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Towards a sustainable whole-farm approach to the control of Ergot

by

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ABSTRACT

The aims of this project were i) to establish whether grass margins act as a source of ergot infection for wheat crops ii) identify low risk grass species for use in margin mixtures and iii) determine whether wheat varieties differ in susceptibility to ergot.

Extensive sampling was carried out on farms to investigate the incidence of ergot in grass margins and compare the infectivity of ergots from different grass species on wheat. Ergots were found in 37 grass species and were more common in areas of weedy grass and natural regeneration than in sown margins. Ergots from different grass species differed in their infectivity for wheat, but there was also variation between ergots from the same grass species. It was concluded that grass species which combine low infectivity for wheat with late flowering should minimise the risk of spread of ergot to adjacent crops. It may be possible to identify ergot populations that are highly infective to wheat by alkaloid profiling.

In epidemiological studies, primary infections in wheat, caused by ascospores, were rare. Secondary infections, caused by conidia, occurred frequently on late tillers of wheat around the edges of plots and along tramlines and also within black-grass-infested areas of the crop. There was no evidence that grass margins produced gradients of infection into wheat crops, such as might be attributable to primary inoculum. They do however contribute to the overall reservoir of ergot inoculum in the arable environment. They may also provide a local source of secondary inoculum from infected grasses and this poses a risk to wheat at the crop / margin interface.

In an investigation of the susceptibility of wheat varieties to ergot, contrasting methods of inoculation were used to reveal a) 'field resistance' i.e. the combined effects of 'escape' and tissue resistance and b) tissue resistance on its own. Flowering traits which might confer escape from infection were also examined. Despite a high degree of variation in field resistance between sites and years, certain varieties showed consistently low levels of infection whereas a number of others tended to be heavily infected. There was also evidence of differences in tissue resistance. No variety was immune, but some exhibited a greater degree of partial resistance than others. This is evidence of genetic resistance to *C. purpurea* in the North European winter wheat gene pool. A range of flowering characteristics that

might influence openness of flowering were identified and assessed. Little consistency emerged, with seasonal variation in temperature or rainfall apparently having a much greater influence than variety. It was concluded that varietal differences in openness of flowering are unlikely to be a major determinant of their relative susceptibility to ergot in the field and that tissue resistance is of more importance.

SUMMARY

In recent years, concern has been mounting about ergot contamination in wheat and the resulting increase in grain loads being rejected. Because of the highly poisonous nature of ergot alkaloids, millers operate a zero tolerance for ergot in wheat. The UKASTA standard for feed grain is 0.001% ergot by weight and there are strict limits on ergot in certified seed. Since cleaning grain to remove ergots is costly and rarely completely successful, emphasis must be on preventing field infections. The increase in ergot has been attributed to various trends in farming systems, including the introduction of grass margins in arable fields, poor control of grass weeds because of herbicide resistance, shorter rotations and the move towards minimum tillage. Successful control of the problem will require a whole-farm, integrated approach.

Ergots, the overwintering sclerotia of the fungus *Claviceps purpurea*, replace the embryo of infected florets of cereals and grasses and are either harvested with the crop or fall to the ground and survive on the surface of the soil. A wheat crop is at risk from several sources of primary inoculum, both from within the cropped area (ergots carried over from the previous season and ergots imported in the seed) and from external sources (field margins, set-aside, non-cultivated land etc.). It is also at risk from secondary inoculum (conidia from infected grasses in the crop or margins and infected wheat plants). The importance of primary compared with secondary infection and the risks from inoculum generated within or outside the crop are unclear. An understanding of the movement of inoculum from field margins to crops is vital if we are to determine whether or not grass margins act as a major source of ergot inoculum for wheat.

There is evidence that different strains of *C. purpurea* occur, which differ in their host range and in their pathogenicity for wheat. If it can be established that certain grass species consistently carry ergot populations that are weakly infective on wheat, there may be an opportunity to reduce the ergot risk of field margins by adjusting their composition in favour of these species. Margin management practices, such as cutting, may also have an effect on ergot development and may provide further options for reducing the risk of spread of ergot from the margin to the wheat crop.

Knowledge about host resistance is extremely limited. Cereal crop species vary in susceptibility to ergot (in order of decreasing susceptibility – rye, triticale, wheat,

barley, oats). Little is known about variation in resistance within species, although certain UK wheat varieties have been reputed to be particularly prone to ergot infection, for example the winter wheat variety Rialto. Infection risk is greatest for species or varieties with florets that gape open during flowering. Florets that remain closed during pollination and for a few days afterwards provide a mechanical barrier to the entrance of spores and escape infection. Susceptibility to ergot infection persists for only a few days after fertilisation and hence factors which delay or reduce pollination are likely to increase ergot infection. In addition to differences in 'escape' there is the possibility that varieties might differ in 'tissue' or 'post-infection' resistance. Plant breeders do not currently screen their potential varieties for ergot resistance, nor do they make any systematic assessment of the flowering characteristics that may be associated with escape. Ergot resistance is not evaluated during official variety trials at the National List or Recommended List stages. This means that farmers do not have the information on which to choose low risk varieties and breeders do not have the knowledge needed to select for improved ergot resistance in their breeding programmes.

A sustainable solution to the ergot problem must be one that minimises the risk of ergot infection in cereal crops whilst retaining the environmental benefits of grass field margins. Key requirements will be (i) to reduce the potential for ergot inoculum production in grass margins by identifying low risk grass species and management regimes and (ii) to integrate wheat variety selection and agronomic practices into effective crop management strategies. The primary aim of this project was to determine whether, and to what extent, grass margins act as a source of ergot infection for wheat crops. This was accompanied by a search for low risk grass species for use in margin mixtures and an investigation of variety resistance as a contributor to the reduction of ergot risk in the wheat crop.

The project was organised into 5 work packages:

WP 1: Grass margin and crop monitoring.

The main aim was to examine the ergot status of representative country stewardship field margins in relation to their grass species composition and management.

WP 2: Evaluating the risk from different grass species

The aim was to determine whether populations of *Claviceps purpurea* supported by different margin grass species differ consistently in their pathogenicity for wheat

WP 3: Epidemiology

This work package aimed to quantify the spatial distribution of *C. purpurea* from primary spread (ascospores) and secondary spread (conidia) and to assess the extent to which field margins contribute to ergot infection in cereal crops.

WP 4: Resistance of wheat varieties to ergot

This work package examined variation in 'field resistance' to ergot amongst UK wheat varieties and the importance of the two components, 'escape' (as conferred by flowering biology) and tissue resistance.

WP 5: Diagnostics

The objective was to develop a PCR diagnostic for quantitative detection of *C. purpurea* for the analysis of spore trap samples collected during the course of WP3.

In WP1, extensive monitoring of field margins and crops across a wide range of regions and margin types was carried out to investigate the ergot status of representative country stewardship field margins in relation to their grass species composition and management. Surveys were carried out on commercial farms during the summers of 2005-2007. A wide range of different field margin compositions and management regimes were covered in different regions to provide contrasting environmental and cropping situations. In addition, sampling was carried out in existing field margin experiments.

Ergot was found widely and in many grass species, both in weed grasses and in sown species in margins. The species affected varied from site to site. The results indicated a high incidence of ergot in East Anglia. This could be due largely to the large number of samples being obtained from East Anglia, but also due to this region being one of the most productive agricultural areas growing a large area of cereals. It appeared that margin age, type of margin and crop cultivation practices (ploughing or minimum tillage) had little effect on ergot incidence in margins. There was evidence that ergot numbers were greater close to the hedge or next to the crop. Very few samples were obtained from within the main body of the margin. In all sampling years, the largest numbers of ergot sclerotia were found in Couch grass, Italian ryegrass, Black-grass, Perennial ryegrass and Cocksfoot. In experimental field margins, ergot became established on sown grass species, but also appeared near the original hedgerow and at the margin of arable cultivation where common grass weeds were not controlled. There was considerable variation in the species affected by ergot.

WP2, carried out in conjunction with WP1, was designed to establish whether ergots from grasses which were found commonly in arable environments were infective on wheat, and whether any low risk grass species could be identified which could be used in margin seeds mixtures. Ergots were collected during monitoring from grass species in wheat growing rotations, including weedy species in crops, grasses in sown field margins, natural regeneration strips or headlands, and a range of other habitats such as paths, verges, banks, hedge bottoms, and rough grassland. The majority of samples were collected in 2004, 2005, and 2007, with relatively few in 2006, giving a final total of 497 and representing 37 host grass species. Of these, 240 samples from 29 species were tested for their infectivity to wheat. The predominant sources of ergot were couch, cocksfoot, black-grass, perennial ryegrass, tall oat grass, Italian ryegrass, timothy, tall fescue and Yorkshire fog. There were large differences between the infectivity of different cocksfoot isolates on wheat. Different sclerotia of cocksfoot ergot from within the same site also varied in infectivity. The population of ergot on cocksfoot should thus be considered as posing a high risk for wheat infection. The flowering period of cocksfoot overlaps with that of wheat, so there is potential for an increase of honeydew on cocksfoot inflorescences which could provide an infection source. Ergots from the weedy species, couch and tall oat grass, also varied in infectivity level to wheat, and some isolates produced many large sclerotia. Some ergots from timothy and tall fescue, both of which are used in margin seeds mixtures, were highly infective on wheat. Meadow fescue ergots were intermediate. Isolates from ryegrass ergots tended to be less infective, and those from Yorkshire fog had consistently low infectivity. A smaller number of ergots were received from other grass species. Of these, sweet vernal grass, and creeping soft grass had ergots with lower infectivity, and may be appropriate for use in margin seeds mixtures. Other grasses eg mat grass and rough-stalked meadow grass had low infectivity but would be of limited use in margin mixtures.

Although initial concern over rising levels of ergot was focused on the role of grass margins, there was no indication from the monitoring that sown margin grasses are the main source of ergots. On the basis of the extensive monitoring carried out during the project, all sown margins represented a lower risk of ergot than unsown areas. The majority of samples originated from areas which had not been sown, and often came from grasses which had been allowed to proliferate in areas such as hedgerows adjacent to margins, and are considered weedy species. The effect that using low risk species in margin mixtures, against a background of naturally occurring higher risk

grasses such as cocksfoot, couch, tall oat grass etc., is not known. However, increasing the level of species such as Yorkshire fog or creeping soft grass in seeds mixtures may help to increase the proportion of ergot in a location which has low infectivity on wheat. During the course of the work, there were no ergots received from crested dog's tail (*Cynosurus cristatus*), even though the species was used in many of the margins surveyed. Repeated attempts to inoculate florets with a number of different isolates failed to produce any ergot sclerotia. This may indicate resistance to ergot, or that the florets exclude infection in some way, but it would seem that crested dog's tail is a promising species for reducing ergot risk. Commercial seeds mixtures for ELS margins already include a high proportion of crested dog's tail, together with red or sheep's fescues, and relatively little of higher ergot risk grasses such as cocksfoot. Such mixtures would thus be unlikely to contribute to increasing ergot risk, and also fulfil desirable environmental requirements.

Selecting some grasses with late heading dates for use in margin mixtures will also help to reduce the incidence of ergot in a crop within a season since the main multiplication period of honeydew will not coincide with the main flowering period of wheat. Combining late heading grasses with species which support non-wheat infective populations should maximise the opportunities for reducing ergot risk from margins. This needs to be balanced against retaining the desired environmental properties. Weedy grasses which have become established in margins still present a potential problem, since it was clear that ergots from species such as couch and tall oat grass could infect wheat significantly. This is in contrast to earlier studies during the 1970s, though number of samples tested then was much lower. Adopting specific management practices such as spraying narrow strips between crop and margin before grass flowering could reduce the threat of ergot spread by direct contact of infected grass ears and prevent the movement of weedy species into the crop

Alkaloid profiling may offer a practical method of characterising local ergot populations. Though the relationship between alkaloid content and infectivity was not completely consistent, with the samples tested here there was a tendency for low infectivity types to have a low ergotoxine content, and for highly infective types to be high in ergotoxine. The effectiveness of attempts to manipulate ergot populations by increasing certain grass species could be judged by alkaloid profiling.

The robustness of information on low risk grass species is necessarily defined by the number of samples received over the period of this project. The table below summarises grass species in terms of risk of ergot transferring to wheat, together with an indication of the basis of the risk data in terms of samples received and tested. A combination of low infectivity, and late flowering, would minimise risk. High infectivity, coupled with early flowering, or a flowering overlap with wheat, would constitute a higher risk.

Species	Flowering time in relation to wheat	Infectivity risk to wheat	Range of infectivity mean sclerotia/ear	Sample numbers on which infectivity risk based
Margin seeds mixtures				
Common Bent	Late	High	3.0 -18.5	2
Crested Dog's Tail*	Overlap	Unknown	-	None received
Cocksfoot	Overlap	High	0.0 - 29.7	64
Meadow Fescue	Late	Intermediate	5.5 - 23.8	5
Sheep's Fescue	Overlap	Unknown	-	None received
Red Fescue	Overlap	Unknown	-	2 - not viable
Smooth stalked Meadow Grass	Overlap	Unknown	-	1 - not viable
Meadow Foxtail	Early	High	33.2	1
Tall fescue	Late	High	2.5 – 20.5	9
Wavy Hair-Grass	Late	Low	0.0	1
Yorkshire Fog	Late	Low	0.0- 11.8	25
Timothy	Overlap	High	0.7- 34.0	10
Weedy and other grasses				
Black-grass	Early	High	5.5 -33.8	5
Couch	Late	High	4.7 – 26.8	15
Tall oat grass	Late	High	0.0 – 21.5	14
Sweet vernal grass	Early	Low	0.0- 0.1	2
Creeping soft grass	Late	Low	0.2-10.0	7
Perennial ryegrass	Overlap	Intermediate	1.3 - 20.0	15
Italian ryegrass	Overlap	Intermediate	0.0 -15.8	6
Meadow brome	Overlap	High	15.8-27.3	4
Annual meadow grass	Early and prolonged	Low	1.6	1

* Crested Dog's Tail highly likely to be low risk since no ergots were produced after repeated inoculations

In WP3, a detailed study of the epidemiology of ergot was undertaken to determine whether grass margins are a significant source of ergot infection for wheat. Ergots over-winter on or just under the soil surface and release spores (ascospores) in the spring to cause new infections. Several days after infection, large numbers of a different type of spore (conidia) are produced in a sweet liquid called 'honey dew' and these spores can spread the infection by direct contact, rain-splash or by insect vectors to new grass or cereal flowers that same season. These 'secondary' infections therefore occur on grasses or cereals flowering several days at least after the initial 'primary' infection.

The first part of the study used different farm sites, often known to have a history or ergot problems. This part studied the timing of release of ascospores (primary inoculum) of *C. purpurea* and the relative importance of inoculum from different sources (field, margin). The second part was designed to measure gradients of infection due to different infection types (primary and secondary), and also to ensure that results would be obtained in case there was poor natural ergot infection rates at the farm sites. This was done using controlled field experiments at Rothamsted Research (RRES).

A quantitative PCR method was developed at NIAB as a tool for the detection and quantification of spore trap samples taken during the epidemiology studies (WP5). Using the sequence data for the 540 base-pair *C. purpurea* species-specific DNA marker, a number of primer pairs were designed. These were assessed for their suitability for use in a real-time PCR assay. Primers 235F and 451R producing a 217bp amplicon were selected and an internal fluorescent hybridisation probe utilising LightCycler red 640 was designed. Use of this probe was found to increase the sensitivity of the real-time PCR reaction 10 fold over use of the primers alone allowing quantification of *C. purpurea* DNA down to 1pg. The developed primers were successfully screened against 30 *C. purpurea* isolates with a range of host and geographic backgrounds.
The primers (235F and 451R) and hybridisation probe were screened against a wide range of ear pathogens and common saprophytes found on both grass and cereal crops to ensure that there was no cross reactivity. Extraction of the pathogen DNA from spore trap tape, passive trap tubes and plant host tissues was optimised. Using artificially inoculated sticky-trap tape to produce a range of infection levels it was established that a single spore could be reliably detected using the system.

Ergot infections at farm sites were rare and at only one location was there a clear difference in infection of wheat with four out of four samples infected next to margins and none within the field. More sites were needed to enable firm conclusions to be made. Ascospores in air were rarely detected, suggesting that they were present in very low concentrations, although the greatest concentration in air was found above natural grassland rather than in an inoculated plot of wheat (on ploughed land). The germinated ergots, which released ascospores were seen mainly in mid-May to early June, which was before wheat flowered but at the time of flowering of grasses like black-grass. In two years out of three, large numbers of ergots were found in the experiments at Rothamsted but these were always on late tillers that would have been infected in late-June to mid-July. These were located mainly around the edges of plots or along tramlines because there are many more late tillers produced in these locations rather than because there was more inoculum or vectors present. There were indications of secondary infections occurring immediately around (within 1 m) inoculated positions but large numbers of other secondary infections that occurred on late tillers are thought to have arisen either from natural inoculum or from vectoring by insects over larger distances. The secondary inoculum (conidia in honey dew) was present for longer than originally thought, and was present on the surface of visible ergots, which were seen to be visited by insects that fed on their surface. Patterns of infection caused by primary inoculum could not be seen because infections caused by ascospores were apparently very rare. This is thought to be similar to patterns of other ascospore-initiated diseases of cereal ears, such as *Fusarium graminearum*, due to widespread dispersal and random deposition of spores.

At other sites, ergot distribution was affected by the presence of black-grass patches being more severe in wheat within the patches This was demonstrated in a black-grass herbicide trial at ADAS Boxworth in 2004 where ergot was much less prevalent in herbicide-treated plots. Where black-grass populations were high, there was only a low population of wheat ears and weak late tillers showed most ergot infection. It was also observed that ergot was highly localised within crops and often occurred in or near tramlines. Steep gradients were found in winter barley in Bedfordshire in 2004. Late tillers showed high levels of ergot close to the wheelings and were a key factor in ergot contamination of grain samples. The field pattern of gradients was confounded by areas of rabbit grazing where late tillers also showed high ergot infection.

The study suggests that margins should not contribute to ergot gradients within a crop due to primary inoculum, only to overall increased production of inoculum. More importantly however, field margins do provide a local source of secondary inoculum from infected grasses and this poses a risk to wheat mainly at the edges of wheat fields where grass ears come into direct contact with cereal ears. This is exacerbated by the increased incidence of late tillers around the edges of crop areas. Indications are therefore that a mown or herbicide-treated strip between the crop and the margin would be advantageous. It is suggested that inspection of crops should concentrate on margins and tramlines, particularly in late tillers and that in addition to margin management and grass selection identified in other work packages, methods to reduce late tillering in crops or to harvest affected areas separately may help to reduce ergot contamination of grain.

In WP4, our aim was to examine variation in 'field resistance' to ergot amongst current UK wheat varieties and to find out whether this is due principally to differences in 'escape', differences in tissue resistance or a combination of the two. This entailed the use of different methods of artificial inoculation to reveal a) 'field resistance' i.e. the combined effects of 'escape' and tissue resistance and b) tissue resistance on its own. Flowering traits which might be associated with escape from infection were also examined.

The investigation of field resistance comprised three experiments, two in summer 2006 one in 2007. In each experiment, 2-row plots of winter wheat varieties were drilled with a 2-row strip of black-grass between them. 46 varieties, comprising lines from the UK Recommended List, together with a number from National List trials and two commercial varieties from each of France and Germany were included. Black-grass strips were inoculated with a conidial spray of a mixture of isolates of *C. purpurea* as the grass came into ear, but before the wheat reached ear emergence, the aim being to allow infection to spread naturally from infected black-grass to adjacent wheat plots. The frequency of ergot infection was estimated in late July by counting the number of visible ergots per plot or the number of heads per plot with visible ergots. Ear samples were taken in August from each plot and threshed carefully to release ergots without loss or damage.

The frequency of ergot infection visible before harvest differed between years, sites and varieties. There were also differences in the total amount of ergot threshed from

ears which had visible ergots and the amount of ergot from ears which were not observed to have ergot infection in the field. There was good correlation within experiments between observed ergot field frequency and data for the amount of ergot threshed from ears which had visible ergots. However, some varieties had appreciable numbers of ergots although infection was not obvious in the field.

While there was generally poor agreement between experiments in the levels of infection on different varieties, it was apparent that certain varieties were consistently relatively little infected whereas others tended to be heavily infected Varieties were thus classified by rank for each character within a trial summed across site-years to produce a mean rank and so look for trends. Least heavily infected varieties included Cordiale, Soissons, Glasgow, Oakley, Malacca, Hyperion, Smuggler, Robigus and Einstein, whilst the most heavily infected included Rialto, Xi19, Mascot, Solstice, Gatsby, Dover, Tommi, Timber and Ochre. This suggests the existence of at least some genetic resistance to *C. purpurea* exists in the North European winter wheat gene pool.

In order to investigate host tissue resistance, ergot spores were introduced directly into the florets, in order to bypass 'escape' factors. Experiments were carried out in four years, 2005, 2006, 2007 and 2008. Ears for inoculation were selected at random from main stems of plants growing in drilled plots on the NIAB trial ground in Cambridge. Small drops of a suspension of ergot conidia were inserted into individual florets to achieve infection of the ovary. This was done at the very early flowering stage, before pollination. At maturity, ears were collected and hand-threshed to remove ergots, which were counted and weighed. In each year, between 4 and 10 varieties were inoculated, giving a total of 14 varieties, 3 of which were common to all 4 years.

The results revealed significant varietal differences in ergot production. Differences were largely consistent over years and environments, indicating genetic differences in quantitative tissue resistance to *C. purpurea*. No variety was immune, but some (e.g. Robigus, Glasgow) exhibited a higher degree of partial resistance than others (e.g Solstice, Xi19).With high inoculum pressure of 10^6 spores / ml, infection success rates were high in all varieties in most situations and there was no discrimination between varieties in terms of the number of ergots produced. Partial resistance was expressed as a reduction in the weight of individual ergots and hence in the total weight of ergot

produced per ear. With conditions less conducive to ergot development, either because of lower inoculum concentration or less favourable environment, infection success rates were lower. Resistance was also reflected in a reduction in the number of ergots produced.

In another experiment inoculation was carried out on emasculated ears of two varieties, Robigus and Solstice. The aims were to examine varietal differences in tissue resistance in isolation from possible resistance-inducing effects of pollen and to investigate the ergot infection window in relation to pollination time. The results confirmed that the difference in resistance between the more susceptible variety Solstice and the less susceptible variety Robigus is a real effect of tissue resistance and not attributable to differential effects of pollen, such as might arise, for example, if the pollen of one variety was more vigorous or was produced more prolifically. The experiment also gave some indication of the window for ergot infection in relation to the pollination event. There was no evidence of inhibition of infection 1day after pollination, but, by 7 days after pollination, infection was almost completely inhibited. The window appears to extend to somewhere between 1 day and 7 days after pollination. In order to produce a more precise estimate it would be necessary to repeat the experiment with additional intermediate inoculation dates.

Of the 46 varieties tested for field resistance, 14 were also tested for tissue resistance. Varieties with the best levels of partial tissue resistance identified here, (Robigus and Glasgow), never became severely infected in the field resistance experiments. A number of varieties that were consistently heavily infected in the field also proved to have poor (Solstice, Xi19, Rialto), or at best moderate (Tommi, Mascot) tissue resistance. Other varieties, which displayed a range of intermediate levels of tissue resistance, gave inconsistent field performance varying markedly between sites and years.

The remaining aim was to quantify whether a variety had an open or closed flowering habit and, by inference, if this could confer any degree of escape from infection by ergot spores. A range of physical flowering characteristics that might influence openness of flowering were identified as being quantifiable and of relevance to potential ergot infection. Four of the characteristics (anther extrusion, anther size, ear density, and blind florets) were assessed in trials in 2005. In subsequent years this was reduced to two, anther extrusion and blind florets. Assessments were made

on a core set of 10 wheat varieties, with 6 additional varieties at some sites in some seasons. Assessments were made in a total of 10 separate field trials, spread over the three years. Using these parameters an open flowering type could be defined as having large anthers with a high degree of extrusion, a lax ear and a high % of blind grain sites after flowering. Conversely a closed flowering variety would have small anthers which were retained in the floret, a compact ear and a lower % of blind florets.

Even making allowance for the high degree of subjectivity in the assessment of some characteristics, it was difficult to see any consistency emerging. Bearing in mind that only the data for anther extrusion and blind florets was recorded in all three seasons, only Cordiale could be considered open flowering and Consort and Robigus closed. It was therefore difficult to identify flowering characteristics which enable a reliable prediction of a variety's tendency to be open or closed flowered to be made. Weather data indicated that seasonal variation in temperature or rainfall can have a much greater influence on this attribute and subsequent escape from ergot infection.

A comparison of flowering characteristics with field resistance data failed to indicate possible associations. For example, the field resistant variety Robigus appeared to have relatively low anther extrusion combined with a low tendency to blind florets, both of which indicate a variety at the low end of the 'open-ness' scale. However, Cordiale, another variety which was never badly infected in the field, had relatively high scores for both of these characters, indicating a variety with a greater tendency to 'open-ness'. To take two other examples, Glasgow (good field resistance) and Rialto (poor field resistance), were both middle of the range for open-ness characters. The conclusion is that varietal differences in open-ness of flowering are unlikely to be a major determinant of their relative susceptibility to ergot in the field and that tissue resistance is of more importance.

Genetic resistance to ergot infection would be attractive to the grower if it effectively removed the risk of grain rejection. Our results suggest that growers immediately have the opportunity to reduce the risk of ergot contamination in their crop through variety selection, although testing to a wider range of *C. purpurea* strains and further commercial experience would be desirable. Selection by plant breeders for the level of resistance shown by the varieties tested would be possible in inoculated field nurseries similar to those developed here. It would be vital to repeat field testing

over a range of years / environments in order to identify lines which show consistently low levels of infection. These lines could then be selected for further testing for confirmation of tissue resistance as described here. For most rapid progress selection at an early stage in the breeding process is desirable. This might not be practicable in inoculated nurseries due to the risk of ergot contamination within seed lots and the need to use black-grass or a similar grass weed as a disease spreader. A solution would be to develop a molecular marker, or markers, for resistance, or susceptibility.

TECHNICAL DETAIL

INTRODUCTION

In recent years, concern has been mounting amongst farmers, millers and the seed trade about increasing ergot contamination in wheat, which has resulted in greater numbers of grain loads being rejected. Statistics issued by DEFRA also indicate an increase in the incidence of ergot in seed crops. Ergot was unusually high in samples of winter cereals tested for certification from harvest 2003. For both winter wheat and winter barley the proportion of samples containing one or more pieces of ergot per 1000g has steadily increased over the last three years to reach 5%. This is the highest incidence of ergot in winter wheat and winter barley in the 11 years that the data have been reported.

Because of the highly poisonous nature of ergot alkaloids, millers operate a zero tolerance for ergot in milling wheat. The UKASTA standard for feed grain is 0.001% ergot by weight. Standard for certified seed set a maximum of three pieces of ergot in a 1Kg sample. Since cleaning grain to remove ergots is costly and rarely completely successful, emphasis must be on preventing field infections. This will require a whole-farm approach as there are potentially many sources of ergot on the farm and simple solutions, such as application of fungicides to the crop at flowering, are not effective (Gladders *et al*, 2001).

The increase in ergot has been variously attributed to recent changes in farming systems, chiefly the introduction of grass margins in arable fields, poor control of grass weeds because of herbicide resistance, shorter rotations and the move towards minimum tillage. Minimum tillage allows ergots to remain on the soil surface where they can readily germinate. In contrast, ploughing buries ergots below 5cm, at which depth they are prevented from sporulating (Bretag, 1981). The continuing trend towards earlier sowing of wheat in the autumn is another factor that would tend to favour ergot. The importance and contribution of these and other factors to the increase in ergot is poorly understood. However, many of these trends in farming are on the increase so ergot problems are likely to become more common unless strategies to improve its management are developed.

Ergots, the overwintering sclerotia of the fungus *Claviceps purpurea*, replace the embryo of infected florets of cereals and grasses and are either harvested with the crop or fall to the ground and survive on the surface of the soil. Usually ergots remain viable for no longer than a year (Taber, 1985), although there have been reports of ergots producing functional perithecia in their second or third season (Weniger, 1924; Kirchoff, 1929).

In spring and early summer, ergots germinate to produce stalked stromata, bearing perithecia containing ascospores (sexually produced spores). Germination is spread over a period of several months (Wood & Coley Smith, 1982) and each ergot can produce a succession of fruiting bodies with up to 60 from a single ergot (Sprague, 1950). Ascospores are discharged forcibly into the air, dispersed by wind currents and provide the primary source of inoculum for infection of open flowers of host species. Hyphae invade and colonise the ovary, grow down to the ovary axis and there establish a host-parasite frontier (Parbery, 1996). The fungus proliferates above this site and produces masses of conidia (asexually produced spores) which are exuded in droplets of honeydew. Conidia are dispersed in this honeydew by rain splash, insect feeding or direct contact, to provide a secondary source of infection (Wood and Coley Smith, 1982). Production of honeydew and conidia ceases when the formation of sclerotia starts.

A wheat crop is at risk from several sources of primary inoculum, both from within the cropped area (ergots carried over from the previous season and ergots imported in the seed) and from external sources (field margins, set-aside, non-cultivated land etc.). It is also at risk from secondary inoculum (conidia from infected grasses in the crop or margins and infected wheat plants). The importance of primary compared with secondary infection and the risks from inoculum generated within or outside the crop are still poorly understood. Primary inoculum is thought to be responsible for infection foci and secondary inoculum for spread around these foci (Mantle, 1988). Little is known about the distances over which ascospores are dispersed. However, there are indications that secondary spread via conidia is relatively localised (Wood and Coley Smith, 1982). Mantle & Swan, (1995) reported a sharp disease gradient caused by secondary infection with disease mainly within 1.5 m of primary foci. The movement of inoculum from field margins to crops needs to be clearly understood in order to determine whether or not margins are an important source of ergot inoculum for wheat.

Black-grass as a weed within the crop is known to be one important source of infection. However, since ergot also occurs in the absence of black-grass, other sources are implicated. Another potential source of inoculum is ergots present in contaminated seed. However, given current seed certification standards and the availability of chemical seed treatments with activity against ergot (Shaw, 1986), this risk should be slight and easily managed.

Because grasses are a major source of ergot infection for wheat (Yarham, 1993), eradication of grasses in headlands, margins and set-aside has traditionally been recommended as an essential part of ergot control strategy. However, this approach is now becoming incompatible with the growing environmental demands being made on farmers. CAP reform is promoting a shift away from payments related to food production towards payments related to environmental initiatives. Such initiatives include the Defra Countryside / Environment Stewardship and Entry Level Schemes, Biodiversity Action Plans, ESAs and the Voluntary Initiative. As a result, the non-crop area on farms is set to increase, in the form of field margins, set-aside and wildlife habitats. The benefits of appropriately managed field margins for biodiversity and wildlife are currently being demonstrated in research projects. Key projects in this area are 'Buzz' (a project operated by Farmed Environment Company and evaluated scientifically by the Centre for Ecology and Hydrology), 'SAFFIE' (a Sustainable Arable LINK project led by ADAS) and 3-D Farming (a Sustainable Arable LINK project led by Rothamsted Research). A variety of margin management options are available to the farmer, from natural regeneration to sowing with different grass / wild flower seed mixtures. It is usually specified that margins should remain uncut until after flowering. The risk is that flowering grasses in the margins will become infected by ergot and act as a source of secondary inoculum for the current wheat crop as well as adding to the reservoir of ergots available in the margin to initiate the next season's infection cycle.

Organically grown wheat crops might be expected to be particularly vulnerable to ergot because of the prevalence of grass weeds, grass margins and other grassy areas on organic farms. Furthermore, ergot in seed for organic production is potentially a greater problem than in conventional seed because of the requirement that a minimum of two generations of seed cannot be treated with conventional seed treatment products. Despite this, what little evidence there is suggests that in general

ergot may be no more of a problem in organic wheat than in conventionally grown wheat. This suggests the importance of mitigating factors such as longer rotation and later sowing in organic situations, both which would reduce the tendency to disease.

There is evidence that different strains of *C. purpurea* occur, which may vary in their host range and pathogenicity for wheat. The fungus has an extremely wide host range amongst wild and cultivated grass species and cereals, infecting about 400 species worldwide (Taber, 1985). The literature on strain specificity within *C purpurea* is extensive and often contradictory. With few exceptions, most strains of *C. purpurea* isolated from one host can pass over to another and vice versa (Campbell, 1957). However, strains have been identified that show adaptation to different host species. In the UK, Mantle *et al* (1977) tested strains from a total of 19 species of grasses and cereals, and found strains that were both highly and weakly pathogenic on wheat. While some grass species, such as black-grass produced ergot strains which were mainly pathogenic on wheat, other grass species, such as Cocksfoot, produced strains that were mainly weakly pathogenic on wheat. In the same study, isolates were characterised according to alkaloid profiles. Consistently different profiles were seen on isolates from black-grass or wheat, compared to those from eleven other grass species.

During the period of the study carried out by Mantle et al. (1977), i.e. 1970-3, it is likely that arable environments contained far smaller grass populations growing in close proximity to wheat due to a) widespread use of herbicides in intensive production and b) removal of margins/headlands/field boundaries. The introduction of sown headlands and margins, as well as natural regeneration, has occurred relatively recently, and may have introduced grass populations which support ergot strains with different pathogenicity profiles to those described by Mantle et al (1977).

If it can be established that certain grass species consistently carry ergot populations that are weakly infective on wheat, there may be an opportunity to reduce the ergot risk of field margins by adjusting their composition in favour of these species. Margin management practices, such as cutting, may also have an effect on ergot development and may provide further options for reducing the risk of spread of ergot from the margin to the wheat crop.

Knowledge about host resistance is extremely limited. Cereal crop species vary in susceptibility to ergot (in order of decreasing susceptibility – rye, triticale, wheat, barley, oats). Little is known about variation in resistance within species, although certain UK wheat varieties are reputed to be particularly prone to ergot infection, for example the winter wheat variety Rialto. Infection risk is greatest for species or varieties with florets that gape open during flowering. Florets that remain closed during pollination and for a few days afterwards provide a mechanical barrier to the entrance of spores and escape infection. Susceptibility to ergot infection persists for only a few days of fertilisation (Willingale and Mantle, 1987). Any factors which delay or reduce pollination are likely to increase ergot infection. Male sterile cereals used to provide hybrid seed are an example of extreme susceptibility because they rely on cross pollination (Done, 1973; Wood and Coley Smith, 1982).

In addition to differences in 'escape' there is the possibility that varieties may differ in 'tissue' or 'post-infection' resistance. This is something about which very little is known. Early indications of varietal differences in tissue resistance are emerging from work at NIAB as part of Defra-funded research on cereal varieties for organic production (OF0330). Plant breeders do not currently screen their potential varieties for ergot resistance, nor do they make any systematic assessment of the flowering characteristics that may be associated with escape. Ergot resistance is not evaluated during official variety trials at the National List or Recommended List stages. This means that farmers do not have the information on which to choose low risk varieties and breeders do not have the knowledge needed to select for improved ergot resistance in their breeding programmes.

A sustainable solution to the ergot problem must be one that minimises the risk of ergot infection in cereal crops whilst retaining the environmental benefits of grass field margins. Key requirements will be (i) to reduce the potential for ergot inoculum production in grass margins by identifying low risk grass species and management regimes and (ii) to integrate wheat variety selection and agronomic practices into effective crop management strategies.

The starting point for this project was to determine whether, and to what extent, grass margins act as a source of ergot infection for wheat crops. In parallel was a search for low risk grass species for use in margin mixtures. The project also investigated the possible contribution of variety resistance to the reduction of ergot

risk in the crop.

The project was organised into 5 work packages :

Work package 1: Grass margin and crop monitoring.
The main aim was to examine the ergot status of representative country stewardship field margins in relation to their grass species composition and management.

Work package 2: Evaluating the risk from different grass species
The aim was to determine whether populations of *Claviceps purpurea* supported by different margin grass species differ consistently in their pathogenicity for wheat

Work package 3: Epidemiology
This work package aimed to quantify the spatial distribution of *C. purpurea* from primary spread (ascospores) and secondary spread (conidia) and to assess the extent to which field margins contribute to ergot infection in cereal crops.

Work package 4: Resistance of wheat varieties to ergot
This work package examined variation in 'field resistance' to ergot amongst UK wheat varieties and the importance of the two components 'escape' (as conferred by flowering biology) and tissue resistance.

Work package 5: Diagnostics
The objective was to develop a PCR diagnostic for quantitative detection of *C. purpurea* for the analysis of spore trap samples collected during the course of Work package 3.

WP 1: Field Margin and Crop Monitoring

Introduction

The objective of field margin and crop monitoring across a wide range of regions and margin types was to establish the ergot status of representative country stewardship field margins in relation to their grass species composition and management. This was achieved through an extensive analysis of farm and experimental field margins to provide evidence on the current ergot status of field margins in relation to the grass species present and management regime.

Materials and Methods

Extensive surveys of field margins were carried out on commercial farms during the summers of 2005-2007. The aim was to identify 40-50 geographic sites where field margins could be sampled, with two fields at each site being sampled, providing a total of 80-100 margins. Sampling of field margins was co-ordinated by Velcourt (R&D) Ltd, with samples collected from a number of contacts within Velcourt Farm Management, Unilever, Farmlink, Frontier, Agrovista and BASF. A broad range of different field margin compositions and management regimes were covered in different regions to provide contrasting environmental and cropping situations.

In addition to field margins on commercial farms, including organic farms, sampling was carried out in existing field margin experiments (SAFFIE project).

Commercial Field Margins

Field margins were sampled twice during the summer month, mid June to mid July and mid August to early September, of each sampling year. The exact method of the sampling procedure can be found in Appendix A. Ergot infection levels were then determined by ergot counts.

For each field site data was collected on the type of margin, age of margin, management, soil type, previous cropping, production type (organic or non-organic) and predominant weed problems. In situations where grass weeds were a particular problem within the crop, additional samples were taken.

Experimental field margins (ADAS)

Three ADAS sites (Boxworth,Cambs, Gleadthorpe, Notts and High Mowthorpe, N. Yorks) with replicated field margin treatments established for the SAFFIE Project (Sustainable Arable Farming for an Improved Environment)) were used to monitor the occurrence of ergot in 2004 and 2005. The species composition of the grass margins in the experiments are given in Tables 1.2, 1.2 and 1.2. A report of the project is available: http://www.hgca.com/cms_publications.output/2/2/Publications/Publication/The%20SAFFIE%20Project%20Report.mspx?fn=show&pubcon=3919.

The plots were 30 m x 6m and had five-fold replication. Ergot assessments were done in July and August/September in 2004 and 2005 using small quadrat counts and more general inspection of the plots.

Table 1.1 Treatments in SAFFIE replicated experiments

Treatment No.	Seed mixture	Management early March
1.	Typical grass as Countryside Stewardship	Cut March
2.	Typical grass as Countryside Stewardship	Soil disturbance
3.	Typical grass as Countryside Stewardship	Selective herbicide
4.	Tussock grass plus broad-leaves/forbs	Cut March
5.	Tussock grass plus broad-leaves/forbs	Soil disturbance
6.	Tussock grass plus broad-leaves/forbs	Selective herbicide
7.	Fine leaved grass plus broad-leaves/forbs	Cut March
8.	Fine leaved grass plus broad-leaves/forbs	Soil disturbance
9.	Fine leaved grass plus broad-leaves/forbs	Selective herbicide

Table 1.2. Tussock Seed Mix in SAFFIE margins Boxworth, Gleadthorpe & High Mowthorpe

% (by wt.)	Sowing rate (kg ha^{-1})	Species	Common Name
4.0	1.4	*Alopecurus pratensis*	Meadow Foxtail
16.0	5.6	*Dactylis glomerata*	Cocksfoot
8.0	2.8	*Deschampsia caespitosa (w)*	Wavy Hair-Grass
20.0	7.0	*Festuca pratensis*	Meadow Fescue
20.0	7.0	*Festuca rubra* spp*. rubra*	Red Fescue
4.0	1.4	*Holcus lanatus*	Yorkshire Fog
8.0	2.8	*Phleum pratense*	Timothy
80.0%	28.0 kg		
100.0%	35.1 kg ha^{-1}	Includes forbs	

Table 1.3. Custom Stewardship Seed Mix in SAFFIE margins.

% (by wt.)	Sowing rate (kg ha^{-1})	Species	Common Name
5.0	1.0	*Agrostis capillaris*	Common Bent
15.0	3.0	*Cynosurus cristatus*	Crested Dog's tail
10.0	2.0	*Dactylis glomerata*	Cocksfoot
10.0	2.0	*Festuca pratensis*	Meadow Fescue
20.0	4.0	*Festuca ovina*	Sheep's Fescue
20.0	4.0	*Festuca rubra* ssp. *juncea*	Slender Red Fescue
20.0	4.0	*Poa pratensis*	Smooth Meadow Grass
100.0 %	20.0 kg ha^{-1}		

Results

Commercial Field Margins

Sample results from 2004 field margins showed very high populations of ergot sclerotia in East Anglia, predominantly Cambridgeshire and Suffolk, and in the South West, Devon and in the North, Cumbria. The highest numbers of sclerotia were found in samples taken from Black-grass, Couch grass and Italian Ryegrass.

Sample results from 2005 showed very high populations of ergot sclerotia in East Anglia, predominantly Cambridgeshire, Lincolnshire and Norfolk. Very low levels of ergot sclerotia were found in Leicestershire. From looking at the margin information supplied with the 2005 samples, it was evident that ergot sclerotia found in margins were situated either close to the hedge or on the edge of the field crop alongside the margins. No apparent differences in number of sclerotia occurred from different cultivation practices or the age of the margin or whether they were in organic or non organic situations. The highest number of ergot sclerotia were found in samples collected from Cocksfoot, Couch grass, Perennial Ryegrass and Italian Ryegrass.

Very few samples were taken in 2006 from margins; however from the samples obtained, high ergot sclerotia numbers were again apparent in East Anglia and North Yorkshire. The highest number of sclerotia appeared in samples taken from Couch grass, Black-grass and Perennial Ryegrass.

The 2007 samples showed high numbers of ergot sclerotia in East Anglia. These sclerotia numbers were obtained from samples of Cocksfoot and Perennial Ryegrass. The margin information that was provided with the samples clearly showed that ergot sclerotia were found very close to hedgerows or next to the field crop alongside the margins. There were no apparent differences in the age or cultivation practices in terms of number of sclerotia.

Ergots were also found in commercial wheat and rye crops and grass margins from the fields selected for spore trapping (see WP3). Haverholme, near Sleaford yielded very high levels of ergot sclerotia from Black-grass samples (36) and from wheat crops where approximately 90 ergot sclerotia were found at one site. Samples from

Rougham showed ergot sclerotia in Italian Ryegrass (10), rather than in the actual rye crop. Leadenhall, near Holbeach, had very low levels of ergot with only one sclerotia being found in Couch grass. The organic farm, Grange Farm, provided a selection of ergot sclerotia from wheat, Black-grass, Couch grass and Italian Ryegrass, however only in moderate numbers.

Experimental margins (ADAS)

SAFFIE margins established in 2001 had low levels of ergot at Boxworth (BX), Gleadthorpe (GT) and High Mowthorpe (HM) when assessed in 2004 and 2005(Tables 1.4 and 1.5).

Table 1.4. Assessments on SAFFIE margins 2004.

Site	Assessment 1	Assessment 2	Ergot
ADAS Boxworth	21 July 2004	24 August 2004	Traces at August assessment in couch, hybrid fescue, black-grass
ADAS Gleadthorpe	19-20 July 2004	24-25 August 2004	None
ADAS High Mowthorpe	30 July-4 August 2004	13 September 2004	Traces in cocksfoot, red fescue, Yorkshire fog (*August only)

No ergot was recorded at Gleadthorpe. At Boxworth, ergot was found in quadrats at the second assessment in couch grass in one plot and on hybrid Fescue and black-grass in two further plots. Five plots showed ergot at the first assessment at High Mowthorpe (only two were in quadrats) and species affected were Yorkshire fog, cocksfoot and red fescue.

Table 1.5. Assessments on SAFFIE margins 2005.

Site	Assessment 1	Assessment 2	Ergot
ADAS Boxworth	19 July 2005	17 August 2005	Traces in black-grass*, cocksfoot, common couch, meadow fescue*, meadow foxtail*, soft brome (*19 July only)
ADAS Gleadthorpe	2 August 2005	3 September 2005	Traces in cocksfoot, common bent*, false oat grass*, smooth stalked meadow grass*, wavy hair grass*, Yorkshire fog (*August only)
ADAS High Mowthorpe Separate fields on different dates	9, 17, 23/31August 2005	23/31 August, 21-23 September 2005	Traces in cocksfoot, red fescue, Yorkshire fog (August only)

In 2005, cocksfoot was affected at all 3 sites, Yorkshire fog at GT and HM, false oat grass at GT but mainly in the original hedgerow, red fescue at HM, common bent, wavy hair grass and smooth-stalked meadow grass at GT, meadow foxtail and meadow fescue at BX. The grass weeds black-grass, common couch and soft brome were affected at BX.

Overall breakdown of samples

Tables 1.6 and 1.7 present an overall summary of the numbers of different host grass species from which ergot samples were collected in each year and a breakdown of the arable environments in which they were found.

Table 1.6. Numbers of different host grass species from which ergot samples were collected.

	2004	2005	2006	2007
Total host grass species	29	22	13	23

Table 1.7. Numbers of ergot samples collected from different arable environments

	2004	2005	2006	2007
Sown grass margins	9	13	6	0
Natural regeneration margins	30	91	21	55
Other – paths, hedges etc	77	80	23	78
Total samples	116	184	50	133

Ergots were found in an extremely wide range of grass species in arable situations, with a maximum of 29 different species in 2004. Although a substantial number of ergots were collected from grasses in naturally regenerated margins, the number detected in sown grass margins was relatively low. Overall, the number of samples collected from grasses in other arable environments, such as paths, hedge bottoms and gateways, outweighed those from margins.

Discussion

The extensive survey of field margins indicated a high incidence of ergot in East Anglia. This could be due in part to the relatively high level of monitoring activity in this region, but is also likely to be due to East Anglia being one of the most productive agricultural areas growing a large area of cereals.

Results from all ergot samples suggested the age of a margin and its species composition (where sown) had little effect on the levels of ergot found in it. Neither was there any evidence of an effect of cultivation practice (minimum tillage or ploughing) in the adjacent cropped area. However, there were clear indications that ergot infestation was higher in grasses in naturally regenerated margins than in sown margins.

Information provided with the samples suggested that ergots collected from margins were located predominantly along the edges, either close to the hedge or next to the crop. Very few ergots were obtained from within the main body of the margin. This suggests the environment at the edges of the margins, or the occurrence of susceptible weed grasses in these areas, could make them more conducive to ergot.

Ergots were collected from an extremely wide range of grass species in arable situations. The largest numbers were found in Couch grass, Italian ryegrass, Black-grass, Perennial ryegrass and Cocksfoot.

In experimental field margins, ergot became established on sown grass species, but also appeared near the original hedgerow and at the margin of arable cultivation where common grass weeds were not controlled. There was considerable variation in the species affected by ergot.

Although grasses growing in field margins may clearly act as hosts to ergot, they represent only a portion of the total grass host area in the arable environment, which also includes, for example, grasses in hedge bottoms, along pathways and on waste areas. This needs to be taken into account in assessing the risk posed by margins themselves.

WP 2 Infectivity of ergots from different grass hosts to wheat

Introduction

A large number of grass species are susceptible to infection by the ergot fungus, *Claviceps purpurea*. The arable environment in the UK contains a wide range of areas where grasses are the predominant plant type, including field margins which are either deliberately sown with seeds mixtures containing grasses, or which are allowed to regenerate naturally with local grass species. Allowing margin grasses to flower is a critical part of the environmental benefits that they can deliver, though it also allows infection by ergot. Changing cultivation practices and loss of effectiveness in some herbicides have also resulted in an increase in weedy grass species within fields. Grass weeds such as black-grass can persist until flowering, giving further potential for ergot infection. There has been growing concern among cereal growers that the increased prevalence of grasses in or around arable fields is responsible for increased ergot contamination in grain. Environmental stewardship schemes which require allowing grasses to flower have thus been suspected of contributing to an increase in ergot, though there is no reason to suppose that grasses encouraged as a result of the schemes influence ergot levels any more than grasses in the general environment, such as on paths, tracks, banks and so on. Previous studies on grass species as potential sources of outbreaks of ergot in wheat showed that several grass species did become infected by *C. purpurea* which was also capable of infecting wheat. However, ergots from other species were found to have low infectivity towards wheat (Mantle *et al*., 1977) .The survey work which lead to these conclusions was carried out in the early 1970s, and thus in a very different arable environment to the present. The work reported here was carried out to investigate the infectivity of ergots collected from grass species in a range of arable environments, and to determine whether some grass species could be regarded as low risk in terms of ability to produce wheat infecting ergots. Such species could be used preferentially in sown grass margins as a means of reducing wheat infecting ergot types within the pathogen population.

Studies by Mantle *et al.* (1977) identified different alkaloid composition in ergots from different grass hosts using qualitative analysis by thin layer chromatography. These differences in alkaloid content appeared to relate to the pathogenicity of isolates to wheat. If these correlations could be substantiated, HPLC methods would have the potential to define risks to wheat of ergot populations in field margins.

Materials and Methods

Sources of ergots

Ergots were collected during a four year monitoring period, 2004-2007. Collection environments were defined as sown grass margins, naturally regenerated field margins, and a number of general arable locations, including weed grasses within crops, paths, tracks and verges, hedge bottoms, banks, gateways, roadsides, meadows and rough grass areas. Nearly all samples were received with their grass host, which was identified before ergots were stored dry in paper envelopes. Digital images of ergots with their grass host were compiled and used by project partners to assist in monitoring.

Production of ergot inoculum

Single ergot sclerotia were surface sterilised in 10% (v/v) sodium hypochlorite for two minutes followed by three rinses with sterile distilled water. Sclerotia were then cut with a sterile scalpel and plated on PDA containing streptomycin. Plates were incubated at 25^0C in the dark. After one week fungal growth was sufficient for sub-culturing to PDA plates. The isolates were stored long term on PDA slopes at 4^0C.

For *in vitro* spore production, isolates were grown on PDA plates at 25^0C in the dark for 2-3 weeks. Sterile distilled water was added to the mycelial mat and agitated using a sterile loop. Conidial spore suspensions were prepared at around $1x\ 10^6$ spores/ml. Tween 20 was added at 0.01% for field inoculations to aid adhesion to the wheat ears.

For *in vivo* spore production, initial conidial cultures from sclerotia were inoculated into florets of their grass host by dipping inflorescences before anthesis in spore suspensions at $1x\ 10^6$ spores/ml. After 14 days honeydew was collected with a plastic

loop and suspended in sterile distilled water. The honeydew was stored at 4^0C short term (up to 12 weeks) or frozen at -20^0C for longer term storage. Prior to freezing glycerol was added to the honeydew samples at 10% of the total volume to prevent spore fracturing.

To produce grass flowers for inoculation, seed of 18 species used in sown agricultural margins was obtained from Advanta Seeds. An additional 7 species of interest were ordered through Herbiseed Ltd. Grass inflorescences were obtained by vernalising pre-germinated seedlings at 5°C in the dark for 6 weeks before growing on in a glasshouse with 16 h days at 18-22°C, 8 h night at 10-12° C. Many species failed to produce flower heads within the time scale needed for production of honeydew, and in the second year of testing, well-established plants of 10 species were obtained from plots at Advanta Seeds, kept outside during the winter, and then moved into a glasshouse during March with 16 h days (18-22 °C) and 8 h nights (10-12° C)

Inoculation of wheat with grass ergot isolates

a) Inoculation of field grown wheat

Strips of winter wheat cv Rialto (1.2 m wide) were established in 2004 and 2005. Ears at GS 58 were selected and inoculated after damaging the florets by pressing ears between two pads, one of which was mounted with sowing machine needles. Spore suspension (adjusted to between 1 and 4 x 10^6 spores/ml) was then sprayed onto the ears with a hand held sprayer at a rate of 10ml per ear. Twelve replicate ears per isolate were inoculated. Isolates were separated by 3 m of crop to avoid cross contamination.

b) Inoculation of glasshouse grown wheat

Spring wheat cv Paragon was used for glasshouse experiments in 2005, 2006, and 2007 to avoid the need for vernalisation. Plants were grown in 25 cm pots with a peat based compost and came into ear after approximately 12 weeks. Ears were inoculated at full emergence, but before anthesis (GS 58). Six wheat ears were inoculated per isolate by inoculating directly into the 2 basal florets of each of 15 spikelets with a hypodermic syringe (generating 180 florets per isolate). Four winter wheat cultivars, Rialto, Einstein, Robigus and Solstice, were inoculated with three isolates (black-grass, cocksfoot and Yorkshire fog hosts) in 2008 in a limited investigation of cultivar x isolate interaction. Three ears per variety/isolate combination were inoculated as for Paragon.

Isolates tested and techniques used

It was not possible to test all the samples submitted, and sub-sets were selected to reflect the most common sources of ergots and some from additional species of interest which are used in grass margins. Within each sub-set, except the cocksfoot isolate comparison, a varying number of black-grass isolates were included of which one was used throughout. Various repeats using the same isolate and inoculation method/environment were carried out during the course of the work. Many of the grass species used in the work did not produce flowers consistently over winter, and thus it was not possible to produce honeydew inocula for most isolates. Mantle *et al*. (1977) reported that pathogenicity was attenuated if *in vitro* spores were used in tests rather than honeydew. However, given the difficulty of producing grass inflorescences encountered here, use of *in vitro* spores was essential. A comparison of honeydew vs *in vitro* spores was therefore carried out for a sub-set of isolates to assess any loss in infectivity. Field inoculations were also compared to glasshouse inoculations for a further sub-set of isolates, since isolate through-put could be greatly increased using glasshouse grown plants if results were comparable. The separate experiments carried out during the course of this work are described in Table 2.1. Isolate numbers are in the format 0n/xxx, where 0n is the year of collection, and xxx is the consecutive number of the samples received in each year. Where multiple ergots from the same host were received from a single location, single ergot isolates are referred to as 0n/xx a, b, c etc.

Table 2.1 Host grass samples and testing methods

Series	Host grass	Inoculation method	Inoculation environment
1	Cocksfoot only	*In vitro* conidia, some with honeydew comparison	Glasshouse
2	Species range	*In vitro* conidia, some with honeydew comparison	Glasshouse
3	Species range	*In vitro* conidia	Glasshouse
4	Within site cocksfoot and other species range	*In vitro* conidia	Glasshouse
5	Species range	*In vitro* conidia	Glasshouse
6	Species range	*In vitro* conidia	Glasshouse
7	Species range (includes repeats of glasshouse set in series 2)	*In vitro* and honeydew comparison	Field
8	Species range	*In vitro* conidia	Field

In each experiment, inoculated ears were harvested after 28 days and hand threshed. The total number of sclerotia per ear was recorded.

Cross infection between ergots from different grass hosts

A small number of cross infection studies between was carried out by dipping newly emerged grass inflorescences in conidial inoculum at 10^6 spores/ml and repeating on the same material at 3-5 day intervals up to four occasions. Inoculum from highly infective cocksfoot isolates, black-grass isolates and wheat isolates was used separately to infect different grass species.

Grass species experiment

A replicated experiment to monitor the flowering and effects of mowing on ergot infection in 12 grass species sown in autumn 2004 at ADAS Boxworth (Table 2.2). It was assessed for three years. The experiment was established in 2004/05 and only smooth stalked meadow grass plots were re-sown in autumn 2005 because of poor

germination in 2004. Black-grass was re-sown in autumn 2006 after light cultivation as plant populations had declined in 2006. There were 6 blocks in the experiment and the same two blocks were subject to each of three mowing regimes each year: unmown, mown once and mown twice.

Table 2.2 . Mowing treatments on grass species at Boxworth 2005-2007.

Treatment	2005	2006	2007
Unmown	-	-	-
Mown once	31 May	31 May	22 May
Mown twice	31 May + 13 July	31 May + 7 July	22 May + 9 July

Comparison of ergot infectivity, alkaloid and AFLP profiles

In another component of work package 2, ergots from various hosts were analysed for alkaloid content. Ergots were either collected from wheat after infectivity studies, or were bulked from original collections from grass samples. Detailed methods for the analyses are presented below. During the course of the project, two HGCA student bursaries were awarded which investigated molecular phenotype of ergot isolates using AFLP analysis. Methods are presented in the individual reports available from NIAB.

Ergot alkaloid analyses

This part of the project involved establishment of HPLC methods to identify the alkaloids in ergots and hence characterise ergot populations. Ergot sclerotia can contain up to 1% physiologically active ingredients (Rottinghaus et al., 1993) including the alkaloids: ergotamine, ergocornine, ergocryptine, ergovaline, ergocrystine, ergonovine, ergosine, ergometrine (Osborne and Watson, 1980). Ergocornine, ergocryptine and ergocrystine are commonly known as Ergotoxine (Osborne and Watson, 1980). However ergotamine is the alkaloid with the greatest biological activity (Lopez et al., 1997) and has been used pharmaceutically for many years.

Chemical background

Ergot alkaloid synthesis

Ergot alkaloids all have an ergoline ring, and it was shown that this ring derived from the condensation of tryptophan with an isoprenoid unit. The second precursor

molecule is an isoprene unit from mevalonic acid, as mevalonate is incorporated into the ergoline ring.

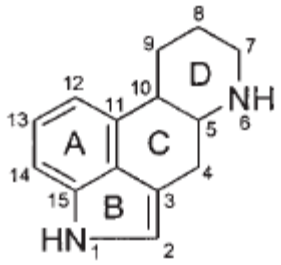


Fig. 2.1 Ergoline ring

Ergot alkaloids can be divided into different types based on the type of substituent on C-8, although this classification can differ between authors: the clavine alkaloids, lysergic acid (Machado 2004) and simple lysergic acid amides, complex ergopeptines (Table 2.3) and ergopeptams (Mukherjee and Menge 1999).

Table 2.3. Ergopeptines that differ at amino acid position I and II. L-proline is normally at amino acid position III (Machado 2004).

	Amino acid position II			
Amino acid position I	L-valine	L-phenylalanine	L-leucine	L-isoleucine
L-alanine	Ergovaline	Ergotamine*	Ergosine	β-ergosine
L-valine	Ergocornine*	Ergocristine	Ergocryptine*	β-ergokryptine
L-2-aminobutyric acid	Ergonine	Ergostine	Ergoptine	β-ergoptine

Note: ergonovine (see Fig. 2.2) and three ergopeptines (*) are available as standards
Ergotoxine refers to a group of alkaloids (ergocornine, ergocristine, ergocryptine and their isomers)

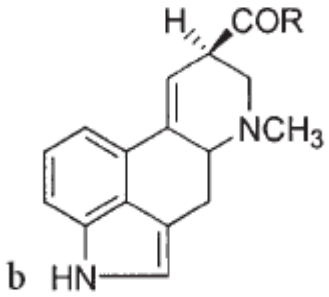


Fig. 2.2 Simple derivatives of lysergic acid
R=OH: lysergic acid
R=NH_2: lysergic acid amide
R= $NHCHOHCH_3$: lysergic acid 2-hydroxyethylamide
R= $NHCHCH_3CH2OH$: ergonovine = ergometrine

Alkaloid toxicity

The pharmacological effects of the various ergot alkaloids and their derivatives are due to the structural similarity of the tetracyclic ring system to neurotransmitters such as noradrenaline, dopamine or serotonin. Two forms of ergot alkaloid poisoning are recognised: gangrenous ergotism (produced after constriction of blood vessels reduces blood flow) and convulsive ergotism (effects on the central nervous system).

Alkaloids reported to have pharmacological activity (summary):

- *Agroclavine* causes uterine contractions – abortifacient. Clavines are found to always accompany lysergic acid derivatives (at least in traces) (Floss 1976), thus if agroclavine is difficult to detect, the presence of lysergic acid derivatives would be an indicator for the presence of agroclavine.
- *Ergotamine* (sometimes in combination with caffeine) constricts cerebral blood vessels. Ergotamine abuse may cause ischaemia and even ergotism (Bigal and Tepper 2003).
- *2-bromo-α-ergocryptine* (synthetic derivative) reduces lactation in women; *ergocryptine and ergocristine* increase dopamine release.
- *ergonovine* (*ergometrine*, *ergobasine*): main constituent of water soluble fraction, responsible for oxitocic (causing uterine contractions, labour inducing) activity (Floss 1976). Can result in nausea and hypertension.

Targeting toxic components

A range of toxic alkaloids are produced in ergots and many are derivatives of lysergic acid (Floss, 1966). Ergocristine and ergotamine are reported to be present in the largest concentrations in wheat and were detectable after processing and cooking (Fajardo et al., 1995). These two alkaloids are likely to represent >50% of toxic alkaloids. Clavine alkaloids (e.g. agroclavine) are produced by *C. purpurea* some other species of *Claviceps* and form a separate group, albeit based on the ergoline ring.

The HPLC technique was established using four standard alkaloids (Table 2.3). Alkaloids were extracted from ergot sclerotia using 80% methanol with 0.1% ammonium hydroxide. The extract was analysed by HPLC using a Gemini C18 column (Phenomenex) with a water / acetonitrile gradient system based on Lehner et al [J.

Mass Spectrometry 40: 1484-1502 (2005)]. Ergot sclerotia contain up to 2% of alkaloids and the method has been used on samples as small as 40mg.

The HPLC technique was established using the alkaloid standards, ergotamine, ergocornine and ergocryptine and the method outlined below. (N.B this method is based on a sample weight of approximately 0.5g, however smaller sample sizes can and were used with scaled down amounts of the other reagents). 33 samples overall were tested from assorted locations and species.

Extraction and HPLC Analysis of Alkaloids from Ergot Sclerotia

The method was adapted from Pazoutova et al. (2000). Pre-cool sclerotia, pestle and mortar at -20'C and grind sclerotia to a powder in the cold pestle and mortar. Transfer the powder to amber extraction vial and add 3ml extraction medium. Wash out the mortar with a further 2ml extraction medium into vial totalling 5ml extraction medium. Purge the mixture with nitrogen and stir for 2 hours in the dark (aluminium foil) the leave overnight in the dark without stirring. Filter through Munktell paper into clean amber vial, and then pass filtrate through nylon filter into another clean amber vial. Evaporate to dryness with nitrogen at room temp. Re-dissolve residue in 0.5ml methanol (or less if less starting material used) and store under nitrogen at -20'C until analysis.

HPLC

The method was adapted from Lehner et al, J Mass Spectrometry (2005) 40:1484-1502

Column: Phenomenex Gemini C18 column and guard

Mobile phases:	A: 0.2% ammonium hydroxide (25%) in water	
	B: Acetonitrile	
Elution solvents:	30% A 70% B to pre-wash. 80% A 20% B to equilibrate	
Elution gradient:	0-5min:	80% A 20% B
	5-40min:	Linear gradient to 30%A 70%B
	40-50min:	Hold at 30% A 70% B
	50-60min:	Return to 80% A 20%B
Flow rate:	0.5ml/min at room temp	

Standard solutions: 0.4mg/ml in methanol, store under nitrogen at -20'C with parafilm wrapping. To make the working solutions take 0.1ml of stock solution and dilute to 2.00ml with methanol, then load 20ul onto HPLC

Sample solutions: take 20ul of extract and dilute to 200ul with methanol, then load 20ul onto HPLC. Run methanol as blank

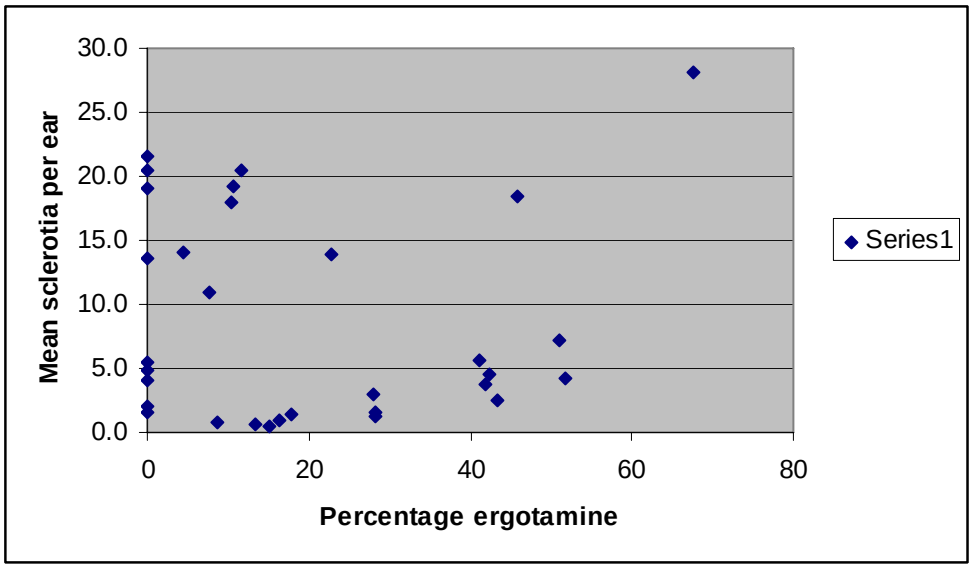
UV Monitoring

Alkaloid absorbance can be monitored at both 310 and 210nm wavelengths as profiles at both wavelengths were very similar in both composition and relative responses. However because there is a tendency for negative peaks to occur at 310nm the chromatograms here were all monitored at 210nm.

Alkaloid standards were first tested with the aim of identifying these alkaloids in the samples– see Chromatogram Appendix E.

With such a large number of samples comes a large number of varying factors. To try and tabulate in some order, Appendix B shows all the samples with the peak occurrence and size in mAU. Because so few standards are commercially available identification of some of the peaks was difficult however ergotamine, ergocornine and ergocryptine we identified and the amounts present in each sample calculated – see Appendix C– this was calculated from the linear relationships obtained by the standards (Appendix D).

Using the area under the peaks the percentage ergotamine in each sample could be calculated, this was then plotted against the mean number of sclerotia found in each ear of the sample;(see over)


30.0
25.0
20.0
15.0
10.0
5.0
0.0
Mean sclerotia per ear
0
20
40
60
80
Percentage ergotamine
Series1

Results

Variation in grass isolate infectivity on wheat

Isolates from cocksfoot showed significant variability in their ability to infect wheat. Some isolates produced numerous large ergots, sometimes infecting a greater number of florets than black-grass isolates under similar experimental conditions, while others produced only one or two small ergots, or none, over all the florets inoculated (Table 2.4).

Table 2.4 Infectivity of cocksfoot isolates (series 1)

Sample No.	County of Collection	Mean number sclerotia / ear
04/35	Suffolk	29.7
05/135 (honeydew)	Bedfordshire	29.5
05/26	Suffolk	28.7
04/12	Cambs	27.2
05/135 (*in vitro*)	Bedfordshire	27.2
05/112	Northants	26.5
05/19	Cambs	26.0
05/157	West Midlands	12.2
04/93c (*in vitro*)	Herts	11.7
04/93c (honeydew)	Herts	4.2
05/15	Notts	1.2
05/130 (honeydew)	Devon	1.2
05/51	Norfolk	0.5
05/130 (*in vitro*)	Devon	0.5
05/40	Warwickshire	0.3
05/158	Yorkshire	0.2
05/115	Leicestershire	0.0
lsd ($p<0.05$)		3.24

There was also significant variation between isolates from many grass species in their ability to infect wheat in the remaining seven experimental series (Tables 2.5, 2.6, 2.7, 2.8, 2.9, 2.10 and 2.11). Numbers of ergots produced by black-grass isolates were consistently high (between 70 and 100% of florets inoculated). Occasionally, some secondary infection took place, giving higher % infection rates. One black-grass isolate (04/41) failed to infect in series 2 (Table 2.5), though it had produced high

numbers of ergots on wheat in other work packages, and the data were omitted. Isolates from Yorkshire fog (*Holcus lanatus*) consistently produced low numbers of ergots. Isolates from tall oat grass (*Arrhenatherum elatius*) were highly variable, with some producing as many ergots as black-grass isolates, while others were much less infective. Tall fescue isolates were generally highly infective. There was some indication that populations of *C. purpurea* from specific locations were less infective on wheat, regardless of host grass origin eg the samples received from Gleadthorpe, Notts (see Table 2.6) from cocksfoot, Yorkshire fog and tall oat grass all had low infectivity. However, in general, numbers of samples received from individual locations were insufficient to confirm the concept of distinct local populations. Moreover, it was clear that, in the case of cocksfoot, where multiple samples were received from a single field in one location, infectivity was highly variable (Table 2.7).

Table 2.5. Infectivity from host species range, glasshouse, including honeydew and *in vitro* inoculum comparison (series 2)

Sample No	Host species	County of collection	Mean sclerotia/ear
05/71 (honeydew)	Meadow foxtail	Cambs	33.2
05/71 (*in vitro*)	Meadow foxtail	Cambs	30.0
05/126	Couch grass	Leicestershire	26.8
05/18	Meadow fescue	Cambs	23.8
05/79b	Meadow fescue	Suffolk	17.3
05/78	Meadow fescue	Wiltshire	16.0
05/160	Tall oat grass	Yorkshire	14.0
05/168	Couch grass	Yorkshire	13.5
05/83	Couch grass	Cambs	13.0
05/96	Tall oat grass	Suffolk	9.8
05/132	Couch grass	Devon	8.7
05/136	Couch grass	Bedfordshire	6.8
05/105b	Tall oat grass	Lincolnshire	4.0
05/91	Tall oat grass	Berkshire	0.7
04/103	Tall oat grass	Cumbria	0.0
lsd ($p < 0.05$)			3.23

Table 2.6 Infectivity from host species range, glasshouse experiment (series 3)

Sample No	Host species	County of collection	Mean sclerotia/ear
04/52	Meadow barley	Cambs	31
04/91 a	Black-grass	Northants	28.2
04/36	Meadow Brome	Suffolk	27.3
04/11	Meadow Brome	Cambs	22.8
05/25	Meadow Brome	Suffolk	21.7
04/76	Tall Fescue	Cambs	20.5
05/163a	Tall Fescue	Yorkshire	20.5
04/110	Sand couch	Norfolk	20
05/60	Bent	Suffolk	18.5
05/33	Black-grass	Lincolnshire	18.3
04/16	Meadow Brome	Cambs	15.8
04/78	Wall barley	Suffolk	15.5
04/80	Wild oats	Cambs	14.2
05/82	Tall Fescue	York	13.2
05/139	Tall Fescue	Norfolk	13
05/90	Creeping Soft Grass	Berkshire	6.8
04/70	Tall Fescue	Cambs	5.5
04/77	Tall Fescue	Cambs	3.2
05/63a	Bent	Cambs	3
05/41	Creeping Soft Grass	Warwickshire	3
05/169	Tall Fescue	Yorkshire	2.5
04/109	Mat grass	Yorkshire	1.5
05/15	Cocksfoot	Notts	0.8
05/16	Yorkshire Fog	Notts	0.5
05/11	Tall oat grass	Notts	0
04/111	Marrram grass	Norfolk	0
lsd ($p < 0.05$)			2.39

Table 2.7 Infectivity from host species range, glasshouse experiment, including cocksfoot samples from a single site (series 4)

Sample No	Host species	County of collection	Mean sclerotia/ear
04/91a	Black-grass	Northants	33.0
05/33	Black-grass	Lincolnshire	26.0
05/130k	Cocksfoot	Devon*	18.8
05/130l	Cocksfoot	Devon	14.5
05/130g	Cocksfoot	Devon	12.2
05/130n	Cocksfoot	Devon	10.5
05/169	Tall fescue	Yorkshire	7.5
06/4	Meadow fescue	Herts	5.7
05/130f	Cocksfoot	Devon	5.2
05/63a	Bent	Cambs	5.2
06/25	Tall fescue	Gloucs.	4.8
06/26	Timothy	N. Yorkshire	4.8
05/130o	Cocksfoot	Devon	2.5
05/130e	Cocksfoot	Devon	1.3
05/41	Creeping soft grass	Warwickshire	0.8
06/18	Yorkshire fog	Herts	0.7
04/109	Mat grass	Yorkshire	0.5
05/90	Creeping soft grass	Berkshire	0.2
05/130c	Cocksfoot	Devon	0.0
lsd ($p<0.05$)			2.19

*Devon isolates from Cocksfoot are single sclerotial isolates from within the same field.

Table 2.8 Infectivity from host species range, glasshouse experiment (series 5)

Sample No	Host species	County of collection	Mean sclerotia/ear
07/107	Timothy	Suffolk	34.0
04/91a	Black-grass	Northants	33.0
07/62	Perennial ryegrass	Norfolk	20.0
07/50	Perennial ryegrass	Suffolk	14.8
07/60	Creeping soft grass	Norfolk	9.5
07/44	Creeping soft grass	Suffolk	2.3
07/87g	Reed canary grass	Bedfordshire	2.3
07/42	Yorkshire fog	Hertfordshire	2.0
07/18	Yorkshire fog	Hertfordshire	1.7
07/36	Yorkshire fog	Somerset	1.2
07/33	Yorkshire fog	Ireland	1.0
07/57	Perennial ryegrass	Suffolk	0.5
07/70	Yorkshire fog	Norfolk	0.3
07/31	Yorkshire fog	Ireland	0.0
07/45	Yorkshire fog	Suffolk	0.0
07/35	Yorkshire fog	Somerset	0.0
07/87e	Reed canary grass	Bedfordshire	0.0
lsd $p<0.05$			2.05

Table 2.9 Infectivity from host species range, glasshouse experiment (series 6)

Sample No	Host species	County of collection	Mean sclerotia/ear
04/91a	Black-grass	Northants	33.8
07/97	Little canary grass	Suffolk	32.8
05/33	Black-grass	Lincolnshire	32.3
07/67	Italian ryegrass	Norfolk	15.8
07/119	Yorkshire fog	Norfolk	11.8
07/23	Tall fescue	Norfolk	10.8
07/116	Yorkshire fog	Yorkshire	10.3
07/115	Creeping Soft grass	Yorkshire	10.0
07/56	Perennial ryegrass	Suffolk	9.7
07/15	Timothy	Cambs	4.8
07/129	Yorkshire fog	Yorkshire	4.5
07/8	Yorkshire fog	Cambs	3.7
07/37	Yorkshire fog	Hertfordshire	1.7
07/4	Yorkshire fog	Bedfordshire	1.7
07/106	Italian ryegrass	Suffolk	1.2
07/127	Creeping Soft grass	Humberside	1.2
07/71	Italian ryegrass	Norfolk	1.2
07/66	Yorkshire fog	Norfolk	0.8
07/61	Italian ryegrass	Norfolk	0.7
07/84	Yorkshire fog	Hertfordshire	0.3
07/47	Rough stalked meadow grass	Suffolk	0.3
07/125	Wavy hair grass	Suffolk	0.0
lsd ($p<0.05$)			2.12

Table 2.10 Infectivity from host species range, field 2006, including honeydew and *in vitro* inoculum comparisons (series 7)

Sample No	Host species	County of collection	Mean sclerotia/ear
05/33	Black-grass	Lincolnshire	22.3
05/24	Tall oat grass	Suffolk	21.5
05/146	Tall oat grass	Lincolnshire	19.8
04/68b	Cocksfoot	Cambs	19.2
05/134	Couch grass	Bedfordshire	19.0
05/148b	Cocksfoot	Lincolnshire	18.4
04/35	Cocksfoot	Suffolk	18.0
05/23a honeydew	Black-grass	Suffolk	16.8
05/126	Couch grass	Bedfordshire	16.1
05/141	Tall oat grass	Lincolnshire	15.5
04/12	Cocksfoot	Cambs	14.9
05/92	Cocksfoot	Norfolk	14.9
05/122	Cocksfoot	Cambs	14.4
05/19	Cocksfoot	Cambs	14.0
05/71 *in vitro*	Meadow foxtail	Cambs	13.9
05/61	Timothy	Cambs	13.6
05/26 honeydew	Cocksfoot	Suffolk	13.3
05/156	Couch grass	Norfolk	12.8
04/48	Cocksfoot	Cambs	12.4
05/26 P	Cocksfoot	Suffolk	11.9
05/148c	Cocksfoot	Lincolnshire	11.5
05/112	Cocksfoot	Northants	10.9
05/83	Couch grass	Cambs	10.9
04/66	Couch grass	Suffolk	10.3
05/160	Tall oat grass	Yorkshire	10.3
04/105	Cocksfoot	Cumbria	9.9
05/23b honeydew	Black-grass	Suffolk	9.8
05/59	Cocksfoot	Suffolk	9.4
05/157	Cocksfoot	West Midlands	8.4
05/96	Tall oat grass	Suffolk	7.9
05/85a *in vitro*	Italian ryegrass	Suffolk	7.9
05/135b *in vitro*	Cocksfoot	Bedfordshire	7.8
05/135b honeydew	Cocksfoot	Bedfordshire	7.5
05/86	Tall oat grass	Suffolk	7.4

05/123	Perennial ryegrass	Leicestershire	7.2
05/109	Timothy	Norfolk	7.1
05/78	Meadow fescue	Wiltshire	7.0
05/111	Timothy	Norfolk	7.0
05/54	Perennial ryegrass	Cambs	6.6
05/23b *in vitro*	Black-grass	Suffolk	6.4
04/50	Couch grass	Cambs	6.2
05/98	Couch grass	Kent	6.1
05/168	Couch grass	Yorkshire	6.1
05/102 *in vitro*	Perennial ryegrass	Norfolk	6.0
05/148a honeydew	Cocksfoot	Lincolnshire	5.8
05/116	Timothy	Leicestershire	5.7
05/104	Tall oat grass	Norfolk	5.7
05/145	Perennial ryegrass	Lincolnshire	5.6
05/23a P	Black-grass	Suffolk	5.5
04/79	Meadow fescue	Suffolk	5.5
05/164a honeydew	Perennial ryegrass	Suffolk	5.2
04/93b	Cocksfoot	Herts	5.2
05/11	Tall oat grass	Notts	4.9
05/132	Couch grass	Devon	4.7
04/62	Tall oat grass	Wiltshire	4.5
04/82	Perennial ryegrass	Cambs	4.5
05/79a	Meadow fescue	Wiltshire	4.3
05/136	Couch grass	Bedfordshire	4.2
05/85b *in vitro*	Italian ryegrass	Suffolk	4.1
05/102 honeydew	Perennial ryegrass	Norfolk	4.1
05/51	Cocksfoot	Norfolk	4.1
05/151	Cocksfoot	Lincolnshire	4.1
04/103	Tall oat grass	Cumbria	4.1
05/164a *in vitro*	Perennial ryegrass	Suffolk	4.0
05/164b	Perennial ryegrass	Suffolk	3.9
05/18	Meadow fescue	Cambs	3.7
05/73	Perennial ryegrass	Cambs	3.6
04/64a honeydew	Perennial ryegrass	Suffolk	3.3
05/158	Cocksfoot	Yorkshire	2.2
05/85b honeydew	Italian ryegrass	Suffolk	2.2
05/16	Yorkshire fog	Notts	2.1
05/40	Cocksfoot	Warwickshire	2.0
05/162	Cocksfoot	Yorkshire	2.0

04/92	Perennial ryegrass	Suffolk	1.8
05/142	Timothy	Lincolnshire	1.8
05/125	Yorkshire fog	Leicestershire	1.8
04/87	Yorkshire fog	Devon	1.7
05/144	Yorkshire fog	Lincolnshire	1.7
04/31	Annual meadowgrass	Suffolk	1.6
04/93c honeydew	Cocksfoot	Herts	1.6
04/93c *in vitro*	Cocksfoot	Herts	1.6
05/119	Yorkshire fog	Leicestershire	1.6
04/64a *in vitro*	Perennial ryegrass	Suffolk	1.5
05/101	Timothy	Norfolk	1.5
05/39	Cocksfoot	West Midlands	1.4
05/131 honeydew	Yorkshire fog	Devon	1.3
05/118	Perennial ryegrass	Leicestershire	1.3
05/131 *in vitro*	Yorkshire fog	Devon	1.1
05/117 honeydew	Cocksfoot	Leicestershire	1.1
05/15	Cocksfoot	Notts	1.0
05/153	Cocksfoot	Cleveland	1.0
05/105b	Tall oat grass	Lincolnshire	0.9
05/117 *in vitro*	Cocksfoot	Leicestershire	0.8
05/115	Cocksfoot	Leicestershire	0.8
05/120	Timothy	Cambs	0.7
05/130 b honeydew	Cocksfoot	Devon	0.6
05/130b *in vitro*	Cocksfoot	Devon	0.6
05/154 *in vitro*	Yorkshire fog	Cleveland	0.5
05/154 honeydew	Yorkshire fog	Cleveland	0.4
05/100	Cocksfoot	Norfolk	0.3
04/112	Yorkshire fog	Sutherland	0.3
05/91	Tall oat grass	Berkshire	0.2
04/108 honeydew	Sweet vernal grass	Cumbria	0.1
04/94b	Tall oat grass	Hertfordshire	0.0
05/58	Tall oat grass	Bedfordshire	0.0
05/85a honeydew	Italian ryegrass	Suffolk	0.0
04/108 *in vitro*	Sweet vernal grass	Cumbria	0.0
04/113	Sweet vernal grass	Sutherland	0.0
lsd $p<0.05$			1.05

Table 2.11 Infectivity from host species range, field 2007 (series 8)

Sample No.	Host grass	County of Collection	Mean number sclerotia / ear
04/91a	Black-grass	Northants	9.0
05/135f	Cocksfoot	Bedfordshire	9.0
05/135d	Cocksfoot	Bedfordshire	7.3
05/33a	Black-grass	Lincolnshire	6.4
05/130k	Cocksfoot	Devon	5.5
05/163a	Tall fescue	Yorkshire	4.6
06/49a	Cocksfoot	Suffolk	4.1
05/169	Tall fescue	Yorkshire	4.1
04/77	Tall fescue	Cambs	3.9
05/130n	Cocksfoot	Devon	1.8
05/41	Creeping soft grass	Warwickshire	1.2
05/135b	Cocksfoot	Bedfordshire	0.7
05/130f	Cocksfoot	Devon	0.6
05/130c	Cocksfoot	Devon	0.1
lsd ($p<0.05$)			2.15

Levels of infection produced by *in vitro* spores were comparable to those produced by honeydew. There was no consistent tendency for lower numbers of ergots to be produced with *in vitro* conidia with the isolates tested here (Table 2.12, data extracted from other tables). Numbers produced by the two types of inoculum were significantly correlated ($r=0.79$, $p=0.05$). In general, numbers of ergots produced in the field experiments were lower than for the same isolates inoculated in the glasshouse (Table 2.13, data extracted from previous tables). This might be expected since the field inoculation technique was less precise in delivering inoculum to a specific number of florets. The inoculation technique also tended to produce a low number of whiteheads, presumably due to excess damage with the sowing machine needles. However, though final ergot counts were lower, clear and significant differences between isolates were observed, and ranking order was comparable to that seen in glasshouse tests ($r = 0.84$, $p = 0.05$)

Table 2.12 Comparison of infectivity of ergot honeydew inoculum and *in vitro* produced conidia

Host and isolate no.	Honeydew	*In vitro*
	Mean number sclerotia/ear	
Sweet vernal grass04/108	0.1	0
Yorkshire Fog 05/154	0.4	0.5
Cocksfoot 05/130 b	0.6	0.6
Cocksfoot 05/117	1.1	0.8
Yorkshire Fog 05/131	1.3	1.1
Cocksfoot 04/93c	1.6	1.6
Italian ryegrass 05/85b	2.2	4.1
Perennial ryegrass 04/64a	3.3	1.5
Perennial ryegrass 05/102	4.1	6
Perennial ryegrass 05/164a	5.2	4
Cocksfoot 05/135b	7.5	7.8
Black-grass 05/23b	9.8	6.4
Cocksfoot 05/26	13.3	11.4
Black-grass 05/23a	16.8	5.5

Table 2.13 Comparison of field and glasshouse inoculations on wheat ears

Host, isolate no. and inoculum type	Field	Glasshouse
	Mean no. sclerotia per ear	
Tall oat grass 05/91	0.2	0.7
Cocksfoot 05/130b *in vitro*	0.6	0.5
Cocksfoot 05/130 b honeydew	0.6	1.2
Cocksfoot 05/115	0.8	0
Tall oat grass 05/105b	0.9	4
Cocksfoot 05/15	1	1.2
Cocksfoot 04/93c *in vitro*	1.6	11.7
Cocksfoot 04/93c honeydew	1.6	4.2
Cocksfoot 05/40	2	0.3
Cocksfoot 05/158	2.2	0.2
Meadow fescue 05/18	3.7	23.8
Tall oat grass 04/103	4.1	0
Cocksfoot 05/51	4.1	0.5
Couch 05/136	4.2	6.8
Couch 05/132	4.7	8.7
Couch 05/168	6.1	13.5
Meadow fescue 05/78	7	16
Cocksfoot 05/135b honeydew	7.5	29.5
Cocksfoot 05/135b *in vitro*	7.8	27.2
Tall oat grass 05/96	7.9	9.8
Cocksfoot 05/157	8.4	12.2
Tall oat grass05/160	10.3	14
Couch 05/83	10.9	13
Cocksfoot 05/112	10.9	26.5
Cocksfoot 05/26 *in vitro*	11.9	28.7
Meadow foxtail 05/71 *in vitro*	13.9	30
Cocksfoot 05/19	14	26
Cocksfoot 04/12	14.9	27.2
Couch 05/126	16.1	26.8
Cocksfoot 04/35	18	29.7

Isolates which showed a wide range of infectivity in the field and glasshouse experiments on Rialto and Paragon showed the same range of infectivity on Solstice, Einstein, and Robigus (Table 2.14) .

Table 2.14 Mean number of sclerotia per ear in four winter cultivars inoculated with three ergot isolates

Host and isolate no	Wheat variety			
	Rialto	Solstice	Glasgow	Robigus
Black-grass 04/91	21.7	15.3	25.7	6.5
Yorkshire Fog 04/112	0.0	0.0	0.0	0.0
Cocksfoot 05/51	2.0	0.3	2.5	0.0

Cross infection between ergots from different grass hosts

There was very limited infection of a number of grass species when infloresences were inoculated with either cocksfoot isolates, black-grass isolates, or wheat isolates (Table 2.15, see over).

Table 2.15 Infectivity of cocksfoot, black-grass and wheat ergot isolates on different grass species

Grass species	Cocksfoot isolates		Black-grass isolates		Wheat isolates	
	No. of heads inoculated	No. of sclerotia	No. of heads inoculated	No. of sclerotia	No. of heads inoculated	No. of sclerotia
Crested dog's tail	8	0	1	0	2	0
Timothy	2	0	1	0	1	0
Sweet Vernal grass	19	0	2	1	1	0
Annual meadow grass	10	0*	4	0*	3	0
Creeping red fescue	5	0	Not tested		Not tested	
Highland bent	5	0	Not tested		Not tested	
Perennial ryegrass	10	0	3	0	4	0
Italian ryegrass	5	0	1	0	5	2
Yorkshire fog	3	0	3	0	Not tested	
Black-grass	2	0	Not tested		Not tested	
Wheat	2	32	Not tested		Not tested	

* honeydew seen on two florets

Grass species experiment

In 2005, data on flowering of grass species was collected and ranged from 6 May for black-grass, meadow foxtail and sweet vernal grass to 10 June for timothy and 24 June for perennial ryegrass. (The cocksfoot did not flower in its first season). Other species flowered at the end of May. Very low levels of ergot were found on perennial ryegrass, black-grass and timothy in 2005.

Ergot was present in perennial ryegrass in the adjacent field margin in 2005, so ergot inoculum should have been available for 2006. The earliest flowering species in 2006 were black-grass, meadow foxtail and sweet vernal grass and these flowered in late April (Table 2.16). A second group included cocksfoot, Highland bent, red fescue and smooth-stalked meadow grass started to flower during mid to late May. Timothy, Yorkshire fog and perennial ryegrass only flowered in 12-18 June, after ear emergence in winter wheat (GS 57 on 5 June).

In 2006 there was limited production of new flowering tillers after mowing on 31 May 2006. The second mowing was done on 7 July 2006 and little regrowth occurred after this in July and early August. Fertile tiller counts were done using small quadrats as individual species matured and on 27 July 2006 in the once-mown plots (Table 2.16). There were no fertile shoots in the twice-mown plots. Mowing once significantly reduced fertile shoot numbers in all species, except for smooth- stalked meadow grass. Perennial ryegrass and Highland bent recovered and produced almost 20% of the unmown shoot numbers.

There were very low levels of ergot in the plots in 2005, but ergot was common in perennial ryegrass in the adjacent field margin in 2005. Wheat ergots buried in the experiment in autumn 2005 germinated during May 2006, but no ergot was found in unmown plots of the test grasses. On 16 August, ergot was found in once-mown plots of false oat grass (12% tillers) and perennial ryegrass (1% tillers) with a trace in timothy (<0.1%). Some ergot was also noted in false oat grass in a twice mown plot.

Table 2.16. Flowering dates of grass species and fertile tiller counts in 2006.

Ref. code	Common name	Species	Date of early flowering	Unmown plots Ears/m^2	Plots mown once Ears/m^2	Plots mown twice Ears/m^2	Mean Ears/m^2
1	Black-grass	*Alopecurus myosuriodes*	25 April	613	34.0	0.0	215.7
2	Cocksfoot	*Dactylis glomerata*	30 May	440	5.6	0.0	148.5
3	Highland bent	*Agrostis castellana*	19 June	1555	235.0	0.0	596.7
4	Crested dog's-tail	*Cynosurus cristatus*	30 May	1541	37.0	0.0	526.0
5	False oat grass/onion couch	*Arrhenatherum elatius*	16 May	490	49.6	0.0	179.7
6	Meadow foxtail	*Alopecurus pratensis*	25 April	120	0.4	0.0	40.0
7	Perennial ryegrass	*Lolium perenne*	19 June	1361	226.0	0.0	529.0
8	Red fescue	*Festuca rubra*	16 May	3725	0.0	0.0	1241.5
9	Smooth-stalked meadow grass	*Poa pratensis*	(16 May)	27	3.6	0.0	10.3
10	Sweet vernal grass	*Anthoxanthum odoratum*	25 April	2994	0.0	0.0	998.0
11	Timothy	*Phleum pratense*	19 June	346	9.6	0.0	118.4
12	Yorkshire fog	*Holcus lanatus*	12 June	1551	5.0	0.0	518.7
	Mean			1230.1	50.5	0.0	426.9
	SED			Mowing 56.9	Species x mowing 197.0		Species 113.8
	F test			<0.001	<0.001		<0.001

Note Sterile brome, annual meadow grass, rough and smooth –stalked meadow were at or near flowering in the vicinity of the trial on 9 May. Wheat was at GS 57 on 5 June

Table 2.17. Effect of mowing on fertile tiller counts in 2007.

Ref. code	Common name	Species	Unmown plots Ears/m^2	Plots mown once Ears/m^2	Plots mown twice Ears/m^2	Mean Ears/m^2
1	Black-grass	*Alopecurus myosuriodes*	941	734	10.4	561.7
2	Cocksfoot	*Dactylis glomerata*	136	16	0.0	50.5
3	Highland bent	*Agrostis castellana*	1474	265	0.0	579.7
4	Crested dog's-tail	*Cynosurus cristatus*	154	326	0.4	160.1
5	False oat grass/onion couch	*Arrhenatherum elatius*	340	39	1.6	126.9
6	Meadow foxtail	*Alopecurus pratensis*	53	3	0.0	18.5
7	Perennial ryegrass	*Lolium perenne*	244	274	1.6	173.3
8	Red fescue	*Festuca rubra*	2034	31	0.0	688.4
9	Smooth-stalked meadow grass	*Poa pratensis*	26	254	0.4	93.3
10	Sweet vernal grass	*Anthoxanthum odoratum*	2061	55	2.0	706.1
11	Timothy	*Phleum pratense*	328	123	0.4	150.4
12	Yorkshire fog	*Holcus lanatus*	1416	394	6.4	605.5
	Mean		767.2	209.5	1.9	326.2
	SED		Mowing 53.8	Species x mowing 186.4		Species 107.6
	F test		<0.001	<0.001		<0.001

Note Sterile brome, annual meadow grass, rough and smooth –stalked meadow grass were at or near flowering in the vicinity of the trial on 9 May.
Wheat was at GS 57 on 21 May

The earliest flowering species in 2007 were meadow foxtail and sweet vernal grass and these flowered from 2 April. Black-grass was flowering on 8 May. A second group including cocksfoot, Highland bent, red fescue and smooth-stalked meadow grass started to flower during mid to late May. Yorkshire fog flowered in early June whilst timothy and perennial ryegrass only flowered from 12 June, after ear emergence in winter wheat (GS 61-65 on 30 May).

Mowing significantly reduced fertile shoots from 767 to 209 per m^2, with the double mowing giving an additional significant reduction to 2 shoots/m^2 (Table 2.17). More species produced fertile tillers after the first mowing than in 2006. This was particularly apparent for black-grass though this was re-sown for 2007 and given some nitrogen fertiliser to provide a good population for ergot infection. Ergot was found in perennial ryegrass on 1.5% ears in the unmown plots and 6.25% ears in the once mown plots on 17 September. This was slightly more than the 31 July assessment (1.5% unmown and 0.5% in once mown plots). One ergot was found in once mown Yorkshire fog on 31 July and 17 September. An analysis of the tiller counts from 2006 and 2007 showed significant effects of year ($P=0.002$), grass species ($P <0.001$) and mowing regime ($P <0.001$). All the interactions between these three factors were also highly significant ($P <0.001$).

HPLC analyses

A small number of wheat and grass ergot samples were examined to develop the methods (Table 2.18). Some of the early results are illustrated in Figs 2.3-2.6. Comparisons are made with the results published by Mantle et al., 1977. Variation is evident and appears consistent with the range reported in the literature.

Table 2.18 Summary of HPLC analyses to September 2006

Source of ergot	No. samples tested	Comments
Wheat	3	Multiple alkaloids present and some variation noted. Ergotamine may be useful indicator alkaloid.
Black-grass	2	Similar to wheat. Ergotamine is third ranking alkaloid as indicated by Mantle.

Cocksfoot	2	Variation noted between Boxworth and Gleadthorpe. Mantle had 6 groups of cocksfoot isolates
Yorkshire fog	1	Gleadthorpe sample similar to cocksfoot sample at same site.
Perennial ryegrass	1	Very distinct with two major alkaloids ergotamine and probably ergocristine from the ergotoxine group.

A range of ergot samples were analysed with emphasis on isolates that have been screened for pathogenicity and had AFLP analyses. The samples did not include weakly pathogenic isolates as they did give sufficient ergots in pathogenicity tests for HPLC analyses to be done. A total of 18 alkaloids were identified in the HPLC but the number and quantities of alkaloids present differed considerably between isolates.

A sample of part grain / part ergots that appeared in some of the wheat variety screening experiments was also analysed. Alkaloids were found to be present in these hybrid grains and they may therefore cause problems in some commercial grain samples.

Results of the analysis of alkaloids in extracts of ergots

Two samples extracted from ergots collected from: 1) spring barley cv. Optic (Suffolk 2007) and 2) hybrid wheat-ergot grains provided by RAGT samples were provided for detailed alkaloid analysis by Dr. Michael Sulyok and Dr Rudolf Krska, Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln) University of Natural Resources and Applied Life Sciences, Vienna Konrad Lorenzstr. 20, A-3430 Tulln, Austria.

Analytical method

An HPLC-ESI-MS/MS based multi-mycotoxin method, with an Agilent 1100 HPLC coupled to an Applied Biosystems QTrap 4000 mass spectrometer was used. Screening and quantification was performed in the Selected Reaction Monitoring (SRM) mode. A detailed description can be found in "Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of

39 mycotoxins in wheat and maize" (*Rapid Communications in Mass Spectrometry* 20,2649-2659, 2006). An extension of this method to 87 analytes including a full method validation in spiked breadcrumbs has been published in *Analytical and Bioanalytical Chemistry* 389, 1505-1523 (2007). The positive confirmation of an analyte is achieved by the retention time and two MS/MS-SRM transitions (yielding 4 identification points according to Commission Decision 2002/657/EC). The method allowed semi-quantitative determination of 106 mycotoxins.

Sample preparation:

The crude extract was diluted 1+1 for qualitative screening using a mixture of acetonitrile/water/acetic acid 20/79/1 (v/v/v). For quantification, the raw extract was diluted 200-fold in order to obtain concentrations in the linear range. A sample of 5 µL of the diluted extract were injected without further pre-treatment into the LC-MS/MS. For external calibration, liquid multi-analyte standards were prepared from stock solutions of the neat compounds that were received in crystalline form from Prof. Miroslav Flieger (Academy of Sciences of the Czech Republic).

Table 2.19. Identification of alkaloids present in extracts from two samples of wheat ergots

Analyte	Conc. (mg/L) in Sample 1	conc. (mg/L) in Sample 2
Ergine + erginine	2.88	1.62
Ergometrine	18.5	30.2
Ergometrinine	2.40	71.8
Chanoclavine	0.80	0.75
Elymoclavine	0.10	0.04
Agroclavine	0.18	0.18
Festuclavine	0.012	0.006
Ergovaline + ergovalinine	6.16	6.32
Ergosine	75.8	116.0
Ergosinine	35.6	49.6
Dihydroergosine	0.21	0.24
Ergocornine	66.0	27.8
Ergocorninine	161.0	54.2
Ergotamine	71.6	61.2
Ergotaminine	24.4	16.1
Dihydroergotamine	0.56	0.29
Ergocryptine	47.4	19.7
Ergocryptinine	120.0	64.2
Ergocristine	46.0	79.6
Ergocristinine	103.0	153.0
Enniatin B	1.16	1.16
Enniatin B1	1.07	3.12
Enniatin A1	0.35	0.14
Enniatin A	0.039	0.010

Both extracts contained large concentrations of ergopeptides, clavine alkaloids and enniatins. The related concentrations (expressed as mg/L raw extract) are given in Table 2.19. Ergocristinine was present at >100 mg/L in both samples whilst ergosine, ergocorninine and ergocryptine were only found in one of the two samples at >100 mg/L. Four other alkaloids (ergometrinine, ergocornine, ergotamine and ergocristine) had concentrations at >50 mg/L in one of the two samples. Eight alkaloids were found at <1 mg/L in both samples.

In the case of chanoclavine, festuclavine and ergocornine, several peaks eluted a bit after the main peak. In case of ergocryptinine, a double peak was observed, presumably due to the presence of both α- and β-ergocryptinine. No peaks were identified in the negative mode.

The two samples showed some differences in the concentrations of some alkaloids. Comparing sample 1 with sample 2, there was almost at 2:1 ratio of ergocornine, ergocorninine, ergocryptine, ergocryptinine, and a 1: 2 ratio for ergocristine and ergometrinine (Table 2.19).

This analytical method did not allow direct comparison with the HPLC method as the ranking of retention times of some of the standards differed. Further investigations using a wider range of standards in the HPLC method is required to identify the 18 peaks

Fig. 2.3. Standard alkaloids used in HPLC analyses

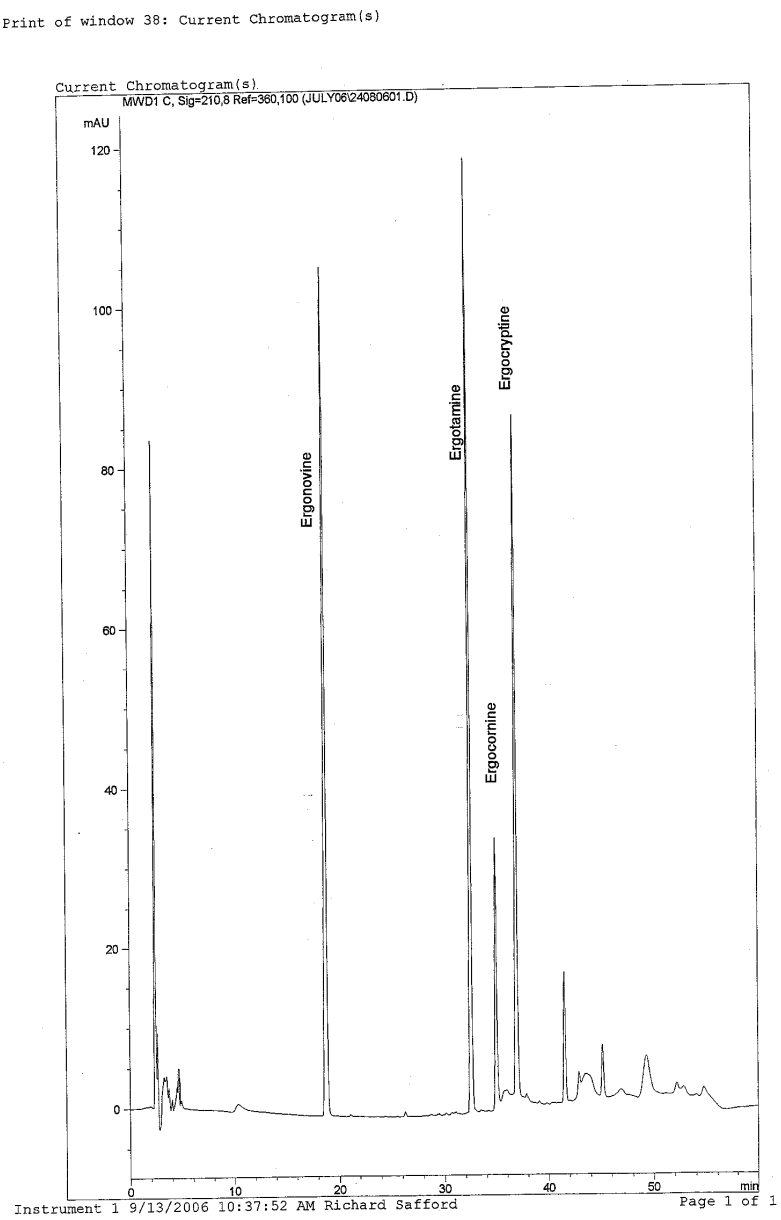


Fig. 2.4 HPLC alkaloid analyses of wheat ergots from Boxworth Cambs and Bacton Suffolk.

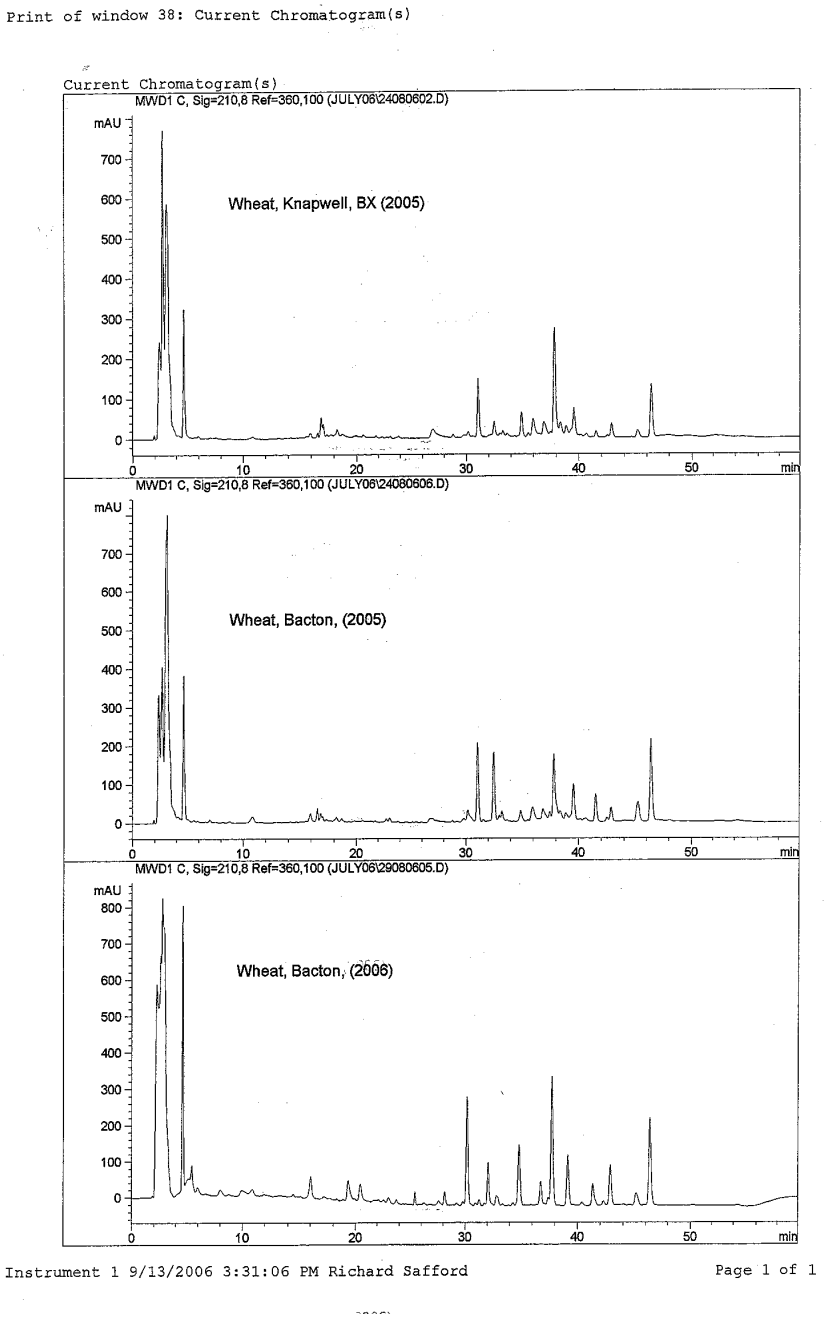


Fig.2.5 HPLC alkaloid analyses of black-grass and perennial ryegrass ergots from Boxworth, Cambs and Suffolk.

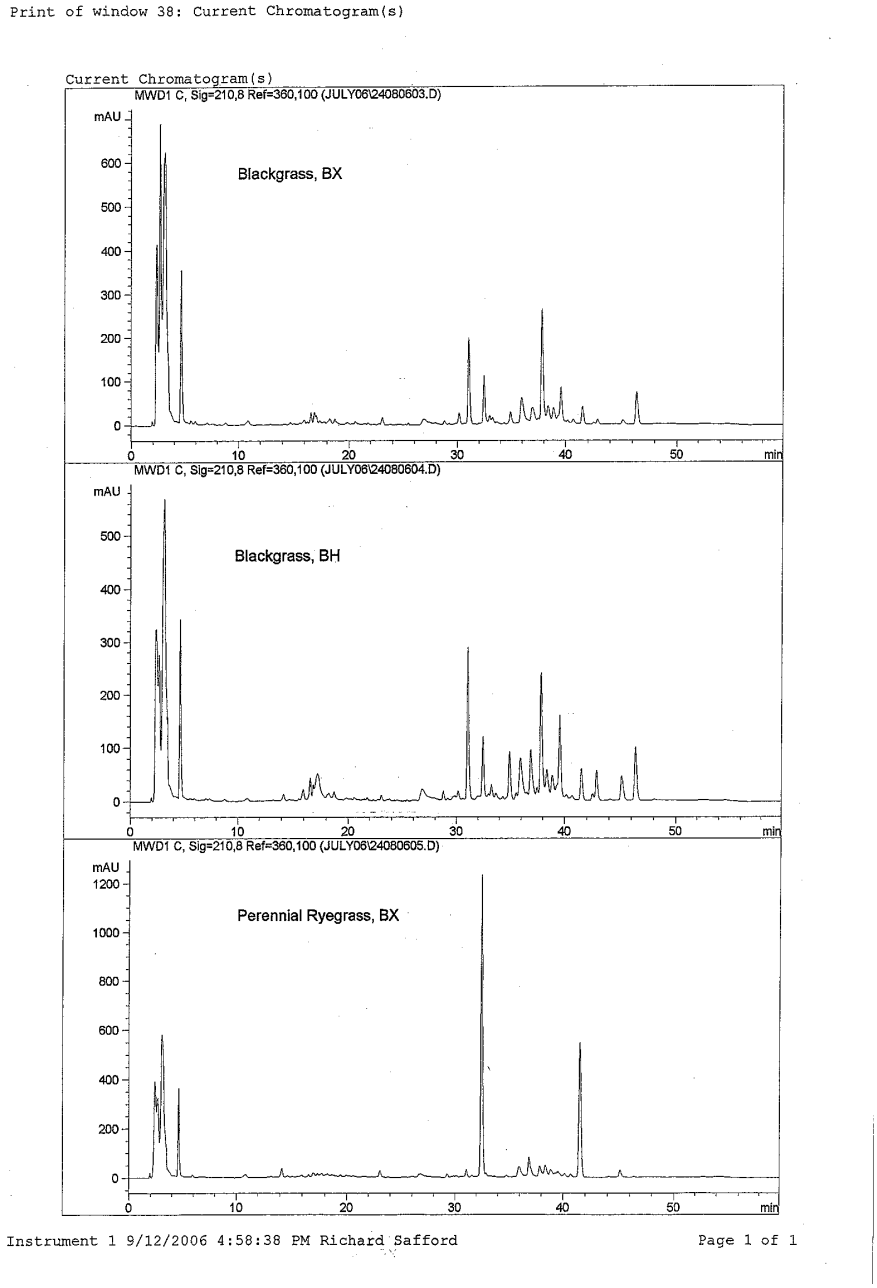
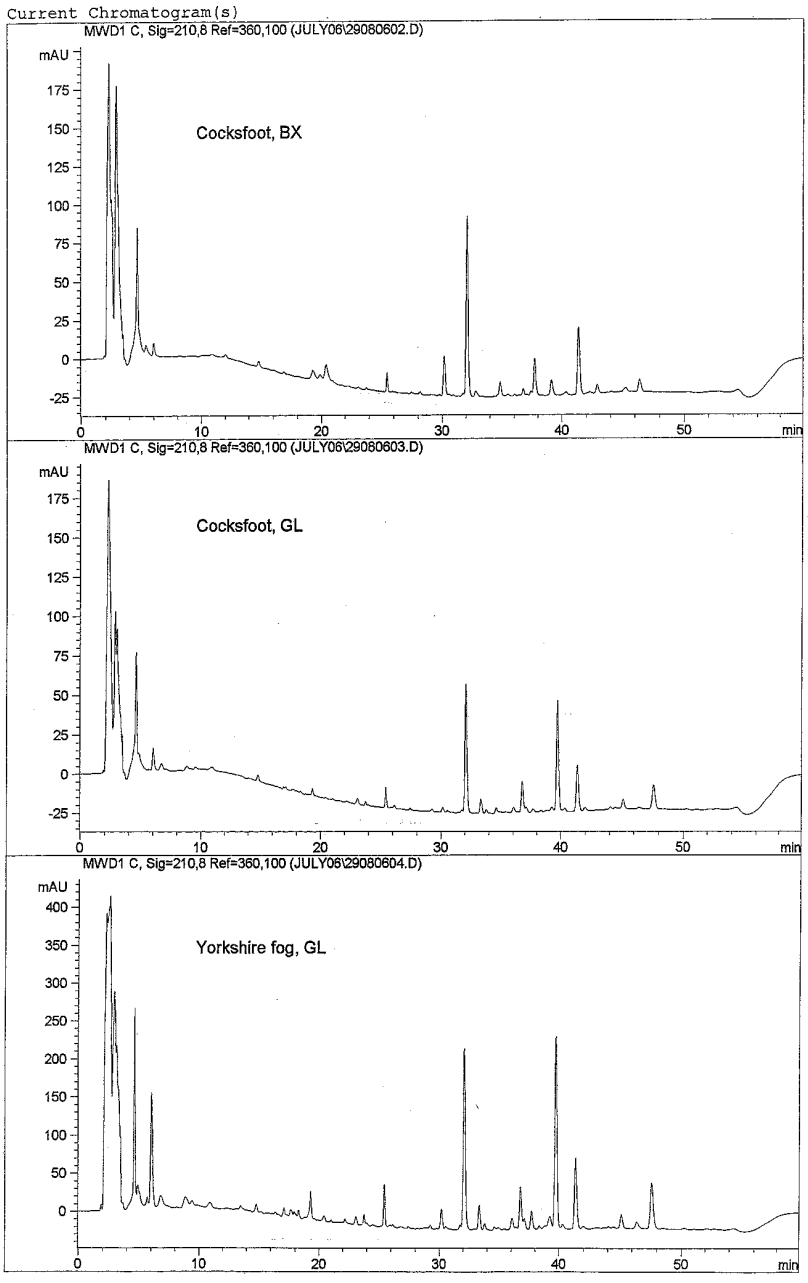


Fig.2.6 HPLC alkaloid analyses of cocksfoot and yorkshire fog from Boxworth, Cambs (BX) and Gleadthorpe, Notts (GL).

Print of window 38: Current Chromatogram(s)



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Comparison of ergot infectivity, alkaloid and AFLP profiles

Non-hierarchical cluster analysis (Genstat 11.1) using the presence or absence of 18 alkaloid compounds did not reveal any tendency for ergots from the same grass species to have similar alkaloid profiles. There was a tendency for isolates with low infectivity on wheat to fall into groups which had no ergotoxine (ergocornine plus ergocryptine) peaks. Quantitative data (% of total alkaloid content) for these two classes also confirmed that low infectivity types had low or nil, ergotoxine content, and tended to have higher ergotamine content. (Table 2.20). Peaks 17 and 18 (see alkaloid results) also tended to be present in the low infectivity types and appeared less frequently in the highly infective types. AFLP analysis indicated that low infectivity isolates tended to form a distinct group, though there was still a degree of overlap (Fig 2.7). Several of the low infectivity data points related to Yorkshire Fog isolates. In AFLP analysis of a wider set of isolates (data not shown) which could not all be tested for infectivity, Yorkshire Fog ergots formed a distinct grouping, with less diversity apparent than within other host sources

Table 2.20 Ergotamine and ergotoxine levels (% total alkaloid) in relation to ergot infectivity level on wheat.

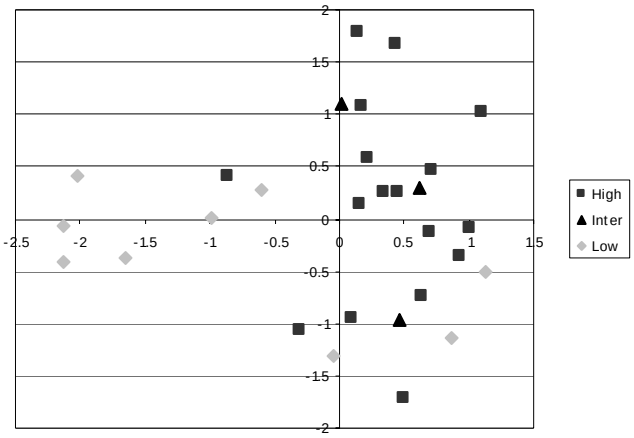
Host grass	Infectivity level	% ergotamine	% ergotoxine
Tall oat grass	I	0	66.90
Couch	H	9.31	48.05
Meadow brome	H	3.36	40.42
Cocksfoot	H	10.30	38.37
Yorkshire fog	L	0	37.19
Tall oat grass	H	0	35.32
Cocksfoot	H	7.68	33.99
Black-grass	H	67.7	32.30
Cocksfoot	H	7.79	28.03
Cocksfoot	H	7.79	28.03
Cocksfoot	H	10.64	27.46
Black-grass	H	0	27.18
Timothy	H	0	23.84
Black-grass	H	0	23.69
Couch	H	0	22.84
Wall barley	H	25.81	19.08
Yorkshire fog	L	0	18.08
Yorkshire fog	L	0	18.08
Italian ryegrass	I	4.16	17.48
Meadow foxtail	H	23.06	16.83
Meadow fescue	H	22.9	16.83
Italian ryegrass	I	0	15.95
Black-grass	H	0	15.79
Cocksfoot	H	4.54	14.46
Cocksfoot	H	4.55	14.46
Meadow brome	H	13.22	13.82
Bent	L	28.02	13.48
Italian ryegrass	I	27.89	13.38
Tall fescue	H	0	11.05
Tall fescue	H	47.24	11.02
Couch	I	0	7.52
Cocksfoot	I	0	6.16
Tall fescue	H	11.64	4.41
Tall fescue	H	11.65	4.41

Table 2.20 contd

Host grass	Infectivity level	% ergotamine	% ergotoxine
Mat	L	28.2	2.32
Cocksfoot	H	40.25	1.94
Black-grass (honeydew)	H	0	0
Black-grass (*in vitro)*	H	0	0
Couch	H	43.04	0
Meadow brome	H	0	0
Perennial ryegrass	I	34.16	0
Perennial ryegrass	I	22.43	0
Perennial ryegrass	I	0	0
Perennial ryegrass	I	26.07	0
Tall fescue	I	43.42	0
Bent	H	45.71	0
Cocksfoot	L	16.31	0
Cocksfoot	L	17.95	0
Cocksfoot	L	8.72	0
Couch	I	51.7	0
Tall oat grass	L	0	0
Meadow fescue	I	41.83	0
Perennial ryegrass	I	42.38	0
Perennial ryegrass	L	28.27	0
Perennial ryegrass	I	51.06	0
Tall fescue	I	0	0
Tall fescue	L	43.43	0
Timothy	I	41.2	0
Timothy	L	13.44	0
Yorkshire fog	L	14.99	0

* H, I, L = high, intermediate or low infectivity, judged from within experiment data.

Fig 2.7 Plot based on principal component analysis showing relatedness between AFLP profiles for isolates with different infectivity levels, high, intermediate and low, on wheat.



Discussion

Samples were received from 37 different grass species, giving a combined total of 497 over the four monitoring years. Of these, 240 ergot isolates were tested on wheat, from a total of 29 grass species. There were large and significant differences in the degree to which *C. purpurea* isolates from different host grass species were able to infect wheat. Samples of ergots tested necessarily reflected the numbers received from different host species, and where only low numbers of isolates were available, it is difficult to reach conclusions on whether a particular species may be a consistently low or high risk for wheat. Low numbers of samples could mean that particular species were not present in the areas monitored, or that ergots were difficult to see (eg in annual meadow grass or fescues) or that there was escape or resistance to infection. One species, crested dog's tail, was known to be present in some of the monitored areas, but no ergots were found, and attempts at infecting the grass with a number of ergot isolates were unsuccessful. This suggests that this species may either resist infection, or avoid it, and its presence in margins would not represent a potential risk to wheat.

A relatively large number of samples were received from Yorkshire fog (*Holcus lanatus*). A total of 28 samples were tested, and all but two either failed to infect wheat, or only produced very low numbers of small ergots. In the two cases where

significant numbers of ergots were formed, these were found to be "half grain : half ergot", suggesting that there was some resistance to pathogenesis. Though a few of the Yorkshire fog samples originated from non-arable environments, the majority came from arable locations in the north and east of England, including East Anglia, and the south west. Seven samples of ergot from creeping soft grass, (*Holcus mollis*) were received and tested. Five had low infectivity, but two produced larger numbers of full-sized ergots.

There was considerable variation within isolates from cocksfoot, couch, and tall oat grass in their infectivity on wheat. Cocksfoot is used in margin seeds mixtures, as well as being common in the natural arable environment, and it was clear the species could be a high risk to wheat. Even within one location, ergot isolates from cocksfoot showed highly distinct infectivity profiles on wheat, with some unable to produce ergots at all, while others produced numerous large fruiting bodies. Couch and tall oat grass are also common arable weedy grasses, and must be considered a significant source of ergot inoculum.

Fewer samples were received from Timothy, but of those tested, there was wide variation in infection levels on wheat, and the species should be considered as high risk. Tall fescue and meadow fescue ergots were also variable in their ability to infect wheat, and though infection levels from meadow fescue isolates were usually intermediate, one or two isolates showed greater ability to infect wheat. Meadow brome, a weed grass of increasing importance, was also a source of ergots which proved highly infective to wheat.

The ryegrasses were a significant source of ergots during the monitoring period. Isolates from both perennial and Italian ryegrasses produced moderate levels if infection on wheat, in general these were not as high as the ergot numbers produced by some cocksfoot isolates. Ryegrasses might therefore tend to select ergot populations which are less adapted to wheat, even though infection could still take place.

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The ability of ergots from one grass species to infect and reproduce on others was investigated in a small experiment. As it was difficult to produce flowering grasses consistently, the conclusions which can be drawn from this work are limited. It appeared that black-grass isolates might have a broader host range than isolates

derived directly from cocksfoot or from wheat. Repeated inoculation of several grass species with a wheat infective cocksfoot isolate mixture failed to produce any sclerotia, though annual meadow grass produced some honeydew with both cocksfoot and black-grass isolates, and the species may be a more important source of ergots than the monitoring results indicate. Comprehensive cross-infection studies were outside the scope of this project, but it would be of interest to investigate whether Yorkshire Fog isolates could infect black-grass, and whether any resulting sclerotia were infective on wheat.

Infectivity studies were carried out with one winter wheat cultivar, Rialto, and one spring wheat cultivar, Paragon. The latter had proved highly susceptible to black-grass isolates in other work packages, and was used to produce florets for inoculation without the need for a lengthy vernalisation period. Using a limited number of wheat genotypes to draw overall conclusions on ergot isolate infectivity assumes that there is no variety x isolate interaction; ie that low infectivity isolates on Rialto would have low infectivity on other wheat genotypes. Though only a small number of additional wheat varieties could be tested, "low infectivity" cocksfoot and Yorkshire fog isolated failed to produce ergots on the additional varieties. The black-grass isolate mixture produced high numbers of ergots except on the cultivar Robigus, as expected from work elsewhere in the project.

The work described here provides the basis for identifying grass species which, though they are infected with ergot themselves, constitute a low risk to wheat because the ergots from them do not infect the cereal. The results give a recent and extensive description of ergot populations in an arable environment which has been subject to a wide range of different influences since the work of Mantle *et al*. (1977). Some significant differences are apparent. In the earlier work, two distinct levels of infectivity on wheat (high and weak) were recognized, whereas in the present work, there appeared to be a continuum. This was especially noticeable within the large number of cocksfoot isolates tested. Mantle *et al*. (1977) tested only four ergot samples from cocksfoot, and found them all to be weakly infective, but in the current work it was clear that highly infective cocksfoot isolates were also present. The two studies rank ergot samples from Lolium species as generally less infective on wheat. In the 1977 study, ergots from tall fescue, tall oat grass, and timothy were all weakly infective, but these all showed both high and low infectivity in the current work. Samples from Yorkshire fog and creeping soft grass (two only tested in each case)

were weakly infective in the 1977 study. Though there are clear differences in the conclusions reached between the 1977 work and the present, it is impossible to determine whether or not the differences are a result of shifts in the characteristics of the ergot population, since the number of isolates tested in the earlier work is comparatively small.

The most consistently low infective ergots in this study were from Yorkshire fog. Using some level of this grass in margin mixtures may help to reduce the proportion of wheat infective isolates in a location. There was a small number of other grasses where infectivity was at a less consistent low level, but which may still tend to support populations which are a lower risk to wheat. These include the ryegrasses and creeping soft grass. Several grass species were represented by only a few, or one, sample, which proved to have low infectivity. These included sweet vernal grass, wavy hair grass, rough meadow grass and mat grass, though some of these will have limited environmental value in sown margins. Crested dog's tail is widely used in margins, and no ergots were found on this species during the four years of monitoring, though this does not guarantee that it is a low risk grass. Only a few samples were received from red fescue, which is also commonly used in margins. Unfortunately, these failed to grow in culture and could not be tested. No ergots were received from sheeps fescue. *Agrostis* species (bent grasses) which are used in some margin mixtures, also produced only a few samples during the survey, but one of these produced relatively high numbers of ergots on wheat.

Late heading grasses where honeydew inoculum increases after the main risk period for wheat infection offer a means of avoiding ergot problems in a season. If the ergots which eventually form on the grass, and fall to the ground, also have low infectivity on wheat, or on an intermediate grass host such as black-grass, then crops in the vicinity the following year will get less infection from the inoculum which eventually arises from the germinating sclerotia. Combining a selection of late heading grasses, together with species which produce low infectivity ergots, should offer an opportunity to suppress ergot inoculum and find appropriate species mixtures to fulfil environmental requirements. A summary of infectivity risk, with flowering periods and number of ergots tested, for grasses which are either used in margin mixtures, or are regarded as weedy species, is given in Table 2.21.

Table 2.21. Summary of infectivity risk and flowering period for selected grass species

Species	Flowering time in relation to wheat	Infectivity risk	Range of infectivity mean sclerotia/ear	Numbers tested
In margin seeds mixtures				
Common Bent	Late	High	3.0 -18.5	2
Crested Dog's Tail	Overlap	Unknown*	-	None received
Cocksfoot	Overlap	High	0.0 - 29.7	64
Meadow Fescue	Late	Intermediate	5.5 - 23.8	5
Sheep Fescue	Overlap	Unknown	-	None received
Red Fescue	Overlap	Unknown	-	2 - not viable
Smooth stalked Meadow Grass	Overlap	Unknown	-	1 - not viable
Meadow Foxtail	Early	High	33.2	1
Tall fescue	Late	High	2.5 – 20.5	9
Wavy Hair-Grass	Late	Low	0.0	1
Yorkshire Fog	Late	Low	0.0- 11.8	25
Timothy	Overlap	High	0.7- 34.0	10
Weedy and other grasses				
Black-grass	Early	High	5.5 -33.8	5
Couch	Late	High	4.7 – 26.8	15
Tall oat grass	Late	High	0.0 – 21.5	14
Sweet vernal grass	Early	Low	0.0- 0.1	2
Creeping soft grass	Late	Low	0.2-10.0	7
Perennial ryegrass	Overlap	Intermediate	1.3 - 20.0	15
Italian ryegrass	Overlap	Intermediate	0.0 -15.8	6
Meadow brome	Overlap	High	15.8-27.3	4
Annual meadow grass	Early and prolonged	Low**	1.6	1

* Crested Dog's Tail likely to be low risk since no ergots were produced after repeated inoculations

** 1 isolate tested, previous work tested two isolates, one high and one low infectivity on wheat

The contribution that sown grass margins may make to the overall ergot inoculum reservoir is not known. With many high risk grasses present in the arable environment, either as weeds or as natural vegetation in headlands, hedge bottoms etc, manipulating sown margins may have little effect. Nevertheless, it offers an on-farm management approach to exert some level of ergot reduction, and potentially to allow the selection of local populations of ergot which have lower infectivity on wheat.

The use of alkaloid analysis to profile ergot populations and predict infectivity to wheat was investigated by Mantle *et al*. (1977). 241 ergot samples were analysed, and nine alkaloid groupings were described. Wheat infective isolates all had high levels of ergotoxine in relation to ergotamine, while weakly infective isolates had high levels of ergotamine in relation to ergotoxine. In the current study, a large number of samples with nil or low ergotoxine content (ergocornine and ergocryptine combined) were weakly infective on wheat, tending to support the earlier findings. Of the twelve cocksfoot samples analysed, all but one of the highly infective types showed a high ergotoxine : ergotamine level. However, not all highly infective samples from other species had high ergotoxine levels, and some had high ergotamine and lower ergotoxine content (eg from bent grass and one black-grass isolate). One Yorkshire fog sample had relatively high ergotoxine, and no ergotamine, but was still not infective on wheat. One sample from meadow brome and one from black-grass which were both highly infective contained no ergotoxine or ergotamine and total alkaloid content was one single unknown product in the case of meadow brome, and two products in the case of black-grass (see detailed results). There did not appear to be any consistent alkaloid profile within a host grass species, but a rather more consistent relationship between the ergotamine and ergotoxine content and infectivity, regardless of host. Even though there were some exceptions, and the total number of samples analysed was limited to 60, it is reasonable to conclude that alkaloid profiling offers some predictive ability of infectivity. However, there is no known functional link between alkaloid profile and infectivity on wheat. The AFLP profiling carried out in ancillary projects also indicated that low infectivity isolates formed a group. Yorkshire fog isolates consistently fell into this group, regardless of the geographic location they were collected from. This strongly suggests that a distinct population of *Claviceps purpurea* exists on this grass, which is not capable of producing large numbers of normal sized ergots on wheat. Low infectivity ergots from other grass species had molecular phenotypes close to those of Yorkshire fog ergots

suggesting that AFLP analysis might also be used to predict the infectivity of isolates. The relationship between infectivity, alkaloid profile and molecular phenotype is worthy of further investigation with more samples.

Work package 3: Epidemiology

Introduction

This work-package was designed to provide epidemiology information to interlink with other work-packages of the project. Information such as the timing of ascospore release could be used to identify which grass species flower at the same time and so have potential to enhance ergot infection in crops by secondary spread within fields or from margins or to build inoculum at margins (in combination with WP2 studies on the susceptibility of grasses and on cross compatibility of infection by different races of *C. purpurea*). Additionally, the timing of ascospore release could identify crop varieties that maximise disease escape due to incompatible flowering date to complement WP4 (resistance of wheat varieties). Quantifying primary inoculum production from different farm habitats augments studies on the spatial distribution of ergot in crops relative to proximity to the field margin undertaken as part of WP1 (field margin/crop surveys). Furthermore, these studies can be made at the same sites to enhance the value of the data by enabling spore concentration to be related to final ergot incidence. The approach taken was to monitor the timing of spore release from fruiting bodies produced by ergots near the soil surface, to quantify spores present in the air in different farm habitats and at different distances from artificial inoculum sources and to measure infection gradients in crops. The overall aims were (i) to identify the extent to which field margins may contribute to ergot infection in cereal crops (using wheat as a model) compared to other sources of inoculum; and (ii) to quantify spatial distributions resulting from primary spread (ascospores) and secondary spread (conidia) of *Claviceps purpurea* in winter wheat. This was arranged as two components of work package 3; 3.1: Spore trapping at a wide range of naturally infected locations to invesitgate a) the timing of release of ascospores of *C. purpurea* and b) the relative importance of inoculum from field and margin; and 3.2: Field experiments designed to quantify dispersal patterns from margins and within fields due to primary and secondary inoculum and to assess the relationship between inoculum exposure and disease in winter wheat crops

The intention of the research was to identify the zone of crop inside the field margin at risk of infection from margin initiated ascospores or margin initiated secondary spread. The results would be used in an ergot management guide to inform growers

on strategies to reduce the impact of ergot in wheat crops with emphasis on margin management.

Much has been reported in the literature about the biology and epidemiology of *C. purpurea*. A vernalisation period of several weeks at 0-10°C is required for germination (Kirchoff, 1929: Mitchell & Cooke, 1968). In the UK, ergots that over-winter at or just below the soil surface, germinate to produce a stalked stroma [germination conditions reported in Kirchoff (1929); Hadley (1968); Mitchell & Cooke (1968)]. Wengiger (1924) reported that in temperate climates, ergots can remain viable for over one year, although later reports suggest ergots survive only one season (Anon, 2002). Ergots can produce a succession of stroma (spore-bearing fruiting body) with up to 60 from a single ergot (Sprague, 1950). Furthermore, Wood & Coleysmith (1982) reported that ergots germinated over a five-month period.

As with most ascomycetes, ascomata, within the stroma mature (after 5-7 days) and release airborne ascospores (the primary inoculum) by active discharge after wetting or in high humidity (Colotelo & Cook, 1977; Hadley, 1968). Alderman (1993) reported that ascospore release showed periodicity with maximum release at 01:00-06:00 hours. In that study, spore release started 2-3 days after rain and continued for up to 16 days, with spore release associated with further rain events. Indeed, Alderman & Barker (2003) found that ergot incidence was related to occurrence of rain during the susceptible period of flower initiation to maturity in many cultivars of Kentucky bluegrass. Ascospore release peaked in late June in Canada (flowering period of winter rye), with fewer spores present in July (flowering period of spring rye) (Brown, 1947). However, in Russia, ascospore release coincided with the end of flowering of rye but in synchrony with late-maturing varieties (Markhasseva, 1936). In southern England, ergots germinated in mid-May and produced maximal ascospore numbers in June (Mantle & Shaw, 1976). In France, late-maturing wheat was reported to be more severely affected than early wheat (Rapilly, 1968).

Infection of the flowers of a compatible grass or cereal [the host range is very large – see Brady (1962)] can only occur before fertilization and results seven - ten days later in the production of conidia (asexually-produced spores), which are exuded from flowers in drops of clear, amber to tan honeydew (Rapilly, 1968; Wood & Coleysmith, 1982). These conidia represent secondary inoculum and can be spread by rainsplash, direct contact or (primarily, according to most researchers) insect-vectors, to infect

other (slightly later-flowering) florets. Many reports suggest a wide range of insects carry conidia [e.g. up to 100% of moths and 75% of flies collected in Kentucky bluegrass fields in NW USA carried conidia (Butler *et al.*, 2001)], but little is reported on disease gradients. One report about infection of male-sterile wheat (therefore more susceptible) suggests a sharp disease gradient caused by secondary infection with disease mainly within 1.5 m of primary foci (Mantle & Swan, 1995).

In addition to conidia production, the fungus continues development into the ergot stage from 12 days after infection of the floret and leading to a mature ergot (type of sclerotia) in place of the seed at harvest (Shaw & Mantle, 1980).

Fungicides have been tested as a means to control floret infection and hence ergot production and some (flusilazole, propiconazole or tebuconazole) were reported to be successful in a *Poa pratensis* seed production field, while other reports suggest insufficient activity i.e. on rye and wheat in England (Anon, 2002). Seed treatment with appropriate fungicide is reported to prevent germination of ergot contaminants in seed with good efficacy (Shaw, 1986; Cagas, 1992).

Cultural practices have been suggested to reduce the impact of ergot – Bretag (1981) reported that ergots buried deeper than 5 cm could not sporulate. Stubble burning was also effective in reducing ergot inoculum, while cutting of grassland before flowering was reported to be a possibility (Jenkinson, 1958; Chester & Lefebvre, 1942). Field margins and volunteers or grass weeds within the crop have been suggested as a source of inoculum e.g. grassy field margins were reported to be the main source of inoculum in Canada (Campbell & Freisin, 1959). All indigenous grasses and forage grasses in England were reported to act as a reservoir of inoculum capable of infecting rye, wheat and barley (Campbell, 1957). Mantle & Shaw (1977) showed that black-grass increased the risk of wheat infection by secondary spread.

Therefore the crop could be exposed to three sources of primary inoculum: from the field itself (residual ergots, ergots in seed); from crop margins; and from external sources other than crop margins (set-aside, margins of other fields, non-cultivated land, other cultivated fields). The crop can also be infected by secondary inoculum derived from infected grass weeds within the field or from grasses in the margins. Reducing primary inoculum should also reduce secondary infection both from initially infected grasses and crop plant to crop plant (early to late flowering tillers).

Materials and Methods

The WP3 study was in two parts. Part (3.1) used different farm sites, often known to have a history or ergot problems. The objectives of this part were to study the timing of release of ascospores (primary inoculum) of *C. purpurea* and to study the relative importance of inoculum from different sources (field, margin). Part (3.2) was done using controlled field experiments at Rothamsted Research (RRES). Part 3.2 experiments were designed not only to provide new information, but also ensure that results would be obtained in case there were poor natural ergot infection rates at the farm sites. The objectives of these experiments were to quantify dispersal patterns from margins and within fields due to primary and secondary inoculum and to assess the relationship between inoculum exposure and disease in winter wheat crops.

Part 3.1 (natural inoculum sources in 2005 and 2006): A number of sites were identified that had a history of ergot problems and many of these sites were also used in WP1 and WP2 to assess the distribution of ergot infection within fields of winter wheat and sample ergots from grass species.

In year 1 (2005) At each site, airborne ascospores were sampled using purpose built passive spore traps. The traps consisted of inert cylindrical collectors (plastic tubes from disposable Pasteur pipettes) mounted vertically on bamboo canes at canopy (ear) height and protected by a small rain-guard (plastic plant pot saucer). As the ascospores of *C. purpurea* were idenifed as being 'sticky' in lab-based pilot trials, the surfaces of the cylinders were not coated with any sticky substance as this was thought to reduce the amount of other particles trapped from the background of airborne particles. The sample tubes were mounted on bamboo canes in clusters of seven per site. Each site was paired as a margin and within-field site, the latter being placed within a crop of wheat at least 30 m from the nearest margin. The exposed passive traps were disassembled, placed in containers (45 ml sample tube) and the exposed collecting surfaces returned in the containers to NIAB for analysis using the PCR diagnostic developed in WP5. The containers were labelled to identify date, location and margin or field. In year 2 (2006) the presence of airborne ascospores was assessed at similar locations by sampling wheat ears in mid-June before any ergots had formed. A hundred ears (approx) were sampled from plants immediately

next to margins and 100 from well into the field (at least 30 m), the latter accessed via a tramline. Each of these 100 ear lots was divided into 4 lots of 25 ears, taken from groups of plants about 1 or 2 metres apart. Each lot was labelled to identify the date, sampler's name and organisation, farm, field, whether 'crop' or 'margin' and a, b, c or d for each of the four sample lots (the four lots were not replicates, as originally intended, as due to costs and time factors they were pooled for DNA extraction). In some cases it was possible to sample different margins of the same field, in which case the margin position (e.g. North, south, east or west) was added to the label and duplicate samples of the 'crop' ears were taken so that there was always a separate pair of samples – 'margin' and 'crop' for each comparison. Samples were sent to Rothamsted in large envelopes, where they were frozen at -20°C, freeze-dried, crushed to a powder and dispatched to NIAB in 45 ml sample tubes.

To generate information on the seasonal and daily timing of primary inoculum, a continuous record of ascospore concentration in air each season during 3 years of the project was made at RRES and at ADAS Boxworth. This was done using 7 day recording Burkard spore traps. The collecting drums were prepared under clean conditions in the laboratory at RRES and transported to the sites in closed containers. The drums were changed weekly and the exposed drums returned to RRES for processing. Exposed tape sections were cut into 96 mm sections (representing air sampled over a two day period), placed in a 45 ml sample tube and taken to NIAB for analysis by qPCR. In year 1 of the project, the trap at RRES was placed in a 4 m diameter fallow area at the centre of a wheat plot 50 x 36 m in size (cv. Rialto). Around the trap, ergots had been seeded in the autumn at eight positions (compass points) approximately 2 m away from the trap. The trap was operated from May to late-June each year. At ADAS Boxworth, the trap was located next to a grassy field margin that had a known history of ergot infection.

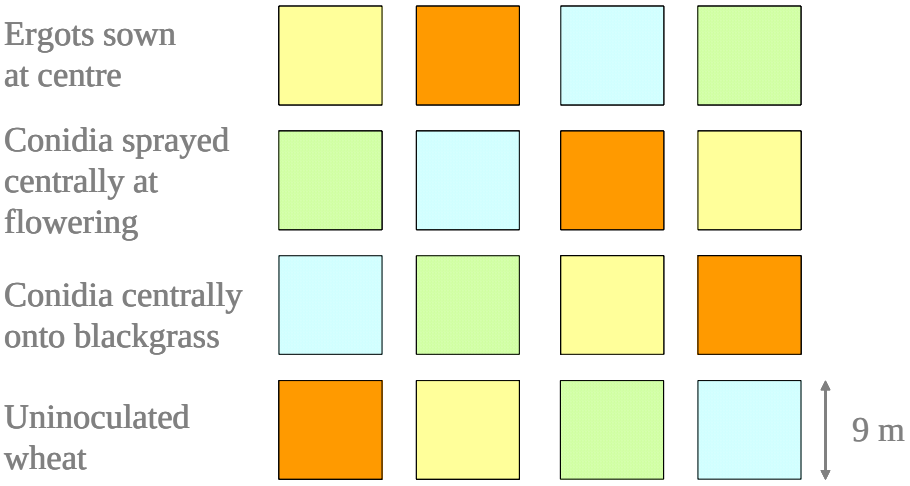
DNA was extracted at NIAB by grinding the spore-tape surface in liquid nitrogen to fracture the spores, followed by a phenol-chloroform extraction method. This was also done at Rothamsted in year three. It was found to be impossible to identify ergot ascospores in outdoor air samples by microscopy as they were very small and there were always large numbers of other spores present.

Part 3.2 comprised field experiments at Rothamsted Research in the final two full seasons of the project. Two experiments per year each used artificially inoculated

sites to study spatial patterns of primary and secondary inoculum spread from small point sources and larger line sources of inoculum.

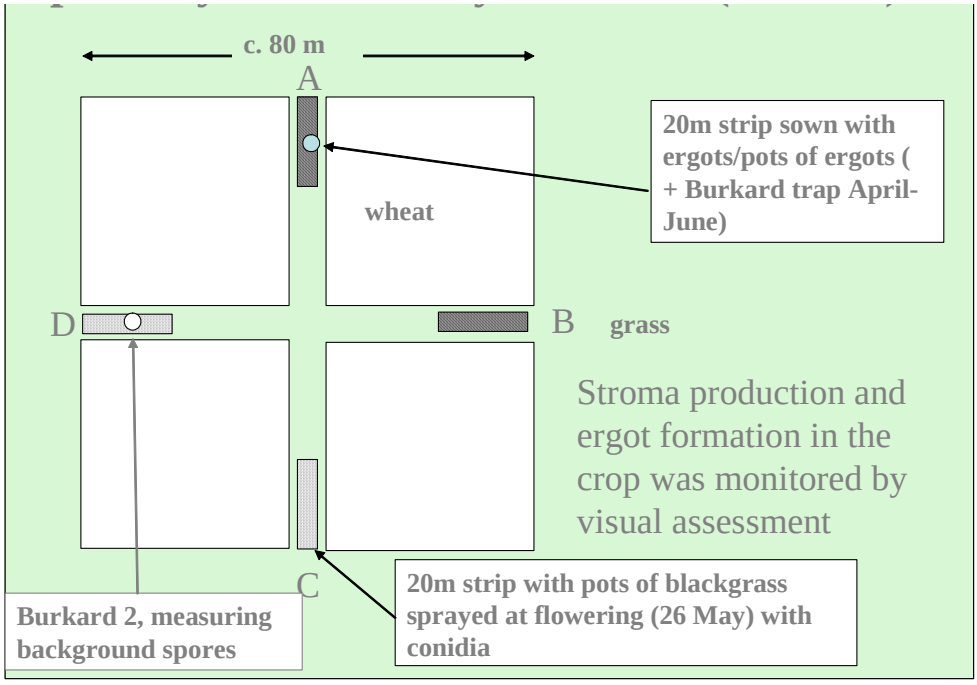
The point-source experiment used 16 plots of winter wheat approximately 9 m x 9 m, surrounded by 3 m fallow rows established in sites that were previously set-aside (Fig. 2.1). With four replicates, the centre of 4 of these plots were seeded with ergots that had been planted in pots of compost in the autumn and kept outdoors over winter until planting-out in March. Each pot had several wheat-derived and several black-grass-derived ergots (exact numbers varied each year). Additional pots of ergots were kept in a cold room at 5°C, occasionally watered with rain water. These were used as a backup in the field experiments in addition to the 'seeded' ergots when the wheat crop was flowering. Four uninoculated plots were used as controls to separate effects of any naturally occurring infection. The other eight plots were not seeded with ergots but were used to investigate gradients of secondary spread caused by conidia. These plots were designed to compare effects of early infection (initiated by infected black-grass) and later crop to crop infection. One set of four plots had a large pot of black-grass planted at the centre and at flowering (mid-May): these were inoculated with a suspension of *C. purpurea* conidia (a mixed suspension of previously frozen conidia produced in vitro or collected from honeydew of infected plants). The conidial suspension was inoculated by a combination of two methods (a) approximately 20 ears were inoculated by injecting several florets per ear with a drop of suspension using a hypodermic needle, and (b) the entire clump of plants at each position was misted with spore suspension using a hand-held sprayer. In the other four plots, at the onset of flowering of the wheat crop, a square 20 cm x 20 cm at the plot centre will be inoculated with *C. purpurea* conidia using the same method. The positions of ergots were mapped before harvest (late-July) in transects through each plot and around the edge of each plot to assess patterns of infection.

Figure 2.1. 2005-6 disease spread from point sources of primary or secondary inoculum compared with background inoculum



The line-source experiment comprised an area of wheat (cv. Xi19) 75 x 75 m, divided into four quarters with a fallow strips approximately 3 m wide (2.2). Lengths of the dividing fallow strips, 16 m long and located towards the outside of the wheat area acted as sources of inoculum having either been sown with pots of over-wintered ergots or pots of black-grass plants in six positions along the 16 m length. The black-grass plants were inoculated with *C. purpurea* in mid-May using the same method as described above. The positions of sown ergots were marked with plastic canes and presence of sporulating fruiting bodies was monitored in May and June.

Figure 2. 2 Design of experiment to study disease spread from line sources of primary or secondary and background naturally occurring inoculum



Additional pots of sporulating fruiting bodies (Fig 2.3), produced in controlled conditions were used to supplement the seeded-ergot inoculum at flowering of the wheat crop. The two strips of sown ergots or black-grass plants were orientated at right-angles to the other strip of the same inoculum type so that whatever prevailing wind direction occurred at flowering would result in inoculum dispersal into the wheat relatively perpendicularly from at least one of the strips. Air was sampled at the centre of one of the ergot-sown strips using a Burkard continuously operating sapore trap in May and June. Additionally, air was sampled at snapshots in time for periods of several to 24 hours, using between eight and twelve rotating-arm traps, located at different positions around one of the ergot-sown areas and upwind of the experiment (Figs 2.4 & 2.5).

Before harvest (late July) the positions of ergots in the experiment were mapped in wheat within 25 cm immediately either side of the fallow strips and around the outside of the experiment, plus in transects and additional sampling positions within the wheat crop. Positions of ergots were discriminated on main or secondary (late) tillers of wheat and on different naturally-occurring or artificially added species of grass.

Figure 2.3. Stroma produced in lab conditions from a cold-treated ergot

Figure 2.4. Spore trap positions, dates and types used in 2006 & 2007 to study disease spread from line sources

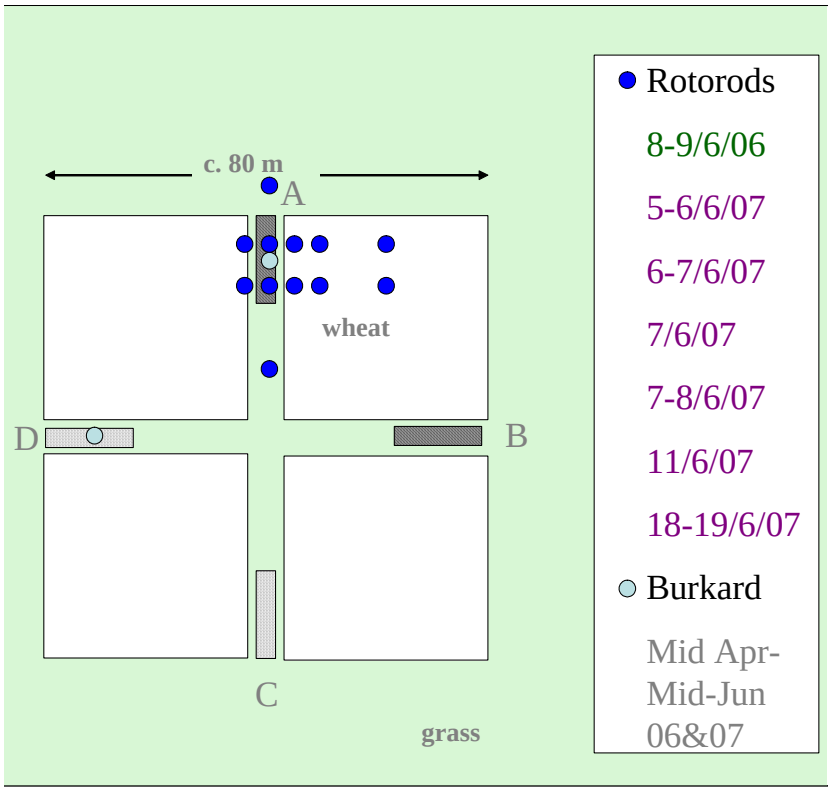
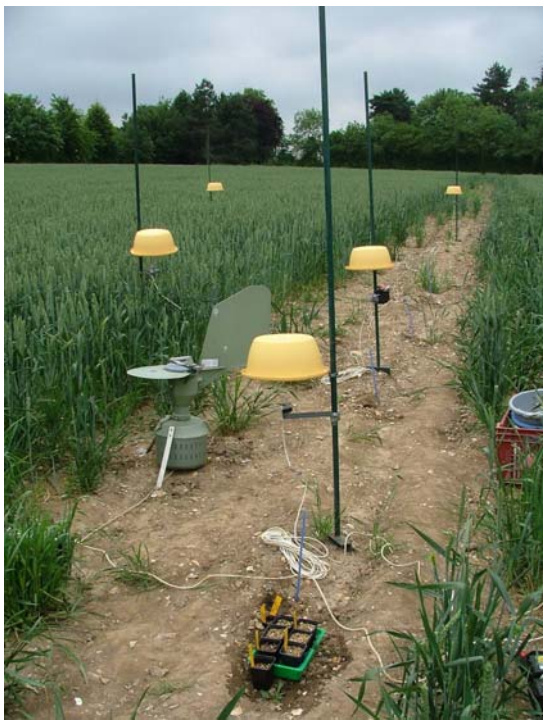


Figure 2.5. A Burkard trap and Rotorod spore traps at different distances from the centre of an ergot-sown line source in June 2007. A tray of supplementary inoculum is visible in the foreground.



Results

3.1a. Strength of inoculum sources.

The passive traps used in the first season were found to be difficult to process for PCR and results from 2005 were unfortunately of limited use because *C. purpurea* DNA was only found on traps from one location (a Velcourt Rye crop). Passive traps at Rothamsted tested negative while samples of barley awns collected from volunteer plants in the same site all tested positive for *C. purpurea* DNA. This DNA could only come from deposited spores. Theoretically, fine structures, such as awns, have a high efficiency of trapping particles in air because particles impact onto them rather than changing direction to flow around the obstruction. The results also indicated a low trapping efficiency of the passive spore traps and so the sampling method was modified for the following season to sample wheat ears at the end of anthesis in June. However, most wheat samples were also clear of any *C. purpurea* DNA. The sites that tested positive for *C. purpurea* in wheat ears were: ADAS Boxworth Extra Farm, both nearest margin (PCR peak-area 0.3) and in crop (peak area 0.5); ADAS Boxworth by field near margin (peak area 2.2), the corresponding within-crop sample was negative; and Rothamsted Research, where all samples were negative out of five natural margins and commercially managed crop areas, except for ears collected from tramlines of an area of wheat (cv. Rialto, 50 x 36 m) that had been seeded at the centre with buried ergots (peak area 0.9). Interestingly ears collected from main tillers in the same field in June were negative. Before harvest, ergots mapped in that plot on 21/7/05 were widespread but almost exclusively on very late tillers, which were mainly in tram-lines and around the edge of the experiment. On average 59 ergots were found per tramline (36 m long), compared to an average of 10.5 ergots per 36m transect through the main crop (almost exclusively on late tillers). A total of 375 visibly infected tillers were detected in total, all but a few of these were on late tillers. This indicated that the distribution of ergots in the crop was mainly down to synchrony of the susceptible crop growth stage with inoculum presence, rather than due to gradients of inoculum.

In June 2006, samples of wheat ears were collected at Rothamsted, ADAS and industry partners, from cereal (wheat) crops next to margins and well into fields (e.g. 30 m into the crop), these were easier to process for quantification of *C. purpurea* DNA by freeze drying and grinding to a powder prior to DNA extraction. All Rothamsted samples were negative. Only one positive sample was found from ADAS

Boxworth (40 acre field, headland A). Velcourt samples in 2006 were all negative (Haverholme and Metheringham sites). Samples from RAGT in 2006 produced one positive sample out of four within-field locations at Fen End Drain, organic site, while the four margin samples were all negative. In contrast, at the RAGT site at Lingwood Middle, four within-crop samples were negative but all four margin samples were positive.

3.1b. Seasonal timing of primary inoculum

The continuously operating Burkard spore traps at ADAS Boxworth were free of *C. purpurea* DNA at all times in 2005, 2006 and 2007. Despite being located next to buried ergots, at Rothamsted, positive samples were found only in four two-daily periods in 2005 (4+5/4/05, 6+7/4/05, 14/6/05 and 17+18/6/05). The latter two positive samples occurred when artificial inoculum had been placed in the field near the spore trap. In 2006 at Rothamsted, positive air samples were obtained from the Burkard trap positioned next to sown ergots (position A in 2.4) continuously over a ten-day period from 23rd May – 1st June 2006 and also on 6+7th June 2006 (in all cases, before wheat anthesis). Samples from the spore trap operating next to black-grass (position D in 2.4, unioculated with ergots) were all negative. In 2007, all Rothamsted samples were negative but this is thought to be due to poor DNA extraction.

Fruiting body production was monitored at sites sown with ergots at Rothamsted. Results are shown in 2.6a & 2.6b. Stroma production peaked on 25th May in 2006, which coincided with black-grass flowering, and had declined almost to zero by the time of wheat anthesis (Fig. 2.6a). In late May and June, it was found that a single warm, sunny day resulted in complete desiccation of any exposed fruiting bodies. In 2007 (Fig. 2.6b), stroma production started later but again stopped around the start of wheat anthesis, except where sown ergot positions were watered.

Figure 2.6a. Stroma production from buried ergots at Rothamsted (line experiment) 2006

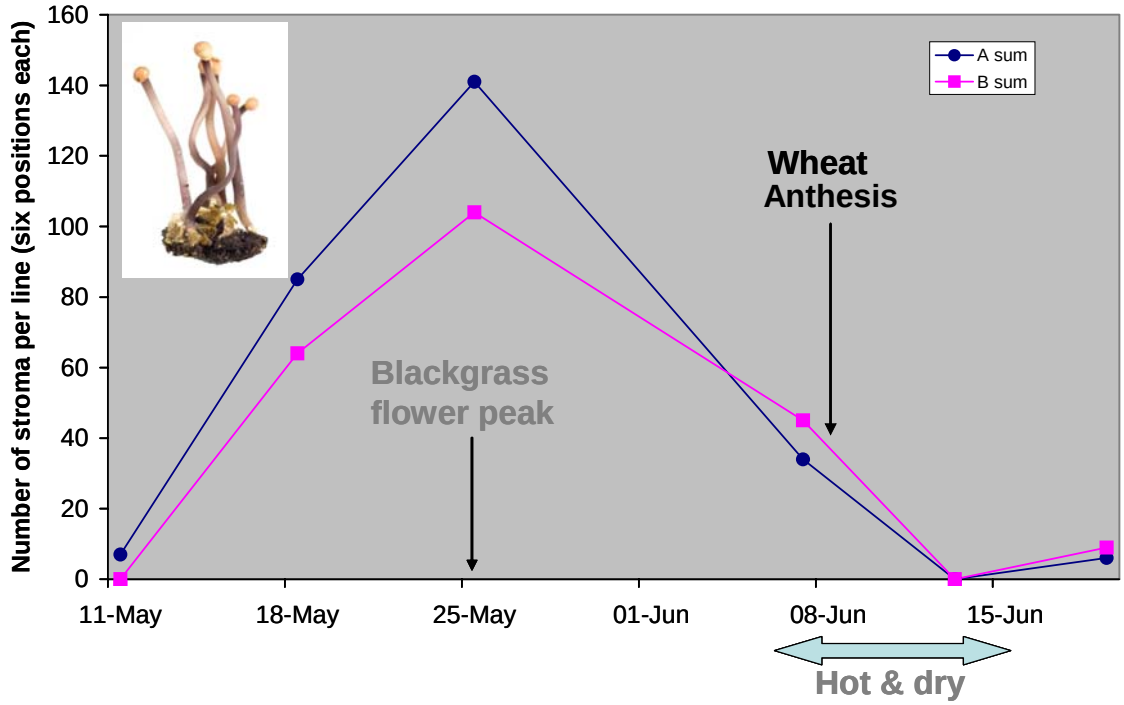
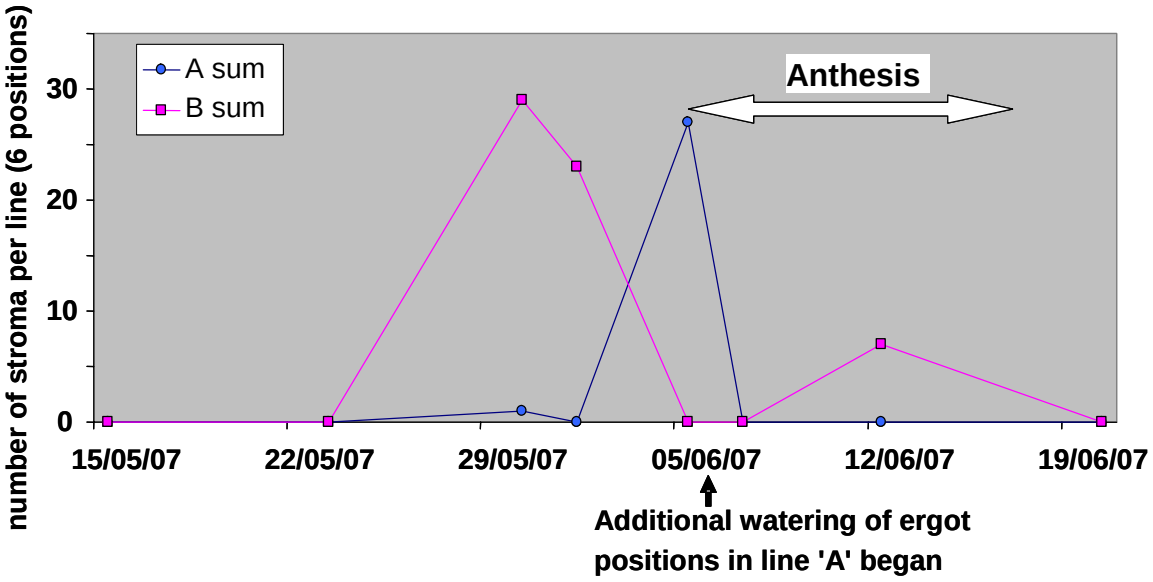


Figure 2.6b. Stroma production from buried ergots at Rothamsted (line experiment) 2007



3.1c. Disease gradients in crops in relation to grass margins, tramlines and black-grass patches.(ADAS).

Grass margins

The presence of disease gradients of ergot in crops adjacent to field margins would provide evidence for spread from infested field margins.

In 2006, ergot was reported at high levels in spring wheat plots near Cambridge adjacent to a grass strip where ergot was present in ryegrass and red fescue. There were weak indications of a gradient from the grass strip into the plots over 10 m (R^2 = 0.34).

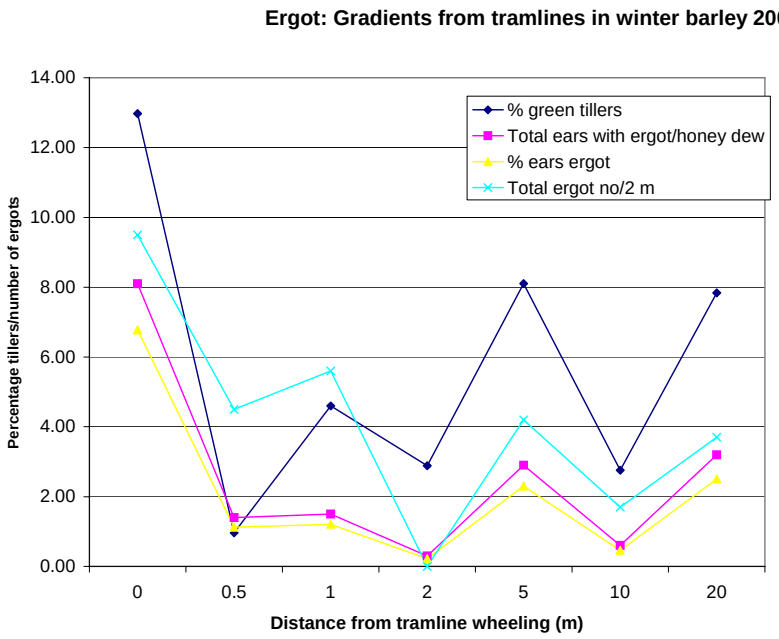
No other clear examples of spread from grass margins were confirmed during the course of the project, though it is clear that ergot can be present in established field margins on a range of grass species.

Tramlines

Ten transects were completed in a mature heavily ergot infected crop of winter barley grown for hybrid seed production in Bedfordshire on 26 July 2004. The number of green and mature tillers of barley and ergot numbers per ear (or presence of honeydew) were recorded in 2m lengths of row at 0, 0.5, 1, 2, 5, 10 and 20m at right angles to the tramline wheelings.

There was a higher percentage of green tillers in the tramline (13%) than in the crop (1-8%) and a higher percentage of tillers with ergot in the tramline (8%) than in the crop (0.3-3.2%) (Fig. 2.7). Ergot numbers were much lower only 0.5 m from the tramline. The number of ergots per 2m row was 9.5 in the tramline and 0-5.6 in the crop. Ergot gradients were less marked than observed previously as there were patches of green tillers within the crop where rabbit damage had occurred earlier in the year. These observations showed the prevalence of ergot along tramlines and the importance of late (green) tillers as source of ergot contamination.

Figure 2.7. Ergot gradients in relation to tramlines in winter barley 2004.



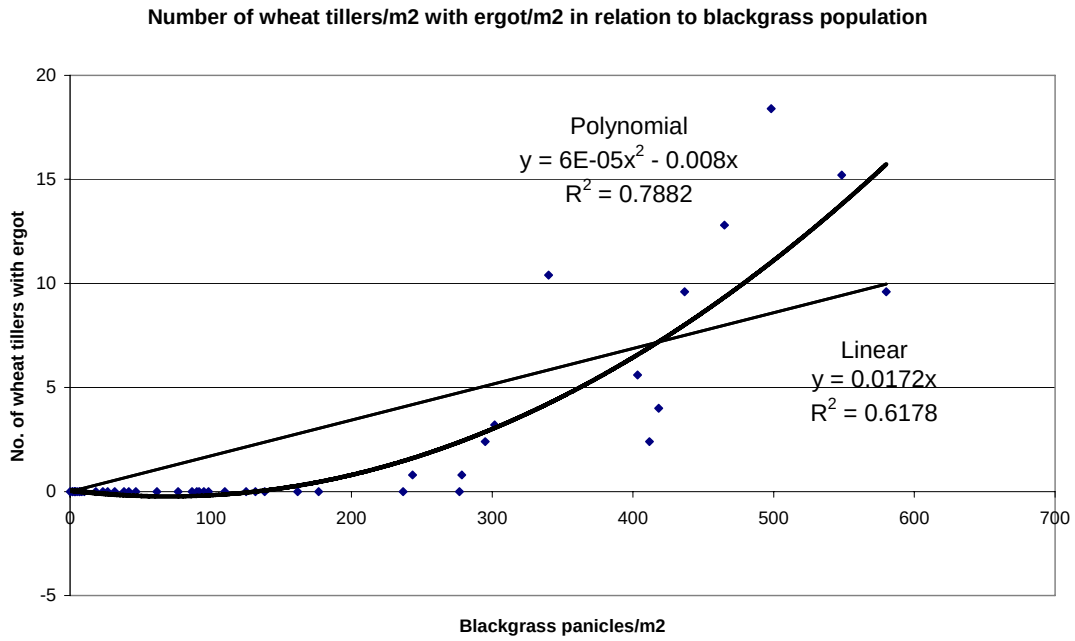
Black-grass infestations

Ergot problems in eastern England are frequently associated with black-grass infestations. At ADAS Boxworth, ergot in commercial wheat crops was associated dense patches of black-grass. Observations were made in selected plots within a replicated black-grass and wild oat herbicide experiment in winter wheat cv. Consort at crop maturity on 5 August 2004. Assessments were made on untreated control plots and on the immediately adjacent plots (mainly herbicide treated plots with low black-grass populations) to examine relationships between black-grass populations and ergot infection. Five quadrats (each 0.25m^2) per plot were used to determine the incidence and number of ergots in mature and green tillers. Panicle counts for black-grass were available from earlier assessments. Plots were combined and ergot numbers per kg wheat grain were calculated from assessments of 0.7-1.0 kg samples per plot.

Ergot was present in black-grass and wild oats within the experiment and wheat was infected, particularly late tillers within dense patches of black-grass. Analyses showed that wheat tiller infection by ergot (as determined by quadrat counts) was associated with black-grass panicle numbers of more than 200/m^2 and this showed a polynomial distribution (R^2 = 0.79) (Fig.2.8). Ergot numbers in harvested wheat samples showed

a positive linear relationship with black-grass panicle numbers (R^2 = 0.52), though some ergot was detected even at very low black-grass populations. This may be due to some secondary spread of ergot to wheat from adjacent plots with heavy black-grass infestation. In general, field observations suggest secondary spread from black-grass occurs over short distances and uncropped pathways only 0.4 m wide appeared to have prevented spread between some adjacent plots.

Figure 2.8 Incidence of ergot in wheat tillers in relation to black-grass population, Boxworth 2004.



No gradients were found in winter wheat growing next to a field with abundant ergot in winter barley in 2004 (see above). Ploughing and weather factors at flowering are thought to have very effective in stopping ergot causing problems despite high levels of inoculum.

3.2a. point-source field experiment at Rothamsted Research

Ergots were mapped in mid-late July through two perpendicular transects per plot and around the edges of each plot. In 2006, ergots were abundant and were found only on late tillers. Over 80 ergots were collected from the site (over 95% on late tillers), while nearby commercial wheat fields had none observed after numerous margins and tramlines were inspected. Conidial suspensions could be produced by placing ergots from late tillers into water in late July, indicating that the 'honey-dew' phase persists

longer than the literature suggests. A wide range of insects, mainly 'house flies' were seen feeding on developing ergots and so potentially could vector the conidia to other hosts. There were no ergots on wheat within the plots. In contrast to 2006, no ergots found on wheat at all in the experiment in 2007, including none on inoculated wheat. In both years, artificial inoculation of wheat with conidial suspensions (which was done on at least two different dates per season) did not produce any infections. Inoculations of black-grass with conidia at plot centres only produced ergots in three positions in 2006 and none in 2007.

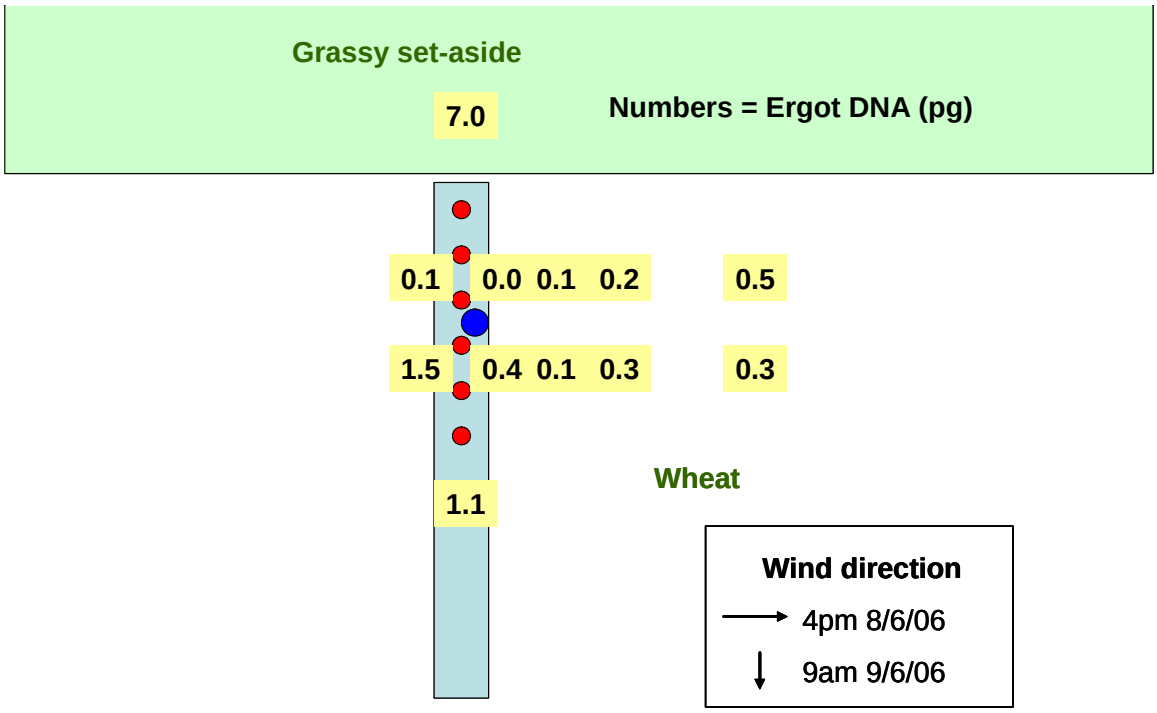
Figure 2.9 Ergots at Rothamsted in July 2006, 99% were on late tillers (left), only 1% were on main tillers (right). The surface coats of ergots were sometimes partially eaten by flies (indicated by arrow in centre picture). Conidia could still be recovered from the ergot on the left.

3.2b. line-source field experiment at Rothamsted Research

Air was sampled for gradients of ascospore inoculum over the period from 4 pm on 8th June 2006 to 9 am on 9th June 2006, which corresponded to early anthesis in the wheat crop. Samples were sent to NIAB for analysis. At this time, the average amount of *C. purpurea* DNA per ascospore is unknown so results are presented as actual amounts of pathogen DNA, which should be proportional to numbers of ascospores. Only a trace of pathogen DNA was found at positions in the wheat crop (<0.5 pg) and slightly more (1.1 to 1.5 pg) was found along the centre and down-

wind of the sown ergot positions (which were also supplemented by artificially produced sporulating ergots) (Fig. 2.10). However, the greatest amount of pathogen DNA (7.0 pg) was found upwind of the experiment in a field of set-aside grassland (Fig. 2.10). This area had not been ploughed in contrast to the wheat experiment.

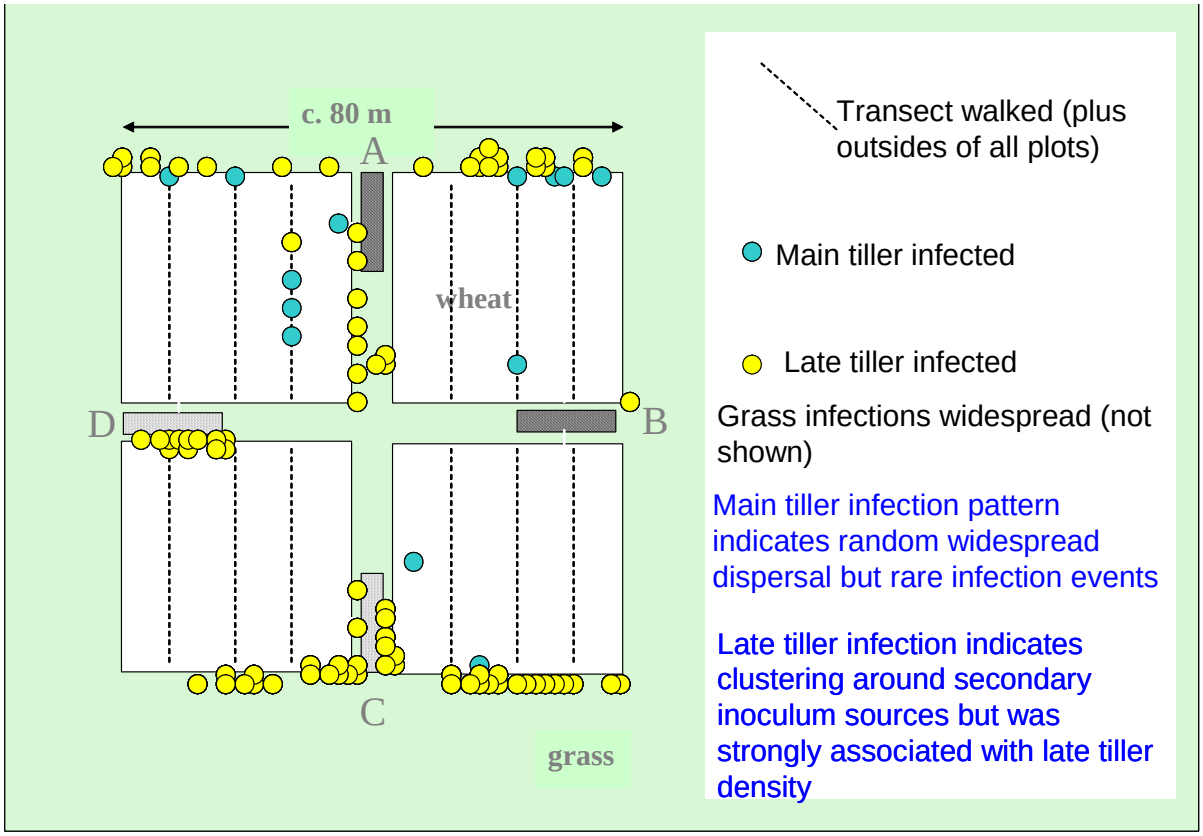
Figure 2.10. Amounts of C. purpurea DNA (pg) obtained from rotating-arm spore traps operated from 4pm on 8/6/06 to 9am on 9/6/06 at Rothamsted



The locations of ergots on wheat and grass weeds were mapped within 2 m of the experiment and along transects and separate sampling points within the experiment. (Fig. 2.11; showing ergots on main and late wheat tillers) on 24 July 2006. Over 200 ergots were found and these were mainly on late tillers (98%). Most ergots on wheat were located along the northern and southern ends of the plot. These ends corresponded to the start and end of seed drill runs and so there were more late tillers here than along the sides of the plot because scattered seed that had germinated produced more tillers in response to less crowding. It also indicates that inoculum or insect vectors were mainly coming from the grassy set-aside around the experiment. However, there were signs of clustering of ergots around artificial inoculum positions within the experiment. There was clustering of ergots around the inoculated black-grass at positions C and D compared to the more central non-black-

grass positions along the same lines. However there was also a lot of ergots along the uninoculated central part of line A. No ergots were found at positions where spore trapping with rotating-arm traps had been made. Infections of main tillers were too sparse to make firm conclusions although there was a tendency for infection along the northern end of the plot, next to the grassy set-aside area. Ergots also occurred on black-grass, ryegrass, and oatgrass in various locations around and within the experiment (positions not shown in Fig. 2.11).

Figure 2.11. Locations of ergots on main tillers and late tillers in and around the line-source experiment in 2006.

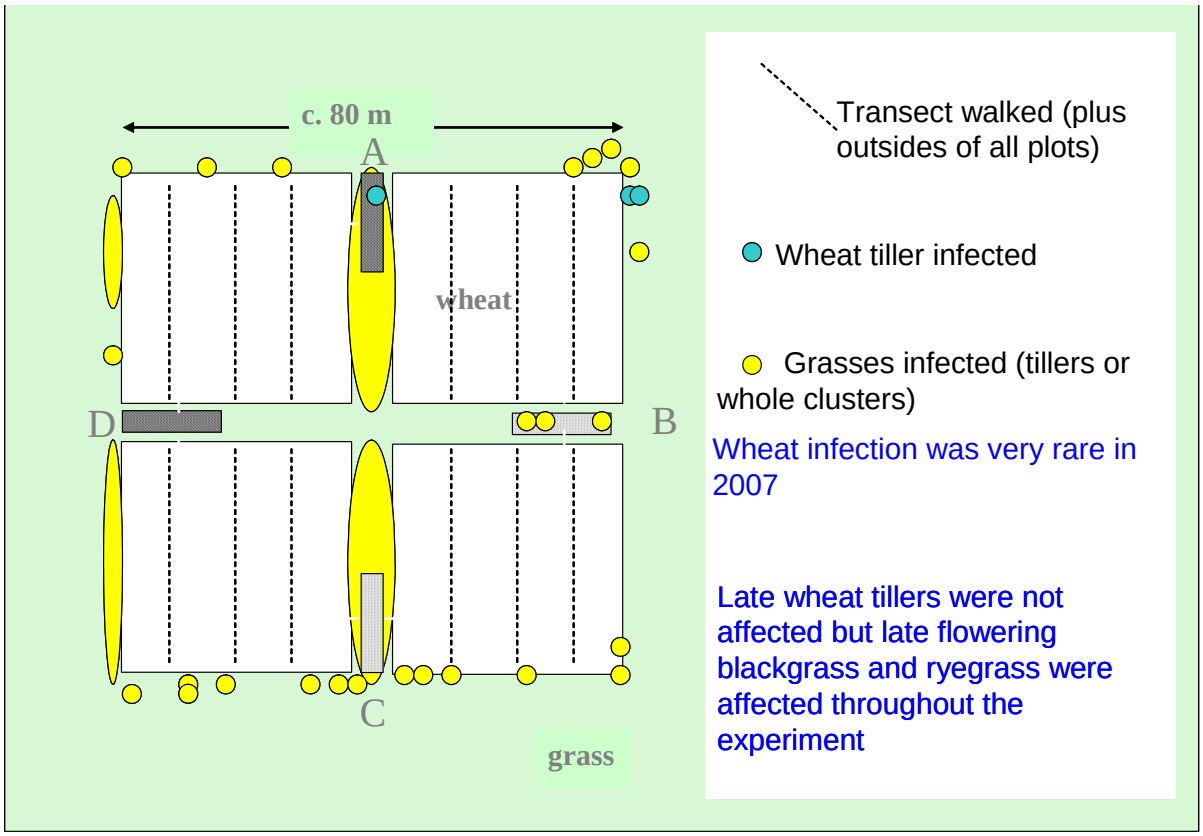


Commercial and experimental wheat fields/plots around the line-source experiment were also monitored for ergot contamination. While dozens of ergots were observed at the line-source experiment site, few or no ergots were found in nearby wheat with the exception of fields to the south-south-west of the experiment (Geescroft, Roadpiece and Highfield), where there is an indication of an infection gradient with all ergots found exclusively on late tillers (10 ergots at 300 m, 4 at 450 m, one at 500 m).

Unfortunately there was no wheat closer than 300 m from the experiment in that direction. In other locations, no ergots were observed on wheat in 2006.

In 2007, wheat infection by ergot in the experiments was very rare – only three infected wheat tillers (four ergots in total) were found in the entire experiment (Fig. 2.12). These were all main tillers and there was no infection on late tillers, although these were present to the same extent as the previous year and were flowering at different times from mid-June to mid-July. In contrast to the late wheat tillers in 2007, grasses, particularly black-grass and ryegrass, which were present around the edges of the experiment, did have a lot of ergot infection. These were flowering at the same time as the late tillers (mid-June to mid-July) that had escaped infection. There was no spatial pattern to infection other than due to the density/occurrence of grass weeds and occurrence of ergots on inoculated black-grass in line 'B'.

Figure 2.12. Locations of ergots on main tillers and late tillers in and around the line-source experiment in 2007.



Discussion

Timing of sporulation V timing of ergot infection

Spores were detected in air before wheat anthesis except on a few days in 2005 when artificially-produced sporulating fruiting bodies had been placed in the experiment in an attempt to produce wheat infections directly from ascospore inoculum. Observations of fruiting body production support the conclusion that ascospores were released ahead of wheat anthesis at Rothamsted. Fruiting bodies were present generally in mid-late May at Rothamsted and were prone to desiccation on dry days in June as day lengths were long, temperatures relatively high and dew periods generally short. With the exception of 2007, the vast majority of wheat infections (around 98% in 2005 and 2006) were on late tillers, which flowered in late June to mid-July. This suggests that wheat infection was mainly by insect-vectoring of secondary inoculum produced on grasses. In 2007, when there were no late tillers infected, there was abundant ergot infection on grasses in and around the line-source experiment. These grasses were flowering throughout the period from mid June to mid-July, yet the succession of wheat tillers around the edges of the experiment that flowered at the same time were not infected. This again reinforces the suggestion that insect vectoring was the main dispersal and infection method, since ascospore inoculum deposited at random onto plants would be expected to infect the susceptible wheat (cv. Xi19) at least proportionally to the level of grass infection. However, the wheat could escape insect vectoring if vectors present in 2007 fed/visited mainly grasses or if strains of *C. purpurea* present were more infective to grasses than wheat. The latter is unlikely because (a) experimentally added inoculum had been mixed ergots derived from wheat and black-grass (b) previous work has shown that strains that infected black-grass were also infective to wheat (Mantle & Shaw, 1977) but this finding has been updated in WP2 of this project. Previous work suggests that ergot infections were more prevalent in late-flowering cereal crops such as rye or late wheat, although these studies were in France, North America and Russia (Rapilly, 1968; Brown, 1947; Markhasseva, 1936). The only previous work on ascospore release and infection timing in the UK was by Mantle & Shaw (1976), who suggested that ergots germinated in mid-May and produced maximal ascospore numbers in June. The present study indicates a slightly earlier release, which may be a result of a current trend towards milder winters, leading to earlier conditioning of the ergots to germinate. In contrast, the timing of wheat anthesis has not advanced as much due to climate change as this is also affected by day-length. Since wetter conditions would

prolong sporulation, it would be interesting to see whether there is a higher incidence of ergot infection per wheat field in western parts of the UK, which is wetter compared to the east.

Spore dispersal processes and spatial patterns

Many biological samples taken from commercial fields and margins around fields in 2005 and 2006 tested negative for *C. purpurea* DNA. This may be because at Rothamsted at least in 2005 and 2006, most (around 98%) ergots were found on late tillers, which were not sampled in the mid-June sampling programme. Although sites prone to ergots were chosen, a larger number of sites and greater number of samples per site was needed to provide statistically valid information on the relative ergot risk within fields compared to next to margins. Only one site showed a substantial difference between crop and margin ergot infection - the RAGT site at Lingwood Middle, where the four within-crop samples were negative but all four margin samples were positive. In inoculated experiments at Rothamsted, the patterns of infection in 2005 and 2006 indicated that the distribution of ergots in the crop was mainly down to synchrony of the susceptible crop growth stage with inoculum or vector presence, rather than due to gradients of inoculum. Ascospore inoculum was only successfully quantified spatially on one period in 2006. This was from 4 pm on 8th June 2006 to 9 am on 9th June 2006, which corresponded to early anthesis in the wheat crop. The amounts of *C. purpurea* DNA at positions in the wheat crop were low (<0.5 pg), slightly higher (1.1 to 1.5 pg) along the central ergot-sown positions (which were also supplemented by artificially produced sporulating ergots), but greatest (7.0 pg) upwind of the experiment in a field of set-aside grassland. This area had not been ploughed in contrast to the wheat experiment. A more extensive sampling regime in 2007 tested negative but this may have been due to poor DNA extraction. New reports about air-dispersed pathogens of cereal ears, such as *Fusarium graminearum*, suggests that spores released in unstable airstreams during the day are uplifted and thoroughly mixed into the air within a region, with deposition occurring downwind at night when a layer of relatively still air develops near to the surface of the Earth (Schmale *et al*. 2006). If this pertains to ergot inoculum, it suggests that spores released from inoculum in margins would be unlikely to create a disease gradient directly, but spores would mix with those in air, produced from other locations such as large areas of grassland and then be deposited downwind at random. However, the occurrence of late tillers at the edges of crops and along tramlines appears to be a highly significant factor in ergot distribution. The fact that these tillers occurred well

after the ascospore inoculum was present, suggests that their infection was mainly due to vectoring of secondary conidia (honey-dew) from infections of earlier-flowering grasses. Strangely this did not occur in the final year at Rothamsted (2007), although grass weeds were heavily infected in that season. It is possible that certain insect vectors prefer particular plants, or else that the grasses in 2007 were more easily infected by ascospores than the wheat (again unlikely if strains that infect black-grass are relatively infective to wheat). Improved methods to sample for ascospores are needed as methods used in this study detected them only at very low concentrations in air, if detected at all. Devices like wet-cyclones that sample at high flow-rates improve the chances of detecting rare airborne spores and spores suspended in the liquid collecting medium can be centrifuged to provide a sample for DNA extraction (West *et al*, 2008; Williams *et al*, 2001). A method to sample awns of cereal plants (especially barley) to detect deposited ascospores could be a cheap alternative, with DNA extraction relatively easy as the entire sample can be ground to a fine powder, rather than having to remove spores from a trap surface.

New Biology

In field conditions, honeydew was rarely seen as obvious drops of liquid on ears. Usually wheat infection was evident by the presence of a moist sticky film on florets, which appeared slightly darker. Conidial suspensions could be produced by placing ergots from late tillers into water in late July, indicating that the 'honey-dew' phase persists much longer than the literature suggests. A wide range of insects, mainly 'house flies', were seen feeding on developing ergots and so potentially could vector the conidia from each original infection to other hosts over a period of weeks rather than days.

Overall conclusions related to original aims of work package

The overall aims were (3.1) to identify the extent to which field margins may contribute to ergot infection in cereal crops (using wheat as a model) compared to other sources of inoculum; and (3.2) to quantify spatial distributions resulting from primary spread (ascospores) and secondary spread (conidia) of *Claviceps purpurea* in winter wheat. In relation to the aims, field margins contribute to ascospore production in a similar way to increasing the overall area of grassland in a system, but since ascospores are thought to be dispersed widely, deposited randomly and to occur largely ahead of wheat anthesis (as indicated by our experiments), margins should not contribute to ergot gradients within a crop due to primary inoculum, only

to overall increased production of inoculum. More importantly by far, field margins do provide a local source of secondary inoculum from infected grasses and this poses a risk to wheat mainly at the edges of wheat fields. This is exacerbated by the increased incidence of late tillers around the edges of crop areas. Data from the line-source experiments (3.2b) at Rothamsted suggests rare and random infections by ascospores (no gradients observed) but clustering of infections due to secondary inoculum occurring within 1 m of the source. More widespread infection thought to be due to secondary inoculum was also apparent but experiments were possibly compounded by super-imposition of natural inoculum from surrounding grassland and effects of late tiller density (which was different at the ends of drill rows compared to along the sides). However, two different mechanisms of secondary infection can be considered – spread by direct contact or local rain-splash, very short distances from infected ears to new ears, and spread by insect vectors over slightly longer distances, with the secondary host species possibly selected by the feeding preferences of the insect vector species. A very poor success of the artificial inoculation using conidia in the point experiment (3.2a) prevented further information about infection gradients from the honey-dew phase being quantified. However, this is likely to vary with location and season according to the weather (which affects direct rain-splashed dispersal) and the prevalence and species of insect vectors present. In commercial farms surveyed (3.1), only one location appeared to have more infection next to margins compared to within the crop but a larger number of samples were needed to enable firm conclusions to be made because ergot infection at natural/commercial sites in the years studied was generally very rare.

Implications to growers

A significant factor clearly evident from the 2005 and 2006 experiments at Rothamsted was the importance of late tillers in the potential contamination of a crop with ergots. For two seasons out of three, the vast majority of ergots occurring in experimental wheat exposed to natural and naturally conditioned artificial inoculum was on late tillers. These tillers, which flowered 2-6 weeks after the main crop, were found chiefly around the edges of plots/fields (i.e. close to margins) and along tramlines. They are thought to be caused by both mechanical damage (usually in tramlines) or grazing by herbivores (usually at crop edges), leading to new tiller production following damage to existing tillers, and by increased light availability low in the canopy at crop margins and tramlines, which promotes an increased production of tillers. The late tillers are thought to be more susceptible to infection due to

reduced pollination incidence and exposure to both late ascospore inoculum and especially secondary conidial inoculum produced from any earlier successful infections. This reinforces the findings at ADAS in 2004, where it was found that late tillers in tramlines and induced in patches of rabbit grazing had increased incidences of ergot. As a result, methods to reduce the occurrence of late tillers, by adjusting sowing density and avoiding damage to plants adjacent to tramlines would be advisable. If a pre-harvest inspection reveals ergot presence in a crop, it may be possible to discard (or harvest separately) the outer rows of a field to prevent the vast majority of ergots from being incorporated into the harvested grain.

Work package 4: Resistance of wheat varieties to ergot.

Introduction

The current state of knowledge about host resistance to ergot is extremely limited. Cereal crop species vary in susceptibility to ergot (in order of decreasing susceptibility – rye, triticale, wheat, barley, oats). However, little is known about variation in resistance within species, although certain UK wheat varieties are reputed to be particularly prone to ergot infection, for example the winter wheat variety Rialto. It is reasonable to suppose that infection risk is greatest for species or varieties with florets that gape open during flowering. Florets that remain closed during pollination and for a few days afterwards provide a mechanical barrier to the entrance of spores and escape infection. Any factors which delay or reduce pollination might be expected to increase ergot infection. Male sterile cereals used to provide hybrid seed are an example of extreme susceptibility because they rely on cross pollination (Done, 1973; Wood and Coley Smith, 1982).

In addition to differences in 'escape' there is the possibility that varieties may differ in 'tissue' or 'post-infection' resistance. This is something about which very little is known. Early indications of varietal differences in tissue resistance emerged from work at NIAB as part of Defra-funded research on cereal varieties for organic production (OF0330).

Plant breeders do not currently screen their potential varieties for ergot resistance, nor do they make any systematic assessment of the flowering characteristics that may be associated with escape. Ergot resistance is not evaluated during official variety trials at the National List or Recommended List stages. This means that farmers have no information on which to choose low risk varieties and breeders do not have the knowledge needed to select for improved ergot resistance in their breeding programmes.

The aim of work package 4 was to examine variation in 'field resistance' to ergot amongst current UK wheat varieties and to try to determine the degree to which this might be attributable to 'escape' (as conferred by flowering biology) or to post infection tissue resistance. Contrasting methods of artificial inoculation were used to reveal a) 'field resistance' i.e. the combined effects of 'escape' and tissue resistance

(WP 4.1) and b) post-infection resistance on its own (WP 4.2). Flowering traits associated with escape e.g. anther extrusion and tendency to produce blind florets, were also be examined (WP 4.3).

4.1 Inoculated field experiments to compare 'field resistance' to ergot of a range of UK wheat genotypes

Introduction

Infection of the developing ear is believed to arise by either air borne ascospores or transfer of asexual conidia produced on alternate hosts such as black-grass, present in or near the crop (Wood and Coley Smith, 1982). Spores germinate in the developing florets and germ tubes penetrate the stigma of the ovule to initiate the infection. As spores need to gain access to the florets before infection can occur resistance observed in the field could be a combination of "escape" and "post infection resistance". Here the combined effects of the two (field resistance) is estimated by inoculating genotypes and observing the sclerotial infection produced.

Materials and Methods

Fungi

Sclerotia of ergot were obtained from samples of either wheat or black-grass from Cambridgeshire, Suffolk, Hertfordshire and Berkshire to represent potential diversity. Isolates of *Claviceps purpurea* were obtained from sclerotia surface sterilized (4 minutes in 5% sodium hypochlorite solution) then washed in 4 changes of sterile distilled water, cut in half and placed on potato dextrose agar (PDA; Merk 39g/l) with antibiotic (125mg streptomycin/l of agar). After approximately 10 days at 25^{o}C in the dark, characteristic colonies were sub-cultured to fresh agar without antibiotic to obtain pure cultures which were identified as *C. purpurea* on the basis of conidial morphology. Five isolates were stored on PDA slopes at 5^{o}C (Table 4.1).

Pathogen inoculum

Isolates of *C. purpurea* were grown on PDA at 20^{O}C in the dark for approximately 14 days. Conidia were scraped from the centre of the colony and suspended in sterile distilled water and used to inoculate glasshouse grown wheat plants (Paragon) to

produce large quantities of spores for field inoculation. In vivo suspensions were thought to be potentially more pathogenic than those produced in vitro.

Seeds of Paragon spring wheat were sown in 20cm (8 inch) plastic plant pots containing peat based compost (Shamrock; General Potting Medium). Pots were watered and placed in a cool, light glasshouse to germinate and grow to maturity between October and April (night temperatures about 10 C; day between 18 - 25C; 16hr day). Ears were inoculated before anthesis with a suspension of individual isolates, using either a hypodermic needle to fill the space between the lemma and palea of each floret (approximately 0.025ml of suspension at $1x10^6$ spores/ml), or by dipping wounded ears in a spore suspension of an individual isolate. Ears were wounded by stabbing several times with a home-made device consisting of seven sewing machine needles set in a rubber bung.

Honeydew appeared after about 10 days and was collected using either a Pasteur pipette or inoculation loop and suspended in tap water. Honeydew was collected every 2-3 days and suspensions stored at approximately 5^OC between collections. Conidial collections were bulked and stored as individual isolates in 10% glycerol at -20^oC. Frozen suspensions were found to retain pathogenicity for at least two years.

Experiments were inoculated using equal amounts of the five isolates with the concentration of each adjusted to 10^6 spores/ml.

Field experiments

Plots of cereals were established in the autumn in 8 site-years (4. 2).and exposed to infection by ergot conidia. Experiments at NIAB consisted of plots approximately 1.2m long and 6 rows wide. The outer 2 rows on either side were test genotypes and the central 2 rows were drilled with black-grass seed. Two experiments at RAGT Seeds Ltd (2006 and 2007) were similar with 6 rows 90cm long again with test genotypes in the outer rows separated by 2 rows of black-grass. In the 2005 experiment variety plots were of 3 rows and black-grass was transplanted at the end of each plot in April. In the 2 experiments at Velcourt plots were 6 x 1.5m with 7 rows. All experiments had 4 replicate blocks.

Genotypes consisted of lines from the UK Recommended List and those being evaluated at NL2 and NL1 in 2006. Two commercial genotypes from each of France

and Germany, one spring wheat and two genotypes of rye were included for comparison (4.3).

Field inoculations

In experiments at NIAB and in 2 at RAGT (in 2006 & 2007) black-grass was inoculated between growth stage (GS) 49-59 (Tottman & Makepeace, 1979) with an equal mixture of 5 isolates of *C. purpurea*. conidia (at 10^6/ml) were either sprayed on (experiments 1,2,3,4,5 & 6; 5mls/plot; twice in experiments 5 & 6) or introduced to heads of black-grass with a 'Mantle Crusher' (a pad of needles dipped in spore suspension (Evans, 2002) experiments 5 & 6; about 3ml/plot). In experiments 7 & 8 wheat was directly inoculated at heading (GS 51-57) by spraying across the plot with a conidial suspension at 10^6 conidia/ml. Mist irrigation was applied (experiments 1,2 &3) from the time of inoculation to appearance of ergots.

Estimation of ergot frequency

The frequency of ergot infection was estimated in late July by counting the number of visible ergots per plot (expts. 5 & 6) or the number of heads per plot with visible ergots (expt. 2). Other experiments failed to produce sufficient disease to warrant attention.

Ear samples, as all ears with visible ergot plus 10 apparently uninfected ears (expt. 2 & 5), and all ears within a 1m length of drill row (expt. 6), were taken in August from each plot. In experiment 6 harvested ears were separated into those with visible ergots and those apparently uninfected. Ears were counted and carefully machine threshed (with low wind setting) to avoid loss of lighter ergots.

From experiment 5 a subset of ears with visible infection were hand threshed and the position of each ergot in the head recorded.

Statistical analysis

Data were analysed by ANOVA for genotype effects within experiments using the GENSTAT statistical package. When significant effects were observed data were re-analysed using a subset of the winter wheat genotypes and comparisons made across experiments.

Data for each trait was converted to 'rank order' and ranks were summed across experiments to obtain average effects.

Results

Numbers of visible ergots before harvest

Ergot infection was visible before harvest in three experiments. Five trials had no apparent infection (Table 4.2) and are not considered further. Frequency of ergot infection differed greatly between years and sites, experiment 2 (2006) being heavily infected, whereas experiment 5 (also 2006) was infected moderately. Genotypes also differed significantly ($P<0.001$) in apparent infection within trials, rye being most infected, particularly the male sterile (4.1).

Ergots in threshed samples

In all experiments two estimates of the number of ergots in threshed samples were made; one in ears where ergot infection was visible before threshing and one in those ears where there were no, or few, visible ergots (Figs. 4.2 & 4.3). Significant differences ($P<0.05$) were found between genotypes in all 3 experiments. There was good correlation within experiments between observed ergot field frequency and data for the amount of ergot from ears which had visible ergots (Table 4.4; $r = 0.71, 0.98$ & 0.47 respectively for the 3 trials). However, some genotypes (codes 4, 21, 24, 41 for example) had appreciable numbers of ergots but these infections were not obvious in the field (compare 4.3 with Fig. 4.1).

In experiment 3 the way in which samples were taken also allowed ergot frequency per weight of grain to be calculated (Figs. 4.4 & 4.5) and the average weight of an ergot (4.6). Again there were statistically significant ($P=0.05$) differences between genotypes but effects were on the borderline of statistical significance for the average weight of an ergot.

Consistency of effects across years and experiments

Correlation analysis suggested low agreement of measured traits between site/years (4.4; values less than about 0.50). However, it was noted than some genotypes were relatively little infected in any experiment (codes 5, 7 for example) whereas others were much infected in one or more experiments (codes 1, 6, etc) for a particular trait. Winter wheat genotypes were thus classified by rank for each character within a trial

(least infected as 'Rank 1'), summed across site-years to produce a mean rank and so look for trends. Data is summarized in Table 4.5 with the 15 best and 15 worst genotypes for each measured trait shade coded.

Position of ergots within heads

In experiment 5 (2006) heads with visible ergot were dissected and the position of ergot within the head noted. Data from all heads was amalgamated and expressed as the frequency of ergots at different points in the head from the bottom (4.7). Ergots were found throughout the head with a slight tendency for few to be found in the lower 15% of the head.

Discussion

Establishment of epidemics

Although there are reports evaluating the resistance of cereals to ergot in the glasshouse (Menzies, 2004; Platford et.al., 1977) to our knowledge this is the first report describing an effective field-based method using sown black-grass (*Alopecurus myosuroides*), a common host of the fungus, to generate an epidemic, although a method using inoculated, transplanted black-grass has been described (Gregory et. al, 1985).

Field inoculated black-grass plants developed ergot (data not shown) and wheat plants growing in close proximity also developed ergot although honeydew, the medium in which conidia are produced on the black-grass, was rarely seen. The frequency of infection on both the wheat and black-grass varied between sites and years (Figs. 4.1 – 4.6) influenced presumably by weather at inoculation of the black-grass and during transfer of the infection to wheat. High humidity is known to favour infection of sorghum by *C. africana* (Wang et. al., 2000) and it is perhaps notable that conditions were relatively cool and wet during flowering in 2006 (experments 2 & 5) compared with 2007. Supplementary irrigation was used only in experiment 2 but could be valuable to enhance success rate. The method described offers a way of exposing wheat genotypes to infection so their propensity to develop infection in the field can be evaluated.

Estimation of infection

Ergot sclerotia became visible in ears towards the end of July in most years. They were conspicuous on rye, particularly the male sterile line, relatively easy to find on many winter wheat genotypes but not visible on others. When ear samples were threshed (by machine or hand) genotypes appeared to differ in the number of sclerotia they had per head (contrast Figs. 4.1 and 4.2). In addition, sclerotia were also found in genotypes which had not appeared infected from observation of the field plots (contrast Figs. 4.1 & 4.3).

In some experiments there was little correlation between this visual field estimate and the frequency of ergots that could be recovered from threshed grain obtained from random samples of ears. This is consistent with the report of Gregory et al. (1985), who obtained the best estimate of ergot infection from assessments of threshed samples rather than from visual estimates in the growing triticale crop. Our results for wheat also suggest that some genotypes have a greater tendency to infection than others. This is consistent with the anecdotal evidence from the seed and milling trade, extension service and those involved with pure stock multiplication (pers. comm.) and is the first report of differences between winter wheat genotypes although differences have been reported in other cereals (Menzies, 2004; Dahlberg et. al., 2001; Gregory, 1985; Platford & Bernier, 1976)

Consistency of effects

Significant differences ($P<0.05$) were found between genotypes for nearly all traits within the three experiments that produced significant amounts of disease (Figs. 4.1 – 4.6). However, correlation analysis showed that consistency between experiments was sometimes poor (Table 4.4) and this was so even for traits where estimation was not subjective (e.g. counts of ergot). This suggests that environmental effects often over-ride any genetic component. Nevertheless, some genotypes (e.g. Rialto, Xi19, Mascot, Solstice, Gatsby, Dover, Tommi, Timber & Ochre) were often amongst the most heavily infected whereas a few others were never badly diseased (Cordiale, Soissons, Glasgow, Oakley, Malacca, Hyperion, Smuggler, Robigus & Einstein) suggesting that some genetic resistance to *C. purpurea* exists in the North European winter wheat gene pool.

Fig 4.1 Frequency of ergot infection in the field; number of infected heads per plot (experiments 2 & 6); ergot per plot (experiment 5)

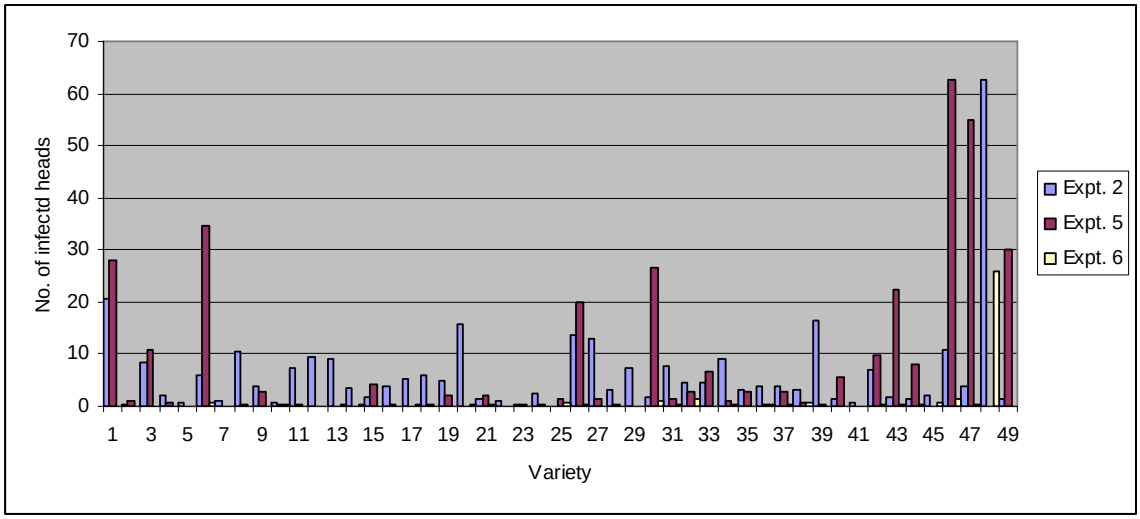


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Fig. 4.2 Number of ergot per plot from ears with visible infection

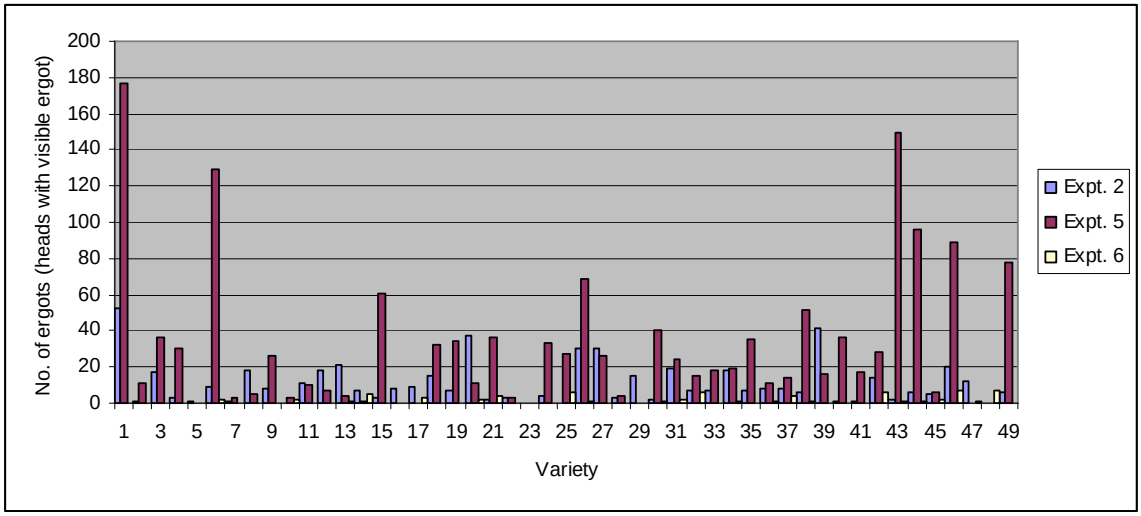


Fig. 4.3 Number of ergot per plot from ears without visible infection

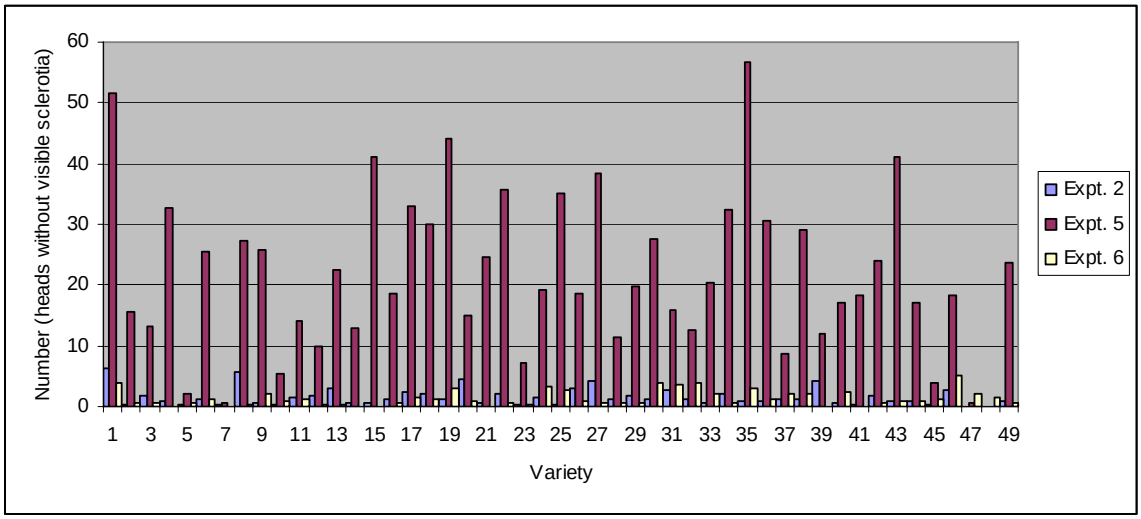


Fig 4.4 Number of ergot per 50g grain; experiment 6

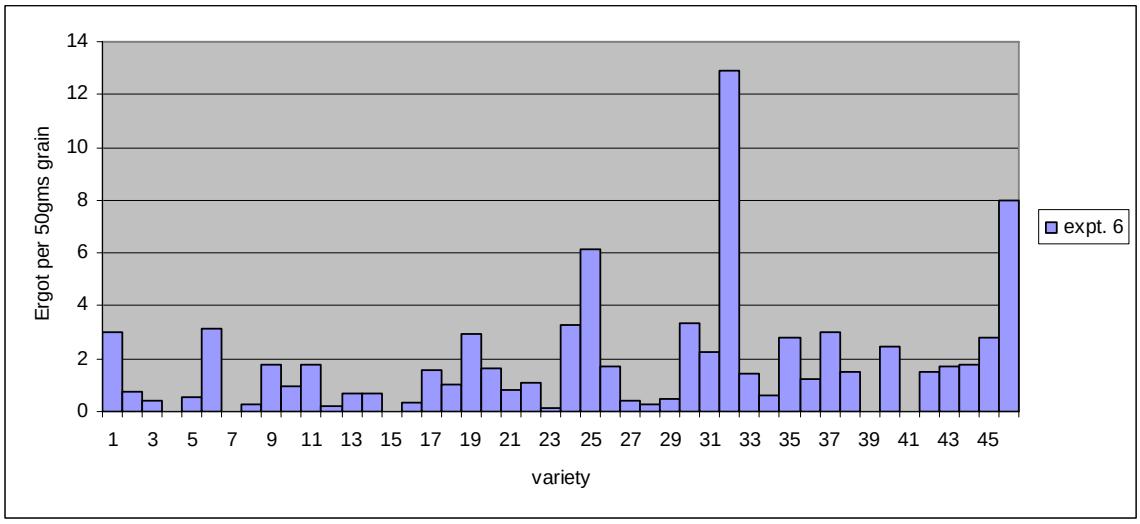


Fig. 4.5 Weight of ergot in 50g of grain; experiment 6

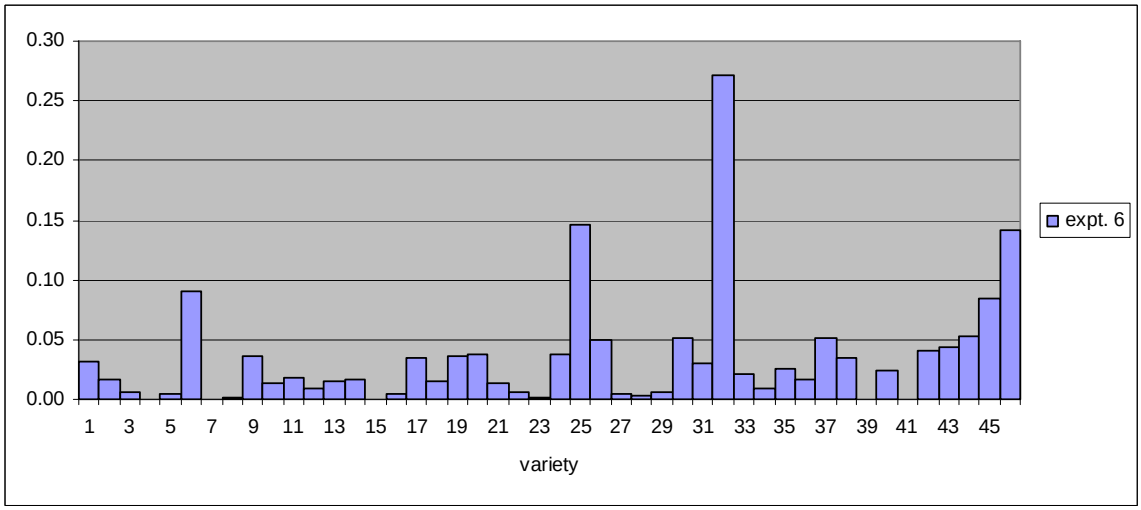


Fig. 4.6 Average weight of an ergot (gms; experiment 6)

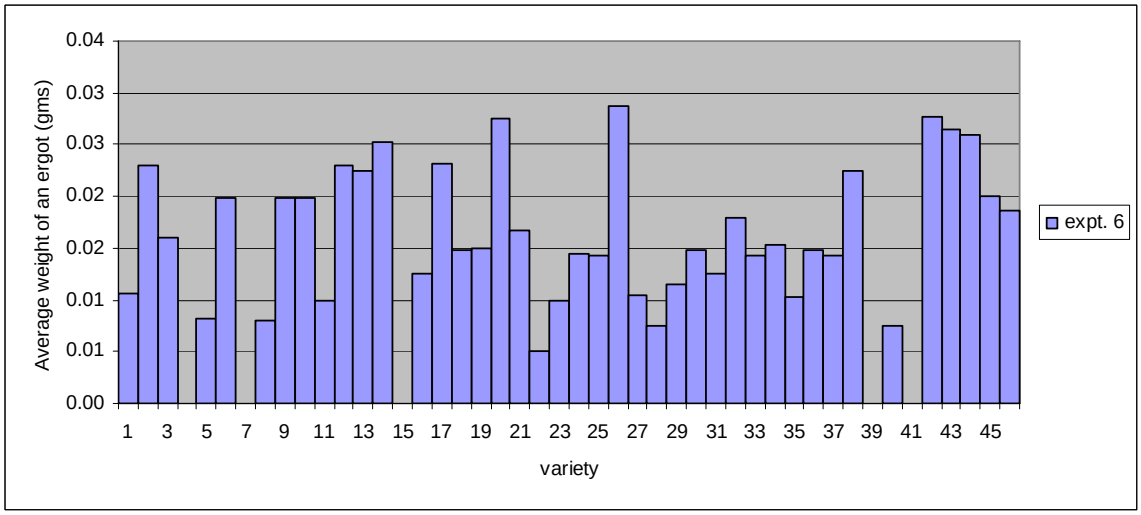


Fig. 4.7 Distribution of ergot within ears; experiment 5

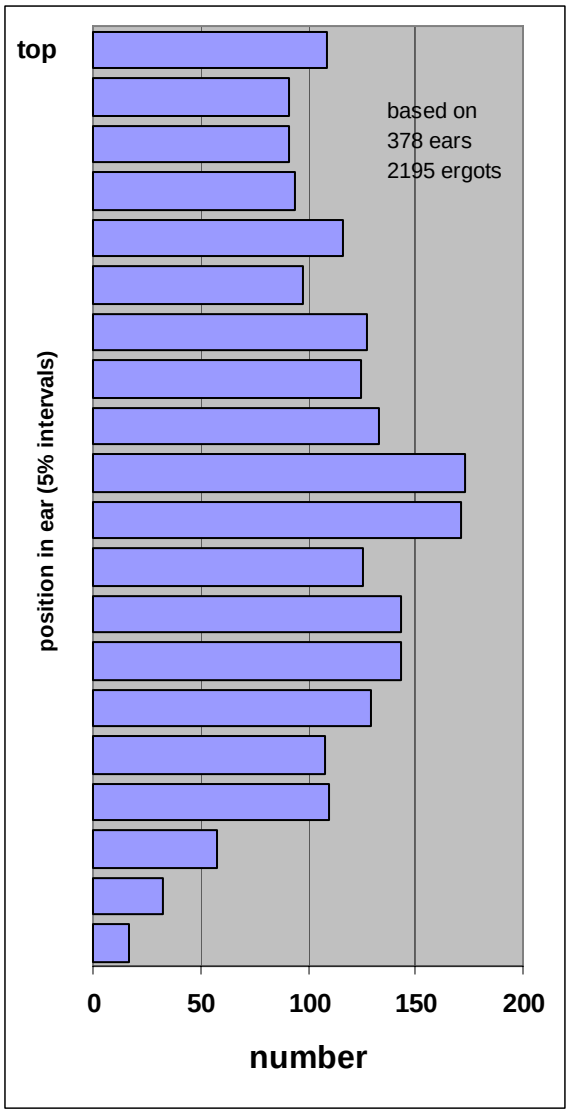


Table 4.1 Origin of isolates used in experiments

Isolate	Host	Location	Date	Source
04-04	black-grass	In barley crop, NIAB, Cambridge	20 July 2004	NIAB
04-29	Wheat cv. Hereward	Little Saxham, Suffolk	25 July 2004	ADAS
04-97	black-grass	Long Hoos IV, Rothamsted Farm, Herts.	7 September 2004	Rothamsted Research
03-20	Spring wheat cv. Chablis	Elm Farm Research Centre, Berks.	2003	Elm Farm Research
03-43	Winter wheat cv, Claire	Elm Farm Research Centre, Berks	2003	Elm farm Research

Table 4.2 Field experiments

	2005	2006	2007
NIAB	Expt. 1 (no data)	Expt. 2 (data)	Expt. 3 (no data)
RAGT	Expt. 4 (no data)	Expt. 5 (data)	Expt. 6 (data)
Velcourt	Expt. 7 (10 no data)	Expt. 8 (no data)	

Table 4.3. Cereal genotypes used in experiments

Code	Genotype	Parentage
1	Xi19	Cadenza x Rialto) x Cadenza
2	Malacca	Riband x Rendezvous x Apostle
3	Hereward	Disponent x Norman
4	Einstein	(NHC 49 x UK Yield Bulk) x (Haven x Clarion)
5	Cordiale	(Reaper x Cadenza) x Malacca
6	Solstice	Vivant X Rialto
7	Soissons	Jena x HN35
8	Dickson	Abbot x Consort
9	Deben	Hunter x Buster) x Wasp
10	Robigus	Z386 x 1366
11	Nijinsky	Claire x Consort
12	Claire	Wasp x Flame
13	Consort	(Riband Sib x Fresco) x Riband
14	Istabraq	Claire x Consort
15	Gladiator	Falstaff x Shannon
16	Smuggler	(6438-88B x Buster) x 6438-88B
17	Access	90-15 X 91-6
18	Napier	Hussar x Lynx
19	Tanker	Beaver x Zodiac
20	Richmond	Brigadier X Flame
21	Welford	CWW92.1/92054
22	Riband	Norman x (Maris Huntsman x TW161)
23	Glasgow	(Ritmo x Z90-2666) x Z 91.11658
24	Ambrosia	(Cantata sib x Genesis) x Pinder
25	Brompton	92-2 / Caxton sib
26	Mascot	Reaper x Rialto
27	Zebedee	Claire x Nelson
28	Hyperion	Aardvark x (Consort x Woodstock)
29	Alchemy	Claire x (Consort x Woodstock)
30	Dover	(Biscay x Aaardvark) x F86Z46-6-2
31	Gatsby	Nelson x Wasmo
32	Kipling	Hunter x 92054
33	Battalion	98ST08 x Aardvark
34	Ochre	98ST31 x Cortez
35	Challenger	Nelson/Wasmo
36	Gulliver	Shamrock x Aardvark
37	Deacon	CPBTW48 x Rialto
38	Timber	Terrier x Hamac
39	Sahara	Savannah x Claire
40	Humber	Anglo x Krakatoa
41	Oakley	(Aardvark (sib) x Robigus) x Access
42	Tommi	Ralf/Astron//Haven
43	Drifter	Ronos x Estica
44	Caphorn	F1(S14579/454[Soissons/Hereward] *Rialto)*Beaufort
45	Apache	(Axial x Camp Remy) x NRPB 8019
46	Rialto	Haven sib x Freso sib
47	Picasso	
48	Male sterile rye	
49	Paragon	CSW1724 x (Axona x Tonic)

Table 4.4 Correlations between site/years

			Experiment 2				Experiment 5			Experiment 6		
			Fc	V	N	wt	Fc	V	N	Fc	V	N
Expt. 2	visible ergots (V)	per plot	0.71									
	non visible (N)	per plot	0.16	0.47								
		weight (wt)	0.29	0.61	0.95							
Expt. 5	Field count (Fc)	ergots	0.28	0.27	0.15	0.12						
	visible ergots(V)	per plot	0.23	0.28	0.17	0.14	0.98					
	non visible (N)	per plot	0.21	0.25	0.28	0.22	0.90	0.89				
Expt. 6	Field count (Fc)	heads	0.52	0.28	-0.08	-0.02	-0.05	-0.08	-0.08			
	visible ergots(V)	per plot	0.28	-0.07	-0.22	-0.15	-0.16	-0.23	-0.28	0.47		
	non visible (N)	per plot	0.50	0.36	0.25	0.34	0.10	0.09	0.10	0.53	0.42	
	random sample	per plot	0.55	0.25	0.04	0.11	0.05	0.01	0.01	0.81	0.68	0.82

Table 4.5 Ergot infection of winter wheat in 3 field experiments (expts. 2,5,6); data expressed as average ranks for each of 3 methods of assessment (light shading: 15 lowest ranking genotypes; heavy shading: 15 highest ranking genotypes)

		Mean Rank			
Code	Genotype	Field counts	Visible ergots	Other ergots	Total
5	Cordiale	1	2	3	6
7	Soissons	3	3	1	7
23	Glasgow	5	1	2	8
41	Oakley	1	8	5	14
2	Malacca	6	5	9	20
28	Hyperion	7	6	10	23
16	Smuggler	9	7	12	28
10	Robigus	13	10	6	29
4	Einstein	11	13	12	36
29	Alchemy	9	9	20	38
12	Claire	12	17	11	40
22	Riband	3	4	35	42
40	Humber	13	11	18	42
14	Istabraq	16	29	4	49
15	Gladiator	16	19	16	51
45	Apache	22	22	7	51
11	Nijinsky	18	14	20	52
24	Ambrosia	8	15	36	59
33	Battalion	27	11	22	60
39	Sahara	26	24	12	62
9	Deben	22	18	25	65
8	Dickson	20	16	33	69
21	Welford	27	35	7	69
3	Hereward	33	27	15	75
18	Napier	15	23	39	77
36	Gulliver	25	25	27	77
35	Challenger	18	21	42	81
13	Consort	29	31	23	83
19	Tanker	24	20	44	88
25	Brompton	30	30	28	88
17	Access	21	25	45	91
44	Caphorn	35	39	17	91
37	Deacon	38	36	19	93
27	Zebedee	31	27	38	96
32	Kipling	43	33	23	99
20	Richmond	32	41	28	101
43	Drifter	39	34	30	103
34	Ochre	36	36	32	104
38	Timber	34	36	36	106
42	Tommi	41	43	26	110
30	Dover	42	32	41	115
6	Solstice	44	44	30	118
31	Gatsby	36	42	40	118
26	Mascot	45	45	34	124
1	Xi19	40	40	46	126
46	Rialto	46	46	43	135

4.2 Direct introduction of ergot inoculum into florets to investigate tissue resistance of wheat varieties

4.2.1 Hypodermic inoculations under field conditions

Introduction

Exposing wheat varieties to ergot spores generated by nearby plants of infected black-grass (**4.1** above), revealed a number of consistent differences between varieties in levels of ergot infestation. However, it is not possible to deduce from these experiments whether varietal differences were primarily the result of escape or of tissue resistance. Escape mechanisms might include aspects of flowering biology which influence the ease with which ergot spores to gain entry to the floret. Subsequently, host tissue resistance may modify infection success and pathogen development within the ovary.

In the experiments which follow, ergot spores were introduced directly into the florets, in order to bypass 'escape' factors and expose tissue resistance.

Materials and Methods

Experiments were carried out under field conditions in four years, 2005, 2006, 2007 and 2008.
Ears for inoculation were selected at random from main stems of plants grown in drilled plots on the NIAB trial ground in Cambridge.

In all experiments, ergot inoculum was inserted into individual florets between the lemma and palea, in order to establish infection of the ovary. This was done at the very early flowering stage, before pollination.

Methods for the production of inoculum, and the isolates used, were the same as in the black-grass spreader field experiments (**4.1** above).

In each year, between 4 and 10 varieties were inoculated, giving a total of 14 varieties, 3 of which were common to all 4 years (Table 4.5).

Table 4.5 Varieties subjected to direct inoculation of florets in the field, 2005-2008.

Variety	Years included	No. years
Robigus	2005, 2006, 2007, 2008	4
Solstice	2005, 2006, 2007, 2008	4
Xi 19	2005, 2006, 2007, 2008	4
Rialto	2005, 2006, 2007	3
Tommi	2005, 2006	2
Drifter	2005, 2006	2
Paragon	2005, 2006	2
Welford	2005, 2006	2
Caphorn	2005, 2006	2
Glasgow	2007, 2008	2
Apache	2006	1
Cordiale	2007	1
Brompton	2007	1
Mascot	2007	1

2005

20 ears (10 from each of 2 replicate plots) of 9 varieties were inoculated over a 4 day period from 31 May to 3 June, depending on the stage of flowering of the variety.

For most varieties, the two outer florets of the 10 central spikelets were inoculated, giving 20 inoculated florets per ear. Inoculum, comprising a spore suspension at a concentration of 10^6 spore / ml, was delivered using a 2ml hypodermic syringe with a 0.8 x 40mm needle. The needle was inserted between the tips of the lemma and palea and the inoculum injected until excess exuded, corresponding to a dose of approximately 0.025ml.

For two varieties, Caphorn and Xi19, inoculation was not limited to the 10 central spikelets; instead, all of the fully developed outer florets were inoculated, resulting in 28-40 inoculated florets per ear.

Ears were removed on 18 July at GS 92 and air dried in paper envelopes in the laboratory, before being assessed for ergot during the week commencing 15 August. Seeds and ergots were removed from each inoculated floret with forceps and the numbers of seeds and ergots per ear counted. The total weight of ergots per rep (10 ears) was determined.

2006

12 ears (3 from each of 4 replicate plots) of 10 varieties were inoculated over a 7 day period from 31 May to 6 June, depending on the stage of flowering of the variety.

The two outer florets of the 10 central spikelets were inoculated, giving 20 inoculated florets per ear. Inoculation methods were the same as those used in 2005.

Plots were mist irrigated from just prior to inoculation until grain ripening.

Ears were removed on 19 July at GS 92 and air dried in paper envelopes in the laboratory. Seeds, ergots and 'semi ergots' (i.e bodies which appeared to comprise some ergot material and some grain material) were removed from each inoculated floret with forceps and the numbers of each counted. The bulked weight of ergots from the 12 ears was determined.

2007

6 ears (3 from each of 2 replicate plots) of 8 varieties were inoculated on 7 June at the early flowering stage.

All those outer florets which were sufficiently developed were inoculated, providing between 28 and 40 inoculated florets per ear. Inoculation methods were the same as those used in 2005.

Plots were mist irrigated from just prior to inoculation until grain ripening.

Ears were removed on 19 July at GS 92 and air dried in paper envelopes in the laboratory. Seeds, ergots and 'semi ergots' (i.e bodies which

appeared to comprise some ergot material and some grain material) were removed from each inoculated floret with forceps and the numbers of each counted. The bulked weight of ergots from each replicate was determined.

2008

10 ears of each of 4 varieties were inoculated at 3 different spore concentrations (the previous standard concentration of 10^6 spores / ml and two reduced concentrations of 10^5 and 10^4 spores /ml). Inoculation took place on 29 May at the early flowering stage (GS 58). The two outer florets of the 10 central spikelets (5 spikelets on each side of the ear) were inoculated, giving a total of 20 inoculated florets per ear. Inoculation methods were similar to those used in 2005.

Ears were removed on 21 July at GS 92 and air dried in paper envelopes in the laboratory. Seeds, ergots and 'semi ergots' (i.e bodies which appeared to comprise some ergot material and some grain material) were removed from each inoculated floret with forceps and the numbers of each counted. The number and weight of ergots were determined for each ear individually.

Results

Results for the 4 years' experiments individually are given in Tables 4.6-4.9.

Table 4.6. Hypodermic inoculations in the field. 2005.

Variety	% inoculated florets producing ergot	*Wt ergot per ear (g)	mean wt of individual ergot (mg)
Solstice	98.0	1.062	54.2
Rialto	95.4	0.939	50.4
Paragon	98.0	0.775	39.5
Drifter	99.3	0.631	32.2
Xi19	92.9	0.579	31.1
Welford	99.0	0.535	27.7
Caphorn	91.2	0.530	29.0
Tommi	89.7	0.477	27.3
Robigus	93.8	0.425	22.7
AnoVar Variety	P<0.05	P<0.001	P<0.001
lsd variety means	5.85	0.1347	6.56

* corrected to 20 florets / ear

In 2005 (Table 4.6) inoculation achieved a very high success rate in all varieties, with between 89.7% and 99.3% of inoculated florets producing ergots. Tommi and Caphorn produced significantly fewer ergots than Solstice, Paragon, Drifter and Welford.

There were highly significant differences in the weight of ergot produced per ear, with Solstice and Rialto producing the greatest weights. Robigus produced the lowest weight, being significantly lower than that of all varieties except Tommi, Caphorn and Welford.

The mean weight of individual ergots varied more than two-fold between varieties, with highly significant differences. Rialto and Solstice produced significantly heavier ergots than all other varieties. Robigus produced the lightest ergots, which weighed significantly less than all other varieties except Tommi, Caphorn and Welford.

Table 4.7. Hypodermic inoculations in the field. 2006

Variety	no. inoculated florets per ear producing ergot*	no. inoculated florets per ear producing partial ergot*	no. inoculated florets per ear producing full or partial ergot*	Wt ergot (including partials) per ear (g)	Mean Wt of individual ergot (including partials) (mg)
Rialto	19.7	0.2	19.8	0.900	45.4
Solstice	19.8	0.1	19.9	0.791	39.7
Xi19	19.9	0.0	19.9	0.734	36.9
Paragon	19.6	0.0	19.6	0.563	28.7
Drifter	19.8	0.0	19.8	0.484	24.4
Tommi	18.8	1.0	19.8	0.407	20.5
Caphorn	20.0	0.0	20.0	0.398	19.9
Apache	18.6	1.0	19.6	0.308	15.7
Welford	19.3	0.5	19.8	0.261	13.2
Robigus	12.3	7.3	19.5	0.184	9.4
AnoVar Variety	P<0.001	P<0.001	NS	n/a**	n/a
lsd variety means	2.88	2.88	-	n/a	n/a

* total no. inoculated florets = 20
** not available, ergots bulked from all ears

In 2006 (Table 4.7), 'partial ergots', i.e. bodies which appeared to comprise a mixture of grain and ergot material, were noted in a few inoculated florets. These were counted separately, but bulked with the full ergots for weighing.

As in 2005, a very high proportion of inoculations resulted in ergot production. Although there was no significant difference between varieties in the total number of ergots produced, Robigus stood out as producing significantly fewer 'full ergots' and more 'partial ergots' than other varieties.

No statistical analysis is available for ergot weights, as ergot samples were bulked for the 12 ears of each variety. Total weight of ergot per ear ranged from 0.184g for Robigus, to 0.900g for Rialto. As in 2005, Solstice was towards the top of the range and Welford towards the lower end. Mean weight of individual ergots followed a similar pattern, with Robigus at 9.4 mg per ergot and Rialto at 45.4 mg.

Nine varieties were common to the 2005 and 2006 experiments. There were highly significant correlations between the two years' results for weight of ergot per ear (r = 0.822) and weight of individual ergots (r = 0.857).

Table 4.8. Hypodermic inoculations in the field. 2007.

Variety	% inoculated florets producing ergot	Wt ergot per ear (g)	mean wt of individual ergot (mg)
Rialto	97.8	0.837	22.6
Xi19	99.6	0.823	20.7
Solstice	98.2	0.672	19.0
Mascot	98.6	0.502	13.6
Cordiale	100.0	0.480	13.9
Brompton	93.9	0.405	11.2
Glasgow	94.6	0.360	9.5
Robigus	97.7	0.295	8.2
AnoVar			
Variety	NS	P<0.01	P<0.01
lsd variety means		0.2227	5.06

In 2007 (Table 4.8) hypodermic inoculations again had a high success rate, with no significant differences between varieties in the proportion of inoculated florets producing ergots. Weight of ergot per ear and mean weight of individual ergots differed significantly between varieties. For both characters, Rialto, Xi19 and Solstice showed significantly more ergot production than Robigus and Glasgow.

Table 4.9. Hypodermic inoculations in field at 3 inoculum concentrations. 2008.

	% inoculated florets producing ergot			Wt ergot per ear (mg)			mean wt of individual ergot (mg)		
Spores / ml	10^4	10^5	10^6	10^4	10^5	10^6	10^4	10^5	10^6
Variety									
Solstice	4.4	34.3	83.5	66.6	394.4	858.6	73.82	59.22	52.60
Xi19	8.9	58.0	88.5	56.7	385.1	586.7	13.41	32.19	33.38
Glasgow	10.0	55.6	84.5	25.8	197.5	289.2	13.55	17.40	17.58
Robigus	1.0	23.5	64.8	3.3	51.7	180.6	16.30	10.76	13.99
AnoVar									
Treatment		P<0.001			P<0.001			P<0.001	
Lsds 5%)									
Average		12.31			111.4			13.59	
Minimum		11.95			108.1			10.46	
Maximum		13.47			121.8			*25.62	

* for comparison Robigus @ 10^4 with other treatments

In 2008 (Table 4.9), the standard inoculum concentration of 10^6 spore / ml resulted in a substantial proportion of inoculated florets (64.8% - 88.5%) producing ergots. This infection rate was somewhat lower than in the previous 3 years. Reducing the inoculum concentration to 10^5 spores / ml led to a significant reduction in infection rate in all varieties (23.5% - 58.0%). At 10^4 spores / ml, infection levels were very low (1.0% - 10.0%).

Significant differences between varieties in infection rate were detected at 10^6 spore / ml, with a lower proportion of ergots being produced in

Robigus than the other three varieties. At 10^5 spores / ml Robigus retained its position as lowest producer of ergots, but Solstice also produced a significantly lower proportion of ergots than Xi19 and Glasgow. At 10^4 spores / ml the differences between varieties were not significant, although they retained the same ranking as at 10^5 spores / ml.

In terms of the weight of ergot per ear, reducing the inoculum concentration from 10^6 spores / ml to 10^5 spores / ml and from 10^5 spores / ml to 10^4 spores / ml led to reductions in ergot yield in all varieties. At the highest concentration, Solstice produced significantly more ergot than Xi19, which in turn produced more than Glasgow and Robigus. At 10^5 spores / ml, Solstice and Xi19 yielded similar amounts of ergot, which were significantly greater than that produced by Glasgow. Robigus yielded significantly less ergot than the other three varieties. At 10^4 spores / ml ergot yield was very low and the differences between varieties were not significant, although they retained the same ranking as at the higher concentrations.

The mean weight of individual ergots was relatively little affected by inoculum concentration. The reduction from 10^6 spores / ml to 10^5 spores / ml had no significant effect on ergot weight. Results for 10^4 spores / ml were erratic because of the very small number of ergots involved and only reached significance in one variety Xi19, in which ergot weight appeared to be reduced compared with the higher concentrations. At the two higher concentrations, the ergots produced by Solstice were significantly heavier ergots than those produced by Xi19, which were in turn significantly heavier than those produced by Glasgow and Robigus. At 10^4 spores / ml the ergots produced by Solstice were substantially heavier on average than those produced by the other varieties, although this result should be interpreted with caution because of the very low number of ergots involved at this concentration. There was a suggestion that Solstice may have compensated for a relatively low infection rate at the lower inoculum concentrations by producing larger, heavier, ergots.

An over-years analysis for the 3 varieties tested in all 4 years is presented in Tables 4.10, 4.117 and 4.12.

Table 4.10 % florets producing ergot following hypodermic inoculation at 10^6 spores / ml

Variety	2005	2006	2007	2008	Mean
Solstice	98.0	99.6	98.2	83.5	94.8
Xi19	92.9	99.6	99.6	88.5	95.2
Robigus	93.8	97.5	97.7	64.8	88.5
Mean	94.9	98.9	98.5	78.9	92.8
AnoVar					
Variety	NS				
Year	P<0.05				
lsds					
Variety means	-				
Year means	12.033				

Table 4.11 Weight of ergot per ear (g) following hypodermic inoculation at 10^6 spores / ml

Variety	2005	2006	2007	2008	Mean
Solstice	1.062	0.791	0.672	0.859	0.846
Xi19	0.579	0.734	0.823	0.587	0.681
Robigus	0.425	0.184	0.295	0.181	0.271
Mean	0.689	0.570	0.597	0.542	0.599
AnoVar					
Variety	P<0.01				
Year	-				
lsds					
Variety means	0.2507				
Year means	-				

Table 4.12 Weight of individual ergot (mg) following hypodermic inoculation at 10^6 spores / ml

Variety	2005	2006	2007	2008	Mean
Solstice	54.2	39.7	19.0	52.6	41.4
Xi19	31.1	36.9	20.7	33.4	30.5
Robigus	22.7	9.4	8.2	14.0	13.6
Mean	36.0	28.7	16.0	33.3	28.5
AnoVar					
Variety	P<0.05				
Year	NS				
lsds					
Variety means	13.39				
Year means	-				

This confirms that, with hypodermic inoculation at a rate of 10^6 spores / ml, inoculation success rate was high in all varieties, with no significant difference in the proportion of inoculated florets producing ergots. However, Robigus produced a significantly lower yield of ergot per ear, with smaller, lighter ergots than the other two varieties. Robigus and Solstice differed by a factor of approximately 3 in these two measures of ergot production.

Discussion

Direct inoculation of florets of a range of UK and European wheat varieties revealed significant varietal differences in ergot production. Differences were largely consistent over years and environments, indicating genetic differences in quantitative tissue resistance to *C. purpurea*.

With high inoculum pressure of 10^6 spores / ml, infection success rates were high in all varieties in most situations and there was no discrimination between varieties in terms of the number of ergots produced. Resistance was recognised as a reduction in the weight of individual ergots and hence in the total weight of ergot produced per ear.

With conditions less conducive to ergot development, either because of a lower inoculum concentration or less favourable environment, infection success rates were lower and resistance could be recognised as a reduction in the number of ergots produced as well as in the weight of ergots. However it is important to note that reducing inoculum pressure beyond a certain point reduced the infection success rate to the point at which ergot numbers produced were too low and their weight too variable to be used as indicators of resistance.

4.2.2. Emasculation and hypodermic inoculation under glasshouse conditions

Introduction

It is generally accepted that susceptibility to ergot infection persists for no more than a few days after fertilisation occurs (Willingale and Mantle, 1987). Evidence for host tissue resistance (**4.2.1**. hypodermic inoculation experiments above) was derived from inoculation of intact florets in which anthers matured normally to produce pollen. Although every effort was made to introduce ergot spores into the floret before anther maturity, the possibility that pollen induced resistance may have been a confounding factor cannot be discounted.

In this experiment, emasculated ears were used to eliminate any possible influence of pollen. The aims were:

1) to examine varietal differences in tissue resistance in isolation from possible resistance-inducing effects of pollen.
2) to investigate the ergot infection window in relation to pollination.

Materials and Methods

Nine experimental treatments and 3 control treatments were applied to 3 replicate ears of 2 varieties. For the experimental treatments, ears of

Solstice and Robigus were emasculated and then either pollinated artificially or left unpollinated. This was followed 1day, 7 days or 14 days later by inoculation with ergot conidia. Non-emasculated ears were inoculated at corresponding intervals after the time at which pollination was judged to have occurred naturally. There were 3 uninoculated control treatments 1) non emasculated 2) emasculated without pollination and 3) emasculated with pollination. The objective of these was to gauge the effectiveness of emasculation and hand pollination processes in the experiment.

18 plants of each variety, sown 3 plants to a pot, were vernalised and grown to the early ear formation stage at RAGT, before transfer to a glasshouse at NIAB on 9 January 2006. Temperature in the glasshouse was maintained at a minimum of 15^{o}C, with natural daylight supplemented by high pressure sodium lamps giving a 16 hr daylength.

Emasculation was carried out when the anthers were still green, before the release of pollen. Just prior to emasculation, top and bottom spikelets were removed from the ear to leave between 14 and 16 spikelets in the middle of the ear. The first two florets of each spikelet were retained and others removed. This left a total of between 28 and 32 florets per ear. Emasculation was carried out by separating the lemma and palea of the floret and removing the anthers, using a pair of fine forceps.

Pollination was achieved by removing a ripe anther from an extra ear of the appropriate variety using forceps, separating the lemma and palea of the floret to be pollinated and depositing pollen on the stigma and internal surfaces of the floret.

Inoculation, 1day, 7 days or 14 days after pollination was carried out using the procedures described above for hypodermic inoculation under field conditions, with a spore concentration of 10^6 spores / ml. Following inoculation, ears were bagged using cellophane crossing packets measuring 55mm x 190mm, which were closed at the bottom with a wire twist.

Ears were harvested on 22 May 2006 and were air-dried in paper envelopes at room temperature. Seeds and ergots were removed from each floret using forceps and counted. Ergots were weighed.

Results

Results for the nine experimental treatments are shown in Tables 4.13 and 4.14.

Controls indicated that the emasculation process was completely successful i.e. no grains were produced in emasculated ears. Hand pollination of emasculated ears had a success rate of around 50% i.e. about half of the emasculated florets pollinated went on to produce grains.

Table 4.13. Number of ergots per ear

Pre-inoculation treatment			Inoculation (days after pollination) 1 day	7 days	14 days
Emasculated	Not Pollinated	Solstice	13.0	12.7	1.0
		Robigus	7.7	2.0	0.0
Emasculated	Pollinated	Solstice	18.3	2.0	0.0
		Robigus	2.0	0.0	0.0
un-Emasculated control		Solstice	9.3	0.3	0.0
		Robigus	3.0	0.0	0.0
AnoVar significance of treatment effect			P<0.01		
lsd (P=0.05)	8.99				

Table 4.14. Weight of ergot per ear (g)

Pre-inoculation treatment			Inoculation (days after pollination) 1 day	7 days	14 days
Emasculated	Not Pollinated	Solstice	3.22	2.22	0.09
		Robigus	1.17	0.10	0.00
Emasculated	Pollinated	Solstice	2.60	0.25	0.00
		Robigus	0.29	0.00	0.00
un-Emasculated control		Solstice	1.91	0.02	0.00
		Robigus	0.38	0.00	0.00
AnoVar significance of treatment effect		P<0.01			
lsd (P=0.05)		1.87			

Emasculated florets pollinated 1 day prior to inoculation with ergot spores produced a similar number of ergots to emasculated florets which were not pollinated (Table 4.13). Numbers of ergots in un-emasculated florets inoculated at the corresponding time were slightly, but not significantly, lower. This is consistent with the observation that seed set in emasculated and pollinated control plants was about half that in un-emasculated control plants, thereby leaving a greater number of unfertilised ovules potentially vulnerable to ergot infection.

When inoculation was delayed until 7 days after pollination virtually no ergots were produced in pollinated emasculated florets, or in un-emasculated florets. However, in un-pollinated emasculated florets, ergot number did not decline significantly over the 6 day period between the first and second inoculation dates.

Inoculation 14 days after pollination failed to produce ergots in pollinated florets and there was negligible infection in un-pollination emasculated florets of Solstice only.

Greater numbers of ergots were generally produced in Solstice than in Robigus, although the difference did not reach statistical significance for every combination of emasculation, pollination and inoculation. This confirms previous results of hypodermic inoculation under field conditions. The differential between the two varieties was clearly expressed in unpollinated, as well as pollinated, florets.

Weights of ergot per ear (Table 4.14) followed a similar pattern to ergot numbers.

Given that 32 florets were inoculated per ear, the proportion of inoculations resulting in ergots under glasshouse conditions in un-emasculated florets with early inoculation was about 29% in Solstice and 9.4% in Robigus. This was considerably lower than the level normally achieved under field conditions, where inoculation success rates over the 4 years of the investigation ranged from 83.5% to 99.6% in Solstice and 64.8% to 99.7% in Robigus.

Discussion

The results indicate clearly that the apparent difference in resistance between the more susceptible variety Solstice and the less susceptible variety Robigus is a real effect of tissue resistance and not attributable to differential effects of pollen, such as might arise, for example, if the pollen of one variety was more vigorous or was produced more prolifically.

The experiment also gave some indication of the window for ergot infection in relation to the pollination event. There was no evidence of inhibition of infection 1day after pollination, but, by 7 days after pollination, infection was almost completely inhibited. The window appears to extend to somewhere between 1 day and 7 days after pollination. In order to produce a more precise estimate it would be necessary to repeat the experiment with additional intermediate inoculation dates.

Comparing ergot infection under field conditions and glasshouse conditions, following identical inoculation procedures, shows that field conditions were more conducive to successful ergot infection. Clearly optimum conditions for ergot infection were not well reproduced in the glasshouse environment. In particular, there was a tendency to overheating during sunny spells, lack of humidity and low light intensity, any of which may have hindered the infection process. It could obviously be important to be able to conduct resistance screening out of season in a glasshouse or controlled environment facility. These observations emphasise the importance of good and appropriate environmental control when working with this host / pathogen combination.

4.3 Examination of varietal flowering traits that may confer escape from infection.

Introduction

This work package aimed to quantify physical characteristics of wheat that could allow varieties to escape from ergot infection. The accepted rhetoric is that infection risk from ergot spores is greatest for varieties (or species) with florets that gape open during flowering (i.e. are 'open flowered'). Conversely, florets that remain closed during pollination ('closed flowering') and, for a few days afterwards, provide a mechanical barrier to the entrance of ergot spores and escape infection. Any factors which delay or reduce pollination are likely to increase ergot infection because of increased gape in an attempt by the wheat to collect pollen.
Plant breeders do not currently screen varieties for ergot resistance, nor do they make any systematic assessment of the flowering characteristics that may be associated with escape. Ergot resistance is not evaluated during official variety trials at the National List or Recommended List stages. This means that farmers do not have the information on which to choose low risk varieties and breeders do not have the knowledge needed to select for improved ergot resistance in their breeding programmes.

Flowering traits such as the timing and duration of anthesis, the degree of floret gape during flowering and the presence of unfertilised (or blind) florets after pollination were identified as likely indicators to quantify a variety's ability to escape ergot infection. Because of the environmental sensitivity of flowering biology the observations were repeated at a number of sites over three seasons.

Materials and Methods

After an initial discussion in March 2005 to identify a range of flowering characteristic that might influence openness of flowering, the following were identified as being quantifiable and of relevance to potential ergot infection:

- *Anther extrusion.* The level to which the anthers extend from the glumes is an important trait used by breeders, particularly those working with hybrid wheat varieties. Anther extrusion is frequently recorded as part of assessments made at heading.
  Anther extrusion is considered to be well correlated with openness of flowering, the presumption being that open flowering varieties - with a high degree of anther extrusion - are more susceptible to ergot. To assess whole ears was felt to be too time consuming but taking selected spikelets only quite practical.
- *Anther size***.** The hypothesis that compact ears produce smaller anthers and lax ears larger anthers, and how this relates to the degree of anther extrusion and openness of flowering could be of relevance. Again, a characteristic that is usually observed during normal heading assessments
- *Blind florets/ear.* Previous observations made on wheat ears as part of the SAL Orange Blossom Midge project suggested that blind florets (unfertilised, no grain developing) could frequently be found after anthesis. This did not appear to reflect any specific pest or pathogen attack and was probably attributable to the floret not producing, or not receiving, viable pollen. There were differences observed between varieties for this character and it was felt this was an important 'background' character to record.

- *Duration of anthesis*. A logical parameter to assess but one that can be strongly influenced by environmental conditions - such as temperature or moisture stress - that could mask any real differences between varieties. Difficult to measure accurately in the field and more reliably recorded under controlled conditions, such as a growth room.
- *Duration of heading*. The time interval between anthesis occurring on the main stem and the secondary tillers clearly governs the time over which the plant is at risk from infection by ergot. Recording the dates on which the first anthers appear on the main stem and last ones can be seen on the smaller tillers would give a good indication of the 'at risk' period. It has to be accepted in the trial situation this is likely to be shorter than in a commercial crop where factors such as tramlines and use of lower seed rates would be likely to give rise to a higher proportion of late tillers.
- *Ear density*. Broadly speaking, wheat can be described as having a lax, medium or compact ear type and this is used as part of a variety's botanical description for National Listing. A common perception is that lax ears are more open flowered, compact ears more closed flowered. Although it was likely there would be a spread of ear types between varieties in early generation material (probably up to NL2) the diversity in Recommended List varieties plus candidates was felt to be quite limited.
- *Glume gape*. This character is frequently cited when considering openness of flowering, particularly when linked to anther size, and there is a perception of differences between varieties. It is difficult to quantify without getting into the realms of microscopic measurements (time consuming), although opening the glumes by thumbnail and making a more subjective assessment of how tight or slack they were could be tried.

After this initial 'brainstorming' the following traits (Table 4.15) were recorded during the first season of the project (2005):

Table 4.15. Traits assessed during 2005			
TRAIT	**ASSESSMENT METHOD**		
	1 – 3 Scale where:		
	1 =	2 =	3 =
Anther extrusion	No anther appearing (fully retained)	Tips of anthers visible but trapped	Anther fully extruded (lost)
Anther size	Small	Medium	Large
Ear Density (Nickerson)	Lax	Intermediate	Compact
Ear Density (NIAB)	Number of spikelets ÷ Rachis length		
Blind florets	Number of empty grain sites per ear as a % of the total number of florets		

Blind florets were assessed by counting the number of empty grain sites on samples taken at early grain fill, deep frozen and assessed during the winter. The other traits were assessed in the field during flowering. Glume gape was considered to be too difficult to easily measure; the duration of anthesis best recorded in a temperature controlled environment where no external environmental factors could influence the process and the duration of heading too time consuming.

Following a review of the assessments taken during the 2005 season, only two characteristics were assessed in 2006: blind florets and anther extrusion. The former used the same method as in 2005 and the latter a revised protocol taking 3 median spikelets and one each in the upper and lower third of the ear (see Appendix F). Ears were air dried and examined at a convenient time during the autumn / winter.
Blind florets and anther extrusion were also assessed in 2007 using the same protocols.

In each of the three seasons a sample of 10 ears were taken from 10 'core' varieties chosen to represent a range of variety characteristics:

- Claire, Consort and Malacca (popular varieties grown on a considerable area commercially).
- Cordiale and Zebedee (earlier maturing, offering the possibility of escape from ergot spores).
- Gatsby and Welford (resistant to Wheat Orange Blossom Midge and different genetic background to many HGCA Recommended List varieties).
- Robigus, Solstice and Xi19 (common varieties to WP4 4.2, inoculation).

Assessments were made on these varieties – and occasionally others, depending on site – at a number of sites detailed in table 4.16 below:

Table 4.16 Locations at which traits were assessed

Year	Location	Anther Extrusion	Anther Size	Blind Florets	Ear Density
2005	NIAB (Field 51), Cambridge	✓		✓	
2005	NIAB (Demo Field), Cambridge	✓		✓	✓
2005	Nickerson, Docking, Norfolk	✓	✓	✓	✓
2006	NIAB, Cambridge	✓		✓	
2006	Nickerson, Docking, Norfolk	✓		✓	
2006	Velcourt, NIAB, Cambridge	✓			
2006	Velcourt, Metheringham, Lincs	✓			
2006	RAGT, Trumpington, Cambridge	✓			
2007	Nickerson, Docking, Norfolk	✓			
2007	RAGT, Trumpington, Cambridge	✓		✓	

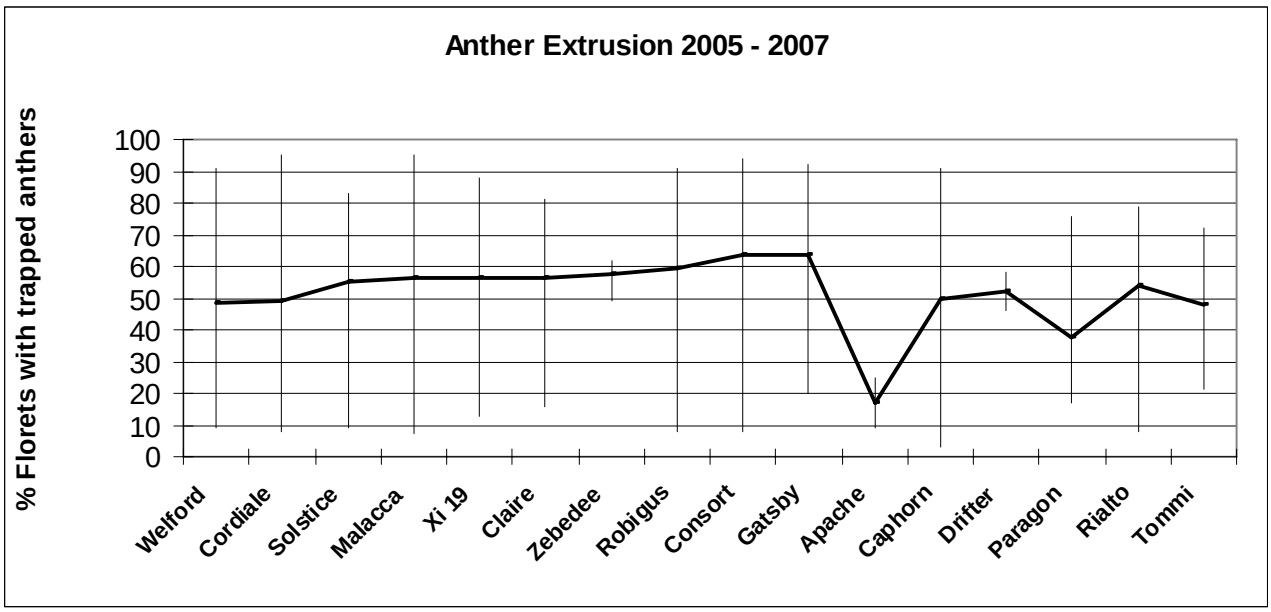
Results

Anther extrusion

Assessments were made on two florets from ten spikelets (see Appendix F) and a figure for the % of florets with trapped (retained) anthers calculated. In addition to the 10 'core' varieties assessments were made on additional varieties at some sites in some seasons.

The data from ten sites over the three years of the project are summarised in Figure 4.6

Figure 4.6 Summary of anther extrusion



(A vertical bar shows the range between sites and seasons for any one variety)

Varieties with a high % of trapped anthers show a low degree of anther extrusion and can be considered to be closed flowering. Conversely, a low % of retained anthers suggests a high degree of extrusion and that a variety is more likely to be open flowering.

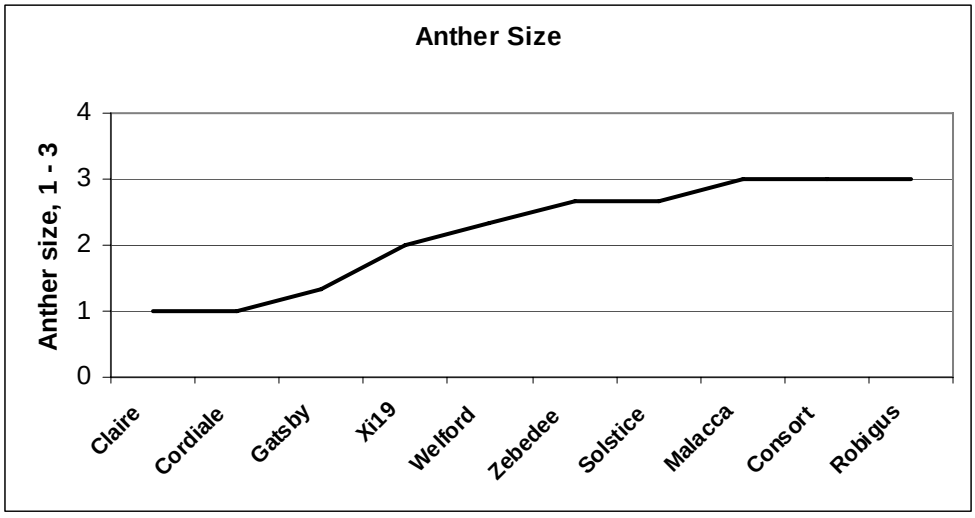
The 'core' varieties are ranked in increasing % of florets with trapped anthers (Welford to Gatsby). The mean figure shows only a relatively small increase from Welford (49%) to Gatsby (64%) with nearly all the varieties showing considerable variation. The data for Zebedee is based on comparatively few sites which explain the apparent lack of variation.

Additional varieties (Apache to Tommi) were assessed at fewer sites and, with the exception of Apache and Drifter, have similar results to the other varieties.

Anther size

Anther size was only recorded at one site in one season (Nickerson 2005) on a 1 (= small) – 3 (= large) scale (Figure 4.7).

Figure 4.7. Anther size assessed at one site in 2005.

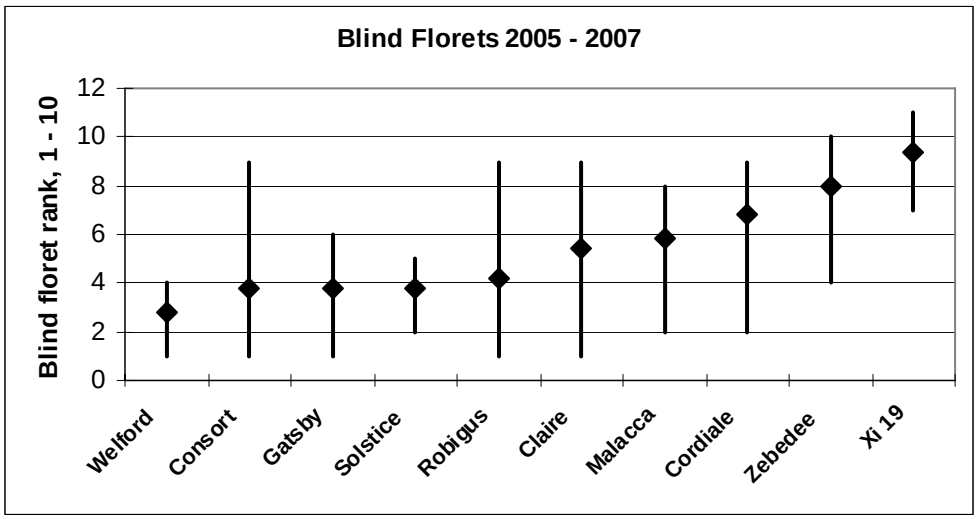


Differences between the varieties were apparent with Claire and Consort tending to have smaller anthers than Consort, Malacca and Robigus.

Blind Florets

Data were available from assessments made in 2005, 2006 ad 2007. Because of differences in assessment method used at different sites the data is ranked using a scale where 1 = a low number of blind florets/ear and 10 = a high number blind florets/ear. The mean result with an indication of variation is presented in Figure 4.8.

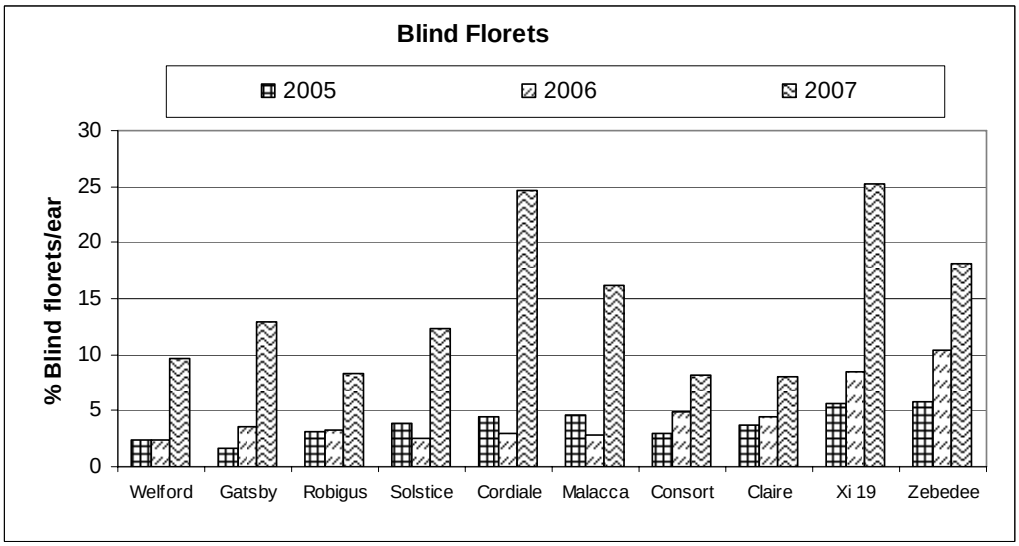
Figure 4.8 Summary of blind floret assessments (by ranking)



Presenting the analysis in this way suggests Welford, Consort, Gatsby and Solstice have fewer blind florets than Zebedee and Xi19. Consort, Robigus, Claire and Cordiale show more variation than Welford, Solstice and Xi19.

There were quite noticeable differences between seasons for this characteristic which become apparent when the data are presented by year (Figure 4.9).

Figure 4.9 Annual variation in blind florets



Overall, season appeared to have a greater influence on the number of blind florets (e.g.2007) than variety, but the graph suggests Claire,

Consort and Robigus have fewer blind florets than many of the other varieties.

At the RAGT site in 2007 a more detailed assessment was undertaken recording the number of blind grain sites in the outer (main) and inner (secondary) florets and expressing these as a % of the total number of florets (Table 4.17).

Table 4.17 Blind grain sites in inner and outer florets – RAGT 2007

% Blind	Solstice	Welford	Robigus	Gatsby	Claire	Consort	Zebedee	Mean
Outer floret	10.1	4.7	3.4	4.7	2.8	2.5	5.1	4.8
Inner floret	14.4	14.7	13.1	21.3	13.1	13.8	31.0	17.3
Inner/Outer	1.4	3.1	3.8	4.5	4.7	5.5	6.1	3.6

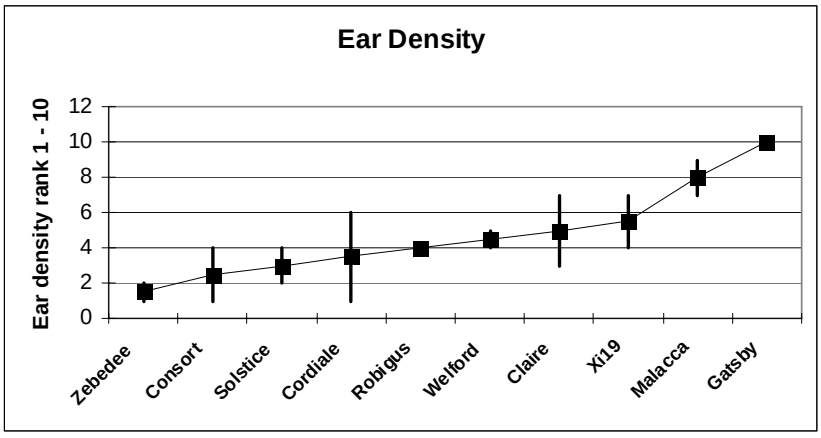
Cordiale, Malacca and Xi19 were not recorded.

For the seven varieties where data is available there tended to be more blind grain sites in the inner florets and this pattern was consistent across all the varieties.

Ear Density

Ear density was recorded at two sites in 2005 only with different methods of assessments used. The data from the individual sites was ranked in increasing order of density using a scale where 1 = most lax and 10 = most compact. An average density figure was calculated for each variety and these are presented in figure 4.11.

Figure 4.11 Summary of ear density at 2 sites, 2005.



(The vertical bars indicate the variation in ear density for an individual variety with a longer bar showing greater variation between sites.)

Although this is rather a crude analysis is does suggest there are differences between varieties with Zebedee and Consort having relatively lax ears and those of Gatsby and Malacca being more compact. Consort, Claire and Cordiale appear to be more variable than the other varieties tested.

Discussion

WP4.3 used selected flowering characteristics of wheat - anther size, anther extrusion, ear density and the proportion of blind florets - to attempt to quantify whether a variety had an open or closed flowering habit and, by inference, if this could confer any degree of escape from infection by ergot spores. Using these parameters an open flowering type could be defined as having large anthers with a high degree of extrusion, a lax ear and a high % of blind grain sites after flowering. Conversely a closed flowering variety would have small anthers which were retained in the floret, a compact ear and a lower % of blind florets.

The data recorded during the project indicates which varieties show these specific traits to a greater or lesser extent. Table 4.18 below summarises this information in an attempt to identify individual varieties with collective flowering characteristics which fit the flowering definition proposed above.

Table 4.18 Tendency to open / closed flowering based on a range of flowering characteristics

	Tendency to open flowering	**Tendency to closed flowering**
Anther extrusion	**Full**	**Trapped**
	Cordiale, Solstice, Welford	Consort, Gatsby, Robigus
Anther size	**Large**	**Small**
	Consort, Malacca, Robigus	Claire, Cordiale
Blind florets	**High %**	**Low %**
	Cordiale, Xi19, Zebedee	Claire, Consort, Robigus
Ear density	**Lax**	**Compact**
	Consort, Solstice, Zebedee	Gatsby, Malacca

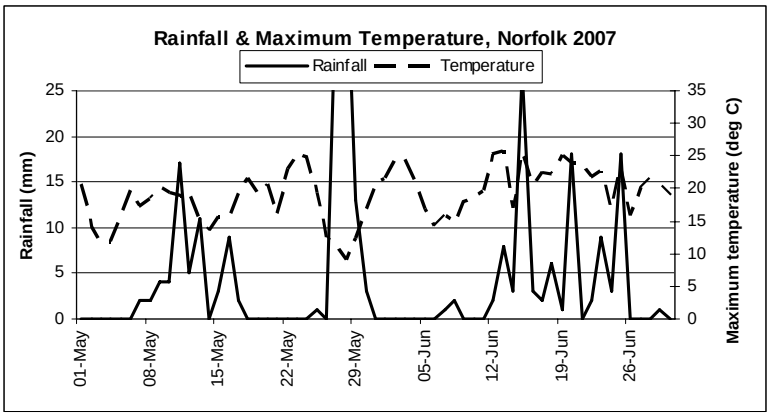
The varieties listed are restricted to those which show the characteristic to a high degree, generally towards the limits of the graphs presented in the results. Even making allowance for the high degree of subjectivity, it is very difficult to see any consistency emerging. Bearing in mind that only the data for anther extrusion and blind florets is taken from three seasons, only Cordiale could be considered open flowering and Consort and Robigus closed.

The results presented for % blind florets show the influence that season has on this characteristic and this is supported by the data for anther extrusion where the majority of varieties show considerable variation between site and season. Reference to the literature (e.g. De Vries, 1970) suggests that many aspects of flowering are influenced by meteorological conditions with temperature and rainfall being particularly important. Weather records are available for most of the sites used and those for the Nickerson site at Docking in Norfolk are presented below

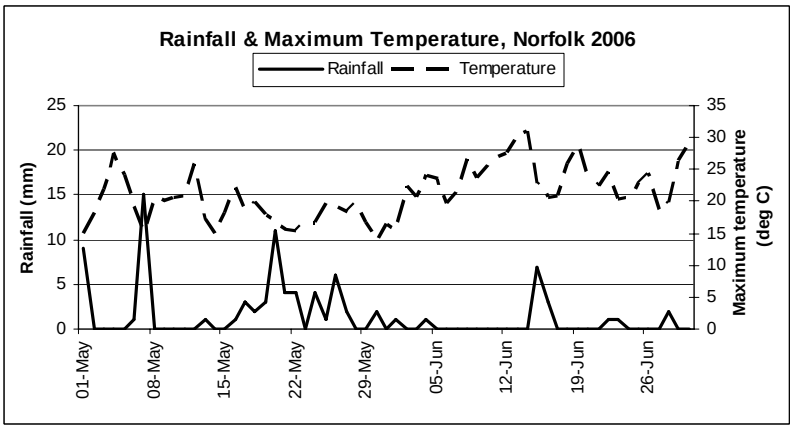
using rainfall and maximum air temperature to illustrate the annual variability (Figure 4.12).

Figure 4.12 Rainfall and maximum temperature Norfolk site

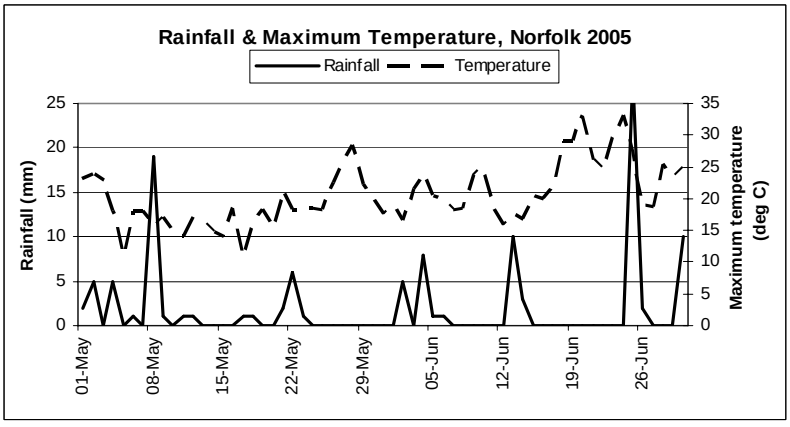
a) 2007



b) 2006



c) 2007



Flowering usually occurs at this location around mid-June. A comparison between 2006 and 2007 shows the large seasonal differences that can occur for rainfall and the fluctuation in air temperature within a single season, at this critical phase in the plant's development.

It is difficult from this work package to identify flowering characteristics which enable a reliable prediction of a variety's tendency to be open or closed flowered to be made. The weather data presented suggests seasonal variation in temperature and or rainfall can have a much greater influence on this attribute and subsequent role in escape from ergot infection.

Overall Discussion for WP4

The aim of this work package was to investigate evidence for varietal differences in 'field resistance' to ergot in wheat and to examine the contributions of tissue resistance and 'escape' to a variety's vulnerability to infection.

The method adopted for the investigation of 'field resistance' was to expose varieties to natural infection from inoculum generated by artificially infected black-grass spreader plots. This not only revealed a high degree of variability in ergot infestation between sites and years, but also inconsistencies in variety effects. Despite this, it was possible to identify a few varieties which never suffered more than slight infection (e.g. Robigus, Glasgow, Cordiale, Soissons, Oakley, Malacca) and others which were consistently heavily infected (e.g. Rialto, Xi19, Solstice, Mascot, Gatsby,).

Investigation of tissue resistance was based on direct introduction of ergot inoculum into individual florets in order to bypass any possible 'escape' mechanisms associated with flowering characteristics. This approach

provided clear evidence of consistent varietal differences in tissue resistance. No variety was immune, but some expressed a significantly higher degree of partial resistance than others (e.g. Robigus v Solstice).

Because of the labour intensive nature of the tissue resistance test, only 14 varieties from the set of 46 tested for field resistance were also tested for tissue resistance, and of these 4 were tested in only a single year. However, it was clear that varieties with the best levels of partial tissue resistance identified here, (Robigus and Glasgow), never became severely infected in the field. No variety was identified as having good tissue resistance but poor field performance. A number of varieties that were consistently heavily infected in the field also proved to have poor (Solstice, Xi19, Rialto), or at best moderate (Tommi, Mascot) tissue resistance. Other varieties, which displayed a range of intermediate levels of tissue resistance, gave inconsistent field performance varying markedly between sites and years.

The term 'escape' has been used here to cover mechanical factors which might influence the ease with which ergot spores gain entry to the floret. Wheat is open-flowering i.e pollen dehiscence starts within the closed floret and continues as the lemma and palea are forced part to allow the extrusion of the anthers. However, field observations suggest that there are degrees of 'open-ness', such that the florets of some varieties gape wider, or remain open for longer, than those of others. Attempts were therefore made to quantify 'open-ness of flowering' based on assessments of anther extrusion, blind florets, anther size and ear density. The results pointed to an over-riding effect of environment on open-ness, for example, many of the varieties examined ranged from as little as 10% anther extrusion at some sites to nearly 100% anther extrusion at others.

A comparison of flowering characteristics information with field resistance data for failed to point to possible associations. For example, the field resistant variety Robigus appeared to have relatively low anther extrusion combined with a low tendency to blind florets, both of which indicate a

variety at the low end of the 'open-ness' scale. However, Cordiale, another variety which was never badly infected in the field, had relatively high scores for both of these characters, indicating a variety with a greater tendency to 'open-ness'. To take two other examples, Glasgow (good field resistance) and Rialto (poor field resistance), were both middle of the range for open-ness characters. The conclusion is that varietal differences in open-ness of flowering are unlikely to be a major determinant of their relative susceptibility to ergot in the field and that tissue resistance is of more importance.

Genetic resistance to ergot infection would be attractive to the grower, particularly if it effectively removed the risk of grain rejection. Our results suggest that growers immediately have the opportunity to reduce the risk of ergot contamination in their crop through variety selection, although testing to a wider range of *C. purpurea* strains and further commercial experience would be desirable.

Selection by plant breeders for the level of resistance shown by the genotypes tested would be possible in inoculated field nurseries similar to those developed in this project. It would be vital to repeat field testing over a range of years / environments in order to identify lines which show consistently low levels of infection. These lines could then be selected for further testing for confirmation of tissue resistance as described here. For most rapid progress selection at an early stage in the breeding process is desirable. This might not be practicable in inoculated nurseries due to the risk of ergot contamination within seed lots and the need to use black-grass or a similar grass weed as a disease spreader. A solution would be to develop a molecular marker for resistance, or susceptibility. It would be valuable to discover whether more than one resistance is present in the current material and if the resistance is different to that already described in spring wheat (Platford et. al., 1977).

WP 5: Development of a real-time PCR diagnostic methodology for quantitative detection of *C. purpurea.*

Introduction

Claviceps purpurea the causal agent of ergot overwinters as sclerotia, which must undergo a period of cold temperatures to break dormancy. During the spring and early summer the sclerotia germinate to produce one or more perithecial stromata from which numerous filiform ascospores are actively discharged. These spores are then air-dispersed over considerable distances before being deposited on to a crop. The indistinctive nature of these spores makes them difficult to identify in the presence of large numbers of non-target spores and debris on spore traps. A species-specific DNA marker (257_{540}) has been cloned and sequenced for *C. purpurea* (Pažoutová and Tudzynski 1999). This 540 base-pair fragment has not been found to exist in closely related *Claviceps* species but is conserved at multiple locations within the *C. purpurea* genome ensuring meiotic stability.
The objectives of our study were to establish a fast and reliable diagnostic test for the presence of *C. purpurea* independently of the presence of disease symptoms that can also be used to quantify airborne spores at infested sites with a real-time PCR assay.

Materials and Methods

Origin and Maintenance of Fungal Isolates
The *C. purpurea* and other fungal isolates used in this work were obtained from geographically diverse areas and host species (Table 5.1). Fungal isolates derived from a single conidia were identified, cultivated and maintained as described by Stevens *et al*. (1998).

Design of diagnostic primers and hybridization probe
Using the 540bp fragment identified by Pažoutová and Tudzynski (1999) a series of primer pairs were designed using preparatory software. These were tested under standard conditions using a standard concentration of

C. purpurea DNA extracted from a sclerotia. Each primer pair was analysed on the basis of amplification and melting curve profile resulting in primers Ergot 235F (5'-GAT ACT TGG CGC AAG GGT TA -3') and 451R (5'- AAT CCT TCC TAT GCC CTG CT-3') which amplified a 217bp fragment being selected. Using a DNA dilution series this primer combination was found to detect 1pg DNA. To further enhance sensitivity a hybridization probe using LightCycler red 640 was designed.

DNA Extraction

DNA extraction were carried out on 20mg of tissue unless otherwise stated, using the DNeasy Plant Mini kit (Qiagen Ltd.) with minor modifications was used. Lysis incubation was extended to 30 minutes and the drying step of the DNeasy Mini Spin Column was extended to 3 minutes. DNA was resuspended in a volume of 50µl.

Real-time PCR

Real-time PCR was performed in a total reaction volume of 20µl. Reaction mixtures consisted of 1.6µl 1.5mM $MgCl_2$; 2.0µl master-mix (FastStart DNA Taq Polymerase and SYBR Green I dye); 1.0µl of each primer; 2.0µl template DNA and 12.4µl water. Amplification was carried out in a LightCycler (Roche) with an initial denaturing step at 95°Cfor 600s followed by 30 cycles of 95°C for 10s, 58°C for 12s and 72°C for 10s. The temperature transition rate was set at 20°C s^{-1}. Acquision of the fluorescence signal was carried out at the end of every extension step. A melting curve was obtained immediately after amplification to distinguish products from non-specific products and primer-dimers. This was done by holding the temperature at 72°C for 10s and gradually increasing the temperature to 95°C at a rate of 0.1°C s^{-1}, with the fluorescence signal acquisition mode set at continuous.

Artificial inoculation of spore trap tapes

Ergot sclerotia were germinated *in vitro* and on production of the stroma section of spore trap tape were place adjacent to the spore bode at a distance of 4cm. Ejected ascospores were allowed to collect on these tapes for a period of 24 hours before being removed. The tapes were

viewed under a microscope at x300 and sections of tape with either 1, 2, 10, 50 or 100 ascospores were cut using a scalpel. The tape was placed in a mortar to which 500µl of lysis buffer was added prior to grinding with a pestle. A small volume of liquid nitrogen was then added. Continue grinding as the buffer thaws until fully melted. The extraction buffer and tape were transferred to a 1.5ml eppendorf centrifuge tube and incubate at 65°C for 30mins, vortexing the mixture every 10mins.

Preparation of spore trap tapes from field experiments

Spore trap tapes were divided into individual days and then divided longitudinally with the first half being used in real-time analysis and the second being stored as a reserve sample for in case visual assessment of the tapes was required. The tape was prepared for DNA extraction as described above.

Preparation of wheat ears

Samples of 25 wheat ears sampled from experimental fields were freeze-dried for a period of no less than 24 hours. Once dry, seeds were ground in a mill into a fine powder. Tissue from ears of the same collection was then pooled and a 20.0mg sub-sample was removed for DNA extraction.

Table 5.1 Details of the *Claviceps purpurea* isolates and other fungal species used in this study.

NIAB	Isolate Identity	Host	Amplification
W04/01	*Claviceps*	Barley	+
W04/02	*Claviceps*	Black-grass	+
W04/09	*Claviceps*	Cocksfoot	+
W04/11	*Claviceps*	Meadow	+
W04/14	*Claviceps*	Rye	+
W04/25	*Claviceps*	Barley	+
W04/37	*Claviceps*	Wild Oats	+
W04/38	*Claviceps*	Canary	+
W04/41	*Claviceps*	Black-grass	+
W04/42	*Claviceps*	Cocksfoot	+
W04/62	*Claviceps*	False Oat	+
W04/70	*Claviceps*	Tall Fescue	+
W04/79	*Claviceps*	Meadow	+
W04/81	*Claviceps*	Timothy	+
W04/86	*Claviceps*	Cocksfoot	+
W04/88	*Claviceps*	Timothy	+
W04/94	*Claviceps*	Tall Oat	+
W04/97	*Claviceps*	Black-grass	+
W04/98	*Claviceps*	Cocksfoot	+
53153	*Claviceps*	Perennial	+
53530	*Claviceps*	Perennial	+
53008	*Claviceps*	Perennial	+
52630	*Claviceps*	Perennial	+
T03/01	*Claviceps*	Triticale	+
T03/07	*Claviceps*	Triticale	+
T03/15	*Claviceps*	Triticale	+
97-1	*Alternaria tenuis*	n/a	-
97-10	*Cochliobolus*	Wheat	-
97-1	*Epicoccum* spp.	n/a	-
98-11	*Fusarium*	Wheat	-
-	*Fusarium*	n/a	-
94-1	*Fusarium poae*	Wheat	-
97-1	*Fusarium* sp.	Bean	-
98-26	*Microdochium*	Wheat	-
98-2	*Septoria*	Wheat	-
-	*Tilletia tritici*	Wheat	-
97-78	*Pyrenophora*	Barley	-
-	*Ustilago nuda*	Barley	-
-	*Penicillium sp.*	-	-
-	*Ustilago tritici*	Wheat	-
97-93	*Pyrenophora*	Barley	-

Preparation of barley awns

Samples of barley awns collected from experimental fields were freeze-dried for a period of no less than 24 hours. Once dry the awns were ground in a mill into a fine powder. A 20.0mg sub-sample was removed for DNA extraction.

Results

Using the sequence data for the 540 base-pair *C. purpurea* species-specific DNA marker, a number of primer pairs were designed. These were arranged in a nested fashion using either a common forward or reverse primer. This would allow the possibility of nested-PCR to be conducted if required. Three of the four primer pairs screened, resulted in the amplification of a single product with no secondary products using the LightCycler (Figure 5.1). Primers 235F and 451R producing a 217bp amplicon were selected and an internal fluorescent hybridisation probe utilising LightCycler red 640 was designed. Use of this probe was found to increase the sensitivity of the real-time PCR reaction 10 fold over use of the primers alone allowing quantification of *C. purpurea* DNA down to 0.1pg (Figure 5.2).

The developed primers have been successfully screened against 30 *C. purpurea* isolates with a range of host and geographic backgrounds. The primers and hybridisation probe have also been tested against a wide range of ear pathogens and common saprophytes found on both grass and cereal crops to ensure that there is no cross reactivity.

Figure 5.1. Melting curve analysis of selected primer pair.

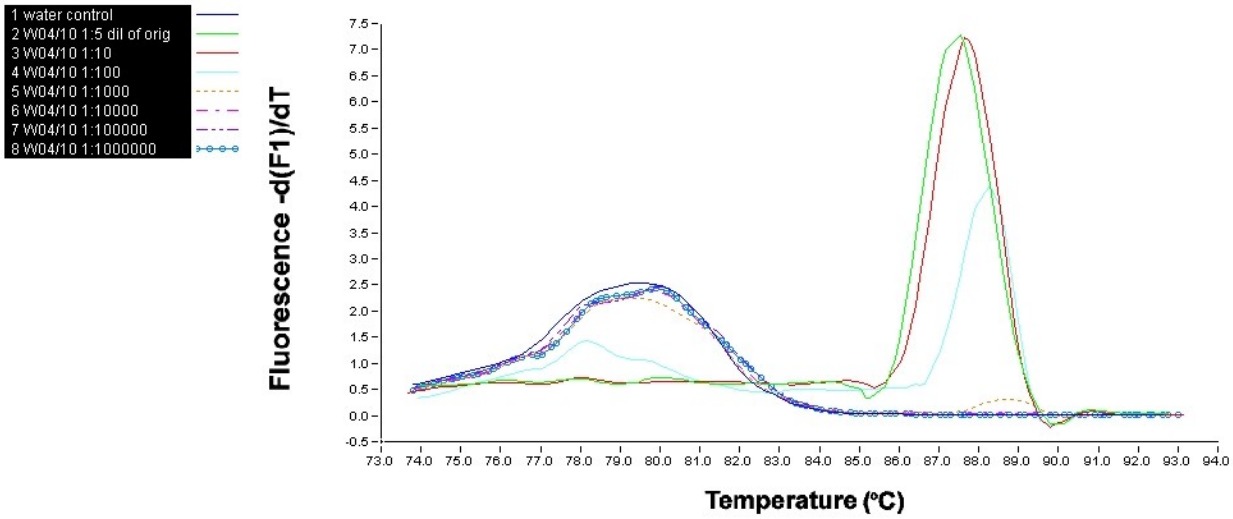
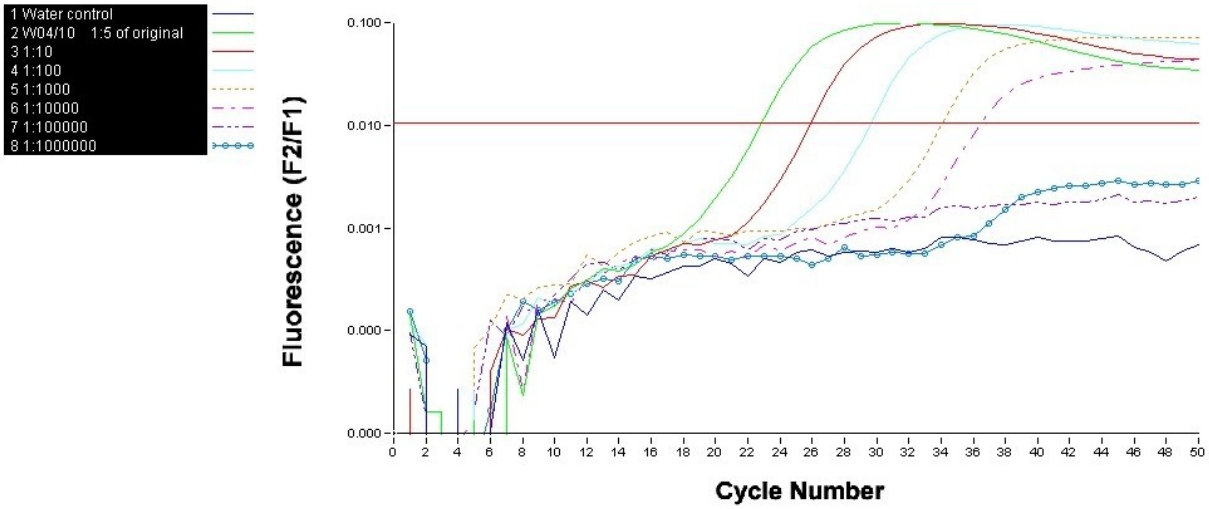


Figure 5.2. Run characteristics of selected primer pair in conjunction with hybridisation probe utilising LightCycler red 640.



Using a previously described method, DNA was extracted from ascospores adhered to cellophane film. Initial results showed that whilst single spores were reliably detected correlation of pgDNA to spore number was poor. It is thought that this is due to the high levels of polysaccharides found in fungal spores not being adequately removed during DNA extraction and subsequently inhibiting the PCR reaction. Extraction of the pathogen DNA from spore trap tape, passive trap tubes and plant host tissues have been optimised. Using artificially inoculated sticky-trap tape to produce a range

of infection levels it has been established that a single spore can be reliably detected using the system developed.

The developed method has been utilised in the detection and quantification of artificial spore traps and in crops samples taken during 2005, 2006 and 2007. Where a specific day was found to be positive using the real-time detection method the reserve portion of the spore trap tape was viewed at x300 under a microscope and the number of ascospores counted. On each occasion only a single ascospore was identified. A further series of days were C. purpurea DNA was not detected using the real-time analysis were also examined but and no ascospores were found to be present.

In 2006 a further series of spinning spore traps which sample a greater volume of air were also analysed using the real-time method. *Claviceps purpurea* DNA was detected on most traps and in some cases high levels of the pathogen's DNA were quantified.

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APPENDIX A

Sampling procedure for each margin.

Each co-ordinator will be issued with a folder containing the sampling procedure, examples of collection envelopes and images of ergot in grass weed samples collected last season.

NB. Prior to sampling it may be worth scouting a few margins or black-grass areas to get your eye in for ergots using the images in the file.

1) Try to select a range of margin types for sampling so that we don't end up with data from just natural regeneration.
2) Use **ONE** survey data envelope per margin (brown envelope in file).
3) A two metre cross-compliance strip with an additional margin will count as **TWO** margins so collect one lot of samples from the cross compliance area and one lot from the additional margin if this is the situation.
4) For **EACH** margin sampled fill in as much of the survey data sheet as possible – location and date are **VERY** important.
5) There will be **TWO** sampling timings – 1) mid-late June to mid July and 2) mid-late Aug to early Sept.
6) Ideally sample the same margins at **BOTH** timings.
7) For each margin sampled:-
    a. Walk approximately 10-20m along the margin before sampling.
    b. Look at a range of grass seed heads in the vicinity to "get your eye in" - use the image cards to help.
    c. Walk steadily down the margin to a max. length of approx. 100m, stopping every 5-10m looking at grass seed heads as you go.
    d. Look at both the right hand and left hand boundaries of the margin as well as the middle.
    e. When you find grass seed heads containing ergots take a sample of several heads (approx. 2-5) keeping some of the ligule and stem attached and place in a self seal bag.
    f. Keep samples from different grass species separate – if in doubt separate.
    g. You may find that a range of grass species have ergots - try to collect as great a range as possible and keep samples separate.
    h. Try to make a note of where the largest number of ergots are found i.e. margin edge closest to the crop etc.
    i. If no ergots can be found or only 1 or 2 grass species seems to have ergot, sample these and record your findings on the data sheet.
    j. Once sampling of the margin is complete seal the large survey bag
8) Collect together all large sampling bags and return to the main coordinator for your organisation.
9) Make sure the samples are dry and stored somewhere free from mice.
10) If you find anything unusual or particularly interesting contact either your company co-ordinator, James Alford (Velcourt Ltd) (01487 773660 – 07771 507295) or Rosemary Bayles (NIAB project coordinator 01223 342211).

APPENDIX B
HPLC results from selected ergot samples

Ergot Sample	Rt(mins) & area under peak (mAU*s)						
Peak no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	15.87-16.02	**21.94-22.92**	**23.52-24.51**	**24.58-25.93**	**26.35-26.41**	**29.16-29.92**	**30.57-31.43**
05/060 Bent				277.16			1495.08
05/063 Bent			1204.19			646.33	
04/091a BG							
04/035 Cocksfoot	1441.37		1068.54			556.22	
04/068b Cocksfoot					641.86		797.87
04/093c Cocksfoot			415.91				
05/015 Cocksfoot		188.27	629.1				
05/019 Cocksfoot	1081.55				422.88		2064.99
05/039 Cocksfoot							
05/112 Cocksfoot					968.60		2989.36
05/115 Cocksfoot		62.33	177.56				
05/134 Couch	2220.62		984.1			926.54	
05/136 Couch			168.71		1258.60		721.82
04/103 False Oat				838.67			
05/011 False Oat		594.30		778.92		773.92	
05/024 False Oat			1180.15			532.61	
04/109 Mat Grass	546.59	388.75	339.01			374.81	206.93
05/018 M Fescue				911.94			1271.63
05/071 M Fox				490.24		2253.20	
04/082 P Rye		865.91	2492.29			982.89	2284.49
05/115 P Rye			1100.9				
05/123 P Rye			1285.99				1128.40
04/070 T Fescue					447.60		
04/076 T Fescue	671.94				820.13		2419.57
05/136a T Fescue				247.56	766.58		358.51
05/169 T Fescue	24.10	18.07	61.21				
04/061 Timothy	1110.34		461.87				
05/116 Timothy					817.41		3135.88
05/120 Timothy			134.96				
05/016 Y Fog		849.66	1906.23			7636.10	
05/154 Y Fog		195.32	249.99				

APPENDIX B contd

Ergot Sample	**Rt(mins) & area under peak (mAU*s)**						
Peak no	**8**	**9**	**10**	**11**	**12**	**13**	
	31.37-32.68 Ergot-amine	**34.17-35.10 Ergo-cornine**	**36.27-36.94**	**37.31-37.95 Ergo-cryptinine**	**38.46-38.85**	**39.30-40.99**	**% Ergotamine**
05/060 Bent	5867.75					1132.22	45.71
05/063 Bent	2580.38		365.47	1241.60	415.23	451.39	28.02
04/091a BG	1195.95	570.73					67.70
04/035 Cocksfoot	2458.90	698.17		8464.93	391.73	502.55	10.30
04/068b Cocksfoot	1473.51	653.88		3149.08		947.34	10.64
04/093c Cocksfoot				114.68		191.12	0.00
05/015 Cocksfoot	1324.59		486.38			1999.35	16.31
05/019 Cocksfoot	734.32	1715.31	1019.76	619.25		1743.54	4.55
05/039 Cocksfoot	114.84					221.13	17.95
05/112 Cocksfoot	1626.01	1108.28		4741.48		2468.08	7.79
05/115 Cocksfoot	206.18		90.72			439.42	8.72
05/134 Couch		2883.44	2457.11	1195.44	1207.79		0.00
05/136 Couch	11488.60					526.82	51.70
04/103 False Oat grass							0.00
05/011 False Oat grass		8403.81	1337.12			673.61	0.00
05/024 False Oat grass		878.46		1084.90			0.00
04/109 Mat Grass	1816.17	149.94			256.62	1602.60	28.20
05/018 M Fescue	9087.73		1347.87				41.83
05/071 M Fox	5116.47			3735.62	1776.08		22.90
04/082 P Rye	17484.00		871.79				42.38
05/115 P Rye	1661.55						28.27
05/123 P Rye	12089.70						51.06
04/070 T Fescue							0.00
04/076 T Fescue	1057.33	401.04				2109.16	11.65
05/136a T Fescue		532.65	504.40				0.00
05/169 T Fescue	203.43						43.43
04/061 Timothy		1732.72	1151.40				0.00
05/116 Timothy	9986.39					2336.38	41.20
05/120 Timothy	378.63					698.94	13.44
05/016 Y Fog		485.9		4467.29	5856.16	694.00	0.00
05/154 Y Fog	484.03					518.7	14.99

APPENDIX B (contd)

Ergot Sample	Rt(mins) & area under peak (mAU*s)							
	14	**15**	**16**	**17**	**18**			
	41.00-41.88	**42.69-42.97**	**43.01-43.85**	**44.88-45.27**	**46.13-47.41**	**Ground sclerotia (mg)**	**Largest Peak**	**% of total AUP**
05/060 Bent	4064.89					0.4997	Ergotamine	45.71
05/063 Bent	1639.43				665.30	0.1912	Ergotamine	28.02
04/091a BG						0.5015	Ergotamine	67.70
04/035 Cocksfoot	2067.67				6227.55	0.5150	Ergocryptine	35.45
04/068b Cocksfoot	1279.58		1011.08		3894.22	0.5093	18	28.12
04/093c Cocksfoot			172.09	967.37		0.1980	17	51.98
05/015 Cocksfoot	1107.04			647.33	1737.77	0.0956	13	57.57
05/019 Cocksfoot	839.85	1802.88	849.35	1757.52	1488.48	0.4914	7	12.79
05/039 Cocksfoot				124.15	179.64	0.1934	13	34.56
05/112 Cocksfoot	1235.53		589.63		5139.47	0.4875	18	24.63
05/115 Cocksfoot				856.57	532.49	0.1183	17	36.21
05/134 Couch		3013.83		2964.72		0.4965	15	16.88
05/136 Couch	8058.66					0.5104	Ergotamine	51.70
04/103 False Oat						0.5253	4	100.00
05/011 False Oat						0.4951	Ergocornine	66.90
05/024 False Oat		653.9			1227.23	0.5200	18	22.08
04/109 Mat Grass		340.84		418.27		0.1809	Ergotamine	28.20
05/018 M Fescue	7334.93			1653.48		0.5345	Ergotamine	41.83
05/071 M Fox	4223.18				4588.30	0.4979	Ergotamine	22.90
04/082 P Rye	14968.2			1306.83		0.4921	Ergotamine	42.38
05/115 P Rye	2004.18			1110.57		0.1812	14	34.10
05/123 P Rye	9175.05					0.5475	Ergotamine	51.06
04/070 T Fescue						0.5017	5	100.00
04/076 T Fescue	1080.58	516.49				0.5046	7	26.66
05/136a T Fescue		714.54	614.03	1081.62		0.5051	5	15.90
05/169 T Fescue	161.63					0.1628	Ergotamine	43.43
04/061 Timothy			1555.65	1253.71		0.5344	Ergocornine	23.85
05/116 Timothy	7206.56			753.98		0.5365	Ergotamine	41.20
05/120 Timothy	445.38			404.16	1268.56	0.2855	13	24.81
05/016 Y Fog					5494.16	0.4920	6	27.88
05/154 Y Fog	888.57			374.29	517.41	0.1210	14	27.52

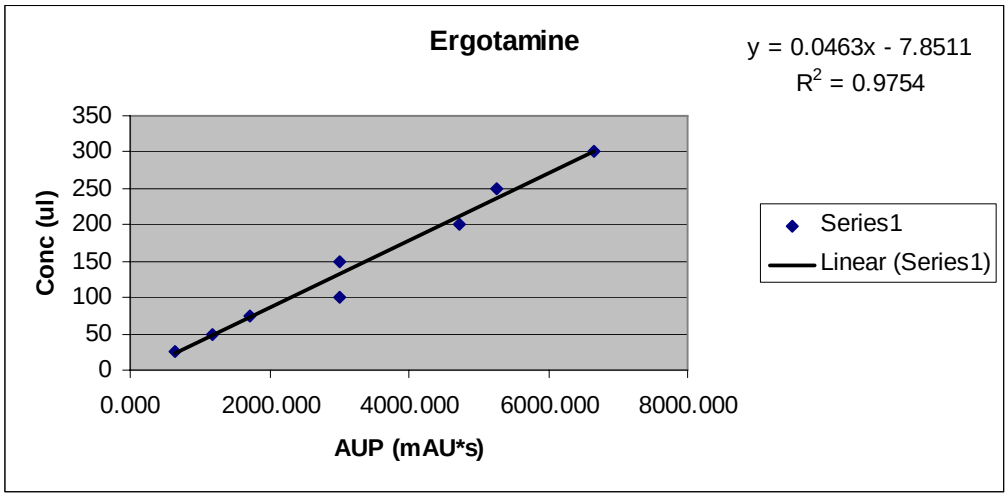
APPENDIX C
(ii) mg ergocornine in samples

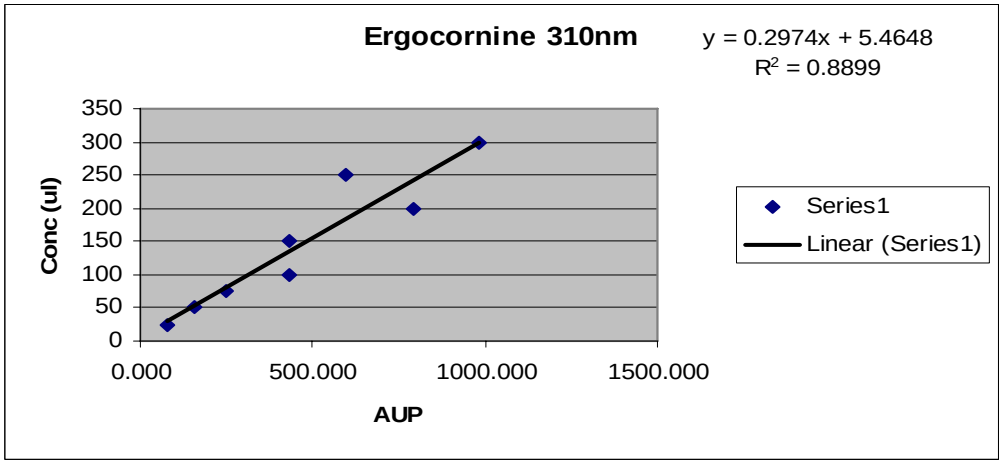
	AUP (mAU*s)	Amt of sample (g)	Conc (ug/ml)	Vol of MeOH (ml)	ergocornine extract (mg)	mg/g	mg/100g
04/091a BG	570.73	0.5015	175.2	0.5	0.088	0.175	17.5
04/035 Cocksfoot	698.17	0.5150	213.1	0.5	0.107	0.207	20.7
04/068b Cocksfoot	653.88	0.5093	199.9	0.5	0.100	0.196	19.6
05/019 Cocksfoot	1715.31	0.4914	515.6	0.5	0.258	0.525	52.5
05/112 Cocksfoot	1108.28	0.4875	335.1	0.5	0.168	0.344	34.4
05/134 Couch	2883.44	0.4965	863.0	0.5	0.431	0.869	86.9
05/011 False Oats	8403.81	0.4951	2504.8	0.5	1.252	2.530	253.0
05/024 False Oats	878.46	0.5200	266.7	0.5	0.133	0.256	25.6
04/109 Mat Grass	149.94	0.1809	50.1	0.2	0.010	0.055	5.5
04/076 T Fescue	401.04	0.5046	124.7	0.5	0.062	0.124	12.4
05/136a T Fescue	532.65	0.5051	163.9	0.5	0.082	0.162	16.2
04/061 Timothy	1732.72	0.5344	520.8	0.5	0.260	0.487	48.7
05/016 Y Fog	485.9	0.4920	150.0	0.5	0.075	0.152	15.2

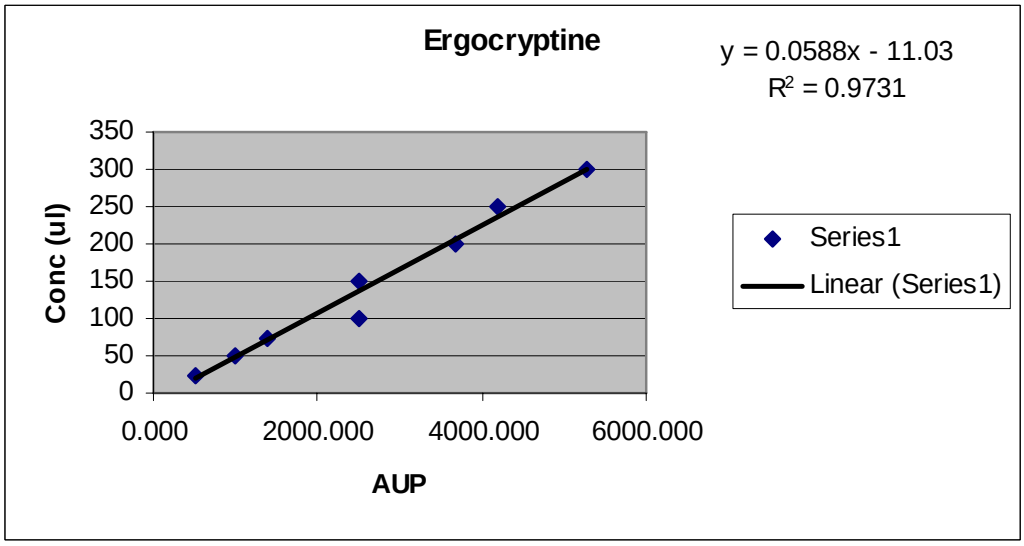
APPENDIX C
(iii) mg ergocryptine in samples

	AUP (mAU*s)	Amt of sample (g)	Conc (ug/ml)	Vol of MeOH (ml)	Ergocryptine extract (mg)	mg/g	mg/100g
05/063 Bent	1241.60	0.1912	84.0	0.2	0.017	0.088	8.8
04/035 Cocksfoot	8464.93	0.5150	508.8	0.5	0.254	0.494	49.4
04/068b Cocksfoot	3149.08	0.5093	196.2	0.5	0.098	0.193	19.3
04/093c Cocksfoot	114.68	0.1980	17.8	0.2	0.004	0.018	1.8
05/019 Cocksfoot	619.25	0.4914	47.4	0.5	0.024	0.048	4.8
05/112 Cocksfoot	4741.48	0.4875	289.8	0.5	0.145	0.297	29.7
05/134 Couch	1195.44	0.4965	81.3	0.5	0.041	0.082	8.2
05/024 False Oats	1084.90	0.5200	74.8	0.5	0.037	0.072	7.2
05/071 M Fox	3735.62	0.4979	230.7	0.5	0.115	0.232	23.2
05/016 Y Fog	4467.29	0.4920	273.7	0.5	0.137	0.278	27.8

APPENDIX D:
Linear Relationships in alkaloid standards

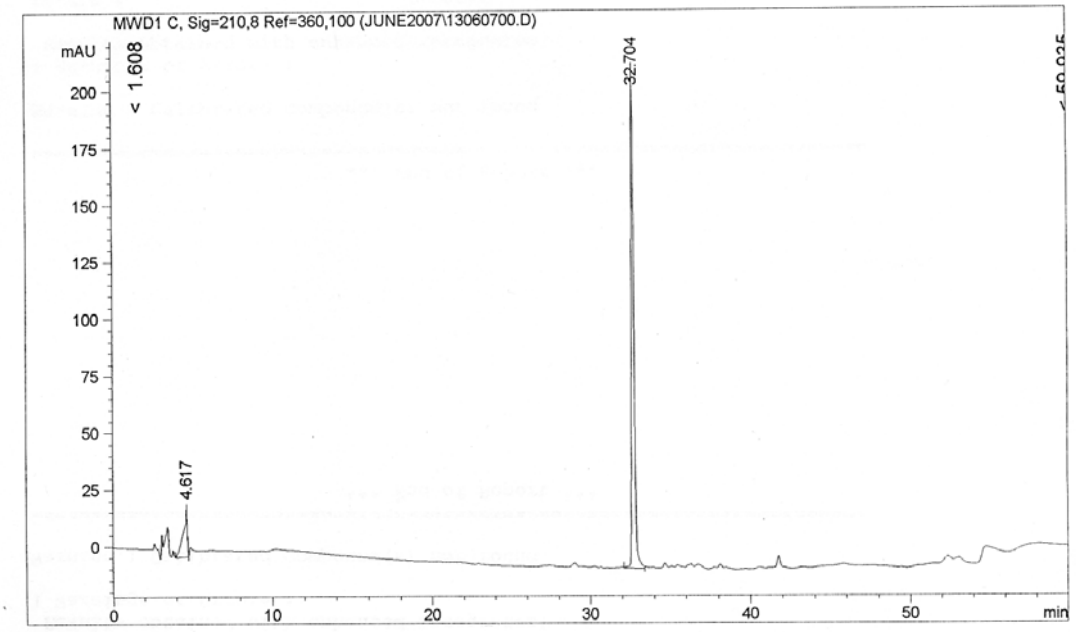




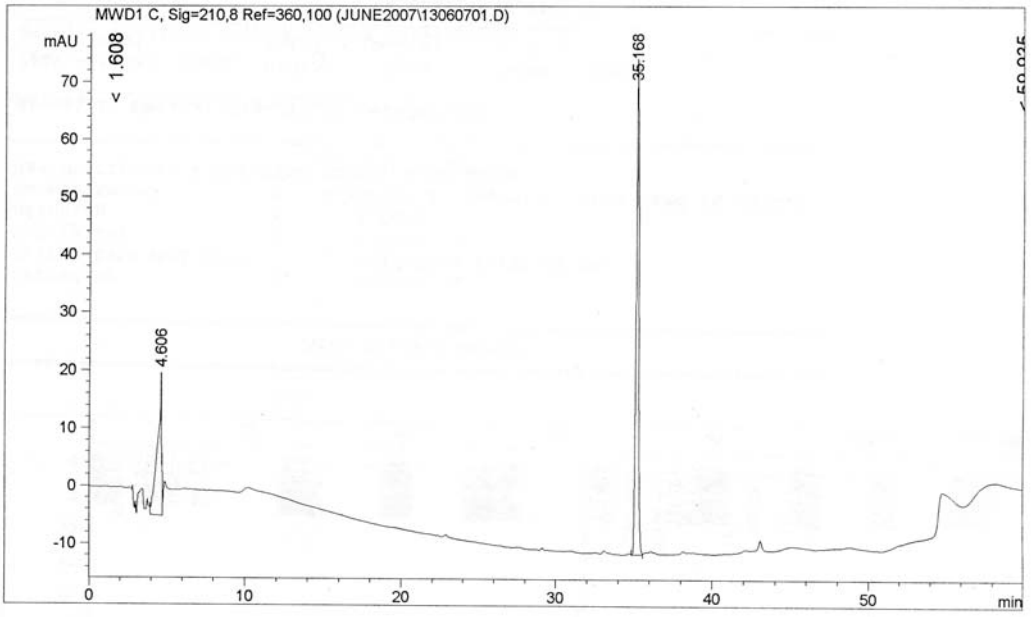


APPENDIX E
Chromatograms

Ergotamine

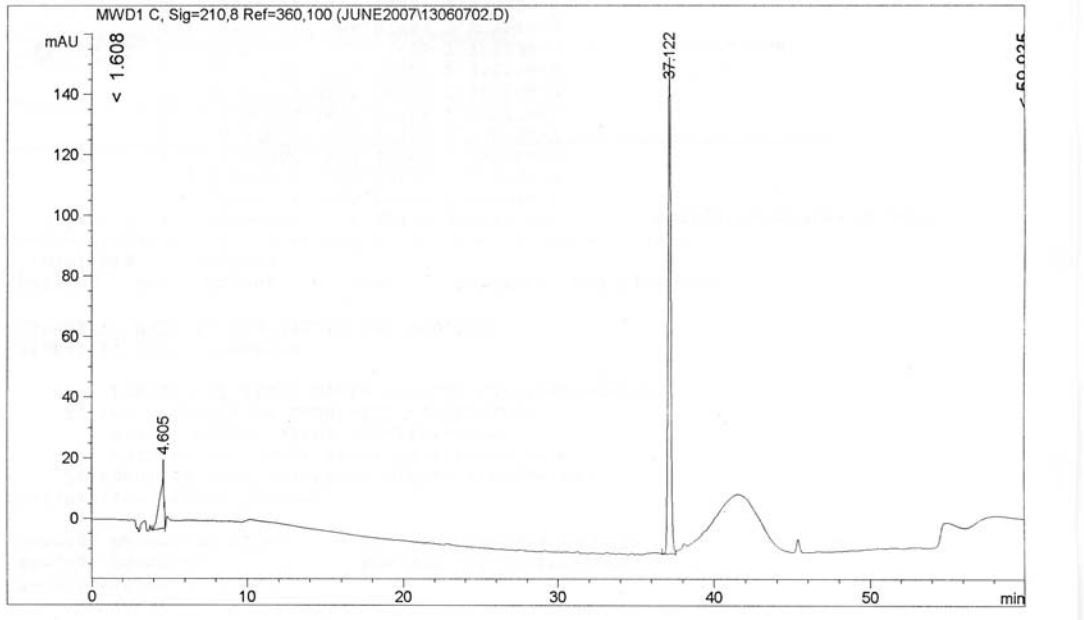


Ergocornine

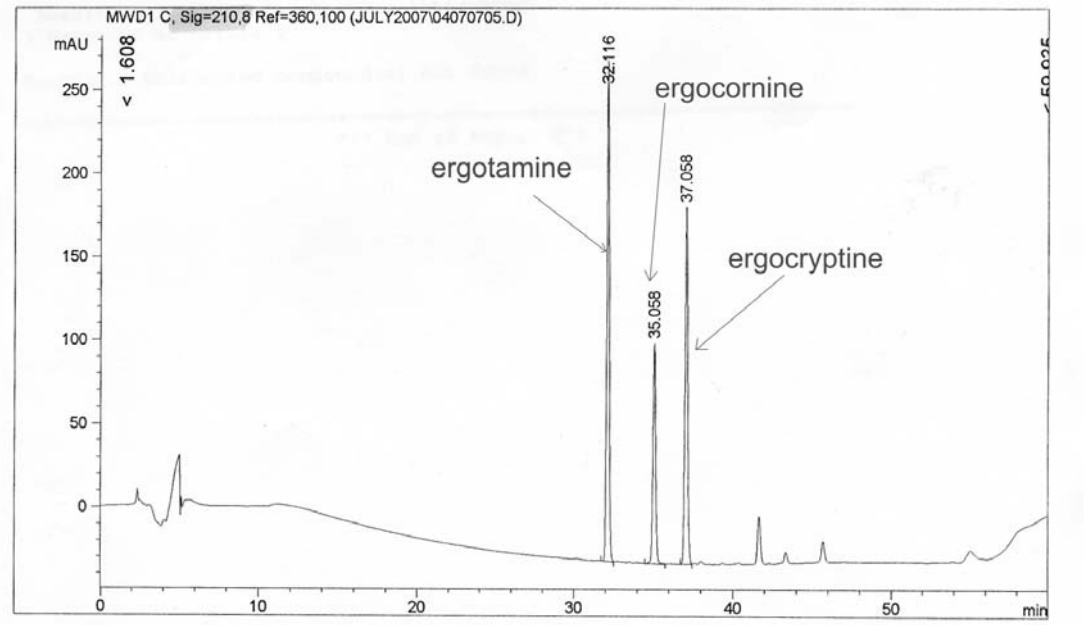


APPENDIX E
Chromatograms

Ergocryptine

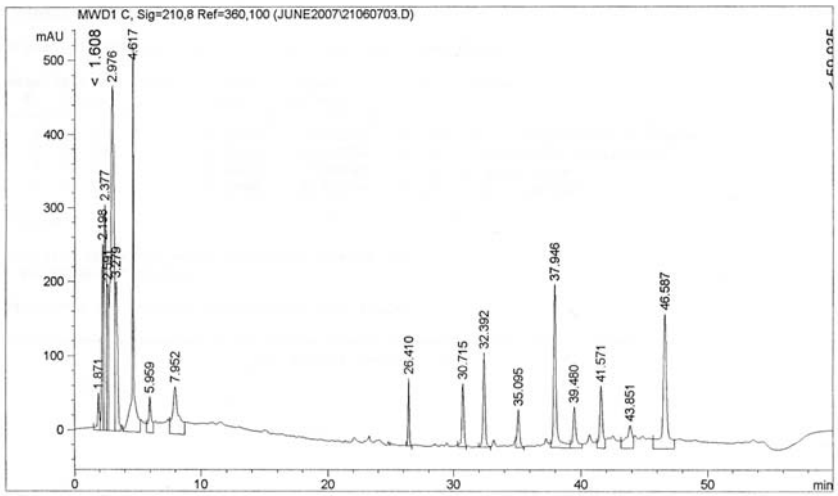


Mixed standards

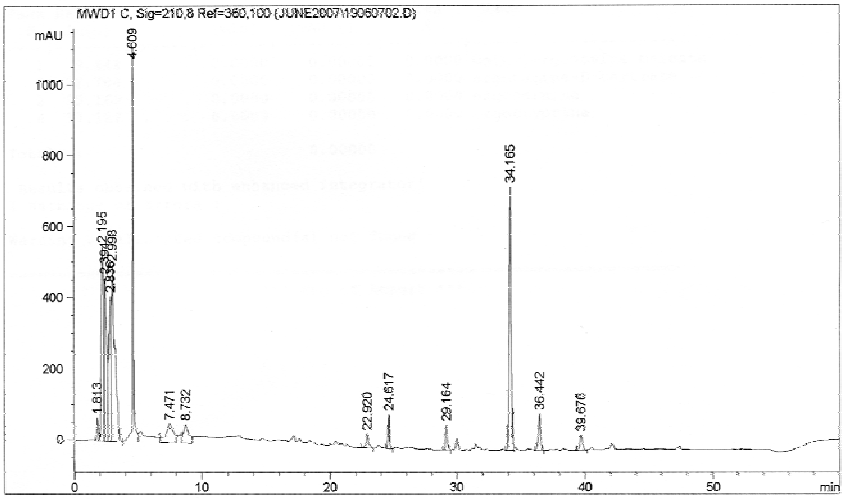


APPENDIX E
Chromatograms

04/068 Cocksfoot

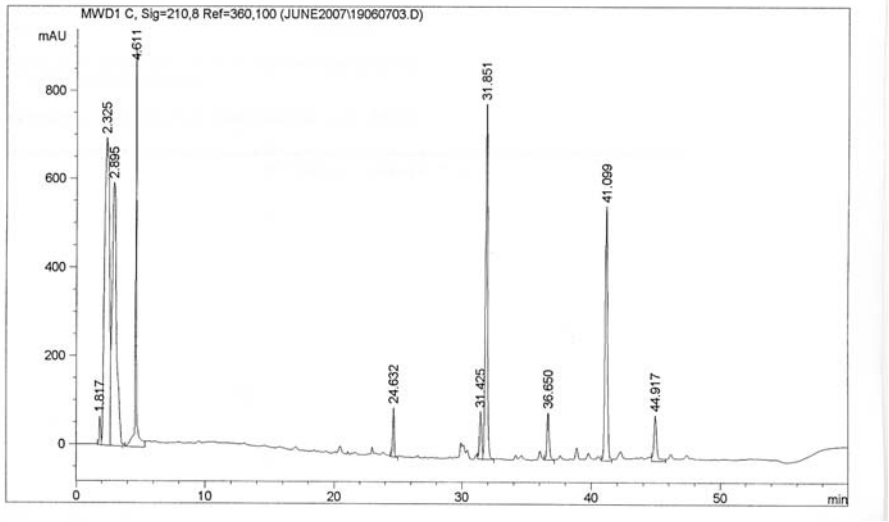


05/011 False Oat Grass

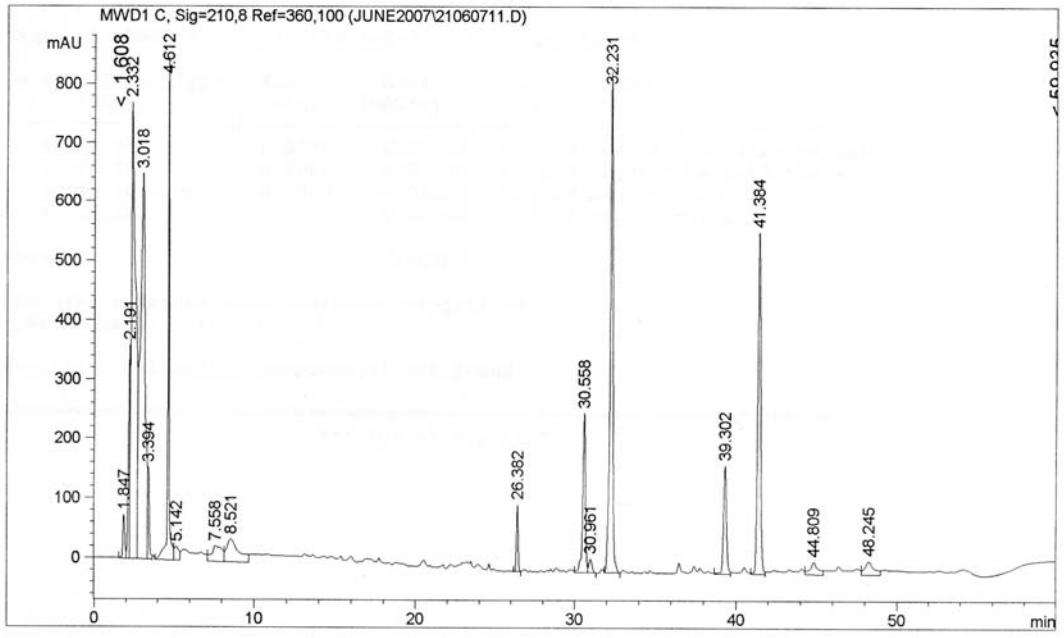


APPENDIX E
Chromatograms

05/018 Meadow Fescue

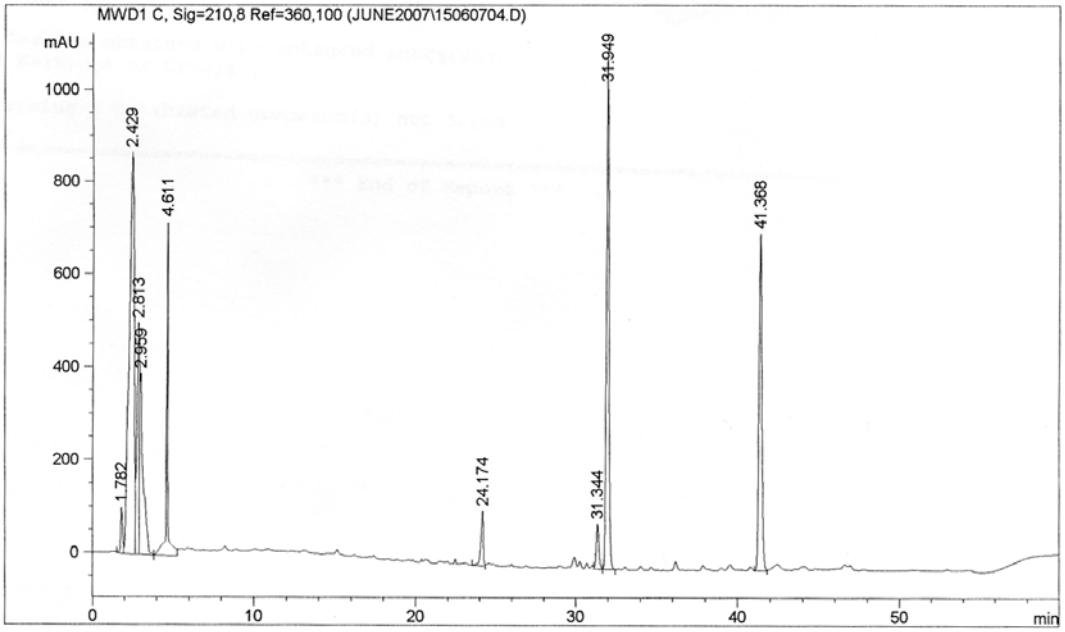


05/116 Timothy



APPENDIX E.
Chromatograms

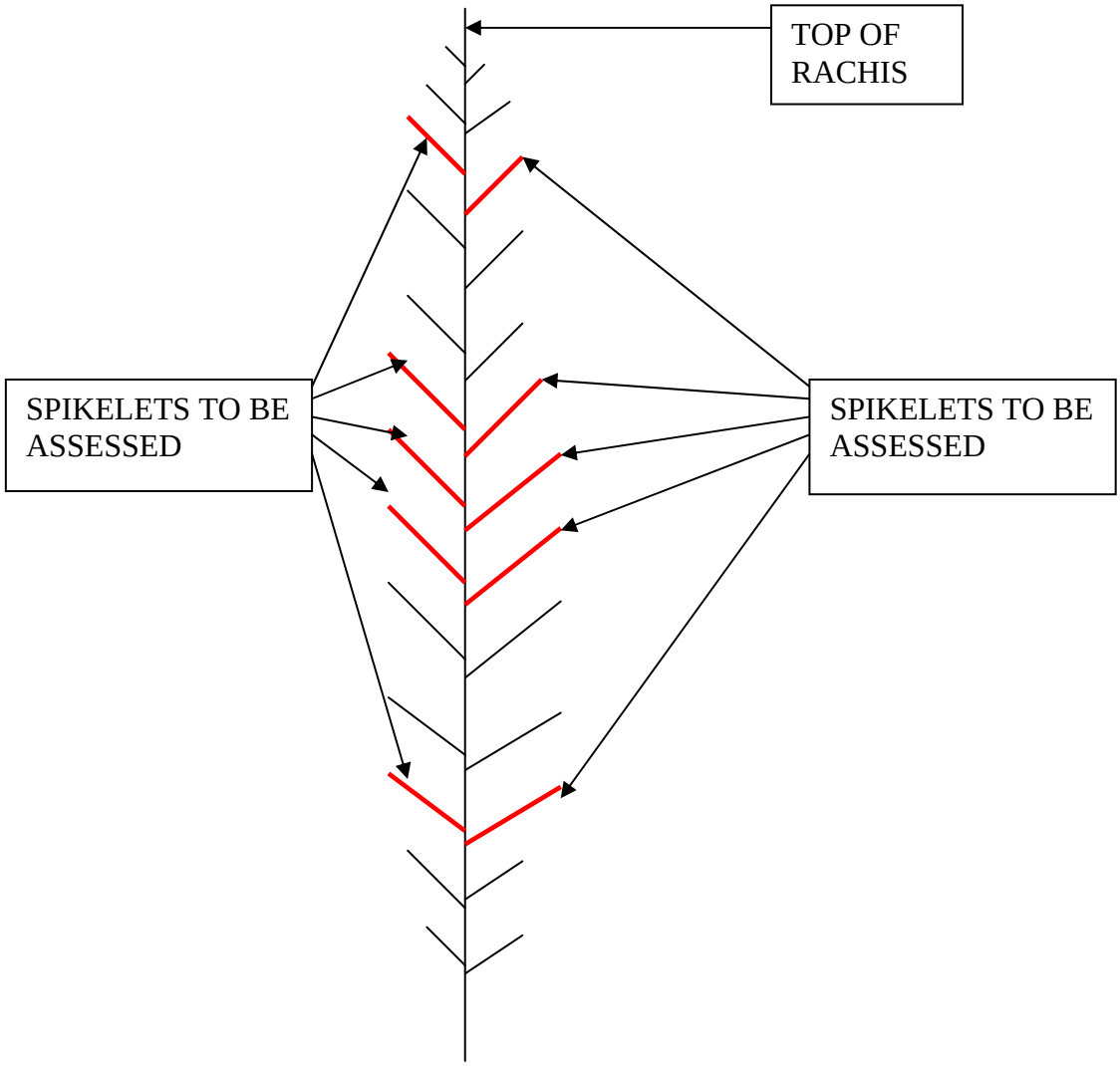
05/123 Perennial Ryegrass



APPENDIX F

Selection of florets for assessment of trapped anthers

10 heads per variety are selected at random for sampling.
Assessments are carried out on the first and second florets of each of 10 spikelets per head, as shown in the diagram below, which shows the side of a wheat head.



Two spikelets are selected near the top of the rachis, six in the middle of the rachis and two at the bottom of the rachis, giving a total of 10 spikelets. With two florets examined per spikelet, this gives a total of 20 florets per head.

The two outer florets of each spikelet (florets 1 & 2, see diagram below) are assessed by separating the palea and lemma with the aid of fine forceps or a needle and counting the number of anthers in the following categories: -

1. Fully retained within the lemma/palea
2. Trapped at the tip of the lemma/palea
3. Fully extruded (lost).

At NIAB, the numbers of anthers in each of the categories 1, 2 and 3 was counted and then expressed as a % anthers extruded (or not) from a potential total of 60 anthers per ear (20 spikelets x 3 anthers). The data from categories 1 and 2 was combined – regarding them both as retained – to give the % of florets with trapped anthers.

Nickerson used a similar approach but assigned an overall score of 1, 2, or 3 to each floret, rather than assessing each anther. As for NIAB, the data for categories 1 and 2 was combined to give an overall % of trapped anthers.

The RAGT data was based on the criteria of A = anther attached, T = anther trapped but not visible and TV = anther trapped and visible. Attached implies the anther was low down, adjacent to the ovule, T that the filament had elongated pushing the anther up but it had not come out of the floret, and was not visible from the outside. TV suggests the anther is visible from the outside (just inside a gaping floret or up to about half extruded) but stuck between lema and palea. As with NIAB and Nickerson, the proportion in categories A and T were combined to give total retained anthers and then expressed as a % of the total anther number.

Diagram of Spikelet.