EMERGING SOIL-BORNE CONSTRAINTS TO IRRIGATED MAIZE: A PYTHIUM–FUSARIUM ROOT DISEASE COMPLEX

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Abstract

A pathogen-selective fungicide trial was established at a site with a history of continuous stubble-retained maize (Zea mays L) cultivation to illustrate the impacts of Pythium, Fusarium and Rhizoctonia root diseases on maize productivity. High soil-borne populations of Pythium and Fusarium were detected at sowing, with no significant differences in their distributions across the site. Fusarium and Pythium inoculum (rhizosphere) increased significantly in the first 12 weeks of crop growth. Whilst no isolates of phytopathogenic Rhizoctonia were recovered from soil or maize roots, 63% and 100% of roots examined were infected by Pythium and Fusarium respectively. Fungicides were therefore ineffective at suppressing rhizosphere pathogen populations and inhibiting root infection and disease development. As a result, there were no significant increases in crop establishment, early crop growth (biomass) or grain yields with any of the pathogen-selective treatments. Sequencing of 5.8s rDNA identified 6 Pythium and 5 Fusarium species, most previously reported as components of a root disease complex contributing to seedling damping off, root and stem rots of maize. Root infections and growth responses of rotation crops grown in the continuous maize soil revealed evidence of host-mediated selection of pathogens with a preference for and greater pathogenicity toward maize and wheat.

Introduction

Irrigated maize crops continue to suffer yield losses thought to be associated with soil-borne diseases and in recent years there have been numerous reports of root rots occurring with increasing frequency and severity. The main reasons appear to be related to the increasing adoption of stubble-retained systems and the switch to less diverse, potentially higher risk cropping strategies (e.g. repetitive maize). If the economic and environmental benefits of this cropping system are to be maintained, there is an urgent need to develop pre-emptive management strategies for these root diseases.

Whilst more prevalent in stubble-retained systems, root disease is also common (although often less severe) in maize crops where the stubble has been burnt or physically removed. It is likely that a complex of root pathogenic fungi are responsible for this disease, with our preliminary research indicating that pathogenic interactions between a suite of soil-borne fungi may be the primarily cause.

Whilst Fusarium and Rhizoctonia are recognised as major pathogen of grain crops, Pythium has also recently received more attention by the industry as an increasingly important soil-borne pathogen. In maize, control strategies for these pathogens are almost exclusively directed at preventing seedling damping-off and as a result, their interactions in developing maize root and stem rots are often underestimated or completely overlooked.

In this context, the aims of this study were to identify the causal pathogens of root rot of irrigated maize, quantify the impacts of the disease on maize productivity and determine the abundance and host-range of these pathogens on crops commonly grown in rotation with maize.

Materials and Methods

Maize cropping site and design of root pathogen-selective fungicide trials

A pathogen-selective fungicide trial was established at Whitton, NSW in October 2004 to illustrate the potential impacts of maize and fungal root diseases on crop establishment and grain yields.
Trials were designed to assess the effectiveness of *Pythium*-(Metalaxyl-M), *Fusarium*-(Fludioxonil) and *Rhizoctonia*-(Tolclofos-methyl) selective fungicides to manage damping-off, root rots and improve crop performance. The trial was sown (cv High Corn 788) on stubble-retained plots that had been cropped to maize for the previous 2 years (i.e. 3rd consecutive maize crop), with N fertiliser applications of 300 kg/ha/year. It consisted of 16 plots (300 m x 5.4 m) and compared maize (Vitavax 220FF-treated) grown in the absence or presence of the 3 additional pathogen-selective fungicides. The trial used a complete randomised block design with 4 replicate plots of each of the 4 treatments.

At sowing (T0) fungicides were applied as a liquidseed dressing at the recommended rates of 70 g (Metalaxyl-M), 5 g (Fludioxonil) and 100 g (Tolclofos-methyl) active ingredient (a.i.) per 100kg seed. An additional treatment was applied at T1 (6 weeks post-emergence) by mixing with the liquid N fertiliser (200 kg/ha) at the rates of 70 g (Metalaxyl-M), 20 g (Fludioxonil) and 100 g (Tolclofos-methyl) a.i. per ha. At T1, crop establishment was measured by taking plant counts along 100 m lengths of the centre of each plot. Biomass production (plant dry weight) was assessed by removing 10 and 4 plants along the same 100 m transect at T1 and T2 (12 weeks post-emergence) respectively.

Quantifying soil-borne inoculum levels of root pathogenic fungi

Soil-borne inoculum levels were quantified twice during the growing season (i.e. at T0 and T2) as described in Pankhurst *et al.* (1995). At T0, 4 soil cores (15 cm diameter x 10 cm deep) were sampled at 50 m intervals from each of the 16 plots, air-dried, sieved and a 100 g sub-sample of the fraction with aggregates <2 mm was retained for analyses. One g samples of each soil were suspended in 100 ml of sterile 1% water agar, shaken for 20 min and 1 ml of soil-agar suspension was streaked onto each of 10 replicate plates of *Pythium*-(VP3), *Fusarium*-(Peptone PCNB) and *Rhizoctonia*-(Ko & Hora) selective media (Singleton *et al.*, 1992). Plates were incubated at 22°C for 3 days, the suspension washed off and the fungal colonies counted. At T2, 4 plants were randomly sampled from each of the 16 plots the rhizosphere soil shaken off and pathogen inoculum levels quantified as described above.

Quantifying root infection frequencies of pathogenic fungi

Plants used for assessments of rhizosphere inoculum were also used to quantify root infection frequencies. Eighteen root segments (each 2-3 cm) showing symptoms suggestive of fungal infection (i.e. brown lesions, lack of lateral and fine roots, cortical pitting) were removed from each of the 4 plants sampled from each plot. If infection symptoms were not apparent, root segments were randomly excised. Segments were washed overnight under running tap water, blotted dry and plated onto the 3 pathogen-selective media (see above). In total, over 1,150 root segments were plated (390 per selective medium) and root disease incidence was expressed as the frequency of infected roots segments. Hyphal tips of root-derived isolates representing the dominant colony types were transferred to half-strength corn-meal agar (CMA) plates and grown at 25°C for subsequent DNA analyses.

Identification of root pathogenic fungi

Fungal isolates derived from soil quantification and root infection analyses were sorted into groups based on similarities in colony morphologies and mycelial growth rates on CMA. Twenty representative isolates (7 *Pythium*, 10 *Fusarium*, 2 *Trichoderma* and 1 *Rhizoctonia*) were selected for and DNA-based identifications using nucleotide sequence data from the nuclear 5.8S ribosomal DNA (rDNA) gene and the adjacent internal transcribed spacer (ITS) regions (White *et al.* 1990).

Isolation of fungal genomic DNA (Harvey *et al.*, 2000) and PCR amplification of ITS-5.8S rDNA sequences (Wakelin *et al*. 2004) were as described previously. PCR products were separated on a 2% TBE agarose gels, stained with ethidium bromide and visualised under UV light. PCR products were purified (Wizard® SV, Promega), cloned into the pGem®-T plasmid vector (Promega) and sequenced in both directions using the M-13 forward and reverse primers (University of Newcastle). Taxonomic identifications were made by comparing DNA sequences on GenBank using the BlastN search.
Defining plant-growth potential in the maize cropping soil: elimination of biological constraints

A glasshouse-based experiment was used to define the growth potential of seedlings of maize and its rotation crops i.e. wheat (*Triticum aestivum* L), canola (*Brassica napus* L) and Adjuki bean (*Vignia angularis* (Willd.) Ohwi & Ohashi) in the absence of soil-borne root pathogens. The impacts of root disease on growth were assessed by growing crops in natural (unsterilised) and g-irradiated (sterilised) maize soil from the Whitton trial site and analysing differences in seedling dry weights.

Ground and sieved (see above) natural and sterilised field soils were mixed with sterile (autoclaved) washed river sand (1:2 w/w), moistened with quarter-strength Hoaglands nutrient solution (Hoagland & Arnon, 1938) to 16% w/w and added to 600 ml non-draining pots at 1125 g pot⁻¹. Four seeds of either maize (*cv* High Corn 788), wheat (*cv* Frame), canola (*cv* Eyre) or Adzuki bean (*cv* Erimo) were sown into separate pots and after germination, were thinned to two seedlings per pot. The randomised complete block design included 10 replicates of each of the 4 crops grown in each soil treatment. Pots were placed in a 20°C water bath in a naturally lit glasshouse (ambient temperature range of 15-22°C) and were watered to their original moisture content (16% w/w) every 3 days with deionised water. At completion (6 weeks after sowing) shoots were dried and weighed and root systems grown in natural soil were assessed for frequencies of *Pythium* and *Fusarium* infection (see above).

Data Analyses

The impacts of soil-borne biological constraints on plant-growth potential were assessed by comparing differences in shoot dry weights of maize and its rotation crops (wheat, canola and Adzuki beans) grown in natural and sterilised Whitton field soils. Field efficacies of pathogen-selective fungicides for controlling maize root diseases were assessed by comparing differences in soil-borne inoculum levels, frequencies of *Pythium* and *Fusarium* root infection, maize crop establishment, biomass production and grain yield. All data were subjected to analyses of variance using GENSTAT 6.1 (Rothamsted, Harpenden, UK). Pairwise comparisons of means were used to compute Fisher’s least significant difference values (LSD) and compare disease control efficacies between treatments.

Results and Discussion

Quantification of *Pythium* and *Fusarium* soil-borne inoculum and root infection levels

There were no significant differences in *Pythium* or *Fusarium* inoculum levels between fungicide treatments at at time of crop sowing (T0), indicating that inoculum that had survived the previous maize crop (i.e. over-winter) had a relatively even distribution across the trial site (Table 1). Notably, *Fusarium* inoculum levels at sowing were up to 40 times greater than those detected for *Pythium*. *Pythium* inoculum increased in the first 12 weeks of maize growth in all treatments with the exception of the *Pythium*-selective fungicide metalaxyl-M (Table 1). The latter resulted in a non-significant decrease (-22%) in *Pythium* inoculum compared with those at sowing, indicating suppression of the pathogen in the maize rhizosphere (Table 1). This suppression was clearly evident in that rhizosphere inoculum levels (T2) in the metalaxyl treatment were significantly lower than those observed in the rhizospheres of untreated (68% less) and Fludioxonil-treated (155% less) maize (Table 1).

Rhizosphere (T2) *Fusarium* inoculum levels were significantly higher than those at the time of sowing (bulk soil) for untreated maize and all pathogen-selective fungicide treatments (Table 1). The largest increase in *Fusarium* inoculum was in the presence of Fludioxonil (+67%), indicating the ineffectiveness of this *Fusarium*-selective treatment, especially later in the development of the crop.

High frequencies of fungal root infection were detected, with an average of 63% of all maize roots infected by *Pythium* and every root segment colonised by *Fusarium* (Table 1). There were no significant differences in root infection frequencies between maize with or without the pathogen-selective fungicides (Table 1), indicating the ineffectiveness of the treatments to prevent root infection under these high soil-borne inoculum pressures.
Table 1. Differences in soil-borne *Pythium* and *Fusarium* inoculum levels at time of sowing (T0, i.e. bulk soil) compared with 12 weeks post-emergence (T2, i.e. rhizosphere soil) and *Pythium* and *Fusarium* root infection frequencies resulting from seed (T0) and soil (T1, i.e. 6 weeks post-emergence) treatments with pathogen-selective fungicides.

<table>
<thead>
<tr>
<th>Fungicide Treatment</th>
<th>Pathogen Inoculum (g⁻¹ soil)</th>
<th>Frequency of Root Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pythium</td>
<td>Fusarium</td>
</tr>
<tr>
<td></td>
<td>Sowing (T0)</td>
<td>12 weeks (T2)</td>
</tr>
<tr>
<td>Untreated</td>
<td>238  ab</td>
<td>348  a (+46)</td>
</tr>
<tr>
<td>Apron</td>
<td>190  b</td>
<td>148  b (-22)</td>
</tr>
<tr>
<td>Maxim</td>
<td>150  b</td>
<td>350  b (+133)</td>
</tr>
<tr>
<td>Rizolex</td>
<td>170  b</td>
<td>222  ab (+31)</td>
</tr>
</tbody>
</table>

1 ApronXL 350ES - *Pythium*-selective (a.i. Metalaxyl-M), Maxim 100FS - *Fusarium*-selective (a.i. Fludioxonil), Rizolex - *Rhizoctonia*-selective (a.i. Tolclofos-methyl).

2 Letters in superscript indicate significant differences in soil-borne inoculum levels among Maize fungicide treatments at sowing (T0, i.e. bulk soil) compared with 12 weeks post-emergence (T2, i.e. rhizosphere soil). *Pythium* (P < 0.05, LSD = 151), *Fusarium* (P < 0.05, LSD = 2627). For clarity, figures in bold indicate a significant difference in inoculum levels of either *Pythium* or *Fusarium* between sowing and 12 weeks post-emergence in the presence of individual fungicides. Figures in parenthesis refer to the % increases (+) or decreases (-) in soil-borne inoculum levels of either *Pythium* or *Fusarium* over the 12 week growth period for each fungicide treatment.

3 Letters in superscript indicate significant differences in *Pythium* or *Fusarium* root infection frequencies among Maize fungicide treatments at 12 weeks post-emergence (T2). Figures in parenthesis refer to the % decrease (-) in either *Pythium* or *Fusarium* infection frequencies for each fungicide relative to the untreated control at 12 weeks post-emergence (T2).

Notably, no Rhizoctonia was isolated from maize cropping soil and only one isolate was recovered from root segments plated onto medium selective for this pathogen.

**Maize crop establishment, biomass production and grain yields**

Seed treatment with *Fusarium* (Fludioxonil) and *Rhizoctonia* (Tolclofos) selective fungicides gave a slight non-significant increase in seedling establishment compared to untreated maize (Table 1). The *Pythium* treatment (metalaxyl) however, resulted in decreased emergence indicating some potential early seedling phytotoxicity when used at this high rate. Whilst these effects on crop establishment were not significant (Table 1), the differences were taken into account by including the data as a covariate in subsequent biomass and grain yield analyses.

Metalaxyl treatment resulted in a non-significant increase in seedling biomass at 6 weeks post-emergence compared with the untreated control, suggesting some suppression of *Pythium* infection in early maize growth. However, this effect was not sustained as evidenced by the lack of any significant disease control (Table 1) and the slight non-significant decreases in 12-week maize biomass and final grain yield (Table 2).
Table 2. Differences in Maize establishment, shoot biomass (at 6 and 12 weeks post-emergence) and grain yields resulting from seed (T0) and soil (T1, i.e. 6 weeks post-emergence) treatments with pathogen-selective fungicides.

<table>
<thead>
<tr>
<th>Fungicide Treatment</th>
<th>Establishment 1 (plants m⁻²)</th>
<th>Shoot Biomass 2 (g plant⁻¹)</th>
<th>Grain Yield 3 (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 weeks (T1)</td>
<td>6 weeks (T1)</td>
<td>12 weeks (T2)</td>
</tr>
<tr>
<td>Untreated</td>
<td>7.28 ab</td>
<td>29.5 ab</td>
<td>157.3 a</td>
</tr>
<tr>
<td>Apron</td>
<td>6.71 b (-8)</td>
<td>30.6 a (+4)</td>
<td>151.5 a (-4)</td>
</tr>
<tr>
<td>Maxim</td>
<td>7.41 a (+2)</td>
<td>26.9 b (-9)</td>
<td>167.6 a (+7)</td>
</tr>
<tr>
<td>Rizolex</td>
<td>7.56 a (+4)</td>
<td>28.5 ab (-3)</td>
<td>164.8 a (+5)</td>
</tr>
</tbody>
</table>

1 Letters in superscript indicate significant differences in Maize crop establishment resulting from seed treatment with pathogen-selective fungicides (P < 0.05, LSD = 0.59). Figures in parenthesis refer to the % increase (+) or decrease (-) in crop establishment relative to the untreated control.

2 Letters in superscript indicate significant differences in shoot dry weights resulting from seed treatment with pathogen-selective fungicides (6 weeks: P < 0.05, LSD = 3.3). Figures in parenthesis refer to the % increase (+) or decrease (-) in shoot dry weights relative to the untreated control.

3 Letters in superscript indicate significant differences in grain yields resulting from seed treatment with pathogen-selective fungicides. Figures in parenthesis refer to the % increase (+) or decrease (-) in grain yield relative to the untreated control.

In contrast to metalaxyl, the Fusarium- and Rhizoctonia-selective treatments decreased biomass at 6 weeks and increased it at 12 weeks (Table 2). Whilst these effects were non-significant, they suggest that these slower growing fungi are involved in root disease expression later in crop growth. Consequently, fungicides selective for these pathogens had a positive effect on T2 biomass production. Neither treatment had an effect on grain yields indicating that as with Pythium control, disease suppression (if any) could not be sustained later in the growing season (Table 2).

Suppression of Fusarium and Rhizoctonia in early maize growth may reduce rhizosphere competition and enhance activity of the faster growing Pythium species, known to aggressively infect younger seedlings. The increased Pythium rhizosphere inoculum levels observed in the presence of the Fusarium- and Rhizoctonia-selective fungicides (Table 1) supports this assumption. Consequently, increased Pythium activity may be responsible for the reductions in maize T1 biomass in the presence of fungicides selective for Fusarium and Rhizoctonia (Table 2).

Collectively, these data indicate that the fungicides used in this study only provide short-term disease control and that their effects are not sustained throughout the season. These analyses imply that maize root diseases result from interactions between pathogens in the rhizosphere that cannot be attributed to individual species.

In addition, the temporal activities of these pathogens vary depending on the growth stage of the crop and therefore have differential impacts over the course of the growing season.

Identification of Pythium and Fusarium species isolated from maize roots

High similarities in 5.8S rDNA-ITS sequences existed between the fungal isolates from maize roots and the worldwide DNA sequence database. In addition to the 7 Pythium and 10 Fusarium isolates sequenced as representative of the morphological groups of these pathogens, the single putative Rhizoctonia and 2 Trichoderma isolates were also analysed.
This provided a phylogenically diverse group of fungi among which to make comparisons, thereby increasing confidence in the use of the data to make rapid and accurate taxonomic identifications.

Figure 1. Phenetic relationships among fungi isolated from maize roots based on DNA sequence homologies of the 5.8S rRNA gene and the adjacent ITS regions.

Six *Pythium* species were identified with an overall average sequence homology of 73% (Figure 1) and all, with the exception of *P. heterothallicum*, had previously been reported as being involved in damping-off, root and stem rots of maize (Van der Plaats-Niterink 1981, White 2000). *Pythium* are Oomycetes and phylogenically are not considered as ‘true fungi’ and as expected, cluster analysis (maximum likelihood) shows that they form a discrete group exhibiting significant sequence divergence from isolates of *Fusarium*, *Trichoderma* and *Rhizoctonia* (Figure 1).

Five *Fusarium* species were identified with an overall average sequence homology of 83% (Figure 1), with only *F. equiseti* and *F. incarnatum* generally not considered as being a major component of the *Fusarium* root, stem and ear disease complex of maize (White 2000). Whilst *Rhizoctonia solani AG-2* is a known pathogen of maize, causing crown and brace rot (White 2000), we did not isolate any *R. solani* strains from maize roots. The sole *Rhizoctonia* isolate from our trial could not be identified to species level and had the highest sequence homology to a non-pathogenic *Rhizoctonia* strain that forms mychorrhizal-like associations with orchids. Similarly *Trichoderma koningii* is considered to be non-pathogenic to maize and is antagonistic to numerous plant pathogenic fungi.

**Plant growth potential and host-mediated selection of maize root pathogenic fungi**

Significant differences in *Pythium* root infection frequencies were observed between maize and it’s rotation crops when grown in soil cultivated to maize for the 3rd consecutive year (Table 3). Canola is known to be highly susceptible to *Pythium* (Harvey 2004) and as expected, had significantly higher infection frequencies than maize, wheat and Adzuki bean.
When compared to maize, infection levels were significantly lower in Adzuki bean (-61%) and whilst not significant, were also lower in wheat (-25%). This implies that continuous maize cultivation had selected for a population of *Pythium* genotypes with a preference for parasitising maize and other graminaceous crops (e.g. wheat) compared with Adzuki bean.

In contrast, there were no differences in *Fusarium* infection levels between crops, with every root segment supporting at least one isolate (Table 3). As indicated previously, infection by *Pythium* is likely to increase susceptibility to *Fusarium* and the high *Pythium* infection rates reported in this experiment would have contributed to *Fusarium* disease incidence. This pathogenic interaction, combined with the extremely high inoculum levels of *Fusarium* in this soil (Table 1), are likely to have overcome any differential crop susceptibilities to *Fusarium*.

**Table 3.** Differences in *Pythium* and *Fusarium* root infection frequencies and shoot dry weights of crop plants grown in natural (unsterilised) and g-irradiated (sterilised) maize field soil.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Frequency of Root Infection 1</th>
<th>Shoot Dry Weight 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Pythium</em></td>
<td><em>Fusarium</em></td>
</tr>
<tr>
<td></td>
<td>Natural Soil</td>
<td>Irradiated Soil</td>
</tr>
<tr>
<td>Maize</td>
<td>0.514 b</td>
<td>1.000 a</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.386 bc (-25)</td>
<td>1.000 a (0)</td>
</tr>
<tr>
<td>Canola</td>
<td>0.814 a (+58)</td>
<td>1.000 a (0)</td>
</tr>
<tr>
<td>Adzuki Bean</td>
<td>0.200 c (-61)</td>
<td>1.000 a (0)</td>
</tr>
</tbody>
</table>

1 For each pathogen, letters in lowercase superscript indicate significant differences in root infection. *Pythium* (P < 0.05, LSD = 0.228). Figures in parenthesis refer to the % increase (+) or decrease (-) in *Pythium* root infection of each crop relative to the infection level in maize.

2 Letters in superscript indicate a significant difference in shoot dry weight among crops grown in natural or sterilised (g-irradiated) maize field soil (P < 0.05, LSD = 0.136). For clarity, figures in bold indicate a significant difference in shoot dry weights for individual crops grown in natural or sterilised field soil. Figures in parenthesis refer to the % increases (+) in shoot dry weights of crops grown in sterilised compared to natural field soil.

Shoot dry weights of both maize and wheat were significantly greater when grown in sterilised soil compared to the natural field soil (Table 3), indicating that the removal of biological constraints to plant growth (i.e. root pathogens) significantly enhanced productivity of these crops. In contrast, whilst shoot dry weights of canola or Adzuki beans increased slightly when grown in sterile compared to natural field soil, the effect was not significant (Table 3). This implies that the indigenous soil-borne pathogen population is more pathogenic toward maize and wheat than it is to canola or beans. Whilst canola was observed to have significantly greater levels of *Pythium* infection than maize or wheat, the lack of a significant growth improvement upon elimination of this pathogen suggests that the *Pythium* population was only weakly or non-pathogenic toward canola.

Collectively, these data indicate that exposure to maize over the last 3 cropping seasons has resulted in host-mediated selection of pathogen populations with a preference for and greater pathogenicity toward graminaceous crops. Opportunities therefore exist to develop root disease management strategies for maize cropping systems, based on integrating more effective fungicide applications and strategic crop rotations to limit the impacts of soil-borne pathogens. This will require a thorough knowledge of the dynamics of these pathogens in maize soils, how they interact across phases of the rotation and the differential susceptibility of rotation crops to diverse pathogen genotypes.
Strategic crop rotation will help restrict large populations of these root pathogens, limit their interactions to avoid severe disease incidence in the current cropping phase and high levels of inoculum carryover in subsequent years. In addition, sustained control of host-adapted pathogen genotypes will remove one of the constraints to increasing the cropping frequencies of the more profitable phases of the rotations (e.g., consecutive maize crops). Achieving this outcome will require developing a greater understanding of the ecology, genetics and pathogenic range of Pythium-Fusarium disease complexes in maize cropping systems and will form the focus of our future research.

References


