SHORT COMMUNICATION

COLONIZATION TYPE OF *GIBBERELLA ZEAE* IN *SORGHUM BICOLOR*

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SUMMARY

The susceptibility of grain sorghum (*Sorghum bicolor*) to colonization by *Gibberella zeae* was assessed by isolation studies involving plants grown in the glasshouse. Studies on infection of sorghum seedlings by *G. zeae* indicated that this pathogen can infect the host at early growth stages and gradually colonize adjacent tissues as an endophyte. The results also showed that roots as well as stem tissues are susceptible to infection. Indeed, the result suggested that the fungus could infect and colonize the proximal parts of roots more aggressively than the leaf sheaths and stem tissues.

Key words: *Gibberella zeae*, grain sorghum, colonization type, seedling infection.

Gibberella zeae (Schwein.) Petch (Anamorph: *Fusarium graminearum* Schwabe), the dominant pathogen causing head blight disease of wheat has been isolated occasionally from sorghum (Reed *et al.*, 1983; Trimboli and Burgess, 1985; Burgess *et al.*, 2002). The field studies reported by Reed *et al.* (1983) indicated that *G. zeae* infects and colonizes sorghum plants frequently late in the growing season rather than in the seedling stage. Moreover, sorghum has been reported as both a host and an important alternative host of *G. zeae* as the fungus has been observed to form perithecia abundantly on senescent sorghum stalks and on sorghum stubble (Goswami and Kistler, 2004; Burgess *et al.*, 2002). It is possible that *G. zeae* is able to infect young sorghum seedlings, develop endophytically within the plant and then cause stalk rot late in the season in a way similar to *Fusarium thapsinum* (Edmund *et al.*, 1970). Alternatively, it may colonize the outer tissues of senescent sorghum stalks from ascospore inoculum or infested crop residues and then form perithecia under favourable conditions when the temperature range is 15-25°C combined with a 24 h period of wetness (Dufault *et al.*, 2002; Parry *et al.*, 1995). As sorghum is commonly grown in rotation with wheat (*Triticum aestivum*) which is a primary host of *G. zeae* (Burgess *et al.*, 1987; Bai and Shaner, 1994; Parry *et al.*, 1995), it is important to understand the relationship between sorghum and this pathogen. Therefore, the study reported in this paper was initiated to test the hypothesis that *G. zeae* is able to infect and colonize young sorghum seedlings, and colonizes throughout the growing period under optimal conditions.

In preliminary experiments to develop the methods, inoculum was prepared in such a way as to be equivalent to inoculum present in the field (Burgess and Griffin, 1968). There were two treatments, inoculated and uninoculated, and two sampling times, 2 weeks (seedling stage) post-inoculation and 4 weeks (booting stage) post-inoculation. There were 20 pots per treatment, with 5 pots allocated to each of 4 blocks. In each block, 5 pots were randomly assigned to be inoculated (using GenStat, 'Generate a Standard Design') and the remaining 5 pots were uninoculated. There were 2 plants per pot. Within each pot 1 plant was randomly assigned to be sampled 2 weeks after inoculation and the other plant to be sampled 4 weeks after inoculation. The experiment was carried out in a controlled-environment glasshouse where the temperature was maintained at 25±2ºC. A data recorder (Gemini Tinytag) was used to monitor temperatures at the soil surface adjacent to the point of inoculation of sorghum seedlings during the experimental period.

A composite sample of 100 seeds of sorghum cv. Pacer was collected and assessed for the presence of *Fusarium* species according to Burgess *et al.* (1994), based on colony pigmentation produced either in the medium or in aerial mycelia, the shape, size, mode of formation of macroconidia and microconidia, the nature of conidiogeneous cells, septation of conidia, chlamydospores, and perithecia if they were produced in the sample. Fourty black cylindrical plastic pots (18.5 cm diameter x 19 cm height), containing 7.97 g cm^{-2} potting mix (Pot N peat, Amgrow, Envirogreen Pty. Australia) and $1.4x10^{-4}$ g cm^{-2} water storing crystals (Rain Saver, Hortex Aust.Pty. Australia), were established 3 days prior to

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sowing. The pots were watered to saturation and allowed to drain freely for 48 h, thus approximating field capacity (Cassell and Nielsen, 1986). A white plastic cover was placed on each pot. Two holes (3.3 cm diameter) were cut in each cover through which seedlings developed. The covers were designed to minimize loss of soil moisture.

Seeds of sorghum cv. Pacer were planted at two opposing positions within each pot, at a depth of 5 mm. Two weeks after germination the plants were supported by a small wooden stake, 2 mm in diameter. A liquid nutrient solution (2.2 g l^{-1}; Aquasol, Hortico Pty, Australia) at the rate 20 ml per pot was added every 7 days during watering. Mature wheat (*Triticum aestivum*) stems, cv. Strezlecki, were cut into 5 cm lengths and placed into a 500 ml beaker containing Potato Agar Broth. The beakers were covered with aluminium foil and autoclaved at 121ºC for 20 min followed by refrigeration overnight at 4ºC. The broth was then drained off and the stems inoculated by mixing with conidial suspension of *G. zeae* (F-13833) in the beaker. The inoculated stems were incubated under fluorescent light for 6 days, then washed by shaking gently in sterile water until all the external fungal mycelium was removed. The excess water was drained off and stems incubated under fluorescent light as described above for a further 2 days to encourage nutrient depletion. The uninoculated stems were prepared following the same procedure as inoculated stems but without inoculation with the pathogen. The stems were then air-dried for 3 days.

The air-dried wheat stem pieces colonized by *G. zeae* were used to inoculate sorghum seedlings 4 weeks after sowing (1 stem piece per plant). One wheat stem piece was placed horizontally on the soil surface so that it was in contact with the stem base of a sorghum seedling. A wooden toothpick was inserted in the soil next to the wheat stem piece to maintain contact of the stem piece with the sorghum stem. Uninoculated (control) wheat stem pieces were also placed next to the sorghum stems in control pots. Parafilm was then placed over the holes in the cover and around the sorghum seedlings to maintain high humidity at the point of inoculation. This inoculation technique was developed to simulate the contact of sorghum seedlings with infested residues under a no-tillage situation in the field. Fourty plants in 20 pots were inoculated and 40 control plants in an additional 20 plots were uninoculated.

Isolations were made 2 weeks (20 plants) and 4 weeks (20 plants) post inoculation (wpi) from both inoculated and uninoculated plants. A mark was made with a black pen adjacent to the site of contact between the stem piece infested with *G. zeae* and the sorghum stem. The plants to be sampled were removed carefully from the soil without affecting the remaining seedlings, and stored in paper bags at 5ºC until plated out (no later than 24 h). The plants were then washed under tap water to remove all soil. Stalk samples 5 mm long were taken from the site of inoculation and 5 mm above it. The leaf sheaths were then removed from the sorghum samples and numbered serially from the outer surface of the plant toward the stem.

A fragment approximately 5 mm in length was excised from each root, adjacent to the crown of each plant. All parts from each section including leaf sheaths, stem and root were surface-sterilized with 2% $NaClO_2$, rinsed with sterile water, air-dried, plated on modified potato dextrose agar (MPDA) and incubated at 25ºC (day) and 20ºC (night) with a 12 h photoperiod (Burgess *et al*., 1994) for 5 days. The components of the basal medium of MPDA were the same as PDA but the concentration of potato broth and dextrose used was halved and only 15 g agar were added.

The colonies that produced red/pink pigment on MPDA were transferred onto carnation leaf-piece agar (CLA) and incubated for 10 days. Colonies of *G. zeae* were identified based on conidial morphology (slender, falcate, 5-6 septate macroconidia, with a tapered apical cell and foot-shaped basal cell, lack of microconidia and chlamydospores) and the production and nature of perithecia (Burgess *et al.*, 1994). Sections from uninoculated plants were excised following the same procedure as for inoculated plants and plated out. Infected wheat stem pieces (inoculum) were also plated onto MPDA to confirm inoculum viability. Analysis of variance (one-way no blocking) was performed using GenStat to analyse isolation frequency of *G. zeae* from sorghum at 2 weeks and at 4 weeks pi.

No *Fusarium* species were isolated from the sample of 100 sorghum seeds that were plated, showing that the seed used was clean. From some of the plants, *G. zeae* was recovered from the sites of inoculation (1st leaf sheath, 2nd leaf sheath, and stem), from the sites 5 mm above that of inoculation (1st leaf sheath, stem) and from the root sites (Fig. 1) at both sampling times (Table 1). The frequencies of isolation (%) from different sampling sites were significantly different from each other for both sampling times ($p = 0.007$ at 2 weeks after inoculation; $p = 0.001$ at 4 weeks after inoculation). The fungus tended to be isolated more frequently from the root sections than the sites of inoculation at both sampling times (Table 1). At the site of inoculation, frequencies of isolation were similar for 1st leaf sheath sections (20%) and 2nd leaf sheath (15%) at both sampling times. At 5 mm above the site of inoculation, fungus was isolated only from 1st leaf sheath at 1st sampling time and decreased at 2nd sampling time.

The frequencies of isolation from the stem sections were similar at the sites of inoculation at both sampling times, whereas 5 mm above the site of inoculation, the fungus was isolated more frequently at 4 weeks after inoculation. The frequencies of isolation from root sites were higher at 4 wpi (65%) compared to 2 wpi (50%)

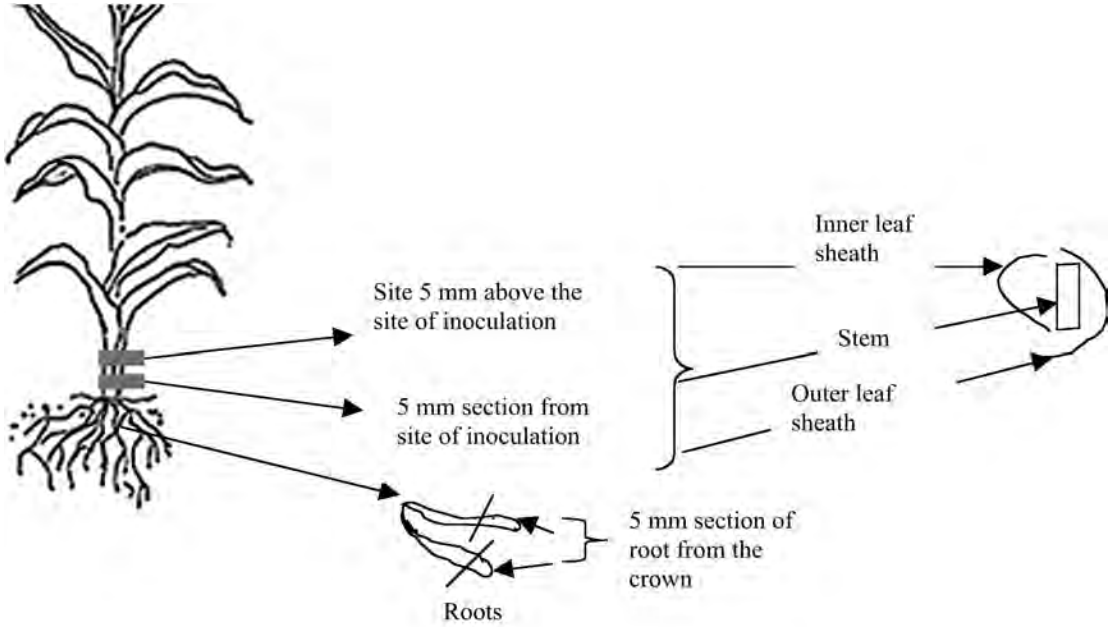

Fig. 1. Diagrammatic representation of sites on stalk and roots of sorghum seedlings from which sections were sampled for isolation of *Gibberella zeae*. Sections were taken from the site of inoculation with a wheat stem piece colonized by *G. zeae*, 5 mm above the site of inoculation and from all roots.

(Table1). *G. zeae* was not isolated from any sampling site of uninoculated control plants.

The results of this study indicate that *G. zeae* can infect sorghum seedlings and plants grown under near optimal conditions in the glasshouse and that it may subsequently colonize stems and roots endophytically over time as no symptoms were produced during the experimental period. Stalk tissues were only colonized to a limited extent during the trial period, up to the boot growth stage.

There are a number of possible explanations for the higher colonization of root sites compared to stalks. Firstly, it is possible that the fungus grew down the surface of the sorghum stem from infested wheat stem piece and thereafter attacked the root adjacent to the crown site from which roots developed. In addition, there may be damaged tissues where roots emerge from the crown, enhancing infection. Secondly, the chemical composition of sorghum roots differs from that of sorghum stems. Wax

Table 1. Frequency of isolation of *Gibberella zeae* (%) from sorghum stalk and root segments at 2 weeks and 4 weeks post inoculation.

Sampling time	Site of inoculation[x]				Site 5 mm above the site of inoculation[y]				Root sites[z]
	1st leaf sheath	2nd leaf sheath	3rd leaf sheath	Stem	1st leaf sheath	2nd leaf sheath	3rd leaf sheath	Stem	
2 weeks (seedling stage)	20b[*]	15b	-	10b	20b	-	-	5b	50a
4 weeks (booting stage)	20b	15b	-	15b	5b	-	-	15b	65a

[x]Leaf sheath and stem segments, 5 mm length, were removed from the site of inoculation, surface sterilized and plated on MPDA.
[y]Leaf sheath and stem segments, 5 mm length, were removed from the site 5 mm above the site of inoculation, surface sterilized and plated on MPDA.
[z]A section, 5 mm in length was removed from each nodal root adjacent to the crown and plated out onto MPDA. Isolation frequency was based on plants with at least one infected root.
- *Gibberella zeae* was not isolated.
[*]Means within each row followed by the same letter are not significantly different from one another at p = 0.05 according to analysis of variance.

and lignin are the two most important structural and chemical components that can differ between sorghum stem and leaf sheath tissues and sorghum root tissues. Lignin concentrations are reported to be higher in stems which contain 0.6% wax (Wall and Blessin, 1970). In leaf sheaths wax deposition varies between 0.8 and 110 µm (Doggett, 1988). In contrast, root tissues of grain sorghum do not contain wax or lignin at the seedling stage, but the tangential wall of the root does become thickened towards grain maturity (Doggett, 1988; Freeman, 1970). Moreover, *G. zeae* is known to produce cell-wall degrading enzymes including polygalacturonases, endo-glucanase, protease and endo-cellulase as well as xylanase (Jenczmionka and Schafer, 2004). Polygalacturonase and cellulase produced by *G. zeae* (Balazs and Bagi, 1997) can easily degrade the cell wall of root cells but not stem cells as they contain a higher percentage of lignin (16%) compared to other parts of the plant (Wall and Blessin, 1970). There is no evidence that *G. zeae* can produce lignolytic enzymes.

The temperature is another factor to consider for infection and colonization. In the experimental period the monthly average temperature (23.9-24.7°C) was suitable for *G. zeae* infection.

Our study has shown that macroconidia and/or mycelium from stubble residues have the potential to initiate infection in sorghum seedlings, as no perithecia were formed on wheat stem pieces during the inoculation period. Based on this, it can be assumed that once the fungus is established at an early stage of growth (seedling stage) through degradation of cell walls, it is likely able to colonize stalk tissues more aggressively at the booting stage. The initial colonization in young plants would provide an advantage over later saprophytic colonizers such as *F. equiseti*, *F. oxysporum*, *F. solani* and *F. sporotrichioides* (Pereyra and Dill-Macky, 2004).

This finding further shows that roots and stalk tissues are both susceptible to infection and colonization by *G. zeae*, but the fungus invades roots more readily than leaf sheaths and stem tissues. Thus young roots may be more susceptible to *G. zeae* than stem and leaf sheath tissues because of the anatomical differences. Infection of seedling roots may also be favoured by root exudates stimulating growth of the pathogen (Vierheiling *et al.*, 1998; Schroth and Hildebrand, 1964).

Further studies, especially the use of *G. zeae* transformed with fluorescent marker genes or PCR-based quantification of *G. zeae* in the plant, are required to clarify the ability of this fungus to infect sorghum plants at different growth stages using infested wheat stems and ascospore inoculum.

ACKNOWLEDGEMENTS

We acknowledge the University of Sydney, Australia for facilitating this research. We also acknowledge AusAid for financial support.

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Received July 15, 2009
Accepted October 4, 2009

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