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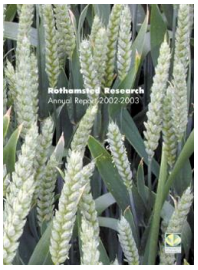
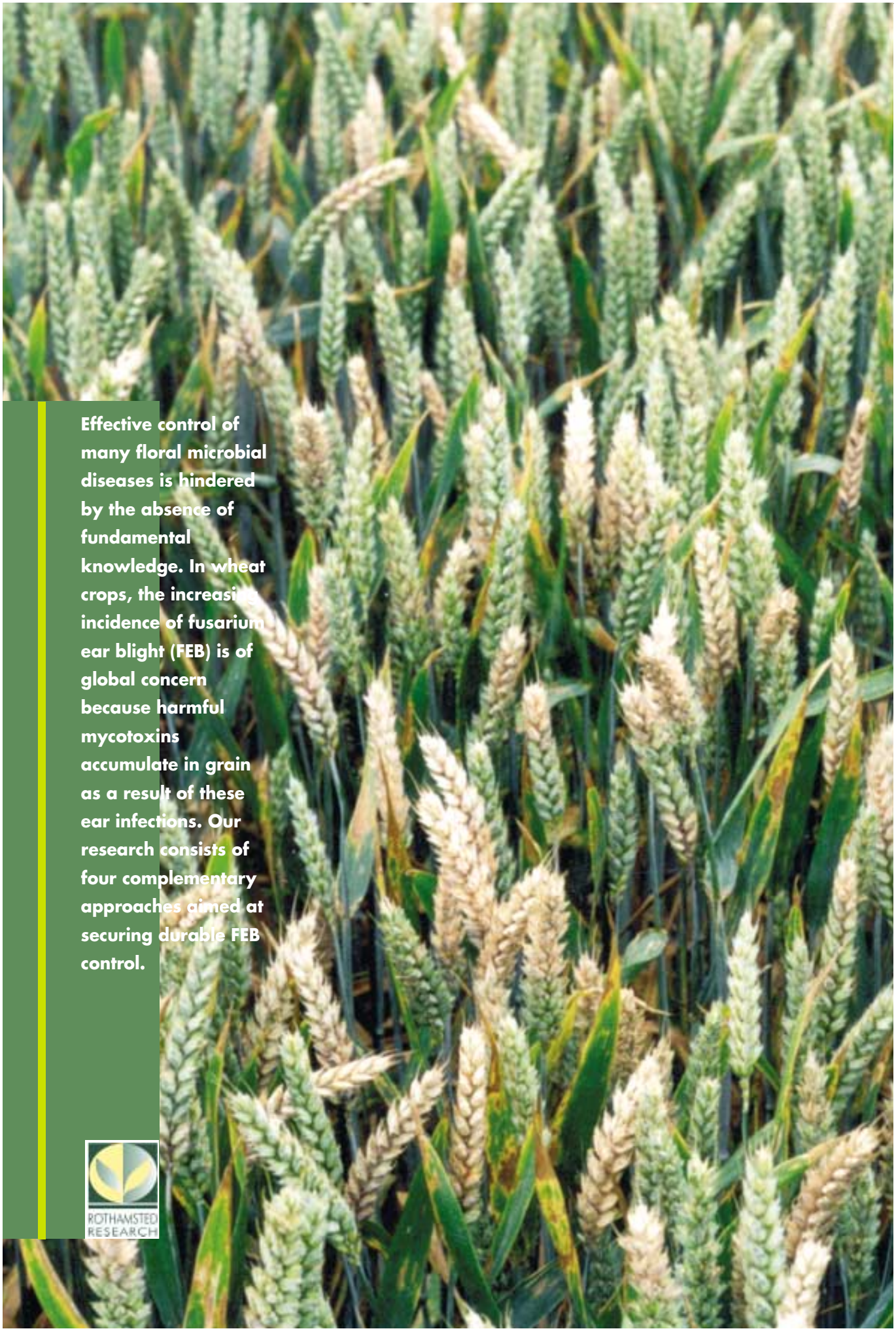
Full Table of Content



Fusarium Ear Blight

Rothamsted Research

Rothamsted Research (2003) *Fusarium Ear Blight ;* Rothamsted Research Annual Report 2002-2003, pp 16 - 19



Strategies for controlling *Fusarium* ear blight disease, an emerging threat to UK cereal crops

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Worldwide, *Fusarium* ear blight (FEB) infections of cereal crops cause considerable losses in grain quality and safety (http://www.scabusa.org). *Fusarium* infections in UK wheat crops have been steadily increasing since the early 1990s, probably due to changes in crop rotations, the introduction of maize into regions where previously only wheat was grown, the use of low/minimum tillage practices and climate change. The two main causative agents are the fungal species, *F. culmorum* and increasingly *F. graminearum* (teleomorph stage *Gibberella zeae*) (Figure 1) (http://www.csl.gov.uk/resdev/AH/PDCP/epid/fusarium/). The disease is primarily monocyclic, with ear infections occurring when moist conditions prevail at anthesis and inoculum is available.

Grain harvested from *Fusarium*-infected ears is frequently of poorer quality (Figure 2) and contaminated with mycotoxins, including the highly toxic trichothecene mycotoxins, such as deoxynivalenol (DON). Mycotoxin contamination of grain presents a serious health risk to humans and animals, and the EU is soon to legislate on the permitted DON levels in food and feed. The brewing industry already has zero tolerance of *Fusarium* mycelium and mycotoxins in cereal grain because these adversely affect the fermentation process. The cellular target site for DON mycotoxin is the peptidyl transferase protein in the ribosome. DON-binding inhibits protein synthesis in eukaryotic cells.

Our research consists of four complementary approaches aimed at securing durable FEB control. These are:
1) increasing our understanding of the epidemiology of the disease under UK conditions;
2) the identification of promising biocontrol species that can restrict infection of wheat ears;
3) defining the *Fusarium* genes required to cause disease and regulate mycotoxin production; and
4) the characterisation of natural wheat resistance mechanisms that can lower mycotoxin levels without compromising grain quality. We have recently shown that *Arabidopsis* floral tissue can be infected by the same *Fusarium* species that attack wheat ears. This model system will be exploited via comparative molecular genetic studies to provide greater insight into FEB.

Figure 1. A UK wheat crop exhibiting severe fusarium ear blight symptoms 6 weeks prior to harvest. (left)

Figure 2. grain recovered from fusarium infected wheat ears is often smaller and shrivelled (right)

Understanding the disease epidemiology

The *Fusarium* species that infect cereals exist saprophytically, on dead crop debris, but can also become pathogenic, causing visible disease symptoms on stem bases and cereal ears. A symptomless state on the surfaces of roots or leaves can also occur. Our epidemiological research has focused initially on understanding the main sources of fungal inoculum and the route of dispersal to the wheat ear. *F. culmorum* population size in soil

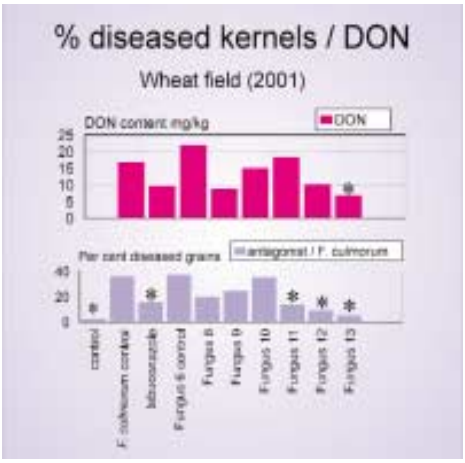

Figure 3. Some antagonist fungal species can control *Fusarium* ear blight as effectively as a conventional fungicide treatment. *Shows significant differences from *F. culmorum* only control

varies erratically, but is greatest after cereal crops and particularly after straw incorporation. A rapid build up in inoculum levels can occur during the summer. Inoculum of *F. culmorum* arises primarily from infested debris within the crop lying on the soil surface. Therefore, burial by ploughing-in should remove such an inoculum source.

Control of *F. graminearum* may be more problematic, because sexually produced ascospores released from infested crop debris are air-borne and potentially dispersed over longer distances. Other researchers have shown that maize is an important source of inoculum for infection of wheat by *F. graminearum* grown in the same or nearby rotations. This situation is likely to occur in the UK, and we have found *F. graminearum* infections to be associated with maize crops. The timing of ascospore production under UK conditions has not yet been determined.

The potential of biological control

FEB has recently been the focus of intensive searches for agents of biological control, particularly in the USA where research has concentrated on strains of antagonistic bacteria. A project funded by the EU aims to prevent *Fusarium* mycotoxins entering the human and animal food chain. It includes the investigation of biological agents as an approach to pre-harvest control.

With partners in PRI (Plant Research International, Wageningen, The Netherlands), ISPAVE (Institute of Plant Protection, Rome, Italy) and EELA (National Veterinary and Food Research Institute, Helsinki, Finland), (www.mycotoxin-prevention.com), our aims were to attempt biocontrol at two vulnerable stages in the natural disease cycle: the production of inoculum (*Fusarium* spores) on crop debris and the ear infection phase. At RRes, we have concentrated on the latter objective. Strains of competitive fungi were chosen as the most promising option and were selected from collections of fungi isolated from various European cereal crops. Candidate strains, identified in semi *in vitro* and glasshouse screens, were tested for efficacy in field trials (Figure 3). Interestingly, different fungal isolates provided the greatest protection at each of the two key stages in the disease cycle where biocontrol was attempted.

Reducing ear infections has proven particularly amenable to biocontrol probably because the susceptible plant tissue, mainly the anthers and young florets, has only recently emerged from the flag leaf and so is substantially free of an established natural surface microbiota. Also, biocontrol is only required for the first 2 weeks after the onset of flowering, whereupon the maturing cereal ear becomes naturally resistant to infection. In the field trials, the best competitors were strains of non-pathogenic *Fusarium* spp. These treatments controlled both ear blight symptoms and mycotoxin production as effectively as a standard fungicide such as tebuconazole. Effective control with the same fungal species was achieved in wheat, barley and oats crops when the antagonist was applied before the pathogen. Various biocontrol fungi are currently being evaluated for their commercial potential.

Targeting *Fusarium* pathogenicity factors

This work is based on the premise that a pathogen mutation that circumvented the effects of an effective fungicide or a transgenic antifungal protein delivered by plant cells would be due to production of a variant pathogenicity factor or loss of such a factor. The suggestion is that such changes would have an effect on the mutant's fitness and ability to cause severe disease

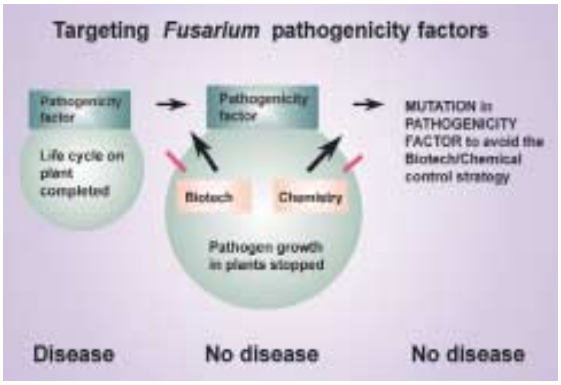

Figure 4. Pathogenicity factors required to cause plant disease are excellent potential cellular targets for disease control

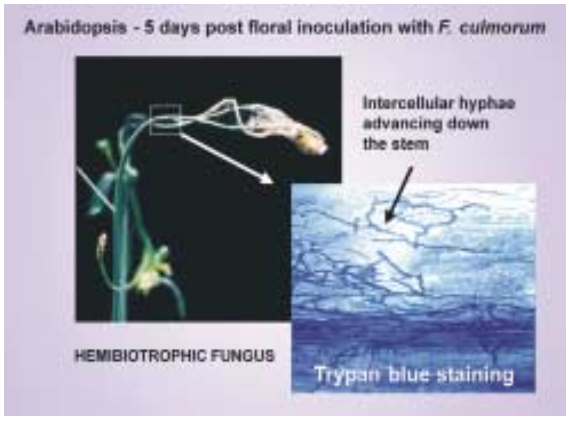

Figure 5. Cereal-attacking *Fusarium* species also cause a floral disease on *Arabidopsis thaliana*, a model non-cereal species

(Figure 4). Hence, the identification of fungal pathogenicity factors provides a target for discovery of novel approaches to achieve durable disease control.

Molecular genetic analysis of the FEB infection process is still fragmentary and understanding is being sought of the *Fusarium* genes required for fungal penetration, cereal ear colonisation and spore formation. It is known that DON production is not essential for *F. graminearum* to cause disease in wheat ears. Our laboratory and others have recently demonstrated that two distinct Mitogen Activated Protein Kinases (MAPKs) Map1 and Mgv1, are independently required for infection and subsequent spread within the wheat ear. In *Saccharomyces cerevisae* (yeast) the homologous protein to Map1 is Fus3/Kss1 which controls the pheromone response leading to mating, whilst the homologue in yeast to Mgv1 is Slt2 which controls cell integrity. In several other phytopathogenic fungal species, proteins sharing homology with Map1 were also found to be essential for plant penetration and/or invasive growth *in planta*. Collectively, these MAPK results suggest the existence of an ancient conserved core signalling mechanism that controls fungal pathogenicity. By targeting this MAPK pathway the control of multiple plant diseases may be achievable.

The resources and techniques available to undertake a large scale exploration of *Fusarium* gene function include the complete *F. graminearum* genome sequence (Whitehead Institute, Cambridge, USA) (http://www-genome.wi.mit.edu/annotation/fungi/fusarium/), various libraries of expressed sequence tags (ESTs) (http://cogeme.ex.ac.uk) and efficient transformation systems to create specific gene knockouts within 4-6 weeks.

Our current research aims to define all the components of the Map1 kinase signalling cascade and identify the downstream cellular targets. In addition, we are undertaking a genetic screen involving random plasmid insertion to isolate other *Fusarium* genes required for wheat ear pathogenicity and *in planta* induced DON mycotoxin production.

Exploiting natural resistance sources and mechanisms in various plants to achieve low DON mycotoxin levels in grain

Two main types of natural resistance to FEB are known in wheat. Type I resistance reduces initial infection incidence but its genetic basis is unknown. Type II resistance reduces the rate of hyphal spread within the ear tissue and is conferred by multiple unlinked loci. Currently, various sources of Type II resistance are being introgressed by breeders into well-adapted genetic backgrounds using marker-assisted approaches. However, this Type II resistance only reduces, but does not eliminate, mycotoxin contamination of grain.

We are attempting to determine which resistant wheat cultivars reduce mycotoxin production and to identify regions of the wheat genome that confer low mycotoxin accumulation. To assist in this research, genetically modified *Fusarium* strains in combination with biochemical analyses are being used to define the exact temporal and spatial patterns of DON biosynthesis in the visibly infected and non-infected parts of the wheat ear. In collaboration with the Crop Performance and Improvement Division, we are also undertaking research to ensure the most promising resistance sources do not adversely affect grain quality.

Recently, we have demonstrated that both *F. culmorum* and *F. graminearum* can infect *Arabidopsis* floral tissue and cause disease symptoms (Figure 5). During these floral infections DON synthesis also occurs. Genetic studies involving a range of *Arabidopsis* mutant lines are being used to define the signalling components controlling plant defence in floral tissue and also *in planta* induction of DON synthesis. Comparative molecular genetic experiments are also being undertaken to ensure that the key findings in the *Arabidopsis* model are relevant to the disease in the economically important cereal crops.