PROJECT REPORT No. 126

SCREENING OF FUNGICIDES FOR THE CONTROL OF ERGOT (*CLAVICEPS PURPUREA*)

JULY 1996

PRICE £3.00

PROJECT REPORT No. 126

SCREENING OF FUNGICIDES FOR THE CONTROL OF ERGOT *(CLAVICEPS PURPUREA)*

by

D. J. YARHAM

ADAS Cambridge, Brooklands Avenue, Cambridge CB2 2BL

This is the final report of a sixteen month project which commenced in July 1994. The work was funded by a grant of £8,398 from the Home-Grown Cereals Authority (HGCA Project No. 0010/1/94).

The Home-Grown Cereals Authority (HGCA) has provided funding for this project but has not conducted the research or written this report. While the authors have worked on the best information available to them, neither HGCA nor the authors shall in any event be liable for any loss, damage or injury howsoever suffered directly or indirectly in relation to the report or the research on which it is based.

Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended nor is any criticism implied of other alternative, but unnamed products.

CONTENTS

	Page
Summary	2
Objectives	2
Introduction	3
Materials and Methods	4
Results	7
Discussion	11
Acknowledgements	13
References	14
Appendix (Husbandry of field trial)	15

SUMMARY

This project investigated the activity of a range of fungicides on the fungus *Claviceps purpurea* which causes ergot in cereals and grasses.In vitro testing showed certain triazole and morpholine fungicides to be superior in their activity against *Claviceps purpurea* to the benzimidazoles which, until now, have been the only materials to be used (allbeit without a manufacturer's recommendation) for the control of ergot. However, bio-assay of ovules dissected from ears four days after fungicides application (at anthesis), failed to detect the presence of the fungicides. This suggests very limited take-up of the active ingredients into the vulnerable organs of the inflorescence.

Of the fungicides tested, the morpholine fenpropimorph and the triazole difenoconazole were particularly effective against isolates of *C. purpurea* from both wheat and barley. Propiconazole was very effective against the barley isolate but not against that from wheat.

No ergot developed, even in the untreated control plots, in the spring wheat field-trial from which the ears were taken for dissection. None of the fungicides applied significantly affected the yield of grain.

The work demonstrated that there are now more effective fungicides than the benzimidazoles for the control of ergot. It did not, however, offer the hope that they are sufficiently systemic to penetrate to ovaries not exposed by the gaping of the glumes. Moreover, the absence of ergots in the inoculated trial clearly demonstrated that the routine application of fungicides against the disease will not always be cost effective even in areas where the risk of infection would normally be considered to be high. If fungicides are to be used against the disease, it will be necessary to develop effective risk assessment and forecasting systems.

OBJECTIVES

1. To test, *in vitro*, the activity of a range of systemic fungicides against the ergot fungus *Claviceps purpurea*.

2. To apply those materials showing most promise in the in vitro tests to field plots of wheat in order to determine:-

   a. whether their systemicity is such that, when applied at anthesis, they can pass into the ovaries

   b. whether they will afford protection against ergot infection in the field

INTRODUCTION

Because of the toxicity of its sclerotia to both humans and animals, *Claviceps purpurea*, the ergot fungus, must be considered one of the major pathogens of cereals, even although it seldom causes significant losses in yield. Contaminated grain stocks are difficult to clean and command lower prices than stocks which are free from contamination. In recent years, the problem has been exacerbated by a reduction in the levels of contamination allowed by the UKASTA standards (Anon, 1989).

Control of the disease currently depends on the use of uncontaminated seed, deep ploughing of fields which have carried infected crops, and the control of the grass weeds on which the pathogen builds up before the cereal crops come into flower. Application of MBC fungicides at anthesis will give some protection against infection but will not control the disease completely. Moreover, because of the difficulty or getting the fungicide to the ovaries (the organs attacked by the fungus), for the treatment to be of any real use repeated sprays have to be applied during the anthesis period.

If an acceptable strategy for fungicidal control of the disease is to be developed the following questions need to be answered:-

i) are any of the newer generation of systemic fungicides effective against the pathogen?

ii) if such fungicides are active against the pathogen, are they sufficiently systemic to be better able than the MBCs to penetrate into the ovaries?

iii) if fungicides are found which satisfy the above criteria, how best may they be used in a field situation to control the disease (more particularly, how best may applications be timed to protect the florets during anthesis)?

iv) assuming that effective treatments can thus be devised, can we improve our forecasting of ergot epidemics so that we may be better able to predict when the application of such treatments will be cost effective?

To obtain answers to questions (iii) and (iv) it will be necessary to work with the most effective chemical available. It was therefore deemed sensible to address only questions (i) and (ii) in this initial study and then, should a suitable chemical be found, to seek a source of funding which would allow this material to be used in subsequent trials aimed at obtaining answers to questions (iii) and (iv).

Initially it had been intended to test the systemicity of the test materials by carrying out bio-assays on excised ovules from a trial at ADAS Arthur Rickwood which was scheduled to be sprayed with a range of fungicides. In the event, however, it was decided to conduct a spray trial specifically for the purposes of this project at ADAS Trawsgoed. This had the advantages that the trial could be inoculated with ergot sclerotia without risking infection of nearby healthy cereal crops, and that the climate of west Wales was likely to be more conducive to the development of ergot than the much drier conditions of the East Anglian fens. It was thus possible to conduct the trial at a site which allowed not only for bio-assays to be conducted but also (in theory at least) for the chemicals to be evaluated for the control of the disease under field conditions.

MATERIALS AND METHODS

A. In vitro testing of fungicides

Isolates of *Claviceps purpurea* were obtained by culturing sections taken from surface- sterilised ergot sclerotia onto potato dextrose agar (PDA). These isolates were then subcultured onto PDA plates amended with the fungicides under test (plus an unamended control plate). A 5mm diameter disc taken (using a sterilised cork borer) from a colony of the original isolate was placed in the centre of each plate at each concentration of each of the chemicals being tested was was replicated on 10 plates randomly distributed in stacks in an incubator.

The subcultures were incubated for 14 days at 21°C after which their diameters were measured, the results being expressed as diameter of colony minus the diameter of original inoculant plug.

It had originally been intended to concentrate the project on the triazole fungicides with their broad spectrum of activity. As the work proceeded however, it was decided to introduce systemic materials from other chemical groups.

Subsequent to the 1995 harvest, a range of the more promising materials was tested against isolates from a fresh sample of ergot obtained from barley.

The of fungicides tested was as follows:-

	Fungicide group	Active ingredient	Product used	Formulation
1.	Benzimidazoles	benomyl	Benlate Fungicide	50% WP
2.		carbendazim	Bavistin	50% WP
3.	Azoles	triadimenol	Bayleton	250 g/l EC
4.		flusilazole	Sanction	400 g/l EC
5.		flutriafol	Pointer	125 g/1 SC
6.		propiconazole	Tilt	250 g/l EC
7.		cyproconazole	Alto	100 g/l SL
8.		tebuconazole	Folicur	250 g/l EC
9.		epoxiconazole	Opus	125 g/l SC
10.		difenoconazole	Plover	250g/l EC
11.		fenbuconazole	Indar	50 g/l EC
12.	Morpholine types	fenpropimorph	Corbel	750g/l EC
13.		fenpropidin	Tern	750g/1 EC
14.	Others	cyprodinil	experimental formulation	75% WDG
15.		iprodione	Rovral Flo	255g/1 SC

The fungicides were first tested at 10mg a.i./l., the concentration being reduced in subsequent tests to 1mg a.i./l, 0.1mg a.i./l and ultimately 0.01mg a.i./l. Except in exceptional cases (where it was desired to keep a material from a particular chemical group in the tests) fungicides which had failed to achieve complete control of the fungus at one concentration were not tested at lower concentrations.

All the initial fungicide sensitivity tests were carried out using isolates made from ergot sclerotia obtained from a 1994 wheat crop in Essex. When the additional chemicals were tested a check was also run on an isolate from rye to make sure that similar results could be obtained using isolates from different sources.

Throughout the in-vitro work, problems were encountered as a result of the growth habit of the *Claviceps* isolates changing with repeated sub-culturing. (The isolates assumed a flatter growth habit with less aerial mycelium than was present in the original isolate.) To overcome this problem fresh isolates were obtained for each set of tests and these were sub-cultured once only onto the fungicide-amended agar plates.

During the 1995 season the only ergots received by the ADAS plant clinic were three sclerotia from a barley crop in Leicestershire (ergot from barley would also be expected to be pathogenic to wheat). It was decided to conclude the work by repeating the sensitivity tests on an isolates from one of these sclerotia. At this stage only the lower rates of the fungicides were used.

B. The replicated trial

The trial area at ADAS Trawsgoed was sown with winter wheat cv. Hunter on 1 December 1994. The area was inoculated on 2 February with ergot sclerotia (a mixture of sclerotia collected in 1993 and 1994) at a rate equivalent to 20-25 sclerotia/plot. Unfortunately the very wet winter weather led to poor emergence of the wheat and a very poor stand in the spring. It was decided, therefore, to capitalise on this situation and to re-sow part of the area with spring wheat - a crop which is normally more prone to ergot than is the autumn sown crop. It was also expected that the presence of both winter and spring wheat on the site would allow the fungus to build up on the former in its sphacelia (conidial) phase and thus increase the likelihood of a significant attack on the spring wheat. Spring wheat cv. Baldus was therefore sown on 24 March 1995. Details of crop husbandry are provided in Appendix I.

The plots were sprayed on 24 June 1995 with six fungicides selected from those tested *in vitro*. As the manufacturers were unwilling for the "straight" material to be used on the trial, fenbuconazole was applied in the formulated mixture with fenpropimorph as "Myriad" (37.5g fenbuconazole + 281 g fenpropimorph / litre). The materials applied were as follows:-

Active ingredient	Product	Rate of application of product
difenoconazole	Plover	0.3 l/ha
epoxiconazole	Opus	1.0 l/ha
tebuconazole	Folicur	1.0 l/ha
fenpropimorph	Corbel	4.0 l/ha
cyprodinil	experimental formulation	0.67 kg/ha
fenbuconazole+fenpropimorph	Myriad	2.0 l/ha

C. The bioassay

Four days after application of the fungicides, eight randomly selected ears were taken from each plot and sent to Cambridge. Ovules were dissected from the bottom, middle and top of each ear and placed on agar plates on which isolates of *Claviceps purpurea* were growing just beyond the advancing edges of the colonies.

The cultures were incubated at 21° C and monitored for any evidence of inhibition of growth as the advancing hyphae approached the ovules.

RESULTS

A. Testing *in vitro*

Table 1 In vitro test of initial range of fungicides at 1 and 10 mg a.i./l using a wheat isolate

Material	10mg a.i./l in agar (26 Jan 95)		1mg a.i./l in agar (23 Feb 95)	
	Diameter (mm) at 14 days	Diameter as % control	Diameter (mm) at 14 days	Diameter as % control
Control	51.00	100.00	65.00	100.00
benomyl	0.00	0.00	25.00	38.46
carbendazim	0.00	0.00	15.00	23.08
triadimenol	10.00	19.61	-	-
flusilazole	0.00	0.00	0.10	0.15
flutriafol	17.40	34.12	-	-
propiconazole	0.00	0.00	12.50	19.23
cyproconazole	10.00	19.01	-	-
epoxiconazole	*	*	0.00	0.00
tebuconazole	*	*	0.00	0.00
difenoconazole	0.00	0.00	0.00	0.00
cyprodinil	0.00	0.00	0.10	0.15
SED	0.292	0.573	0.214	0.329

* not represented in test

It was of interest that the isolates obtained in January and February grew much more rapidly than those obtained later in the year. No explanation for this phenomenon has been found.

Table 2 Additional chemicals tested at 1 and 10mg a.i./l on wheat and rye isolates

Diameter of colony (mm) after 14 days

Material	10mg a.i./l in agar (5 May 95)		1mg a.i./l in agar (13 June 95)
	Wheat isolate	Rye isolate	Wheat isolate
Control	29.80	32.50	37.00
fenbuconazole	0.51	1.46	0.00
fenpropimorph	0.01	0.00	0.00
fenpropidin	5.52	6.02	-
iprodione	10.70	16.30	-
SED	1.567	1.681	1.199

Table 3 Additional chemicals tested at 1 and 10 mg a.i./l on wheat and rye isolates

Diameter of colony as percentage of diameter on unamended agar after 14 days

Material	10mg a.i./l in agar		1mg a.i./l in agar
	Wheat isolate	Rye isolate	Wheat isolate
fenbuconazole	1.6	4.1	0.00
fenpropimorph	0.3	0.0	0.00
fenpropidin	18.4	19.5	-
iprodione	36.8	51.3	-
SED	5.19	4.42	-

Table 4 In vitro tests at 0.1 and 0.01mg a.i./l (28 June 1995) on wheat isolates

Material	0.1mg a.i./l in agar		0.01mg a.i./l in agar	
	Diameter of colony (mm)	Diameter as % control	Diameter of colony (mm)	Diameter as % control
Control	35.00	100.00	35.00	100.00
tebuconazole	0.00	0.00	28.00	80.00
epoxiconazole	0.00	0.00	20.00	57.14
difenoconazole	0.00	0.00	7.00	20.00
fenbuconazole	0.10	0.29	13.00	37.14
fenpropimorph	0.00	0.00	0.10	0.29
cyprodinil	15.00	42.86	47.00	134.29
SED	<0.001	<0.001	<0.001	<0.001

Table 5 In vitro tests on barley isolates (24 November 1995)

Material	0.1mg a.i./l in agar		0.01mg a.i./l in agar	
	Diameter of colony (mm)	Diameter as % control	Diameter of colony (mm)	Diameter as % control
Control	31.0	100.00	31.0	100
benomyl	0.0	0.0	31.0	100
carbendazim	0.1	0.3	27.0	87.1
flusiliazole	0.0	0.1	11.0	35.5
propiconazole	0.0	0.0	5.0	16.1
tebuconazole	0.0	0.0	26.0	83.9
difenoconazole	0.0	0.0	17.0	54.8
fenbuconazole	0.1	0.3	19.0	61.3
fenpropimorph	0.0	0.0	10.0	32.3
fenpropidin	0.0	0.0	27.0	87.1
cyprodinil	5.0	16.1	29.0	93.5
SED	<0.01	<0.01	<0.01	<0.01

B. Bioassay tests

In the event, no zones of mycelial inhibition development around any of the embryos plated out onto agar. Occasional cultures showed slight, indentation of the leading edges of the isolates as they approached the embryos but this was almost invariably the result of the presence of bacterial (very occasionally, fungal) colonies developing from the embryos themselves. The only exception to this was seen on the cyprodinil plates where a few colonies showed varying degrees of transitory emargination around the ovules in the absence of obvious contamination of those embyos by any micro-organism. There was no close association between the emargination seen and the position of the ovule in the ear though it seemed to occur most frequently around ovules taken from the middle of the ears. The degree of emargination was very variable - from obvious to scarcely noticeable - and no objective measurements were made of it.

C. The field trial

No ergot was found in any of the plots either of winter or of spring wheat.

Table 6 Results of the field trial

	Yield spring wheat (t/ha) (85% DM)	Specific wt (kg/hl)
Control	3.37	79.62
tebuconazole	3.24	80.77
epoxiconazole	3.28	76.37
difenoconazole	2.97	77.92
fenpropimorph	3.23	81.30
fenbuconazole + fenpropimorph	3.31	81.27
cyprodinil	3.24	78.43
SED (18 df)	0.261	2.017 (skew)
CV (%)	11.4	2.6

DISCUSSION

The absence of ergot in the field trial was disappointing but instructive. It underlined the point that, even when sclerotia are known to be present on the soil, and even when an autumn sown crop is present to "bulk up" the disease before the spring crop flowers, ergot may not develop in the crop. The disease is very weather dependent, infection being favoured by high average relative humidifies (>74%) and low maximum temperatures (< 19° C) (Marshall, 1960). Hot, dry weather desiccates the stromata developing from the germinating sclerotia, dries up the conidial honeydew and reduces the period of host susceptibility by favouring efficient pollination (Shaw, 1986). The trial was deliberately situated in what is normally one of the wetter parts of Britain yet, even here, conditions did not favour ergot infection in 1995. It is of significance that the only cereal ergots received in the ADAS plant clinic in 1995 (those used in the final bio-assay) were from an irrigated crop of barley. The irrigation would have favoured infection in an otherwise unfavourable season.

There is obviously no case, even in what could be conceived of as high risk situations, for the routine application of fungicides for the control of ergot.

The trial at Trawsgoed, though not designed primarily as a yield trial, was taken to yield. None of the fungicides applied gave a significant increase in the yield of grain. This is likely to have been due, in part, to the absence of serious foliar disease in an abnormally dry season. Moreover, although anthesis has been regarded as the optimal time for the application of fungicides to control ergot, it is later than the optimal time to apply the main fungicide to wheat to control foliar diseases. Thus, while there is no case for routine application of a fungicide specifically to control ergot, neither can one justify delaying the main foliar fungicide treatment in case ergot occurs.

The in vitro sensitivity tests showed a number of fungicides to be considerably more effective against *Claviceps purpurea* than the benzimidazoles which, to date, have been the only materials used (albeit without label recommendation) for the control of the disease. Our inability detect the materials in the ovules by bio-assay four days after spraying suggests, however, that these newer materials are little or no more systemic than the old. The emargination of the colonies occurring around a few of the ovules taken of ears which had been sprayed with cyprodinil was the more surprising as the material was not among the more effective in the in vitro sensitivity test. The fact that the phenomenon involved primarily ovules from the middle of the ears suggests that the spray had penetrated to the florets between glumes which happened to be gaping at the time of application.

The greater effectiveness of the newer materials is encouraging though they still need to be tested under field conditions. If such testing is to be carried out, it is suggested that the trials should be conducted on irrigated crops (preferably in situations where an infestation of black-grass is present to bulk up the inoculum) and on sites inoculated with fresh ergots from the previous season's crop. As rye is particularly susceptible to the disease, especially in the wetter south-west of England, consideration should also be given to conducting trials on this crop.

Of the chemicals tested, some appeared to show differences in their activity against different strains of ergot. Propiconazole was outstanding in this respect, being weak in its effects on the wheat isolate but very active against the isolate from barley. For further testing it would obviously be wise to concentrate on fungicides which showed more consistent activity. Fenpropimorph was outstanding in this respect and, of the azole materials, difenoconazole looked particularly promising.

However active a material may be shown to be against the ergot fungus, if it is to be used cost effectively there is a need to develop:-
(a) a method of risk assessment based on site factors (presence of the disease in previous crops, presence of weed grasses, weather conditions earlier in the season etc.) and
(b) a set of criteria for spray timing based on the growth stage of the crop and the weather prevailing at the time.

ACKNOWLEDGEMENTS

The author would like to thank Mrs Christine Hewett (ADAS Cambridge) who carried out the sensitivity testing of the ergot isolates, Mr Tony Lathwood (ADAS Trawsgoed) who conducted the field trial and Mrs Amanda Jones (ADAS Cambridge) who analysed the data.

REFERENCES

ANON. (1989) New arrangements for moisture and ergot, Agricultural Supply Industry 19 (23) 4.

DICKENS, J.S.W. (1974) Ergot of Cereals and Grasses Advisory Leaflet 584, Ministry of Agriculture, Fisheries and Food, Agricultural Development and Advisory Service, London.

MARSHALL, G.M. (1960) The incidence of certain seed-borne diseases in commercial seed samples, II Ergot, *Claviceps purpurea* (Fr,) Tul. Annals of Applied Biology 48 19-26.

SHAW, S. (1986) Evaluation of Baytan for control of ergot contaminants in cereal seed, Pflanzenschutz-Nachrichten, Bayer 39 (1) 47-70.

WOOD, G. & COLEY-SMITH, J.R. (1980) Observations on the prevalence and incidence of ergot disease in Great Britain with special reference to open flowering male-sterile cereals. Annals of Applied Biology 21 41-46.

Further reading:-

HGCA Research Review No.25: Ergot of cereals a literature review and survey of incidence in treated grain by D J Yarham

APPENDIX

Husbandry of field trial

30 November:	Trial marked out and soil sample taken.
1 December:	Trial sown as blocks 3m x 40m to be divided into 3m x 5m plots (plus end-of-block discards).
2 February:	Trial site inoculated by scattering ergot sclerotia - equal weights being applied to each plot.
20 March:	2m x 40m of each of the strips sown on 1 December were sprayed with glyphosate to leave I m x 40m winter wheat.
24 March:	Sprayed strips harrowed and sown with spring wheat.
27 March:	Fertiliser applied at rate equivalent to 75kg N/ha, 60 kg P/ha, 90 kg K/ha.
24 June:	Fungicides applied to crop at anthesis.
28 June:	Plots sampled and ears sent to Cambridge for bio-assay.
17 July:	Trial examined for ergot - none found.
19 August:	Trial harvested.