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Integrated control of Fusarium ear blight

by

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1 INTRODUCTION

Fusarium ear blight (FEB) is a significant disease of small grain cereals and has been reported throughout the world. The disease is caused mainly by five mycotoxigenic species, *Fusarium culmorum*, *F. graminearum*, *F. avenaceum*, *F. poae* and *Microdochium nivale*. These fungi survive and sporulate on crop residues, and infect subsequent crops at the flowering stage. High temperature and humidity play an important role in infection. Initial symptoms of FEB commonly appear 4-5 days after infection. Symptoms are generally the same in all small grain cereals and are initially similar for all the species causing FEB in the UK. Small, water-soaked brownish spots develop at the base or middle of the glume or on the rachis. Infected spikelets then senesce and take the typical colour of ripe ears in contrast with the green uninfected ears.

Grain harvested from FEB-infected ears is often small and shriveled and may contain mycotoxins (e.g. deoxynivalenol) produced by the fungi. The risks connected with the consumption of contaminated food products by livestock and humans must therefore not be ignored. In June 2005 the EC advised action to be taken on trichothecenes and proposed regulatory limits, which will apply from 1 July 2006 (EC regulation No 856/2005). For deoxynivalenol (DON) the limits include 1250 ppb for unprocessed cereals, 750 ppb for cereal flour and pasta, 500 ppb for retail products such as bread, pastries, biscuits, cereal snacks and breakfast cereals, and 200 ppb for baby food.

Due to change in agricultural practice (no-tillage) and in climatic conditions, FEB and therefore, the contamination of grain with mycotoxins, are posing an increasing risk to animal and human health. The availability of control measures remains limited. Control options consist of reducing the amount of inoculum available to cause the disease. Cultural practices such as crop rotation can reduce the carry-over of spores or other survival structures between seasons. Once the crop is exposed to the pathogen, a further set of

control strategies must be considered, including genetic resistance, chemical treatment and biological agents. Breeding programs have greatly aided in the eradication of very susceptible varieties. However, the resistance appears to be in most cases under polygenic control making the development of resistance cultivars with suitable agronomic and quality traits a challenge. Therefore, such efforts do not offer an immediate protection against FEB. At the present time, there is no fungicide to control FEB with a 100% efficacy. Among the active ingredient in the tested fungicides, tebuconazole has been reported the most effective for controlling FEB (Magan *et al.*, 2002; Homdork *et al.*, 2000; Menniti *et al.*, 2003; Simpson *et al.*, 2001; Cromey *et al.*, 2001 and 2002).

In most cases, inadequate control of FEB by chemicals is due to incorrect timing of application. Timing of fungicide application is indeed crucial for effective FEB control. Infection usually occurs during mid-anthesis, the period between growth stages 65 to 71 being the most susceptible for FEB infection (Lacey, Bateman and Mirocha, 1999). The efficacy of fungicides also depends on the timing of infection. Matthies and Buchenauer (2000) found that fungicide applications early post-infection, 2 days after inoculation, provided the most effective control against FEB while pre-inoculation and late post-inoculation applications (9 days after inoculation) were less effective.

Micro-encapsulated fungicides could prove effective against FEB. They could indeed increase the length of activity of the active ingredient but also reduce cost as smaller quantities of fungicides would be needed and there would be no need for multiple applications. Over 100 micro-encapsulation processes have been described, amongst them, the micro-encapsulation of active ingredient in yeast cells (Pannell, N.A., European patent 242135). The technology uses strains of the yeast *Saccharomyces cerevisiae* commercially available in the baking and brewing industries. The viability of the yeast cells is not required but intact cell membranes and cell walls are essential for efficient encapsulation. The process uses only water, yeast and the active

ingredient to be encapsulated. The yeast cells are dispersed in water with top stirring. The active ingredient is then added to the dispersed yeast cells and the suspension mixed until the majority of the active ingredient has entered the cells. Encapsulation levels generally attain 30 to 40 % (w/w) but can sometimes reach 80 % (Nelson and Crothers, 2003). Yeast cells containing the active ingredient are then washed with water or another appropriate solvent and spray dried. The technology has been used successfully in the food industry. Encapsulated essential oils and synthetic flavours have successfully been encapsulated and are released from the capsule on contact with the moist tongue surface without the yeast cell being disrupted.

A UK-based company, Micap plc. developed and provided a microencapsulated formulation, containing the active ingredient tebuconazole, using the technology described above. The formulation is available as a powder that can be used readily for seed treatments or mixed in water for foliar treatments. The yeast cells are 5 to 10 µm in diameter, which make them small enough so that the formulation do not clog the equipment used during the spraying process.

The project aimed at testing this novel tebuconazole-encapsulated fungicide provided by Micap plc. The efficacy of the microencapsulated fungicide to control FEB was evaluated *in planta* and compared to that of various foliar fungicides. The study also aimed at isolating and screening biological control agents in the view of an integrated approach to control FEB. Chemical and biological treatments were tested in controlled environment before being assessed for their efficacy in the field.

2. METHOD

The same procedure was followed for all experiments. Treatments were applied at full ear emergence (GS59). At mid-anthesis (GS65), the ears were challenged with spore suspensions of *F. culmorum* at a concentration of 10^6

spores mL^{-1}. Fourteen days after artificial inoculation, the disease incidence (% of infected ears) and severity (% of infected spikelets in diseased ears) were recorded. When ready, the grain was manually harvested and threshed. The thousand grain weight was determined. The grain was then separated into 3 categories: healthy looking grain, infected grain or TSK (tombstone kernel) and heavily infected grain or failed grain, and the proportion of each grain category recorded. Finally, the contamination of the grain with the mycotoxin deoxynivalenol (DON) was measured using an ELISA (enzyme-linked immunoassay) kit.

3. EVALUATION OF MICAP, A MICROENCAPSULATED FUNGICIDE

Micap plc. initially provided a product containing 19% of the active ingredient tebuconazole. Its efficacy was evaluated on ears of wheat cv. Charger (susceptible to FEB) in a controlled environment. Folicur was used for comparison. Micap 19% was prepared in water to contain the same concentration of tebuconazole than Folicur. The two fungicides were sprayed at rates equivalent to 0.5 L ha^{-1} Folicur.

Disease incidence and severity were greatly reduced by both fungicides (Figure 2 and 3). Moreover, the effect of Micap 19% on both FEB incidence and severity was similar to the commercial fungicide Folicur. Both formulations reduced the disease incidence by 75% and the disease severity by over 65%.

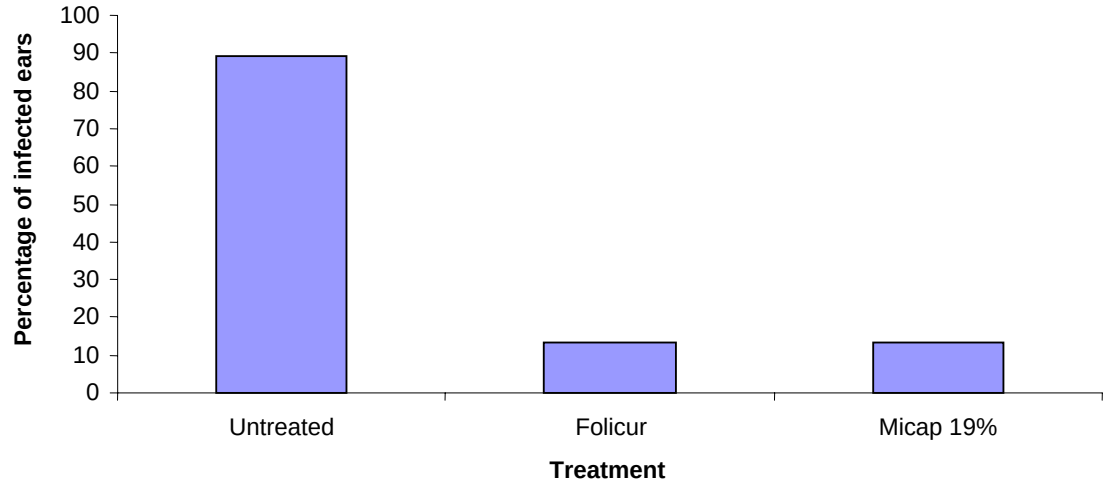

Figure 1. Effect of tebuconazole and microencapsulated tebuconazole on disease incidence.

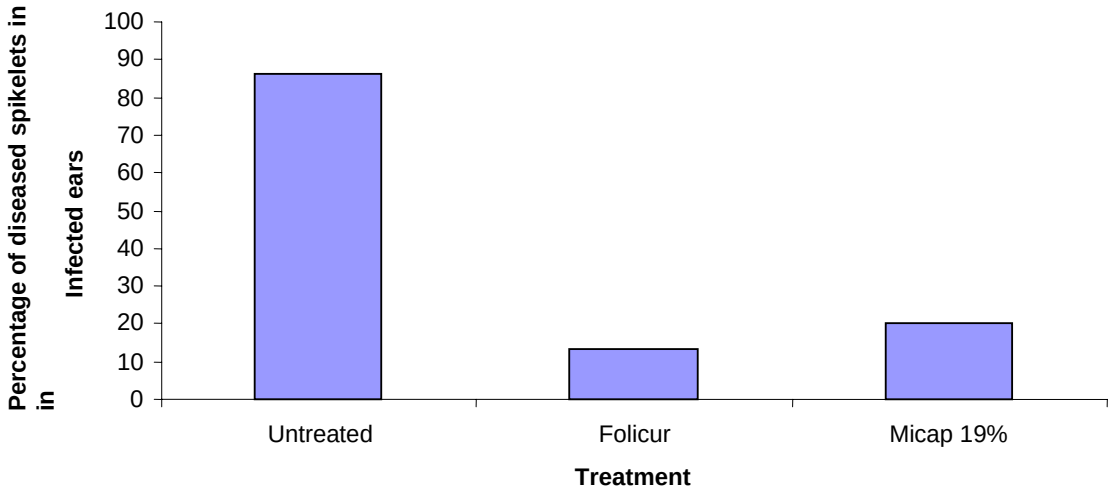

Figure 2. Effect of tebuconazole and microencapsulated tebuconazole on disease severity.

The thousand grain weight was increased after treatment with both formulations, with Micap 19% giving the best result.

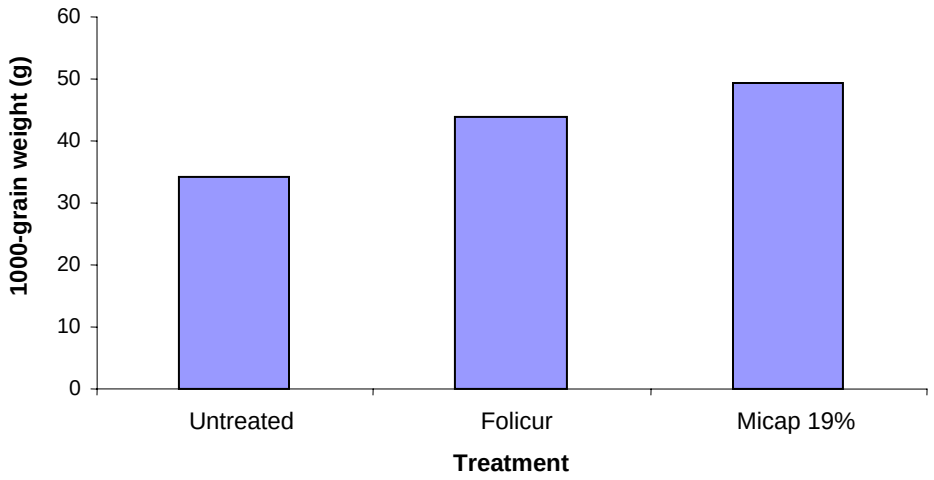

Figure 3. Effect of tebuconazole and microencapsulated tebuconazole on thousand grain weight.

The proportion of healthy looking grain was more than doubled after treatment with both tebuconazole treatments. The lowest proportion of TSK was observed after treatment with the encapsulated fungicide. However, the percentage of failed grain recorder after treatment with Micap 19% was higher than the untreated control while no failed grain were recorded amongst the grain treated with Folicur.

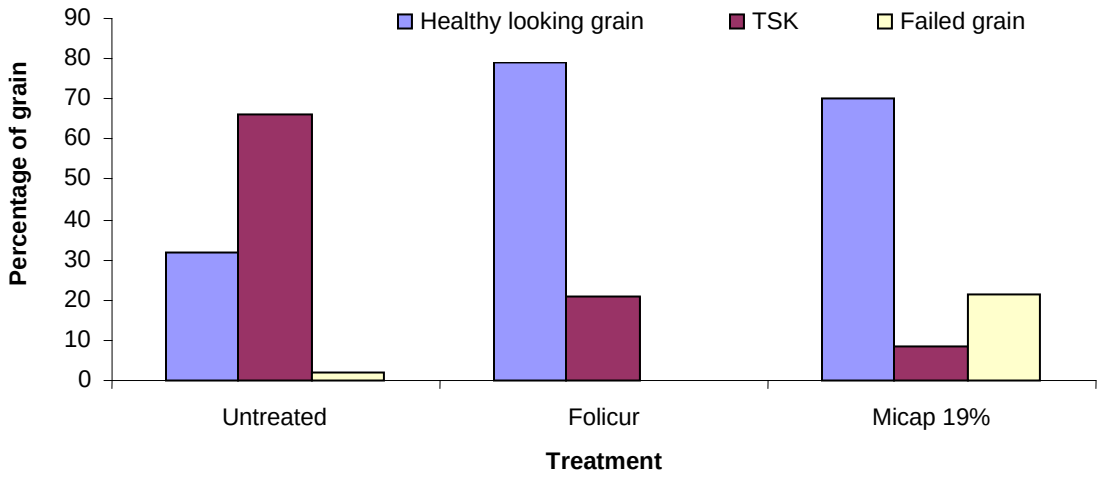

Figure 4. Effect of tebuconazole and microencapsulated tebuconazole on the proportion of healthy looking grain, infected grain or TSK (tombstone kernel) and failed grain.

The contamination of grain with DON was greatly reduced by both formulations. The levels recorded in healthy looking grain after treatment with Micap 19% and Folicur were 90 ppb and 580 ppb respectively. Both treatments reduced the contamination of the grain to an acceptable level (maximum limit in flour is 750 ppb).

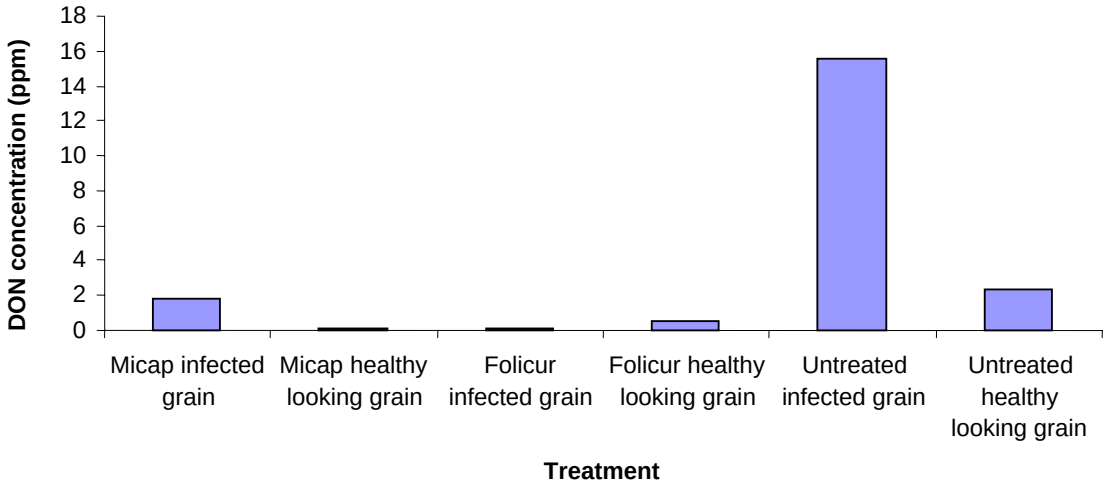

Figure 5. Effect of tebuconazole and encapsulated tebuconazole on the contamination of grain with the mycotoxin DON.

Micap plc. provided 2 more products containing 9.6% and 21.7% of tebuconazole. The 3 products were assessed *in planta* (results not shown). Micap 21.7% was found to control best FEB and was then compared to fungicide treatments recommended by Dupont. In this experiment, two wheat cultivars were used. The winter cultivar Charger was on the HGCA recommended list for 2003/04. This cultivar rated 4, in a scale from 1 to 9, for its resistance to FEB, making it the most FEB susceptible cultivar of the HGCA recommended list. The winter wheat cv. Centrum was also used. This German cultivar was identified in the Monsanto genotype screening for its low disease score.

Table 1 summarises the treatments and application rates used during the experiment.

Table 1. Active ingredient, product name and application rate of treatments used in the experiment.

Product name	Active ingredient (a.i.)	Application rate
Charisma®[a]	Flusilazole and Famoxadone	**1 L ha^{-1}**
Proline®[b]	Prothioconazole	**0.5 L ha^{-1}**
Punch® C[a] +	Flusilazole and Carbendazim	**0.4 L ha^{-1}**
Proline®[b]	Prothioconazole	**0.25 L ha^{-1}**
Charisma®[a] +	Flusilazole and Famoxadone	**0.5 L ha^{-1}**
Proline®[b]	Prothioconazole	**0.25 L ha^{-1}**
Charisma®[a] +	Flusilazole and Famoxadone	**0.5 L ha^{-1}**
Talius[a]		**0.25 L ha^{-1}**
Folicur®[b]	Tebuconazole	**0.5 L ha^{-1}**
Micap 21.7%	**Tebuconazole**	**0.5 L ha^{-1}**

[a] manufactured by DuPont
[b] manufactured by Bayer CropScience

The effect of fungicide treatment, sprayed on wheat cv. Charger and Centrum, on FEB incidence can be seen in Figure 6. Only the two tebuconazole formulations were able to substantially lower the incidence of FEB compared to the untreated controls. Micap 21.7% reduced FEB incidence by 82% on wheat cv. Charger and by 86% on wheat cv. Centrum while Folicur reduced FEB incidence by 81% on wheat cv. Charger and 84% on wheat cv. Centrum. Incidence of the disease was generally higher on wheat cv. Charger than on cv. Centrum.

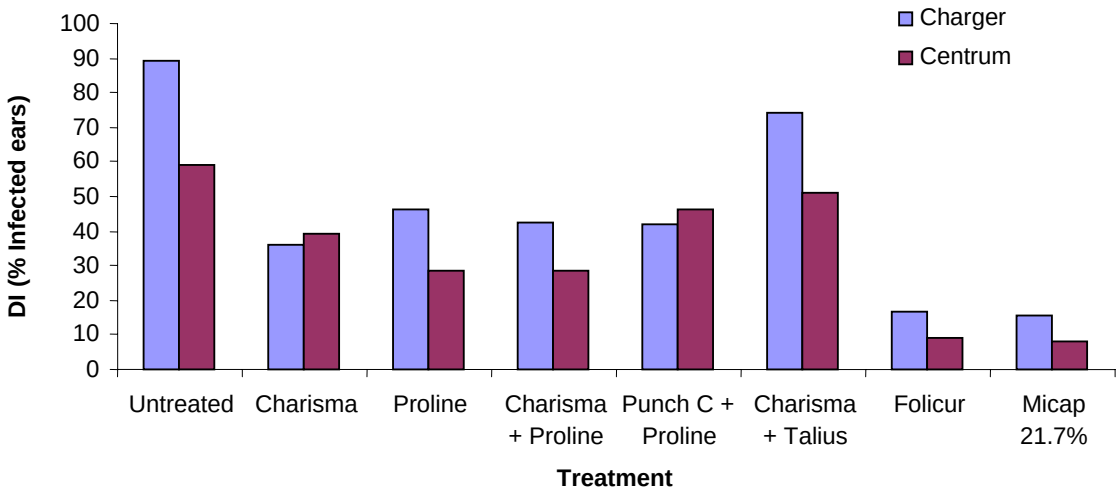

Figure 6. Effect of fungicide treatments on FEB incidence on wheat cv. Charger and cv. Centrum.

The results for the disease severity recorded on both cultivars are presented in Figure 7. Again, tebuconazole-based fungicides were the most effective. Micap 21.7% reduced severity by 89% on cv. Charger and 71% on cv. Centrum compared to the untreated controls. After treatment with Folicur, disease severity was lowered by 79% on wheat cv. Charger and by 46% on wheat cv. Centrum.

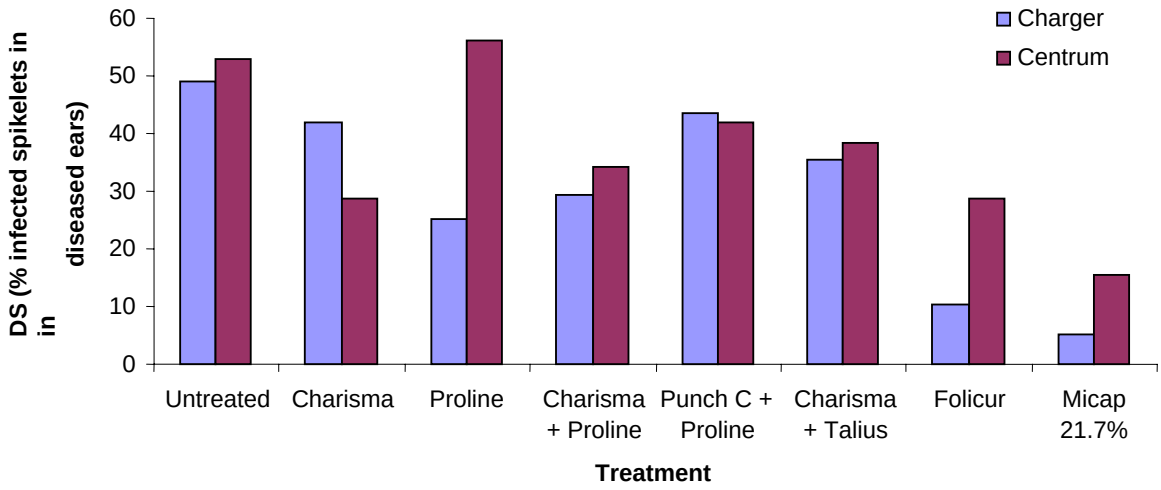

Figure 7. Effect of fungicide treatments on FEB severity on wheat cv. Charger and cv. Centrum.

Figure 8 shows the effect of fungicides on the TGW. All treatments improved the TGW to some extent.

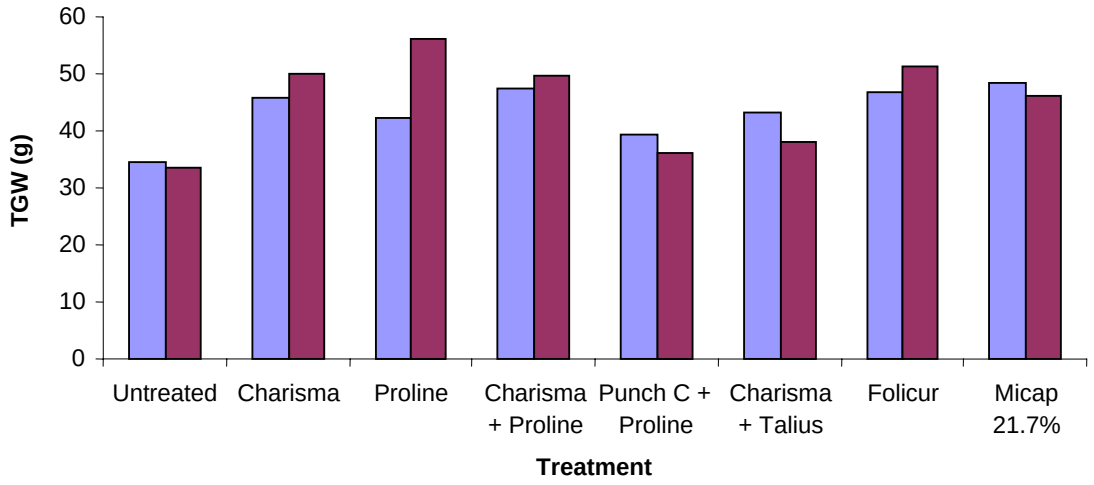

Figure 8. Effect of fungicide treatments on TGW of wheat cv. Charger and cv. Centrum.

All treatment except the tank mix Punch C + Proline reduced the proportion of TSK and increased the percentage of healthy-looking grain when sprayed on wheat cv. Charger (Figure 9). Application of Charisma gave the best results.

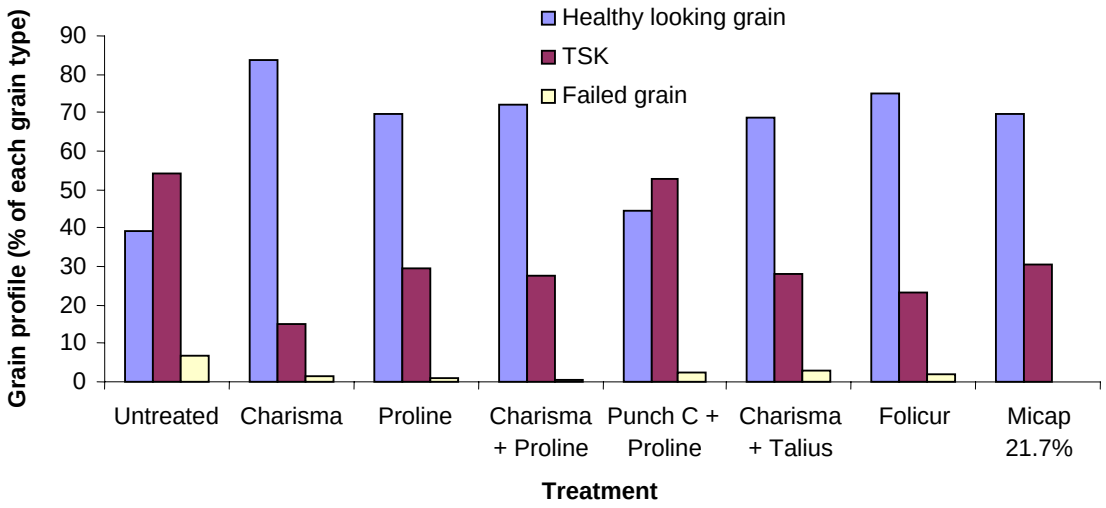

Figure 9. Effect of fungicide treatments on grain profile in wheat cv. Charger.

All treatments increased the proportion of normal grain on wheat cv. Centrum (Figure 10). However, proportion of TSKs in treated ears remained high, particularly after treatment with Charisma + Talius and Micap 21.7%. .

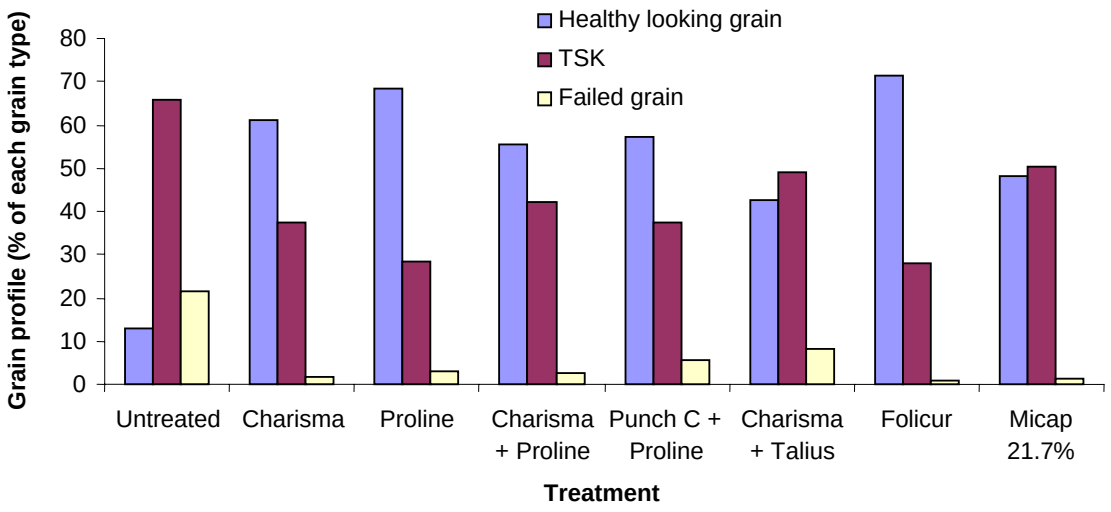

Figure 10. Effect of fungicide treatments on grain profile in wheat cv. Centrum.

DON contents in healthy looking grain and infected grain of wheat cvs. Charger and Centrum are presented in Figure 11 and 12. None of the treatments were able to control the contamination of the grain with the mycotoxin DON I wheat cv. Charger.

Only tebuconazole-based fungicides reduced DON content in both grain types compared to the untreated control in wheat cv. Centrum. Micap 21.7% gave the best results, reducing DON level to 0.017 mg Kg^{-1} or 17 ppb in healthy looking grain. This corresponds to a level well under the limit of 700 pb imposed by the EC. Contamination of grain of wheat cv. Centrum was much lesser than the contamination of DON recorded in grain of wheat cv. Charger.

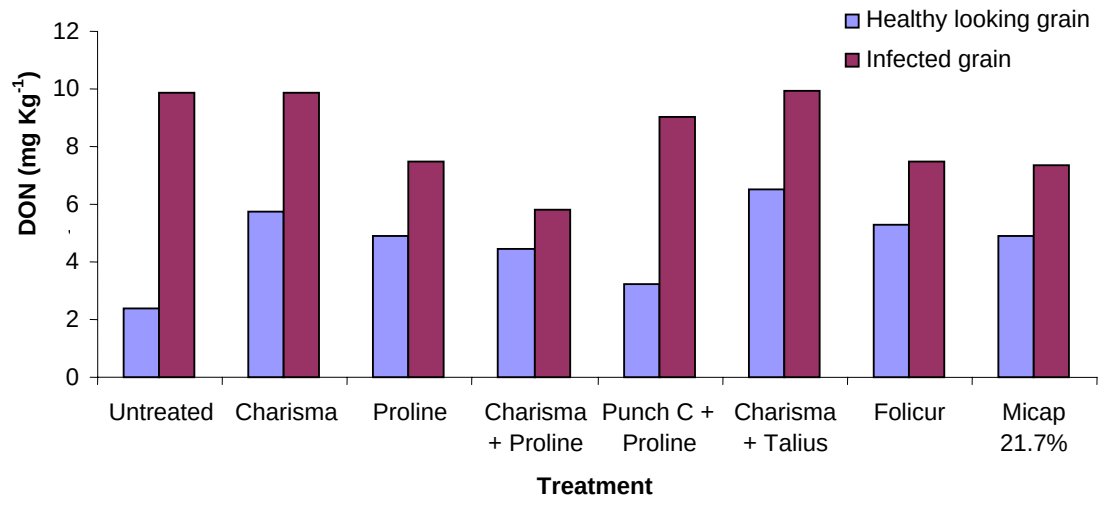

Figure 11. Effect of fungicides applied at GS59 on DON concentration in grain of wheat cv. Charger.

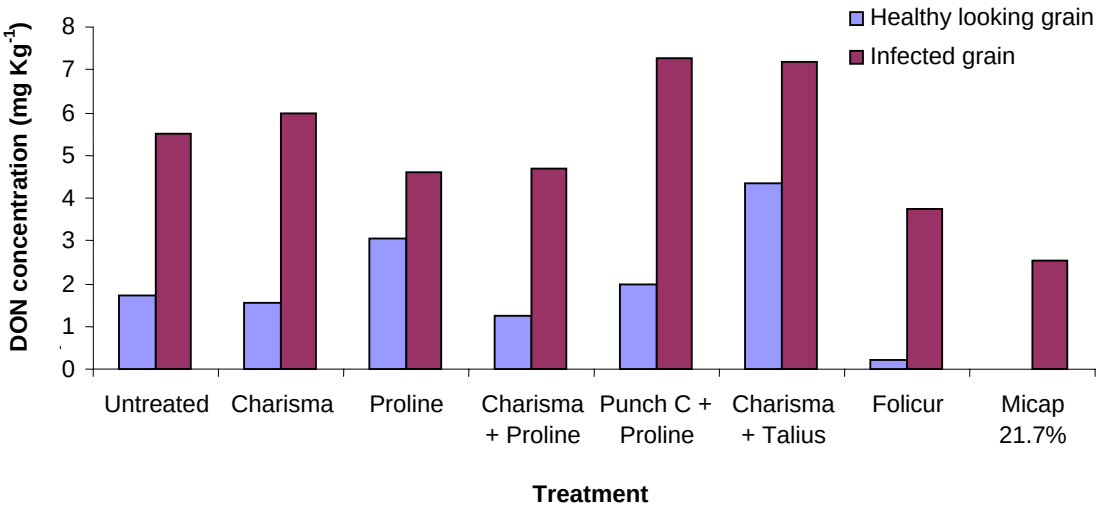

Figure 12. Effect of fungicides on DON concentration in grain of wheat cv. Centrum.

4. EFFICACY OF BIOLOGICAL CONTROL AGENTS TO CONTROL FEB

Bacteria were isolated from leaves, stems, and ears of wheat growing in Sutton Bonington (Nottinghamshire, UK). They were tested in the laboratory for antifungal activity against *F. culmorum* and *F. graminearum*. Fifty-five bacteria were isolated. After *in vitro* screen, 3 bacteria were selected for *in planta* tests. These were *Pseudomonas fluorescens* (M39), *Bacillus subtilis* (M22) and *Bacillus licheniformis* (M2). The bacterial isolate *B. subtilis* Bs 3.64 with proven biological activity against various fungal pathogens was used to compare with the bacteria isolated from the rhizosphere and phyllosphere of field wheat. Bs 3.64 was selected because this agent is already available as a formulated product, Botokiller, which has previously been shown to have activity against FEB of wheat (Troth, 2003). This biological formulation, containing sticking agents and nutrients, is commercialised in Japan, under license by Idemitsu, for the control of botrytis in aubergine and tomato.
All biological treatments reduced disease incidence (Figure 13) and severity (Figure 14) compared to the untreated control. *B. subtilis* M22 reduced the disease incidence by 34% and the disease severity by 61%, while *B. licheniformis* reduced the disease incidence and severity by 27% and 56% respectively, compared to the untreated control. *P. fluorescens* M39 reduced the disease incidence by 34% compared to the untreated control, but did not reduced the spread of the fungus in the ear as well as the Bacillus strains. Botokiller gave the best results, reducing FEB incidence and severity by 39% and 65% respectively. However, none of the biological treatments reduced the disease incidence and severity as efficiently as Folicur, which reduced both incidence and severity by over 70%.

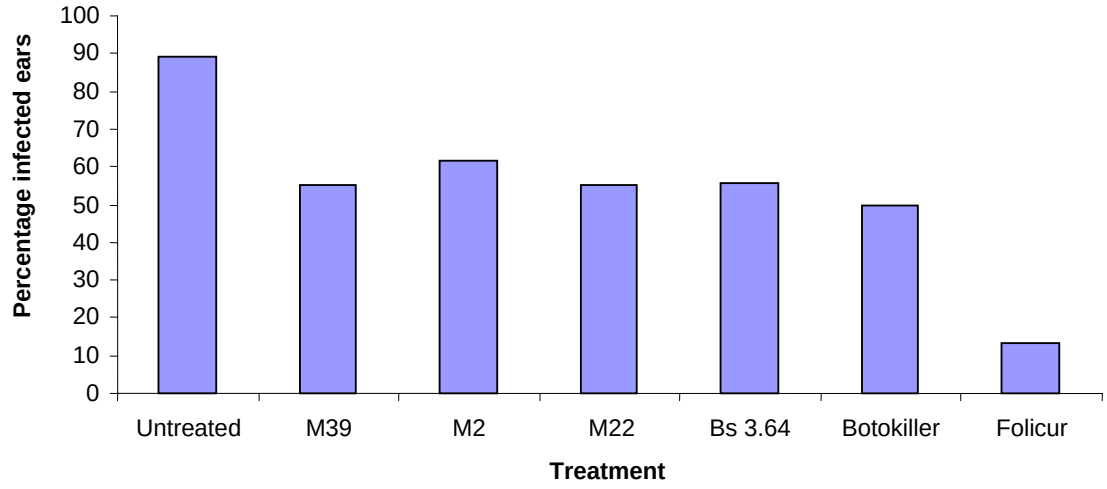

Figure 13. Effect of biological treatments and Folicur on disease incidence.

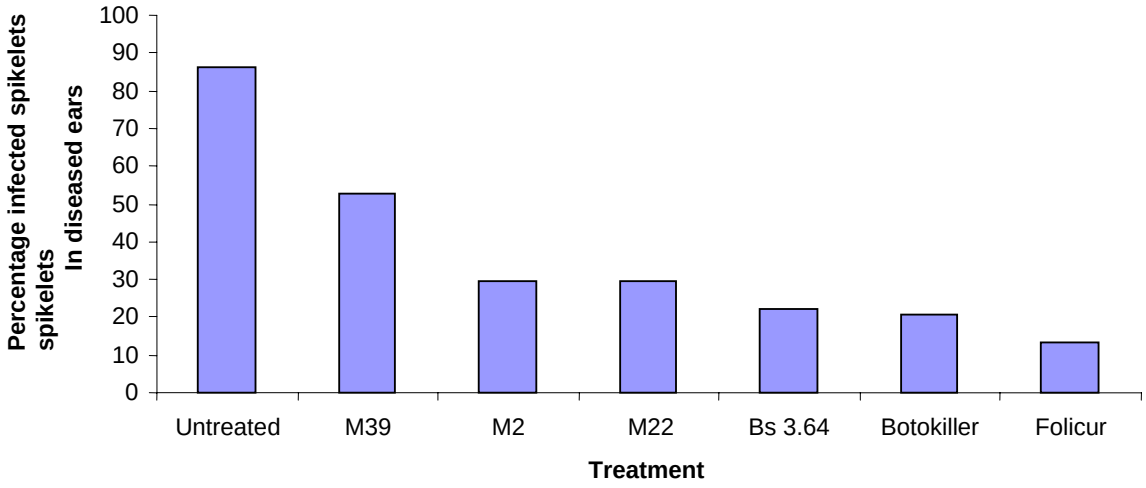

Figure 14. Effect of biological treatments and Folicur on disease severity.

The proportion of healthy looking grain was increased after application of all treatments (Figure 15). However, the number of failed grain was high for all treated grain except for grain from wheat treated with Bs 3.64.

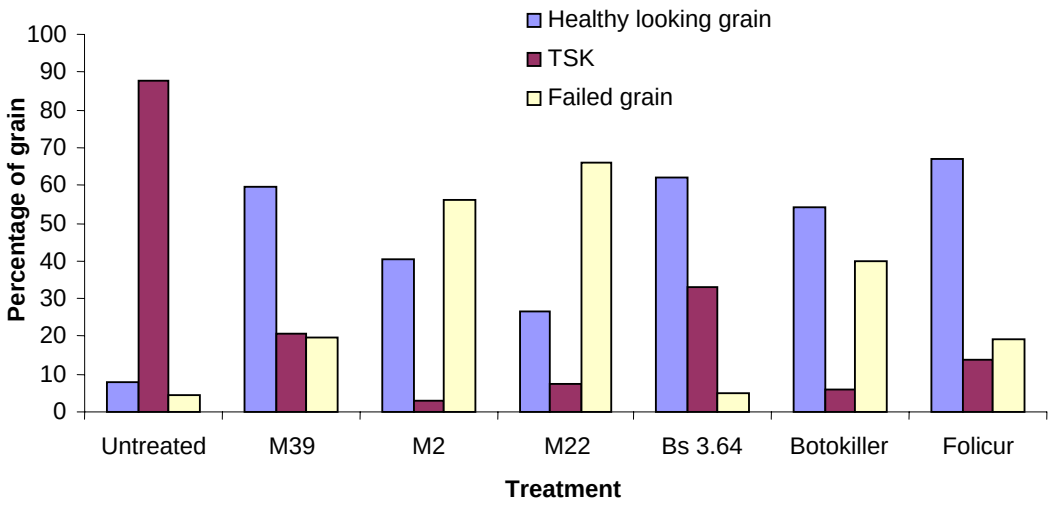

Figure 15. Effect of biological treatments and Folicur on the proportion of healthy looking grain, infected grain and failed grain.

All treatments increased the thousand grain weight. Treatment with Botokiller was the most efficient, increasing the thousand grain weight by two-fold compared to the untreated control.

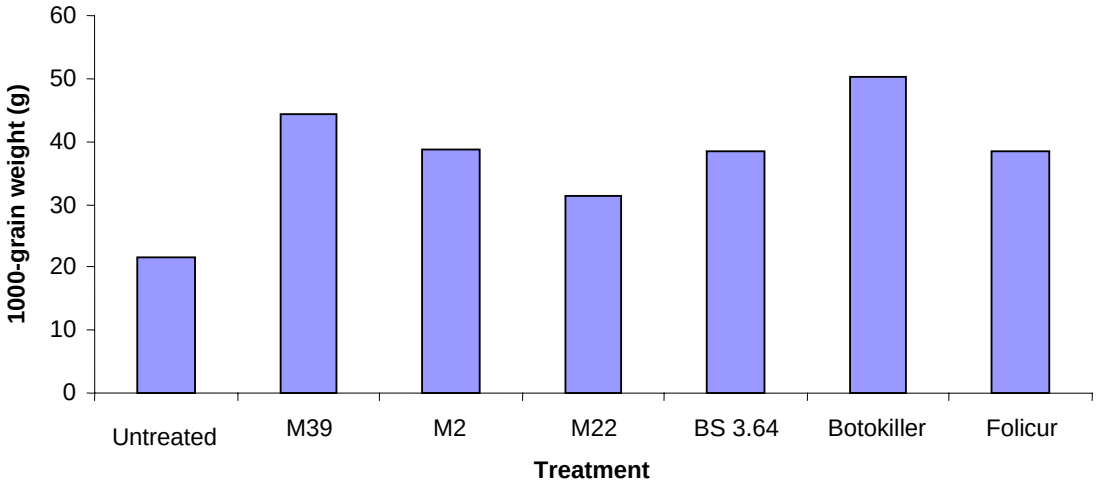

Figure 16. Effect of biological controls and Folicur on the thousand grain weight.

All treatments greatly reduced the contamination of the harvested grain with DON compared to the untreated control. *B. licheniformis* M22 was the most effective, reducing DON content in grain by 98%.

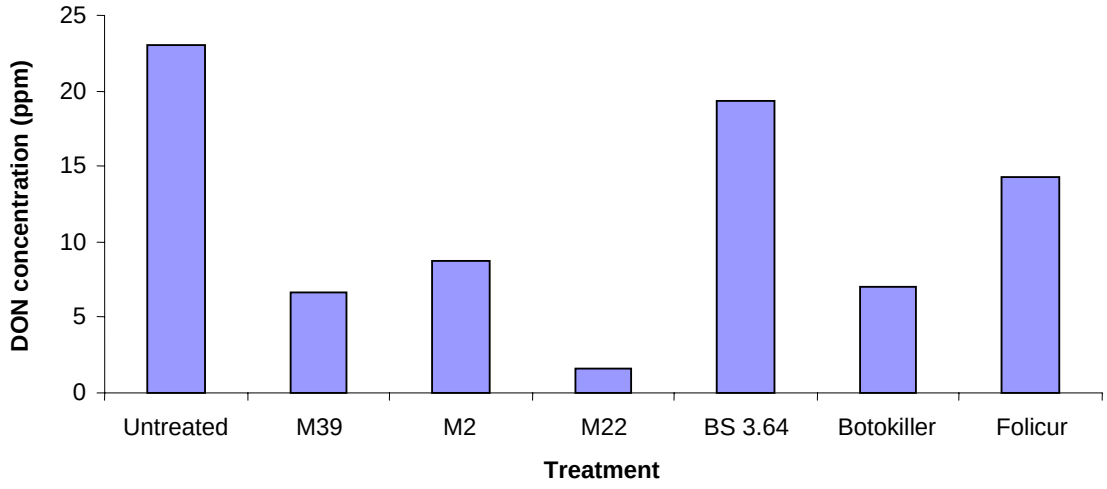

Figure 17. Effect of biological controls and Folicur on DON contamination of grain.

5. EVALUATION OF MICAP 21.7% AND BOTOKILLER UNDER FIELD CONDITIONS

The encapsulated fungicide Micap 21.7% and the commercial biological formulation Botokiller were tested for their efficacy in the field. Wheat cv. Charger was grown in Sutton Bonington (Leicestershire, UK). Table 2 summarises the treatments applied and the application rates used during the field experiment.

Table 2. Product name, active ingredient and application rate of treatments used in the field trial.

Product name	Formulation	Active ingredient (a.i.) or Biocontrol agent (BCA)	Application rate (g a.i. ha^{-1})
Folicur®[a]	Emulsion	Tebuconazole (250 g L^{-1})	125[c]
Micap™	Powder	Tebuconazole (21.7%)	125
Botokiller®	Powder	*Bacillus subtilis*	200

[a] manufactured by Bayer CropScience
[b] under license by Idemitsu
[c] half manufacturer's recommended dose rate

The disease incidence and severity data are presented in Figure 18 and 19. Overall conditions of the test favoured significant FEB pressure. Incidence of FEB was indeed very high with 95.3% of untreated ears showing disease. No symptoms were observed on ears from untreated non-inoculated plots, indicating that there was no natural infection and that the artificially inoculated pathogen did not spread to the untreated non-inoculated control plots.

Treatments only reduced disease incidence slightly. Folicur, Micap and Botokiller reduced FEB incidence by 14.0%, 18.7% and 9.3%, respectively, compared to the untreated control.

34.3% of spikelets in diseased ears from untreated control plots showed FEB symptoms. Folicur, Micap and Botokiller treatments reduced the disease severity by 7.1%, 8.6% and 4.6%, respectively, compared to the untreated control.

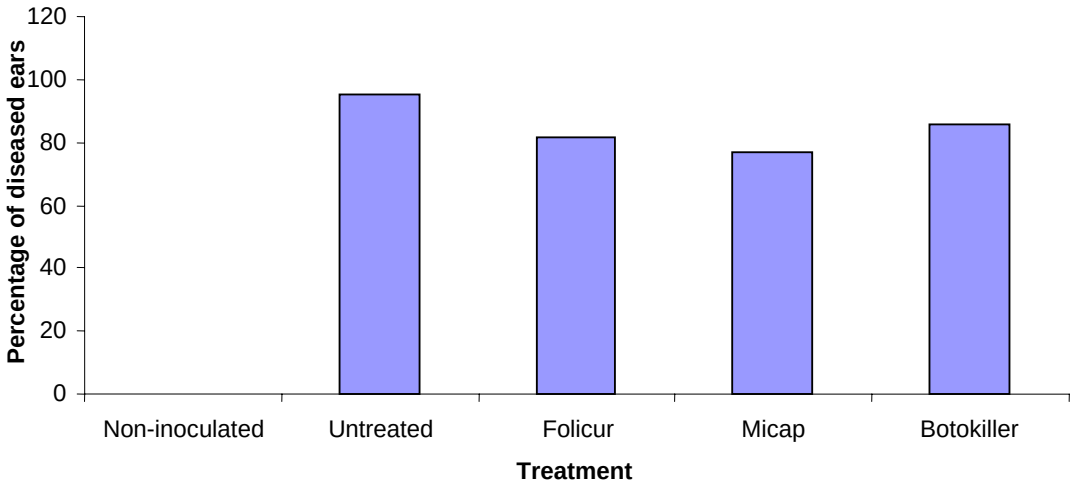

Figure 18. Effect of Micap 21.7% and Botokiller on disease incidence under field conditions.

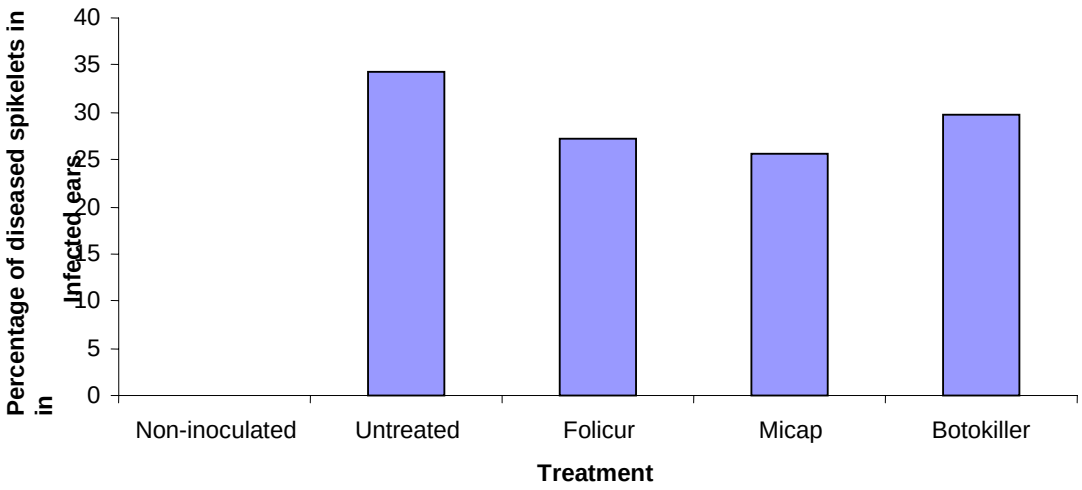

Figure 19. Effect of Micap 21.7% and Botokiller on disease severity under field conditions.

Neither treatment nor infection significantly affected the number of grain per ear (Table 3). This might be explained by the fact that all grains, including TSK and failed grains, were taken into account.

Both Folicur and Micap treatments resulted in increased TGWs compared to the untreated control and the biological treatment. However, the increase produced by the application of the two fungicides was 54% lower from the non-inoculated control. The biological treatment did not increase the TGW compared to the untreated control.

Table 3. Effect of Folicur, Micap and Botokiller on FEB yield parameters.

Treatment	*Number of grain per ear*	*TGW (g) (manually harvested grain)*
Untreated, non-inoculated	52.9	40.3
Untreated, inoculated	48.9	21.9
Folicur	51.6	25.9
Micap	50.5	26.3
Botokiller	55.4	21.0

None of the treatments significantly increased the proportion of normal grain compared to the untreated control (Figure 20). Ears sprayed with a preventive application of Folicur, Micap and Botokiller did not significantly produce less TSK compared to untreated ears.

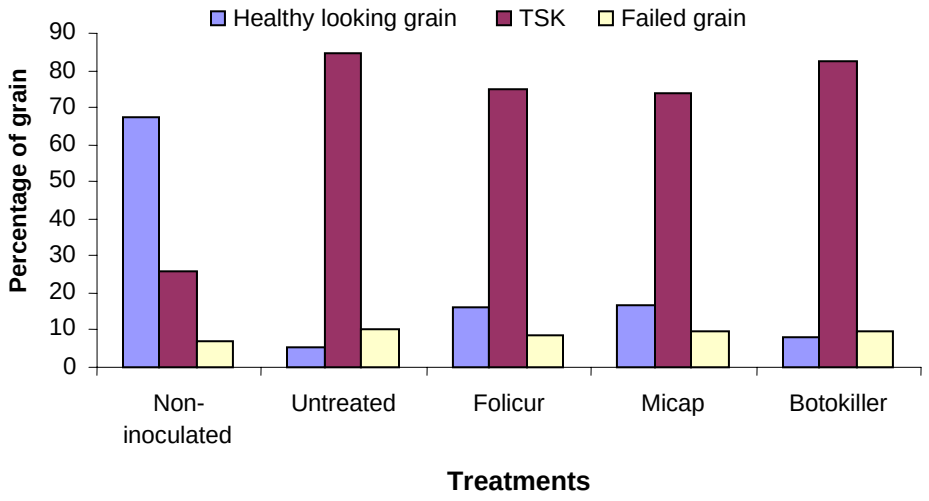

Figure 20. Effect of Micap 21.7% and Botokiller on the proportion of healthy looking grain, infected grain or TSK and failed grain.

There was no DON contamination of grain from untreated non-inoculated plots.

DON concentrations of 0-9.4 mg kg^{-1} were recovered from the infected harvested grains with the highest value obtained from diseased grain treated with Folicur. This fungicide resulted in a 25.3 % increase in DON content in diseased grain compared with the untreated control. Micap and Botokiller led to lower DON accumulation in infected grain than Folicur, however they were still higher than the untreated control: 9.0 mg kg^{-1} for Micap, 8.5 mg kg^{-1} for Botokiller, compared to 7.5 mg kg^{-1} for the untreated control.

All healthy looking grain samples showed high contamination with the lowest concentration obtained from untreated, Micap and Botokiller treated grain: 3.2 mg kg^{-1} for untreated and Micap treated grain, and 3.4 mg kg^{-1} for

Botokiller treated grain. Folicur (6 mg kg^{-1}) increased the concentration of DON by 80 % compared with the untreated control.

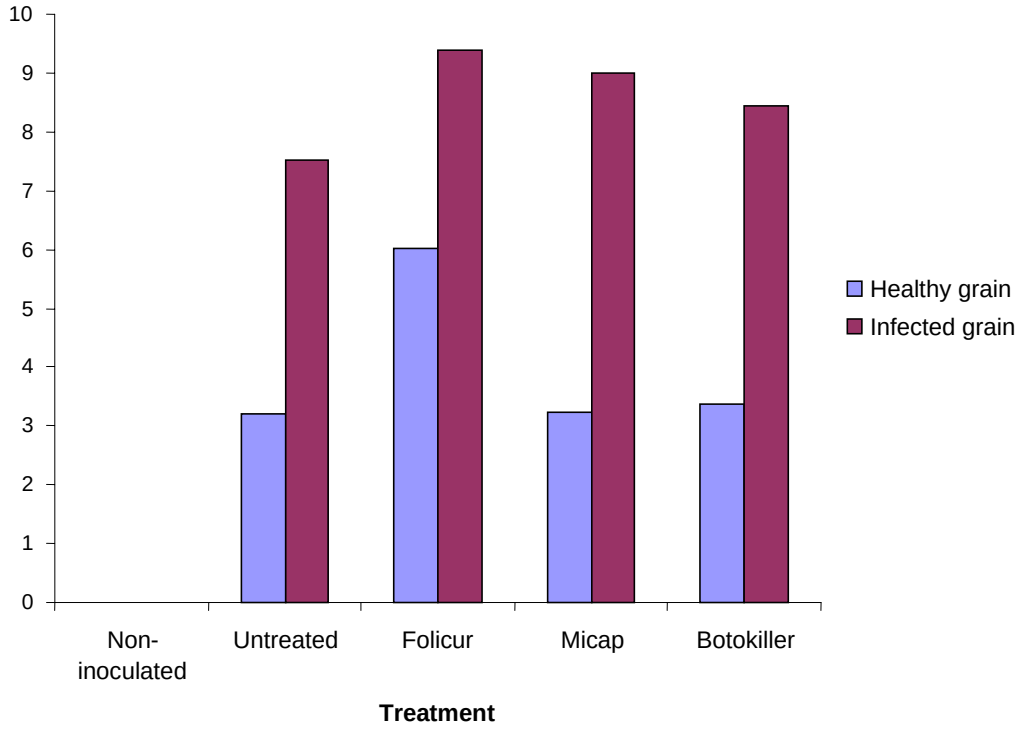

Figure 21. Effect of preventive application of Folicur, Micap and Botokiller on DON contamination of grain.Average values from two replications of ELISA analysis.

4. DISCUSSION

This study demonstrated the effectiveness of the encapsulated tebuconazole formulation to control the disease. When compared to various fungicides, the Micap formulation protected the ears against infection and DON contamination best. It is interesting to note that in this study, the two tebuconazole formulations performed better than prothioconazole (Proline) which has previously been shown to give better control than tebuconazole (Suty-Heinze and Dutzmann, 2004). The encapsulated fungicide is very promising for FEB control since it presents the possibility for the slow release of the active ingredient tebuconazole on wheat ears. Moreover, encapsulated fungicides present important advantages over liquid formulations: the active

ingredient is protected against environmental degradation, such formulations can reduce phytotoxicity and the contamination of the environment, but also the conversion of liquid materials into powders makes encapsulated formulations more convenient to handle.
The difference between using a susceptible cultivar and a moderately resistant cultivar was not evident in this study except on the contamination of the grain with DON. When application of tebuconazole-based fungicides was combined with the use of cv. Centrum, the concentration of DON in grain was substantially reduced.

Three bacterial isolates showed some activity against FEB. However, they were not as effective as tebuconazole. A second experiment (results not shown) also showed the instability of the activity of the biological control agents.

Micap 21.7% and Botokiller were not successful at controlling the disease in the field. Many factors may explain the poor efficacy of treatments reported in the present study. Factors such as heavy infection pressure, wheat cultivar susceptibility, wheat at susceptible growth stage, optimal temperature and humidity conditions, all play an important role in the intensity of FEB infections. In this study, all these factors were present. The crops were artificially inoculated with a pathogenic and toxigenic *F. culmorum* strain and, therefore, the infection pressure was high. The artificial inoculation occurred at the beginning of flowering, period during which the crop is the most susceptible to *Fusarium* infection. Moreover, the wheat cultivar used in the field trial was highly susceptible to FEB. In addition, the environmental conditions during the trial were particularly favourable to the development of FEB disease. The day following artificial infection, the air was moist and the plots were misted with water in the evening to further enhance infection. During the next two days, important thundery rainfall occurred and humidity levels remained very high. Day temperatures ranged between 19-26°C.

Consequently, FEB was severe in all plots where conidial inoculum had been applied to ears.

Reference:

Suty-Heinze A. and Dutzmann S. (2004). Fusarium head blight: an additional strength of prothioconazole. *Pflanzenschutz-Nachrichten Bayer*, **57**: 265-282.