmission of diseases to subsequent or adjacent cereal crops.

## 2. Material and Methods

### *2.1. Isolation and Identification of Microbial Species*

#### *2.1.1. Soil Samples*

Soil samples used in this research were obtained from University College Dublin's research farm at Lyons Estate, Newcastle, Co. Dublin, Ireland. The samples were collected from a number of different fields within the farm which had different cropping and agricultural management histories (Table 1). A total of 16 management plans were sampled with three distinct soil samples collected from each management history using a soil corer. The three samples from each site were combined to form a single composite sample for each management history. Soil samples were stored at 4 °C prior to analysis.

Table 1: Agricultural management plans from which soil samples were collected

| Sample number | Crop | Cropping history | Sample collected |
|---|---|---|---|
| SS1 | Barley | No slurry applied | Soil only |
| SS2 | Barley | Biodiversity study | Soil only |
| SS3 | Barley | Slurry applied | Soil only |
| SS4 | Barley | No slurry + slurry (mix) | Soil only |
| SS5 | Wheat | Stubble | Soil only |
| SS6 | Wheat | Current crop | Soil only |
| SS7 | Grassland | Permanent | Soil only |
| SS8 | Grassland | Reseeded | Soil only |
| SS9 | Maize | Soil only | Soil only |
| SS10 | *Miscanthus* | Soil only | Soil only |
| SS11 | Willow | Soil only | Soil only |
| SS12 | Barley | Stubble only (no slurry) | Debris only |
| SS13 | Barley | Stubble only (slurry applied) | Debris only |
| SS14 | Wheat | Stubble only | Debris only |

| | | | |
|---|---|---|---|
| SS15 | Maize | Stubble only | Debris only |
| SS16 | *Miscanthus* | Stubble only | Debris only |

Soil samples were prepared according to a similar method to that used by Khan et al. (2004). Briefly, 5 g of each composite soil sample was added to 100 ml of sterile water and agitated for 5 min before 100 µl aliquots were spread onto potato dextrose agar (PDA) containing streptomycin as a bacterial growth inhibitor. Four replicates of each soil sample were prepared of which two were incubated at 15 °C and two were incubated at 25 °C. Fungal and oomycete growth was monitored daily and any emerging colonies were sub-cultured onto fresh PDA plates and returned to their original incubation temperatures to obtain pure cultures through repeated sub-culturing and incubation.

*2.1.2. Seed Samples*

The seed samples used in this study were obtained from the Plant Health Laboratory, Department of Agriculture, Food and the Marine located at Backweston Campus, Celbridge, Co. Kildare, Ireland. A total of 42 seed samples were analysed which included four different seed types: 21 barley samples, 19 wheat samples, 11 oat samples, and 1 tritosecale sample (Table 2).

Table 2: Seed type, cultivar and origin of seeds tested for presence of fungal and oomycete species

| Seed Sample | Seed Type | Cultivar | Irish County of Origin |
|---|---|---|---|
| SC1 | Barley | Saffron 2008 | Wicklow |
| SC2 | Barley | Camion 2008 | Louth |
| SC3 | Barley | Snakebite 2008 | Louth |
| SC4 | Wheat | Alceste 2008 | Dublin |
| SC5 | Wheat | Einstein 2008 | Dublin |

| | | | |
|---|---|---|---|
| SC6 | Wheat | ELS/05/43 | Dublin |
| SC7 | Wheat | Expert 2008 | Dublin |
| SC8 | Wheat | Raffles 2008 | Louth |
| SC9 | Barley | Cocktail 2008 | Louth |
| SC10 | Barley | Maglay 2008 | Louth |
| SC11 | Wheat | Sahara 2008 | Louth |
| SC12 | Wheat | Granary 2008 | Louth |
| SC13 | Wheat | Byron 2008 | Louth |
| SC14 | Wheat | Alceste 2008 | Cork |
| SC15 | Wheat | Alchemy 2008 | Cork |
| SC16 | Wheat | Lion 2008 | Cork |
| SC17 | Barley | Centurion 2008 | Cork |
| SC18 | Barley | Maglay 2008 | Cork |
| SC19 | Barley | Maglay 2008 | Cork |
| SC20 | Barley | Snakebite 2008 | Louth |
| SC21 | Wheat | Byron 2008 | Kilkenny |
| SC22 | Wheat | S5311 | Kilkenny |
| SC23 | Wheat | Granary 2008 | Kilkenny |
| SC24 | Wheat | Trappe | Kilkenny |
| SC25 | Barley | Prestige 2008 | Cork |
| SC26 | Barley | Riviera 2008 | Cork |
| SC27 | Barley | Anakin 2008 | Cork |
| SC28 | Barley | Snakebite | Kilkenny |

| | | | |
|---|---|---|---|
| SC29 | Barley | Cocktail | Kilkenny |
| SC30 | Oats | Huskey | Kilkenny |
| SC31 | Barley | Sequel 2008 | Waterford |
| SC32 | Oats | Huskey | Kilkenny |
| SC33 | Oats | Barra | Kilkenny |
| SC34 | Oats | Corrib | Waterford |
| SC35 | Oats | Huskey | Waterford |
| SC36 | Oats | Huskey | Waterford |
| SC37 | Oats | Huskey | Waterford |
| SC38 | Oats | Corrib | Waterford |
| SC39 | Oats | Corrib | Waterford |
| SC40 | Oats | Huskey | Waterford |
| SC41 | Tritosecale | Fidelio | Waterford |
| SC42 | Wheat | Lion | Meath |

The seed samples were analysed as described by the International Seed Testing Association (ISTA 2008). Briefly, the seeds were pre-treated by immersing in NaOCl solution (1% available chlorine) for 10 min before rinsing with sterile distilled water. The seeds were then aseptically transferred to PDA containing streptomycin as a bacterial growth inhibitor and the plates were incubated at 20 °C for seven days.

*2.1.3. Morphological and PCR Analyses*

Morphological analysis of colonies isolated from soil and seed samples was conducted in a similar manner to that used by Khan et al. (2004) by taking a small scraping of the colony, placing it on a glass slide, and examining the cells at 400x magnification on a light

microscope. Spores and mycelia were captured and analysed using Olympus Image Analysis Software (Olympus, UK).

DNA extraction was conducted using a similar method to that used by Doohan et al. (1999). Briefly, fresh mycelium was placed in a 2 ml Eppendorf tube before being freeze dried for 24 hours. A metal ball bearing was added to each tube and the samples placed in a tissue lyser for 1 min; the ball bearings were then removed and 1 ml of 65 °C CTAB (hexadecyltrimethylammonium bromide) buffer was added and the tubes vortexed gently before being placed in a thermal shaker at 65 °C for 10 min. An equal volume of 1:1 mixture of phenol:choloform/isoamyl alcohol (24:1) was added and the sample vortexed and centrifuged. The aqueous layer was removed and transferred to a new tube where a further volume of chloroform/isoamyl alcohol was added and the sample centrifuged again. The aqueous layer was removed and transferred to a new tube and a quantity of -20 °C isopropanol equal to 70% of the sample volume was added and the samples placed in a -20 °C freezer for 20 min. The samples were centrifuged two additional times before the alcohol was removed and the pellets were dried and dissolved in Tris EDTA (10 mM Tris-HCl, 1mM EDTA) buffer.

PCR analysis was used to confirm morphological identification of the fungi and oomycetes. PCR reactions were performed in volumes of 50 μl and contained DNA from appropriate reference isolates as a positive control (10ng $\mu l^{-1}$); 10x PCR buffer (Invitrogen, UK); 100 μM each of dATP, dCTP, dGTP, and dTTP; 100 pM of relevant species-specific forward and reverse primers; 0.8 units of *Taq* DNA polymerase (Invitrogen, UK); and 1.5 mM $MgCl_2$. Reaction mixtures were overlaid with mineral oil and amplification was performed in a DNA engine (MJ Research, USA). 10 μl aliquots of the amplification product were electrophoresed through a 1.5% agarose gel prepared using a 1x TAE buffer (40 mM Tris

base, 1 mM EDTA, and 20 mM acetic acid) and which contained 0.05 mg ethidium bromide per 100 ml TAE buffer. The resulting PCR products were visualised on a G: Box Chemi transilluminator (Syngene, USA).

The following PCR primers were used in this study: *F. poae* (Fp82 F/R) (Parry and Nicholson 1996); *F. culmorum* (Fc01 F/R) (Nicholson et al. 1998); *F. graminearum* (Fg16 F/R) (Nicholson et al. 1998); *Microdochium nivale* var*. nivale* (Y13N F/R) (Nicholson et al. 1996); *M. nivale* var. *majus* (MnM2 F/R) (Nicholson and Parry 1996); *R. secalis* (RS8 F/RS1 R) (Lee et al. 2001); *R. cerealis* (Rc1 F/R) (Nicholson and Parry 1996); *P. ultimum* (K1 F/K3 R) (Kageyama et al. 1997); *T. acuformis* (TY16 F/R) (Nicholson et al. 1997); and *S. tritici* (MAT1-1 F/R) (Waalwijk et al. 2002). Reference isolates obtained from the UCD isolate collection (School of Biology & Environmental Science, University College Dublin, Ireland) and from the isolate collection held by Teagasc (Oak Park Crops Research Centre, Carlow, Ireland) were used as positive controls in the PCR analysis; their details are listed in Table 3. The identity of *Ascochyta graminicola*, *Cladosporium herbarum* (synonym: *Mycosphaerella tassiana*), *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*), and *Sordaria fimicola* was determined using morphological analysis only.

Table 3: Reference isolates used in the identification of species present in soil and seed samples

| Isolate code | Geographic origin | Species |
|---|---|---|
| FCF200 | UK | *Fusarium culmorum* |
| CC339B | Ireland | *F. poae* (synonym: *F. sporotrichiella var. poae*) |
| O68 | Italy | *F. avenaceum* (synonym: *Gibberella avenacea*) |

| | | |
|---|---|---|
| M7A | Ireland | *F. avenaceum* |
| M7B | Ireland | *Microdochium nivale* var *majus* |
| S048/1/N | UK | *M. nivale* var *nivale* |
| L18C | Ireland | *F. graminearum* |
| SS10 | Ireland | *Septoria tritici* |
| WAT8 | Ireland | *S. tritici* |
| SS13 | Ireland | *S. tritici* |
| PU-1 | n/d[a] | *Pythium ultimum* |
| RSO-1 | n/d | *Rhizoctonia solani* |
| GG-1 | n/d | *Gaeumannomyces graminis* (synonym *Ophiobolus graminis*) |
| DT-1 | n/d | *Dreschlera teres* (anamorph: *Pyrenophora teres*) |
| RS-1 | n/d | *Rhynchosporium secalis* |
| TA-1 | n/d | *Tapesia acuformis* |
| RC-1 | n/d | *R. cerealis* (synonym: *Ceratobasidium cereale*) |
| AG-1 | n/d | *Ascochyta graminicola* |
| IS14 | Ireland | *F. culmorum* |
| IS20 | Ireland | *F. graminearum* |
| IS13 | Ireland | *Alternaria alternata* |
| IS12 | Ireland | *Cladosporium herbarum* |
| PHL45 | Ireland | *M. nivale* var *majus* |

| | | |
|---|---|---|
| PHL67 | Ireland | *M. nivale* var *nivale* |
| PHL34 | Ireland | *M. nivale* var *nivale* |
| PHL59 | Ireland | *M. nivale* var *majus* |
| PHL60 | Ireland | *F. culmorum* |
| PHL31 | Ireland | *F. poae* |
| PHL05 | Ireland | *F. poae* |
| PHL11 | Ireland | *F. graminearum* |
| PHL62 | Ireland | *F. graminearum* |
| PHL23 | Ireland | *A. alternata* |
| PHL06 | Ireland | *A. alternata* |
| PHL53 | Ireland | *Bipolaris sorokiniana* |
| PHL48 | Ireland | *B. sorokiniana* |
| PHL66 | Ireland | *C. herbarum* |
| PHL52 | Ireland | *Sordaria fimicola* |

[a] n/d = not determined

*2.2. Detached Leaf Test*

The response of *M.* x *giganteus* to soil- and seed-borne fungi and oomycete species was assessed using a detached leaf test. A total of 19 species (37 isolates) were chosen for the detached leaf test (Table 4). The species investigated were selected on the basis of (i) their known pathogenicity towards cereal crops in Ireland; (ii) previous reports of their ability to cause infection of *M*. x *giganteus* biomass; or (iii) their common presence in Irish tillage environments.

Fungi were grown on PDA and *P. ultimum* was grown on corn meal agar; all plates were incubated at 20 °C. Inoculants for the detached leaf test were produced by harvesting conidia/sporangia from the surface of sporulating cultures into sterile distilled water. The spore concentration was calculated using a haemocytometer (Hycor Biomedical, UK) and was diluted using 0.2% (v/v) Tween 20 (Sigma, UK) to give a final concentration of $1 \times 10^5$ $ml^{-1}$.

Table 4: Species used in detached leaf and whole plant testing of *M*. x *giganteus.* Number of isolates of each species used in the detached leaf testing is detailed in parentheses.

| Species | Detached leaf testing | Whole plant testing |
|---|---|---|
| *Alternaria alternata* | ✓ (3) | |
| *Ascochyta graminicola* | ✓ (1) | |
| *Bipolaris sorokiniana* | ✓ (2) | |
| *Cladosporium herbarum* | ✓ (2) | |
| *Drechslera teres* | ✓ (1) | ✓ |
| *Fusarium avenaceum* | ✓ (2) | |
| *F. culmorum* | ✓ (3) | ✓ |
| *F. graminearum* | ✓ (4) | ✓ |
| *F. poae* | ✓ (3) | |
| *Gaeumannomyces graminis* | ✓ (1) | |
| *Microdochium nivale* var. *majus* | ✓ (3) | ✓ |
| *M. nivale* var. *nivale* | ✓ (3) | ✓ |
| *Pythium ultimum* | ✓ (1) | |
| *Rhizoctonia cerealis* | ✓ (1) | |

| | | |
|---|---|---|
| *Rhizoctonia solani* | ✓ (1) | |
| *Rhynchosporium secalis* | ✓ (1) | ✓ |
| *Septoria tritici* | ✓ (3) | ✓ |
| *Sordaria fimicola* | ✓ (1) | |
| *Tapesia acuformis* | ✓ (1) | |

Response of the cultivar *M.* x *giganteus* towards known cereal pathogens and other fungal and oomycete species was assessed using a modified method of Brennan et al. (2005). *Miscanthus* leaves were cut into 4 cm segments and surface sterilised in NaOCl solution (1% available chlorine) for 10 min before being rinsed in sterile distilled water. Two segments were then placed on the surface of 0.5% (w/v) water agar containing 10 mg $l^{-1}$ kinetin (Sigma, UK). The leaf segments were pierced on the midrib of the adaxial surface and inoculated with a 15 μl droplet of spore suspension. Control conditions were obtained by inoculating with 15 μl of 0.2% (v/v) Tween 20 solution. All plates were incubated at 20 °C and the leaves were examined four times post-inoculation (on day three, day five, day seven, and day ten) and the extent of infection, if any, was determined by measuring the length and width of each lesion and subsequently calculating the total area of leaf infection. Each inoculation treatment was replicated four times and the experiment was conducted twice.

Normal data distribution (lesion growth rate, LGR) was confirmed using Minitab© 15.0 statistical software (Minitab Ltd., UK). The correlation coefficients between replicates were determined using the Pearson Product Moment Coefficient. Analysis of variance incorporating Tukey's pairwise comparison at the 5% level of significance was performed using SPSS© 18 statistical software (SPSS Inc., USA).

*2.3. Whole Plant Test*

A whole plant test based on a modified method of Brennan et al. (2005) was used to evaluate the response of *M.* x *giganteus* to fungal isolates. The rhizomes used in this assessment were obtained from a two year old plot on the UCD research farm located at Lyons Estate, Newcastle, Co. Dublin, Ireland. The rhizomes were planted in 14 cm diameter pots with a mixture of 80% sterile compost and 20% sterile soil. The plants were grown to 3-4 leaf growth stage prior to inoculation.

Isolates of seven fungal species (Table 4) were chosen for the whole plant test based on known economic importance in the Irish cereal industry and their common presence in Irish agricultural soils (O'Sullivan 2004; Dardis and Walsh 2002). Inoculants for the whole plant test were produced by harvesting conidia/sporangia from surfaces of sporulating cultures into sterile distilled water. The spore concentration was calculated using a haemocytometer and was diluted using 0.2% (v/v) Tween 20 to give a final concentration of $1 \times 10^5$ $ml^{-1}$. Leaves on each of the test plants were pierced in the midrib of the adaxial surface and inoculation with 10 ml of spore suspension was conducted using a hand-held nozzle sprayer; each plant was inoculated with a single microbial species. Control plants were sprayed with 0.2% (v/v) Tween 20 solution. The plants were enclosed in a polythene bag for 48 hr to increase humidity and promote disease development. Following inoculation, the treatments were randomly arranged in a glasshouse. Plant response was determined by recording the lesion growth rate in cm $day^{-1}$ on the first three leaves of each plant on days 7, 14, and 21 post-inoculation: the length and width of each lesion were measured to determine the total area of leaf infection. Each treatment was replicated three times and the experiment was conducted twice.

Normal data distribution of LGR was confirmed using Minitab© 15.0 statistical software. The correlation coefficients between replicates were determined using the Pearson Product

Moment Coefficient. Analysis of variance incorporating Tukey's pairwise comparison at the 5% level of significance was performed using SPSS© 18 statistical software.

## 3. Results

### *3.1. Isolation and Identification of Fungal Species*

Species diversity in soil in this study was low: only four fungal genera were identified. Of the sixteen soil samples analysed fungi were only identified in eight samples. PCR and morphological analyses identified ten isolates belonging to four genera: *F. graminearum* (3 isolates), *F. culmorum* (1 isolate), *C. herbarum* (2 isolates), and *A. alternata* (4 isolates). No one species was present in all of the soil samples examined (Table 5).

Table 5: Species isolated from soil samples

| | Species isolated | | | |
|---|---|---|---|---|
| Management history | *Fusarium graminearum* | *Fusarium culmorum* | *Cladosporium herbarum* | *Alternaria alternata* |
| Barley - soil (no slurry) | | | | ✓ |
| Barley - soil (biodiversity study)* | | | | |
| Barley - soil (slurry applied)* | | | | ✓ |
| Barley - soil (slurry/no slurry mix) | | | | |
| Wheat stubble – soil | | | ✓ | ✓ |
| Wheat current crop - soil* | | | | |
| Grassland (permanent) - soil** | | | | |
| Grassland (reseeded) - soil* | | | | |
| Maize - soil** | | | | |
| *Miscanthus* – soil | | | ✓ | ✓ |

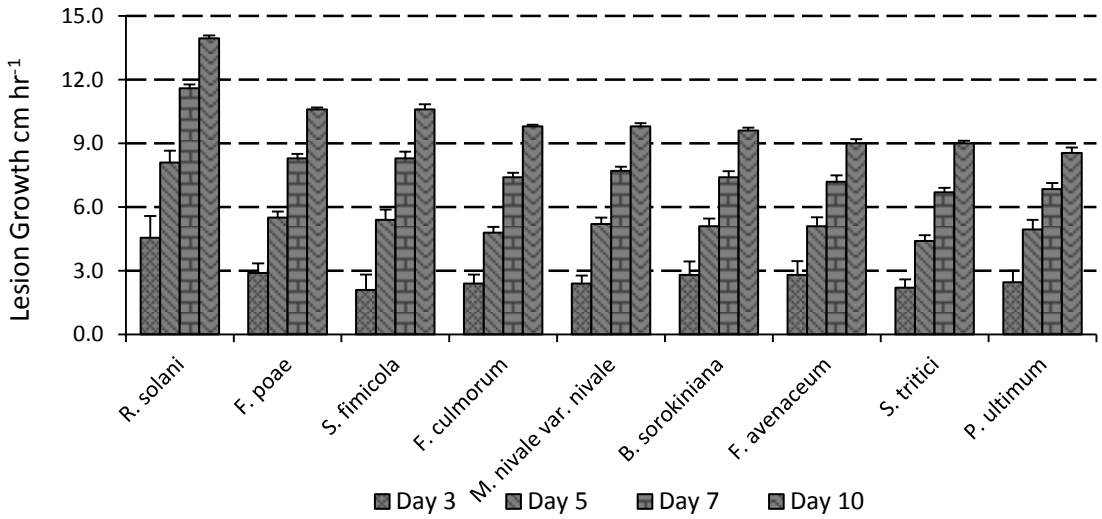
| | | |
|---|---|---|
| Willow – soil | | ✓ |
| Barley stubble (no slurry) – debris | ✓ | |
| Barley stubble (slurry applied) - debris** | ✓ | |
| Wheat stubble - debris** | | |
| Maize stubble - debris* | | |
| *Miscanthus* stubble – debris | ✓ | |

* = fungal species present but identity not confirmed; ** = *Fusarium* species present but identity not confirmed

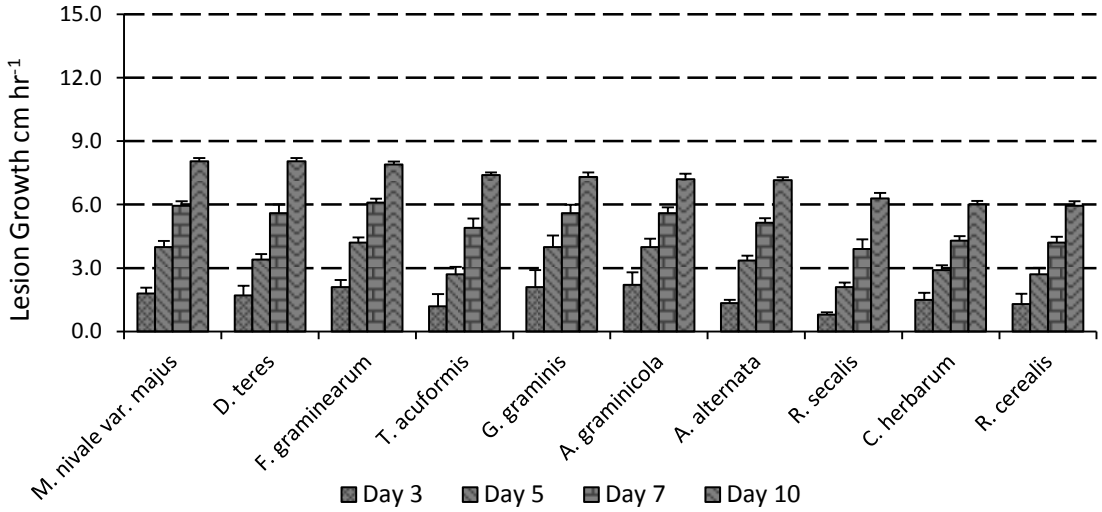
A greater level of species diversity was recorded from seed samples than from soil samples: 68 isolates belonging to six distinct genera were identified: *Alternaria* spp., *Bipolaris* spp., *Cladosporium* spp., *Fusarium* spp., *Microdochium* spp., and *Sordaria* spp. The most abundant species present was *M. nivale*; this fungus was present in 24 of the 42 seed samples. *A. alternata* was the second most prevalent species identified and was recorded in 13 of the 42 samples. *F. graminearum, F. poae*, and *F. culmorum, C. herbarum*, *B. sorokiniana*, and *S. fimicola* were also isolated from the seed samples.

*3.2. Detached leaf tests*

The disease symptoms caused by the fungal and oomycete isolates varied and included brown lesions, premature necrosis, and water-soaked patches. In general all 19 pathogens caused some level of infection in the detached leaf assay (Figure 1).




(a)




(b)

Figure 1: The response of detached *M*. x *giganteus* leaves to 19 fungal and oomycete species 3, 5, 7, and 10 days post inoculation, respectively: (a) depicts the nine more aggressive species and (b) depicts the ten less aggressive species.

The greatest level of disease symptoms was observed on leaves inoculated with *R. solani*, *F. poae* and *S. fimicola*. *R. solani* showed the greatest level of disease symptoms on day 3 with its rate of infection decreasing over time (mean LGR day 3 = 4.6 cm day$^{-1}$, day 10 = 2.4 cm day$^{-1}$). *F. poae* also displayed this trend (mean LGR day 3 = 2.9 cm day$^{-1}$, day 10 = 2.3 cm day$^{-}$

$^{1}$) as did *B. sorokiniana* where day 3 mean LGR (2.7 cm day$^{-1}$) was greater than day 10 mean LGR (2.2 cm day$^{-1}$). *S. fimicola* showed a peak in mean LGR on day 5 (3.3 cm day$^{-1}$) and then declined over the rest of the incubation to a mean LGR of 2.3 cm day$^{-1}$ on day 10. Conversely, *R. secalis*, which caused the least visual disease symptoms, followed a trend of increasing mean LGR over the incubation period (mean LGR day 3 = 0.7 cm day$^{-1}$; day 10 = 2.4 cm day$^{-1}$). This trend was also observed for *T. acuformis* (mean LGR day 3 = 1.3 cm day$^{-1}$; day 10 = 2.5 cm day$^{-1}$).

Three days post inoculation there were significant differences between the LGRs of the greatest and least aggressive species ($p$<0.05); this difference was also observed five days post inoculation ($p$<0.05). At seven days post inoculation, *R. solani* had caused significantly greater levels of disease symptoms than seven of the nine least aggressive species ($p$<0.05), however ten days post inoculation there were no significant differences between the 19 species.

*3.3. Whole plant tests*

Visual disease symptoms observed on the *M.* x *giganteus* leaves in this experiment included brown lesions and necrosis. Only three of the seven fungi investigated caused visual disease symptoms on the leaves: no visual disease symptoms were observed on plants inoculated with *D. teres*, *S. tritici*, *R. secalis,* or *M. nivale* var. *nivale*.

The level of infection among the three fungi increased steadily to day 14 post-inoculation; a reduction in lesion growth rate was noted between day 14 and day 21 (mean LGR day 14 = 0.7, 0.5, and 0.4 mm day$^{-1}$, respectively; mean LGR day 21 = 0.3, 0.2, 0.2 mm day$^{-1}$, respectively) (Figure 2). Of the three species, *F. culmorum* caused significantly more visual disease symptoms on the *M. giganteus* leaves throughout the experiment ($p$<0.05); *F. graminearum* caused slightly, though not significantly ($p$>0.05), more disease symptoms

than *M*. *nivale* var *majus*. Despite the reduction in LGR at the end of the experiment, this trend was still evident with a higher mean LGR on day 21 for *F. culmorum*, than *F. graminearum* and *M. nivale* var. *majus.*

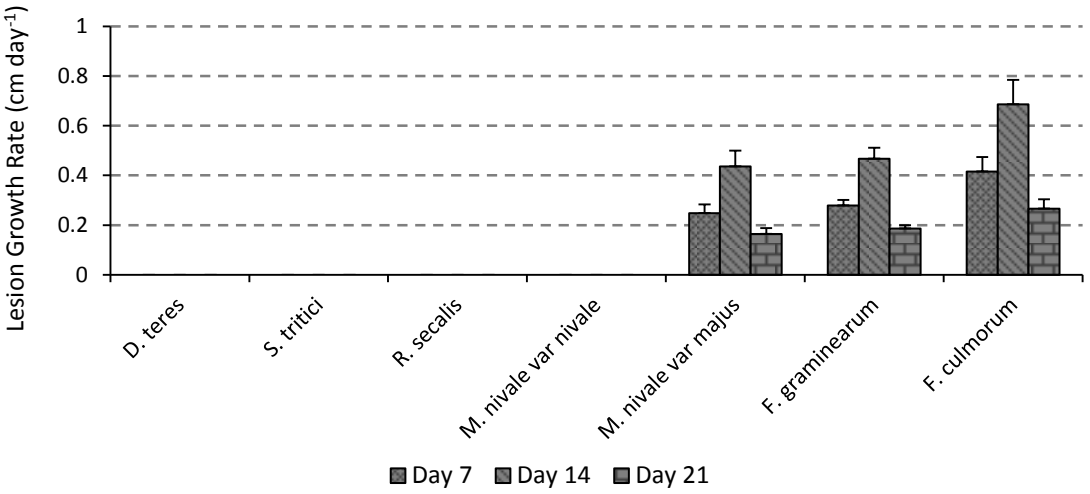



Figure 2: The response of whole *M*. x *giganteus* plants to 7 known cereal pathogens 7, 14, and 21 days post-inoculation, respectively.

**4. Discussion**

A variety of fungal genera were isolated from soil samples including *Fusarium*, a known cereal pathogen. Three of the eight samples in which fungi were present contained stubble, known to be an overwintering habitat for many cereal pathogens including *Fusarium* species (HGCA 2008). *F*. *graminearum* was isolated from *Miscanthus* stubble which is of particular interest to this study: although this does not infer that the fungus infected the living plant it shows that it is capable of colonising *Miscanthus* debris which may have implications for the transfer of diseases from *Miscanthus* stands to adjacent or subsequent cereal crops.

In the detached leaf tests all 19 species investigated caused some level of visual disease symptoms with *R. solani*, *F. poae* and *S. fimicola* causing the greatest disease symptoms. The five causative agents of FHB which were assessed (*F. culmorum, F. graminearum, F. poae, F. avenaceum,* and *M. nivale*) each caused similar levels of visual symptoms on the *M.* x

*giganteus* leaves. Both *R. solani* and *Fusarium* spp. have been described as having the ability to cause “tremendous economic damage” (Liu et al. 2011). These results are therefore of great importance: the observation that detached *Miscanthus* biomass may act as a reservoir for cereal pathogens could have serious implications for the transfer of disease to subsequent or adjacent cereal crops. *Fusarium* has been associated with root rot of *Miscanthus* (Goßmann 2000) as well as with blight of cereals (Brennan et al. 2003; Brennan et al. 2007) and so may be transmitted through *Miscanthus* debris. Three of these five species also caused infection in living *Miscanthus* tissue during whole plant testing. This result contributes to the argument that *Miscanthus* could act as a “disease bridge” for cereal diseases and transmit pathogens to subsequent or adjacent cereal crops.

*R. secalis* and *S. tritici*, both of which are known cereal pathogens in Ireland and caused increasing levels of visual disease symptoms on detached leaves, did not cause disease symptoms in *Miscanthus* leaves on whole plants. These results suggest that there is no immediate threat to *Miscanthus* yields in Ireland from these common fungal cereal pathogens. This may be financially significant as these pathogens have shown increasing resistance to pesticides (O'Sullivan 2004) and could prove costly to control over the lifespan of *Miscanthus*, had the opposite results been observed.

**5. Conclusions**

Combining the results of the detached leaf and the whole plant assays and the results of species isolation from soil and seed samples, it becomes apparent that *Fusarium* is capable of colonising *Miscanthus* debris and of negatively influencing *Miscanthus* biomass development. Under the conditions investigated, this suggests that *Fusarium* species may become important pathogens of *Miscanthus* plantings in Ireland.

These results will also have implications for cereal crops grown subsequently or adjacent to *Miscanthus* stands as *Fusarium* spp. are known causal agents of blight in cereals. These results indicate that not only could *Miscanthus* yields be vulnerable to *Fusarium* diseases but it may also act as a "disease bridge" allowing the transmission of cereal pathogens from bioenergy crops.

**6. References**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**7. Table and Figure Captions**