

**Septoria Leaf Blotch (SLB) caused by *Mycosphaerella graminicola* (Mg) is economically the most important foliar disease of wheat in the UK<sup>1</sup>.**

**SLB causes extensive yield loss through reduction in green leaf area.**

During the late 1990s strobilurin fungicides were used extensively alongside triazole fungicides to give excellent disease control. In 2002, resistance to strobilurins was detected in *Mg*<sup>2</sup> and has become widespread throughout the UK; control now relies heavily on the triazoles. Recent studies suggest that triazole efficacy is eroding<sup>3</sup>.



Septoria leaf blotch on winter wheat at Terrington, Norfolk.

Four reported resistance mechanisms:

- Alteration in the target CYP51 protein
- A reduction in triazole accumulation through increased active efflux
- Alteration in sterol biosynthesis
- Increased expression of *CYP51*.

Additive combinations of these mechanisms occur in the *Mg* population

### Project aims:

Elucidation of mechanisms in UK field populations of *Mg* that cause insensitivity to triazole fungicides.

Correlation of biological characteristics (e.g. fitness costs) with resistance, which will aid risk assessment and resistance management.

### Selection for resistance

Selection for resistant *Mg* isolates occurs when infected wheat is treated with triazole fungicides. This experiment was designed to measure the extent to which selection occurs.

### Methods

Isolates of *Mg* from epoxiconazole treated (with four sprays of 0.25 Lha<sup>-1</sup>) and untreated winter wheat, cv. Consort, were collected from a site in Terrington, Norfolk.

EC50 values were determined for isolates using a range of epoxiconazole concentrations.

### Results and future work

Mean EC50s for treated and untreated isolates were similar and show no significant differences (fig.1).

Isolates with significantly reduced sensitivities were identified, in treated and untreated plots, for further study using molecular assays. This will show the prevalence of resistance mechanisms.

A two amino acid deletion in the CYP51 protein, ΔY459/G460, has been identified in isolates with a triazole insensitivity<sup>4</sup>. A bidirectional, multiplex, allele-specific PCR assay has been used to distinguish isolates with this deletion and to determine its prevalence in Terrington (fig.2).

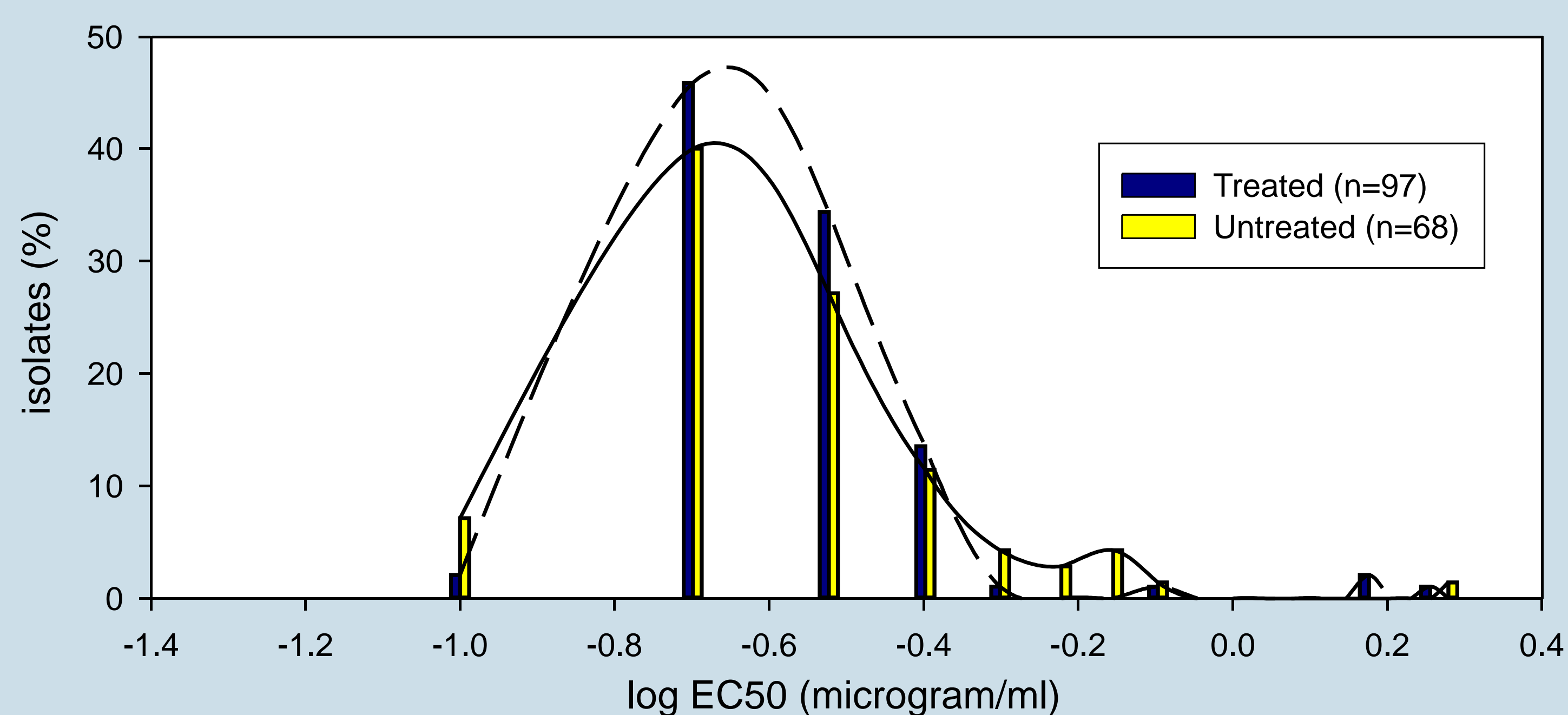


Figure 1  
Comparative distribution of EC50s of individual *Mg* isolates to epoxiconazole between treated and untreated plots. No selection has occurred in the treated

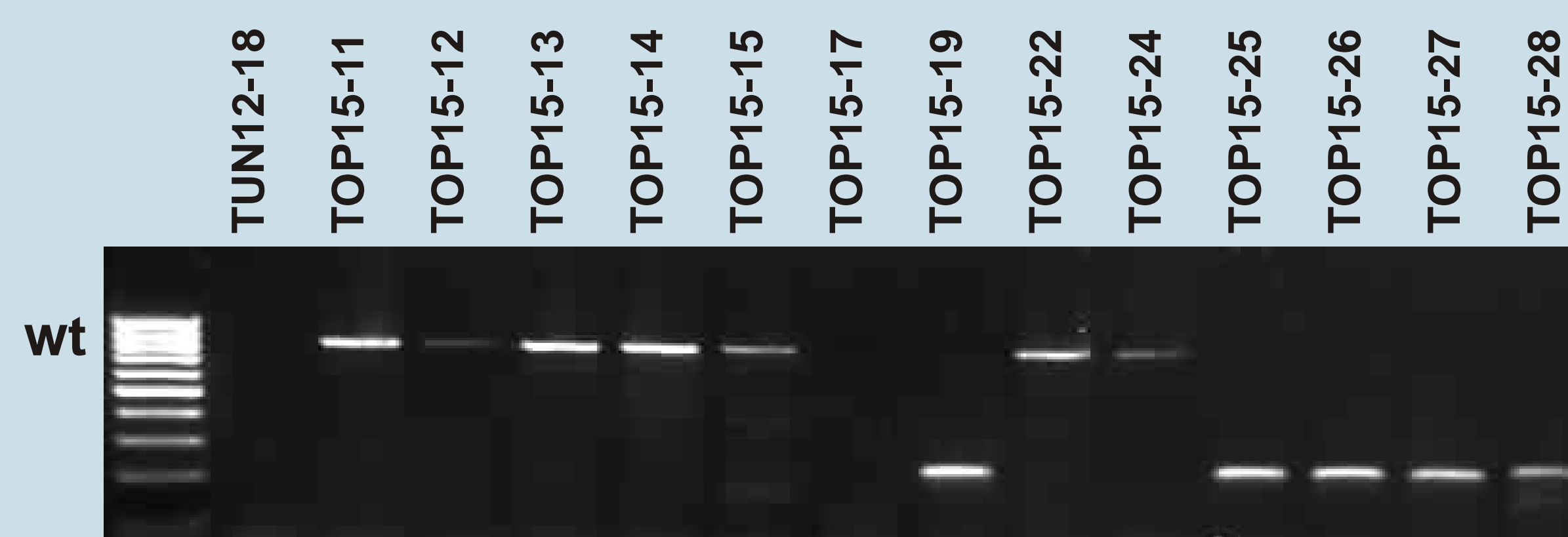


Figure2  
Allele specific PCR assay.  
Wild type (wt)  
Deletion ( )

### Discussion

A reduction in sensitivity of the treated population was not evident from the results obtained. Other studies have shown efficacy is eroding. Therefore, it is likely that features of the experimental procedure (dose rate, application number etc) reduced the potential for selection. Future experiments will provide optimum selection pressure to promote survival of resistant isolates.

### 2005 Field Trial Selecting for isolates with reduced sensitivities to triazole fungicides

- Plots were inoculated with three resistant and one sensitive isolate.
- Quantification of each isolate after treatment, using molecular markers, will determine the ratio of resistant: sensitive isolates on each plot and isolate survival rate at differing selection pressures.
- High throughput testing, using a discriminatory fungicide dose, will be used to define the mean sensitivity of isolates in each plot.
- Fungicide treated 'bait' plants will be used to capture resistant isolates toward the end of the season.
- Individual isolates with interesting characteristics will be identified and used in further detailed studies.

### References:

1. Fraaije, B. A., Lucas, J. A., Clark, and Burnett, W. S. (2003) In Proceedings of the BCPC Conference - Crop Science & Technology, Vol. 2, pp. 689-694.
2. Hardwick, N. V., Jones, D. R. and Slough, J. E. (2001) Plant Pathology, 50, 453-462.
3. Mavroidi, V. I. and Shaw, M. W. (2004) Crop Protection, 24, 259-266.
4. Cools, H. J., Fraaije, B. A. and Lucas, J. A. (2005) In: Dehne, H.W., Gisi, U., Kuck, K.H., Russell, P.E. and H. Lyr eds, Modern Fungicides and Antifungal Compounds IV. (in press)

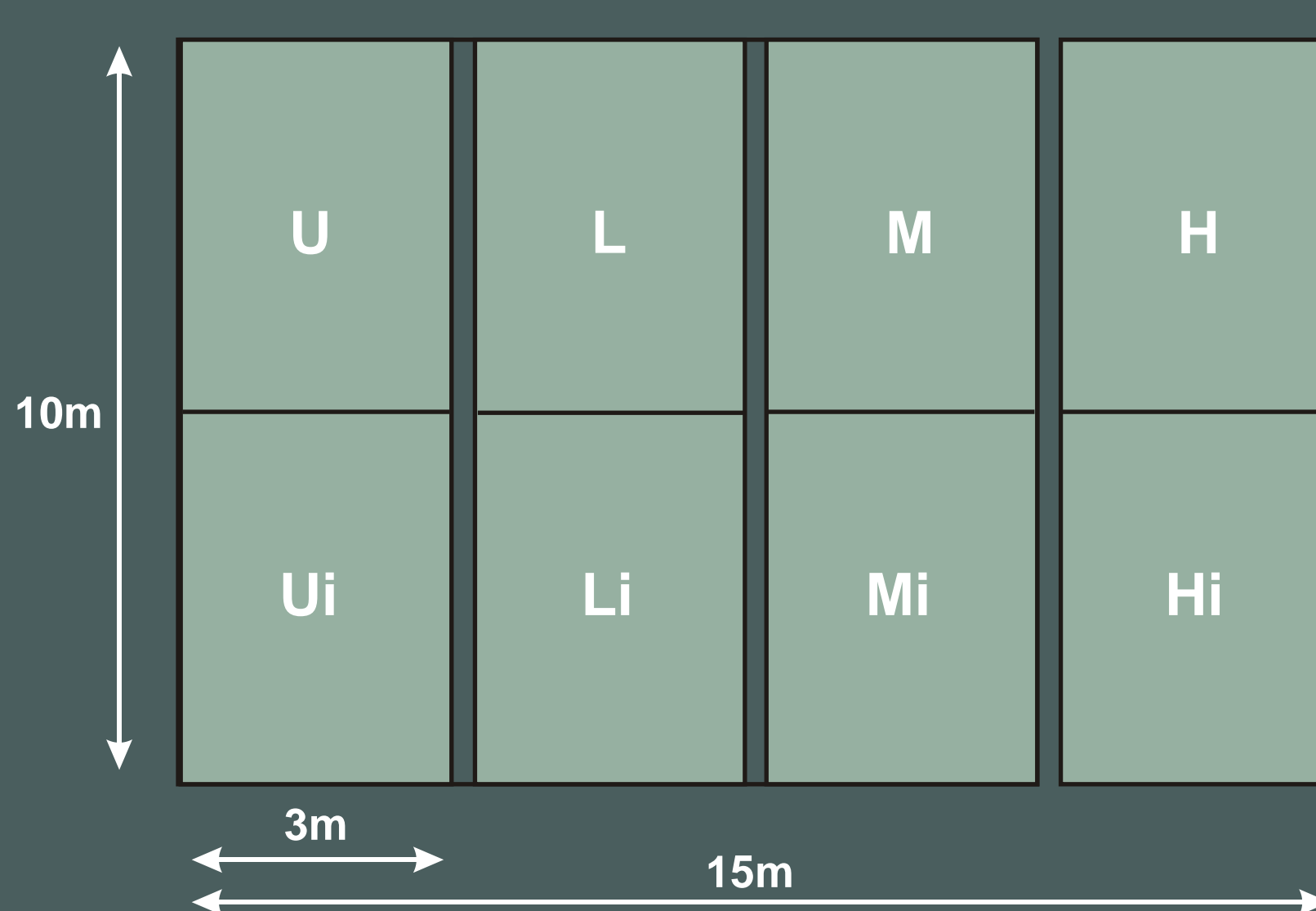


Diagram of 2005 field trial

Three treatments of:

- U- Untreated
- L- Opus @ 0.125 L/HA
- M- Opus @ 0.25 L/HA
- H- Opus @ 0.5 L/HA
- i- inoculated

