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STERILITY IN WINTER WHEAT: REVIEW OF OCCURRENCE IN DIFFERENT VARIETIES AND POSSIBLE CAUSES

by

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1. Introduction

Sudden reductions in fertility leading to major reductions in final yield are not uncommon in wheat, both in the UK and elsewhere. The most notable and recent example of this occurred in the mid-1980's with the variety Moulin where sterility induced by the particular environmental conditions at that time caused reductions in yields of over 70 percent. However, there have been other recent years when sterility leading to reduced seed set is believed to have affected other wheat varieties. The majority of these effects have proved to be minor, the overall influence on yield being negligible. However, in a few instances, failures have been large. This has led to worries that another Moulin episode may be about to hit UK agriculture. The most recent alarm occurred at one trial site near Aberdeen in Scotland in 1997 where the yields of several varieties and lines were drastically reduced.

An HGCA funded investigation was carried out by the Scottish Agricultural College (SAC) into the extent and magnitude of the sterility at two Scottish sites, Aberdeen and Kelso, and its relationship to final yield. At both sites, a strong correlation was found between the level of seed setting and final yields. At Aberdeen, percent fertility as measured by seed setting was as low as 30 percent in one variety but even at the more fertile site, percent's were down to 50 percent (Hoad, *et al.*, 1999). This has prompted the questions "To what extent are final yields vulnerable to fluctuations in percent fertility?" and "Are such sub-clinical levels of sterility/fertility important to the stability of the wheat crop generally?". This is apart from the potentially catastrophic events that might occur if the large reductions in fertility that happened at Aberdeen in 1997 were to follow the Moulin example and occur more widely. Also, were the Aberdeen and Moulin events, separated by 10 years, related in any way? Or, were they completely unrelated and have different genetical and/or environmental causes?

The answers to such questions are important to the future of the wheat crop in the UK.

For these reasons it was thought necessary, whilst continuing with the experimental work on the relationships between fertility and final yield, to review the whole field of fertility in wheat. Fertility problems are not confined to the UK and such problems occur world wide. What is the experience of wheat growers in other countries and can their findings and opinions be relevant to the UK? One aim of the review will therefore be to search for evidence of such events in other countries. Another will be to re-look at the events leading up to and beyond Moulin. What were the weather conditions at the time? Were they abnormal and why was it that Moulin and no other variety turned out to be so vulnerable? What has happened during the intervening years up to and including the problem results occurring at Aberdeen in 1997? A major purpose of the review will be to examine potential genetical and environmental causes of infertility to see whether it is possible to identify the most likely basis for the effects observed in winter wheat in the UK.

The ultimate aim of the review will be to assess whether there is a potential problem of fertility waiting to emerge in the wheat crop of the UK, and if so what should be done about it. It is hoped that, as a result of the review, recommendations can be made for introducing additional safeguards during the testing of future varieties, providing guidance in planning breeding programmes and highlighting important areas that need to be researched.

2. Origins of sterility

There are three origins of sterility in wheat:

1. Male Sterility. This is the most common. Usually, there is a failure to produce sufficient viable pollen but associated with this can be a stunted development of the anthers themselves, and in extreme cases, a complete failure of anthers to develop.

2. Female Sterility. This is rare but as the description of the genetical causes of sterility indicates on page 37, the rather drastic removal of whole chromosomes can give rise to female sterility, sometimes without affecting male fertility. There are no reports of female sterility occurring under field conditions in the UK.

3. Asynchrony. On occasions an asynchronous development can occur between the anther and stigma so that the stigma, for instance, may no longer be receptive at the time the anthers are ready to dehisce with viable pollen. When this occurs, ears often gape and are prone to out-crossing. A critical factor in determining whether asynchrony will result in a failure to set seed is the length of time the stigma maintains its receptivity and the pollen its viability. If these are both a matter of several days then there is a good chance of overlap and successful fertilizations will occur. If they are short then the chances are reduced. There is little information about the length of these periods in wheat but it is thought that they vary from one variety to another. Many breeders believe that asynchrony occurs frequently in wheat and can be brought on when the crop is subjected to stress about the time of flowering. It could well be imagined that a variety with a small window of receptivity could easily have fertility problems when exposed to say a sudden frost in late May or early June. Unfortunately, there is little knowledge about the extent of the differences in the durations of receptivity between varieties. Nor to what extent selection may be changing this character. This might be particularly the case in hybrid wheat breeding where longevity of pollen and asynchrony may have positive advantages, and this could inadvertently feed into conventional breeding programmes.

The sterility problems discussed in this review are thought to be due to male sterility, certainly this is known to be the case for Moulin and is most probably the same at Aberdeen. Several of the other examples of infertility presented have also been established as being due to male sterility. However, many instances, are not clearly described and induced asynchrony cannot be ruled out as being a contributory cause to the infertility reported. There is a need to examine the control of this character in the future and whether it has important consequences for infertility.

3. The history of the variety Moulin

3.1 Origin

The variety Moulin was bred at the former Plant Breeding Institute and was submitted for National List trials in 1981 and 1982 followed by Recommended List trials in 1983. Moulin was derived from a complex cross:-

Moulin = {Yecora X Ciano 67} X {Maris Widgeon X Hobbit sib}

> Yecora = Ciano sib/3/Sonora 64/Klein Rendidor//Siete Cerros 66
>
> Klein Rendidor (Argentina) = Klein 33[*Ppd1*]/Sinvolocha[*Ppd2*]
>
> Ciano 67 = Pitic 62/Chris/Sonora 64
>
> (*Ppd1* and *Ppd2* refer to day-length insensitive genes thought to be present in these varieties)

An important and possibly crucial feature of this cross is the use of unadapted germplasm stemming from CIMMYT, Mexico, i.e., Yecora X Ciano 67. The possible significance of this is discussed later.

3.2 Behaviour of Moulin in trials

Throughout its progress in NL and RL trials, Moulin was plagued with unacceptably high levels of off-types although it gave high yields and was of outstanding bread-making quality. It was widely recognised at the time that the off-types were due to the tendency for this variety to out-cross. For this reason Moulin was not given National List status in 1983 although at the time it was accepted and grown commercially in France and Belgium. This decision was appealed against in 1984. At the Tribunal, a low level of out-crossing in this variety was acknowledged by the appellant, but they maintained that, under the conditions of commercial seed production, this was unlikely to lead to unacceptable levels of off-types because most out-crossing would occur within the variety. In contrast, the system of assessment for distinctness, uniformity and stability (DUS) used for registration purposes at the time of Moulin would tend to encourage out-crossing with other varieties leading to an enhanced and, as far as registration was concerned, misleading level of off-types. This argument was accepted by the Tribunal and Moulin was National listed and then added provisionally to the Recommended list in the Autumn of 1984 for the 1985 list.

In 1985, Moulin performed poorly in NIAB trials around the country and was 8 percent down in yield compared to previous years. This poor performance is indicated in Fig 1 where the deviations in yield between the site mean and that of Moulin and two other varieties, Brock and Boxer, are shown. Clearly, Moulin under-performed for almost all of the 22 sites. These results were circulated prior to the Autumn drilling of that year, and a warning note about the possible problems with Moulin sounded in the press by the National Seed Development Organisation and by NIAB in their provisional recommendation for 1986.

NSDO in their press release commented upon the poor seed set in 1985, resulting in a reduced number of grains per ear as being responsible for the lower yield. The reason for this was unknown because in previous years Moulin had yielded well with excellent quality. However, it was thought that "low temperatures coinciding with a critical development stage played a part, and that the pollen of Moulin was less effective when flowering time coincided with cold wet weather". NIAB encouraged growers to exercise restraint with Moulin until the problems of 1985 had been resolved. They also issued a Press Notice which stated:

"The Council of the NIAB wish to draw the attention of growers to the disappointing performance of Moulin in 1985 when yields were well below the level expected. The reduced yields appeared to be linked to poor grain set in the ears. The reasons for this are not known, but possibly adverse weather at a critical growth stage played a part. In many affected crops, sprouted grains were evident and therefore quality in Moulin as well as yield was disappointing."

In 1986, Moulin performed reasonably well as is illustrated in Fig 2. Yields were more or less close to the site means, and there was little evidence of reduced seed set. However, virtually all seed stocks developed in 1985 for 1986 were down-graded because of off types due presumably to the out-pollinations that had occurred in 1985. In their review of the variety in the Autumn of 1986, NIAB was still worried about the variety and opted to be cautious and removed it from the Recommended List after completing its two years in the Provisional Recommended category. This was despite the fact that flour millers and bread makers spoke highly of the variety and were encouraging growers. There was much low-priced down-graded seed available for sale in the Autumn of 1986.

About 100,000 acres of Moulin in the UK and a large area in France were sown in autumn 1986. In the following year, male sterility leading to drastic losses in yield was widespread in the UK and France. Fig 3 shows this in stark detail for the UK and demonstrates that Moulin under performed to the tune of 20 to 40 percent of the mean for all 16 NIAB trial sites. Some farmers reported that their yield returns from Moulin were less than one ton per acre compared to an expected three to three and half tons. Some crops were so poor that they were used for silage. There was widespread alarm which resulted in many growers instigating legal actions against their seed suppliers and the breeder PBI. Questions were also raised about the role of NIAB and whether it had done enough to alert growers to the potential problem with Moulin.

[insert fig 1 here]

[insert fig 2 here]

[insert fig 3 here]

3.3 Scotland

In 1985 trials were carried out separately by SAC in Scotland and not, as is the case today, as part of a UK trials system co-ordinated by NIAB. Although there was not much interest in a quality wheat for Scotland, Moulin featured in three trial sites but, as in the NIAB trials further south, the yields were disappointing.

Table 1. Yield results at three Scottish sites in 1985. The yields are expressed as a percentage of controls for fungicide treated (F^+) and untreated (F^-) plots.

Variety	Haddington		Oldmeldrum		Lockerbie	
	F^+	F^-	F^+	F^-	F^+	F^-
Avalon	126	107	156	113	121	103
Mercia	135	117	165	117	128	117
Moulin	112	82	166	107	79	68

The causes of the lower yields of Moulin were not investigated. In 1986, the yields of Moulin were satisfactory but by then there was a general awareness of the fertility problems of Moulin so further trials with the variety were discontinued. The SAC results therefore parallel those obtained from further South.

3.4 Symptoms associated with the loss of fertility

One of the first signs are gaping ears which on closer examination reveal poor anther development, often giving small pale anthers with little if any pollen. In some instances, cross-pollination takes place, after which gaping ears close, resulting in some seed set. Often this is a consequence of out-pollination.

3.5 Causes of the sterility

Following the disastrous collapse of Moulin in 1987, PBIC instigated a research project to determine the conditions that were responsible for inducing the sterility in Moulin. This was paid for by PBIC and undertaken by the then Institute of Plant Science Research, now subsumed into the John Innes Centre. As a consequence of this research, PBIC use routinely a test for the Moulin effect on all their varieties and lines that have been submitted to national list trials in the UK, France and Germany. The test involves the imposition of low light intensities and temperatures at or about microsporogenesis (P I Payne, PBIC, personal communication). Other breeding companies, e.g., Elsoms Seeds and Nickersons, have also instituted tests which involve reducing light levels usually by erecting screens to produce shading in the field at certain stages of development. The majority of breeding companies however rely on their multi-site testing to pick up any prospective Moulin effect. Given the history of Moulin which was screened widely before its release in 1985, such an approach would seem to be unwise.

The only published work on the effects of environmental variables on the induction of pollen sterility in the variety Moulin was undertaken by the French. They showed that low irradiance rather than low temperatures at about the time of meiosis produced marked reductions in pollen viability in Moulin compared to the variety Pernel (Demotes-Mainard, *et al.*, 1996). This supports the reports of the PBIC work.

3.6 Conditions

It would be impossible in a review such as this to research all aspects of weather differences on a day by day basis at all the sites in which Moulin failed or partially failed in the years 1985 and 1987 and then to compare them with the weather patterns in a good Moulin year such as 1986. I have therefore concentrated on the weather records for May and June collected by IACR-Broom's Barn, Higham, Bury St.Edmunds, Suffolk over the years 1981-1999. The months of May and June were chosen because they straddle with some margin of error both the early stages of meiosis and right through to the completion of microsporogenesis, the period generally acknowledged as containing the sensitive stage or stages during which male-sterility is induced. The mean air temperatures, mean amount of sunshine per day and total rainfall for these two months are presented in Figs. 4, 5 and 6.

Considering 1987 first, the most dramatic of the Moulin years, temperatures were low and much below average in both May and June and these were complemented by similarly low sunshine levels. Rainfall was also very high and much above average in June. Broom's Barn from 1987 onwards have published a summary of the weather for each month. The following is what they had to say for May and June in that year.

May: "The warm weather which gave rise to the record temperatures in April quickly disappeared during early May. Temperatures for May were 1^{o}C below average with both sunshine and solar radiation 5 percent below average. Rainfall was average but occurred mainly in the form of showers making May more typical of a warm April."

June: "1987 has given rise to the wettest June ever recorded (to 1987 that is, 1997 was even higher, Author) at the station by a margin of some 23mm with a monthly total two times the June average. Reflecting the overcast nature of the month both sunshine and solar radiation were 50 percent and 25 percent below the averages expected. Most temperatures were 1^{o}C below average."

[insert fig 4 here]

[insert fig 5 here]

[insert fig 6 here]

In 1987 therefore both low temperatures and sunshine levels occurred in May and June. The more marked effect, along with an unprecedented high rainfall, took place in June. In 1985 however, below average drops in temperature and sunlight also happened although not to the extent of 1987. This might account for the less dramatic failures of Moulin in 1985 compared with 1987. By contrast, temperatures and sunshine levels were higher in 1986, being either close to average or above. This is the year when Moulin returned to its previous high yield levels.

There is thus a pattern discernible amongst the weather data from Broom's Barn which fits the earlier reports by NSDO and NIAB of cool, cloudy weather being responsible for the adverse behaviour of Moulin in 1985 and 1987. It also ties in with the results of the French experiments and the reports of the PBIC test. Perhaps the best predictors are the June data which show the largest differences between 1986 and the disaster years of 1985 and 1987. It would of course be valuable to analyse these data further to look at day to day differences to pin-point the critical stages more precisely and to try and show that these effects occurred at other sites in the UK but as already mentioned this would be an enormous task, more suited to a special study of weather patterns and crop development.

3.7 Moulin since 1987

Moulin has been grown in PBIC trials as small plots in Cambridge from 1987 onwards. No effects on male sterility similar to those found in 1987 have been observed (Information provided by PBIC). This is rather remarkable because the weather patterns emerging from the Broom's Barn data (Figs 4, 5 and 6) indicate that critical conditions occurred not just in 1985 and 1987, but also in 1991 and possibly also in 1995. As will be mentioned in the next section, some reports of male-sterility were reported in these years but, because Moulin had been withdrawn from commercial growing, in varieties other than Moulin.

3.8 Post-Moulin, 1987 onwards.

No dramatic collapses in fertility on the scale of Moulin have occurred since 1987. However, some instances of what might be regarded as isolated outbreaks have been observed by breeders in their trialling systems. At Nickersons Seeds Ltd., at Woolpit in Suffolk, close to Broom's Barn, the low temperatures and levels of sunshine were also recorded in 1991 during May and June. Observations on breeding material, advanced lines as well as established varieties listed some as showing symptoms of male-sterility (W.J. Angus, personal communication). On the other hand, NIAB at Cambridge, also close by, gave no indication of sudden failures in yield in their RL and NL trials for 1991. However, no observations were made, as indeed is the case to-day, at the time of anthesis to see whether there was a low level of sterility occurring in the trials, and which because it was at a low level could be compensated for without adversely affecting yield. The major difference between 1991 and 1985 and 1987 is the absence of the variety known to be susceptible to these conditions, Moulin.

4. Trials at Aberdeen and Kelso in 1997

In 1997 dramatic reductions in yield were observed in the official trial sites in Scotland, particularly at Aberdeen and to a much lesser extent at Kelso. In this case, SAC undertook a study of the causes and were able to establish that low seed set was responsible for the reduced yields. By taking ear samples, percent fertility scores were obtained at both Aberdeen and Kelso. These were correlated between sites although the mean fertility at Kelso was higher than at Aberdeen. Similar correlations were also found for fertility with yield, again at both sites (Hoad *et al.*, 1999). A large proportion of the yield differences could therefore be explained by variation in percent fertility.

Observations at Aberdeen at the time of flowering highlighted gaping ears which were prevalent in the varieties giving reduced fertility. This suggested that pollen fertility was the cause, although no close examination of the pollen was undertaken. The marked reductions in yield were most pronounced at Aberdeen and occurred in all trials, RL, NL1 and NL2 (see Appendix A). Varieties such as Equinox, Pentium, Caxton as well as a number of unnamed lines were badly affected.

Examination of the RL residuals - a measurement of variety x site interaction - for yield across all 24 sites pin-points Aberdeen as the site with the greatest number of and largest interactions (see Appendix A). The residuals for the Kelso site were by comparison negligible and not very different from the values obtained at other sites. Similar marked interactions due to major reductions in yield occurred within the NL1 and NL2 trials at the Aberdeen site. It would appear that although fertility levels were correlated between the two sites, the levels of fertility at Kelso were not reduced sufficiently to prevent some compensation taking place between yield components to minimise the effects of reduced grain set.

Aberdeen was therefore the site in 1997 which stood out as giving anomalous and rather dramatic yield results. All other sites including Kelso gave only a few significant negative and positive residuals and their incidence did not fall into any pattern. Similar results occurred for the trials in 1995 and 1996, but in these cases the Aberdeen site fitted the general pattern and was no different from elsewhere. In 1998, the pattern was again similar but the results from the Aberdeen site were not available.

The full data relating to these sites over years are given in Appendix A.

4.1 Comparisons with Moulin

Many of the varieties and lines grown at Aberdeen in 1997 were linked to Moulin in their parentage (Hoad, *et al.*, 1999). This is brought out in Appendix B where the parentages are described. In some cases, Moulin was a direct parent, but more often than not, Moulin was used as a grand parent or great grand parent. Haven, for instance, features frequently as a parent but was derived from a three way cross (Hedgehog x Norman) x Moulin. About half of the genes in Haven could therefore be derived from Moulin. Similarly if Haven was then used as a parent in a single cross then a quarter of the genes in any derivative from the cross could be from Moulin. This of course ignores selection so that the actual number of Moulin derived genes could be more or less than these proportions. However, it is possible, using these purely chance proportions, to derive a crude "Moulin" score for each of the varieties and lines grown at Aberdeen and test to see whether there are any indications of a relationship with yield or the difference between the yield at Aberdeen and the overall mean across all sites. When this is done, there is no correlation between the "Moulin" score and these two parameters, nor indeed the "Rendezvous" score (another variety used frequently as a parent and thought to contribute genes for sterility) in either the RL or NL2 trials . However, in the NL1 trial the correlation between the Moulin score and both parameters is highly significant (r = - 0.377 ± 0.123, P 0.01-0.001 for yield, and r = 0.434 ± 0.087, P < 0.001 for the difference in yields) (see Appendix B). The main reasons for this are the three lines provided by Hybritech, all of which have Moulin in their immediate parentage and gave dramatically low yields at Aberdeen. When these three "outliers" are removed from the analysis, the correlations are no longer significant (r = - 0.058 ± 0.136, P 0.69 for yield, and r = 0.150 ± 0.135, P 0.23 for the yield differences) (again, see Appendix B).

In the one trial therefore, there is some evidence that Moulin may be responsible for the lowered yields, whereas in the other two trials the evidence is non-existent. This latter result of course does not completely rule out a connection with Moulin or the genes responsible for the Moulin effect. Those varieties not reacting adversely in Aberdeen and having Moulin in their parentage may have lost the critical genes as explained above, whereas those varieties reacting adversely but not connected to Moulin may have received the critical genes through an alternative route. An example of this might be Pentium whose parentage is Rendezvous X Talon, neither of whom show any link with Moulin or its parents.

A major difference between the results in 1997 and those in 1985 and 1987 is the widespread failure of Moulin in the earlier years whereas, for current varieties, only one site appears to be affected in 1997.

4.2 Environmental conditions.

Meteorological data obtained at Craibstone near to the Aberdeen site indicate that mean temperatures in May and June 1997 were respectively, 8.5^{o}C and 11.6^{o}C. Both these figures are below the suspected male-sterile inducing temperatures recorded further south for the critical Moulin years. Rainfall figures in both months were very high, 135 and 148mm in May and June respectively. Both these figures are higher than the highest rainfall figures registered at Broom's Barn in June 1987 for the Moulin years. This was regarded as one of the wettest June's on record so that, on this criterion, the two months in Scotland were extremely wet!

Unfortunately sunshine levels were not recorded at Craibstone so that a full comparison with the records collected in the south cannot be made. Nevertheless, the temperatures and rainfall at Craibstone are consistent with those that occurred in 1985 and 1987 at Broom's Barn and which are being held responsible for inducing male sterility in Moulin. Also, records of mean temperatures and rainfall taken at Craibstone during May and June from 1990 onwards point to 1997 being an abnormal year with temperatures being lower and rainfall, in particular, being well above average (see Figs. 7 and 8).

4.3 Screening

Apart from perhaps Hereward, none of the PBIC varieties at Aberdeen in the RL trial gave anomalous yields. However, in the NL1 and NL2 trials several of the lines originating from PBIC are affected and give reduced yields but not as seriously as some varieties in the trial. The results of these trials for the PBIC submissions are shown in Tables 2 and 3.

PBIC have stated that all the lines had been through their Moulin test so this would seem to imply that the effects observed at Aberdeen are not due to Moulin and a different syndrome is involved. Or, alternatively, their test is unreliable. PBIC when questioned about this, indicated that their test always involves Moulin and Mercia as controls and they look to see whether sterility is significantly lower than that of Mercia. Usually Moulin gives about 8 percent and Mercia 90 percent fertility in tests. In the tests carried out for the NL1 and NL2 submissions, none of the lines were anywhere near to Moulin, but some were significantly lower in fertility than Mercia. When these differences were compared with those observed at Aberdeen, nine of the lines agreed i.e., had low or high fertilities in both the test and at Aberdeen, and five did not (Peter Payne, personal communication). This therefore suggests that the variation observed at Aberdeen is not related to Moulin and that the reduced fertilities detected in the test are not related either. It further suggests that the low light and temperatures used in the Moulin test are not the same as those inducing the sterility conditions at Aberdeen.

[insert fig 7 here]

[insert fig 8 here]

Table 2: PBIC lines in NL1 trials 1997: calculated as for the NL2 trial data above. Only treated plots were harvested for yield

Variety	**Overall mean**	**Aberdeen**
CWW 96/47	95	90
CWW 96/48	98	86
CWW 96/119*	90	41
CWW 96/17	108	114
CWW 96/20	102	95
CWW 96/21*	95	53
CWW 96/39*	89	56

* Lines doing badly at Aberdeen

Table 3: PBIC lines in NL 2 trials 1997: yield of grain at 15% moisture expressed as a percentage of the mean of Brigadier, Hereward, Rialto, Hussar and Riband

Variety	**Overall mean**		**Aberdeen**	
	untreated	*treated*	*untreated*	*treated*
CWW 95/41	118	104	116	110
CWW 95/57*	103	91	79	75
CWW 95/9*	100	103	62	83
CWW 95/16*	106	99	81	89
CWW 95/23*	104	97	79	81
CWW 95/26	117	102	110	106
CWW 95/33	100	100	92	103

* Lines doing badly at Aberdeen

4.4 Other differences from Moulin

Moulin before it was provisionally recommended had a history of giving off types and these were recognised during DUS trials. This was accepted as being due to its tendency to outcross. That the cause was male sterility was not acknowledged until after recommendation. By contrast, for the varieties and lines behaving anomalously at Aberdeen in 1997 there was no prior evidence either at DUS or more generally that off-types were a feature of their behaviour.

4.5 Relationship to Moulin

Apart from the three lines in NL1, there would appear to be no direct genetical connection with the 1997 results at Aberdeen and Moulin.   However, some connection cannot be ruled out entirely. If the interaction involving Moulin had a genetical basis which was polygenic then some of the genes implicated in the interaction may still be passed on and be present in the varieties and lines being studied at Aberdeen in 1997.

4.6 Cause of the 1997 result at Aberdeen

It is impossible to be definitive about this. The evidence that there is little direct genetic link between the earlier Moulin results and those at Aberdeen suggests that a different cause is responsible. However, there is a striking similarity with the weather data deduced to be responsible for the Moulin effect and that occurring at Aberdeen in 1997. The three Hybritech lines are most probably responding as Moulin might have done to these conditions. On the other hand, the PBIC screening results which were designed to simulate these conditions were incapable of recognising the sensitivity that appeared amongst their lines at Aberdeen. This suggests that a different genetic base than Moulin was responsible. It further suggests the involvement of a different component of the environment than lowered sunshine levels and temperatures.

At the moment therefore there is no clear answer to this question of causation. Was Aberdeen though a "one-off"? This could be the case since a similarly extreme episode had not been identified previously or has since. On the other hand, the relationship between fertility scores and yield at Kelso was the same as that at Aberdeen. This suggests they are connected and that they have a common genetical basis, unrelated to that of Moulin. The Aberdeen results cannot therefore be just considered as a "one-off" that can safely be ignored.

5. Sterility problems in other countries

5.1 France

As already mentioned, reduced fertility arising from male sterility problems were observed in Moulin in France at the same time as in the UK. Indeed, because Moulin was licensed for sale in France before 1984, instances of out-crossing in this variety were widely being reported before this weakness was recognised in the UK. INRA research workers observed examples of male sterility in soft-milling bread wheats in 1973. These instances seemed to follow on abnormally low temperatures (average temperatures below 10^{o}C for several consecutive days) in the spring, coinciding with stages just before meiosis. Varieties originating from the USA and carrying the dwarfing genes from Norin 10 appeared to be the more vulnerable, as well as lines selected in France and derived from inter-specific crosses with *Aegilops ventricosa*. In 1984, similar cool conditions occurred in Northern France and varieties such as Moulin and Pernel were affected (Doussinault, *et al.*, 1988).

Since the demise of Moulin, there have been reports of isolated cases of reduced fertility in French grown varieties but nothing on the scale of Moulin in 1987.

5.2 Northern Europe

Moulin type problems have not been observed in Denmark, Sweden and Germany. This includes successful material which has been derived from Moulin. At the time the weaknesses of Moulin were identified, Moulin had not percolated through to commercial growers in this region and so the problems with this variety further south were never observed. For a period after 1987, Moulin was grown in breeders' trials for a few years but no instances of sterility were observed.

In Germany, the *Rht* genes derived from Norin 10 have rarely been exploited - possibly because varieties with these genes are too short. There is a similar story further south in Austria, Hungary and Italy where breeders have not found them to be particularly valuable. This is in contrast to the UK, Denmark, Holland, Belgium and northern France. If sterility problems are associated with the introduction of Norin 10 germplasm then this could be a factor in the absence of such problems in some of these countries.

5.3 North America

The variety Norin 10 from Japan was first exploited by Orville Vogel in 1948-50. Early selections from this line were dogged with male-sterility problems but he was able to select away from this condition. Vogel's Norin 10 derivatives were used as the gateway to the introduction of the dwarfing genes into many breeding programmes world-wide so it is possible that this has been the route via which environmentally induced male-sterility has travelled. However, in the Pacific North West where Vogel did his early work there have been no further reports of sterility problems amongst varieties that have subsequently been released (R. Allen, personal communication).

Further to the west in Oregon, problems of male-sterility have been observed in winter wheats and triticales that were day-length insensitive. When these were planted in mid-October, they emerged in early April and were frequently found to be male-sterile. If planting is delayed then spike development is delayed also, so that emergence occurs in late April to early May. Such lines do not appear to suffer as much from male-sterility. Growth room experiments and further field observations confirmed that temperature about 10^{o}C at the time of microsporogenesis was associated with the male-sterility. The latest example of this problem concerns a Kansas variety which is partially day-length insensitive, which in Oregon flowers around mid-April and is plagued with male-sterility. In Kansas however, sterility is never observed possibly because spike development is delayed due to lower temperatures and microsporogenesis is not reached until temperatures are greater than 10^{o}C (R. Metzger, personal communication).

The parentage of these varieties is not known to the reviewer but the presence of day-length insensitivity suggests that CIMMYT wheats carrying the Norin 10 genes are involved.

Further east in the USA, some problems with semi-dwarf wheats were experienced. Varieties of this type were more sensitive to environmental problems than adapted germplasm and this often manifested itself as male-sterility. However, these symptoms have not been observed in adapted breeding material for well over 20 years (R. Busch personal communication). In North Dakota and Minnesota, the tall spring wheat variety Waldron released in 1969 gave massive sterility in its first year which seemed to be associated with cool temperatures and cloudy conditions at about the early booting stage.

5.4 Australia

Hardly any instances of environmentally induced male-sterility in wheat have been observed in Australia where temperatures can be high during ear development and at the time of meiosis. However, male-sterility was recorded in NW Australia under irrigated conditions some 25 years ago in which the material was from early Norin 10 derivatives (Beech and Norman, 1966).

5.5 Asia

Sub-tropical and tropical countries of Asia have reported several instances of male-sterility in wheat. Severe sterility problems have been observed in many parts of Nepal. Factors suggested as being responsible for the sterility included low boron, low temperature during reproductive development, waterlogging at flowering, low soil nitrogen and hot, dry winds (Subedi, 1992; Misra *et al.*, 1992). It was reported that sterility was reduced by the application of boron (Sthapit, 1988). Bangladesh has experienced major losses in yield due to sterility. Again it is thought that boron may be responsible because application of boron has been shown to give significant improvements in yield. Male-sterility is widespread in West Bengal in India. Again this has been attributed to low boron. Certain varieties are noted for their susceptibility. According to Tandon and Naqvi (1992) sterility can be found in Assam, Orissa and along the Indo-Nepal border as well as in West Bengal. Although wheat growing in

Thailand is minimal, sterility appears to be a major problem with varieties such as Inia 66 being very susceptible whereas Sonora 64, another CIMMYT wheat, is less so.

In China, severe sterility problems have been observed in Yunnan Province bordering Burma and Vietnam. It is thought that boron deficiency was responsible although application of boron did not always solve the problem. In northern China also cases of sudden drops in fertility have been recorded. In 1998, the winter wheat variety, Jing He No3, was partially male-sterile in Beijing although it had performed satisfactorily in previous years. Male-sterility is not uncommon in breeders experimental fields and such affected lines are discarded. It is thought that low light intensities and cold temperatures around about the time of meiosis is the environmental cause although some varieties are more sensitive to these conditions than others which form the majority (Zhiyong Xin, personal communication).

5.6 South America

Sterility problems have been reported in Brazil (da Silva, *et al.*, 1980). Again boron seems to be implicated because treatment with boron increased grain set and yield. In Argentina, breeders are not aware of any male sterility problems. Temperatures and levels of sunlight are usually high around about meiosis in most wheat growing areas. On occasions temperatures can be very high for short periods. This can cause sterility in some genotypes which would be selected against in the development of breeders' lines. Segregating material involving Norin 10 frequently shows partial sterility under experimental conditions (E. Suarez, personal communication). Conditions in Chile can mimic closely those occurring in the UK. However, Moulin was grown in trials in 1992, 1993 and 1994 without any signs of sterility. In breeding material, sterility of terminal spikelets are frequently observed. This is often associated with brittle awns. Both these features are symptomatic of boron deficiency, an element which is low in the soils of Chile. To correct this, boron is added as a fertilizer but even so deficiency symptoms still occur and appear to be triggered by weather conditions, particularly dry conditions around about the time of heading. Although sterility levels differ between lines and individual plant segregants, there appears to be no particular parentage implicated (C. Hewstone, personal communication).

5.7 Summary

This survey of sterility problems in wheat outside the UK has pin-pointed several possible environmental causes.

1. Low and high temperatures.
2. Low light.
3. Low boron levels.
4. Droughted and water-logged conditions.

There does not seem to be a particular type of germplasm involved although the incidence of CIMMYT wheats associated with sterility failures appears to be quite high. This may, however, just be a reflection of the widespread use of these wheats in developing countries.

6. Possible causes of sterility

6.1 Genetical

6.1.1 *Rht* genes

As already mentioned, the two genes, *Rht1* and *Rht2* for semi-dwarfism, were introduced into world wide agriculture via the Japanese variety Norin 10 when Vogel in Washington State, USA, crossed it with the local variety Brevor 14. Many of the lines from this cross were sterile or partially sterile but he was able to get away from this in later generations by selection. There were also unpublished reports that Norin 10 itself suffered from infertility problems and observations at meiosis recorded that univalents or unpaired chromosomes were often seen (Author). This chromosomal instability at meiosis could have been responsible for the infertility. No meiotic analyses were carried out on the sterile segregant lines that Vogel rejected so it is not possible to establish whether infertility and meiotic stability were associated in his material. Nor, from this evidence, is it possible to associate the *Rht* genes directly with the sterility. This comes from other sources, the most direct of these being the study of isogenic lines carrying each of the *Rht* genes, separately and combined, as well as a more extreme dwarfism determined by *Rht3*, an allele of *Rht1*. These lines have established, beyond any doubt, that the outcome of the activities of these genes in the wheat plant is to increase the numbers of grains that are set within the ear without increasing the numbers of spikelets or florets - in other words to increase the fertility within an ear (Gale and Youssefian, 1985).

Because of competition within the ear for assimilates, a consequence of the greater seed set is a corresponding reduction in grain size. If assimilates are limiting then seed size may suffer to the extent that the full potential of the increased fertility is not realised and overall ear yield decreases compared to lines carrying the tall alleles. When assimilates are not so limiting the reverse can take place and yield increases up to 20 percent over the tall controls have been obtained (Gale & Youssefian, *loc. cit.*). It is this latter situation which is responsible for the successes of the semi-dwarf wheats in many parts of the world including the UK. However, in some environments, the former conditions apply and yields may actually drop compared to the tall controls (Kertesz, *et al.*, 1991). In some circumstances also the increased fertility does not materialise and yields are affected adversely.

Experiments at the former PBI have shown that lines isogenic for *Rht3* were almost completely sterile under summer glasshouse conditions, the tall isogenic being fertile. Pollen viability was not tested but anthers were poorly developed and pollen was difficult to obtain so that it was impossible to use homozygous *Rht3* plants as male parents. When grown in the field, however, the *Rht3* plants were fertile and indistinguishable from the control. The effect was partly background dependant since some varietal isogenics were not as sterile as others (Anon, 1981). It was thought that the higher temperatures in the glasshouse were responsible for the effect. To test this further, experiments with isogenic lines carrying *Rht1*, *Rht2* and *Rht3* along with their tall controls were undertaken in controlled environment conditions in which plants were subjected to temperatures of 27 to 30^{o}C between the period from flag leaf emergence to ear emergence. These showed that all three dwarf isogenics suffered greater fertility reductions than the tall controls (Anon, 1985).

These results were used to suggest that the incorporation of the Norin 10 genes, *Rht1* and *Rht2*, should be restricted to those areas where these critical developmental stages are not regularly subjected to high temperature stress. It was argued that the known distribution of these genes in currently grown varieties in Europe supported this. In the UK where such high temperature stresses are unlikely, most varieties carry one or other of the *Rht* genes. The same applies to Northern France, Belgium, the Netherlands and Denmark. Countries of continental Europe as well as those of lower latitudes, on the other hand, use these genes only rarely, favouring either a less potent allele originating from Saitama 27 or the dwarfing genes introduced by Strampelli from Akakomugi (Worland, 1986). An apparent exception to this trend is the spring wheats of Spain and Portugal which are predominantly CIMMYT in origin and therefore carry the genes *Rht1* and *Rht2*. However, such wheats are usually sown as winter wheats to avoid the high temperatures of the summer months.

Isogenic lines carrying these insensitive dwarfing genes have been grown widely in many European countries and in general the results support the adaptive model stated above. These include trials in Hungary, Poland, and Germany - all of which gave yield figures for the dwarf isogenics lower than their taller controls, despite the opposite effects on ear grain numbers (Kertesz, *et al.*, 1991; Borner *et al*, 1993; Miazga, *et al.*, 1994).

There appear to be no studies of whether lines carrying these genes are sensitive to low temperatures or environmental stresses generally. More particularly, the types of conditions that appear to trigger the Moulin effect, except in one instance, have never been fully tried on this kind of isogenic material. The one instance refers to unpublished work at JIC using single chromosome substitution lines, in which chromosomes of the CIMMYT semi-dwarf Ciano 67 (incidentally one of the parents of Moulin) replace homologues in the variety Chinese Spring. When these were grown under low light conditions in a winter glasshouse, all the lines were fertile apart from Ciano 67 which was sterile and behaved just like Moulin (A.J. Worland, personal communication). Because one of the fertile substitution lines carried *Rht2*, it could be concluded that *Rht2* was not directly responsible for the sterility of the donor variety, Ciano 67. Evidently at least two interacting genes, on separate chromosomes, must be involved. Although *Rht2* can be ruled out as being the sole genetic cause of the environmentally induced sterility, it could nevertheless still be one of the genes implicated in the interaction.

Both genes from Norin 10, the gene *Rht3*, and $Rht1^{S}$ from Saitama 27 are insensitive to applied gibberellic acid (GA) whereas most mutant dwarf plants respond to such treatment. GA insensitivity is associated with high levels of GA in plant tissues and is thought to be due to an inability to utilise both endogenous and applied GA. GA is involved in anther development and applications of GA at critical stages of its development can bring about male-sterility (Radley, 1980; Colombo and Favret, 1996). Anything which can change GA levels or availability during these critical stages of anther development could be expected to cause sterility problems. There could therefore be a physiological link between the insensitive *Rht* genes and male-sterility. Treatment of *Rht* isogenics with the chemical hybridising agent, ethrel, an ethylene releasing compound causing male-sterility, gave increased sterility of dwarf lines compared to the tall controls (Keyes and Sorrells, 1990). This was interpreted to mean that GA may exert a stabilising effect on pollen development so that a block in its availability would produce a greater response to gametocidal agents. This however disagrees with Colombo & Favret (*loc. cit*) who found that both GA insensitive and sensitive wheat lines were equally responsive to applied GA leading to increased sterility.

Whatever the explanation, there does seem to be a strong connection between the expression of the insensitive *Rht* genes and fertility. The increased fertility potential that these genes can allow certainly seems to be a very important part of the increased yields obtained by varieties that carry them. Indeed, many would say that this is the prime cause of their effectiveness rather than conferring lodging resistance. On the down side though, it would appear that their exploitation could cause varieties carrying these genes to be more vulnerable to environmental variables, making them ill-adapted for use in certain environments. Because these variables are not properly understood at the moment, it is not possible to state that they are involved in the sudden drops in fertility observed in Moulin in the 80's, and more recently in Scotland. The fact that most varieties in the UK carry such genes would tend to suggest that they are not involved, otherwise more varieties would have succumbed. However, the possibility that they are part of a genetic interaction with other genes in the background cannot be ruled out at present.

6.1.2 *Rht* genes in other plants

Rht genes, both insensitive and sensitive, are found in many plant species, and have been studied in maize, tomato and, more recently, in *Arabidopsis*. Three dwarf lines in Maize, each carrying different GA insensitive genes, are all found to be difficult to maintain in the homozygous state, because of pollen sterility (N. Harberd, personal communication). In tomato, GA sensitive mutants, deficient in GA, possibly because a gene for GA synthesis has been deleted, gave poor flower and anther development leading to sterility. The same story occurs for GA sensitive mutants in *Arabidopsis* (Koorneef and van der Veen, 1980). GA insensitive lines in this species when grown in a winter glasshouse were partially sterile. Under summer conditions with temperatures much higher and with greater light, these lines were fertile (N. Harberd, personal communication).

6.1.3 Male-sterile genes

A single recessive male-sterile gene, *ms1*, located on chromosome 4B has long been recognised in wheat (Driscoll, 1975). A dominant allele, *ms2*, has been reported in China (Liu and Deng, 1986) and is located on chromosome 4D. Whether this is related to *ms1* or is a functionally different gene has yet to be established.

Almost every chromosome affects fertility so that many genes could be potentially involved in controlling this character. This is based on the behaviour of monosomics (lines lacking a single chromosome) which are available for each of the twenty one chromosomes of wheat in a number of varieties. In a monosomic series of the variety Bersee, 18 monosomics had reduced percent seed set compared with the euploid variety itself. This of course combines both female and male fertilities and it is not possible to separate the two without direct observation. However, Sears in his classic study of the aneuploids of the variety Chinese Spring (Sears, 1954) found that all the nullisomics, i.e., plants lacking a homologous pair of chromosomes, of homoeologous group 2, i.e., 2A, 2B and 2D, were female sterile but male fertile; but all the six nullisomics of groups 4 and 5 were male sterile and female fertile, whereas the six nullisomics of groups 3 and 6 were partially female and male sterile. In only one instance, nullisomic 3B, was a possible cause, asynapsis, of the sterility identified. Many of the genes detected through aneuploid studies are therefore almost certainly responsible for the variation in male-sterility observed within the wheat crop.

6.1.4 Nuclear-cytoplasmic interactions

The exploitation of cytoplasms taken from related species to wheat, notably *Triticum timopheevi*, has been a common area of investigation in hybrid wheat breeding. The "alien" cytoplasm induces 100 percent male-sterility which can be restored to fertility by one or more restorer genes in the pollen parent of a hybrid. Frequently restorer genes are obtained from the species that donated the cytoplasm. Restorer genes have also been identified in bread wheat itself through the transfer of wheat into an "alien" cytoplasm which still results in a male-fertile phenotype. However, by removing or substituting, usually from another related species such as *Triticum macha*, the existing restorer gene in *aestivum*, the line becomes male-sterile. This points to the possibility that many of the male-sterile genes identified through aneuploidy in wheat are in fact restorer genes interacting with the *Triticum aestivum* cytoplasm. At least five major genes for restoration have been identified and many modifiers have been suggested (McIntosh, 1988).

Many of the problems associated with exploiting a cytoplasmic-restorer system in hybrid wheat breeding are to do with the environment. Either the degree of male-sterility or restoration can be less than the sought after 100 percent expressivity. The primary cause of this failure is changes in the environment. Studies of F1 hybrids in a range of environments showed widely different degrees of restoration. The particular components of the environment which are responsible are unknown but it is thought that temperature differences between sites are implicated.

The origin of the cytoplasm of *Triticum aestivum* is likely to be a goat grass, *Aegilops speltoides* or a species very closely related. Also, because the origin has supposedly been monophyletic, the cytoplasms of the majority of wheat varieties are identical. It is only recently that different cytoplasms have been introduced primarily for the development of hybrids. In a few instances, "alien" cytoplasms have been introduced as a by-product of wide-crossing undertaken for other reasons. The best example of this is the variety Rendezvous which resulted from a crossing programme in which *Aegilops ventricosa* had been used as a source of eyespot resistance. It is possible therefore that in this variety a different spectrum of restorer genes may be involved, and this presumably could have different consequences on interactions with the environment. This could lead to a different pattern of male-sterility emerging in this variety compared to other varieties. This would also apply if Rendezvous were used as a female parent in the development of other varieties.

6.1.5 Hybrid wheat

Interesting observations, possibly relevant to the problem of environmentally induced sterility, have emerged from attempts to develop hybrid wheats. This is probably because they also involve the induction and manipulation of sterility followed by controlled out-crossing. Thus some hybrid wheat breeders believe that the selection of improved restorer lines gives an increase in pollen production leading to enlarged anthers and a tendency for these to extrude prior to dehiscence. Apart from the extrusion these changes could be important in achieving more stable seed setting and fertility in varietal production. Accumulation of restorer genes could thus be an important way of removing problems of fertility in the future. This may be relevant to the view held by some French wheat breeders that German varieties are usually better pollinators than those developed in England (A.A. Pickett, personal communication). Selection of lines of wheat which are more easily sterilised with chemical hybridising agents (CHAs)seems to correlate with those lines which are prone to environmentally induced sterility. Moulin, for instance, was regarded as a good female for use in hybrid wheat breeding, because it was easily sterilised with CHAs (W.J. Angus, personal communication). These observations would seem to be worthwhile pursuing experimentally because they could lead to useful diagnostic tests for varieties susceptible to sterility.

6.1.6 Pollen killer

In the 1960s, distorted segregations of known single genes for disease resistance were found to be due to a single linked gene, *Ki*, on chromosome 6B affecting pollen. Pollen carrying the dominant gene, *Ki*, caused the abortion of pollen carrying *ki*, the recessive gene—hence the name, "*killer gene*". Genes that are linked to *Ki* suffer distorted segregation because of this effect. The degree of distortion is related to the tightness of the linkage. Evidence was obtained that *Kiki* microspores abort in *KiKiki* trisomics but not in *Kikiki.* In *KiKikiki* tetrasomics the high rate of abortion leading to total failure of anther dehiscence indicates that most or all *Kiki* pollen is killed. It appears from this that killing is an effect of maternal tissue on microspores and that its severity depends upon the relative dosage of *Ki* and *ki* (Loegering and Sears, 1963).

It is not known how widespread is this phenomenon. It is thought that there are several allelic variants at the *Ki* locus, and that background modifiers as well as environmental variables could influence the degree of killing that might occur.

It is unlikely that pollen killer is solely responsible for the Moulin behaviour and is most probably not even involved. However, it is worth recording if only to underline the extraordinary complexity of the mechanisms that can influence pollen viability and behaviour.

6.1.7 Low temperature pairing

Cytological examination of the events leading up to pollen dispersal has been carried out for *Ms* lines, cytoplasmic male steriles and heterozygous *Ki* genotypes. In all instances, it is the final stages of pollen formation which appear to be affected rather than earlier meiotic events during which chromosome misbehaviour can readily be recognised. Meiosis seems to be normal. For the *Rht* isogenics detailed investigations do not seem to have been undertaken so that an effect at meiosis cannot be ruled out. However, in one instance effects on pollen meiosis have been observed leading to complete male-sterility. Nullisomic 5D plants in the variety Chinese Spring when grown at 20^{o}C give normal meiosis but when meiosis takes place at 12^{o}C, chromosome pairing fails at metaphase and the plants are completely asynaptic. This gives plants which are completely male sterile (Riley, 1966). Further investigations have shown that duplicate genes, *Ltp1* and *Ltp2*, are involved, one on chromosome 5A, the other on 5D (Hayter and Riley, 1967; Chapman and Miller, 1981). The presence of only one *Ltp* gene is sufficient to give normal pairing but if the recessives of both genes are present then at temperatures less than 15^{o}C at meiosis the chromosomes become asynaptic and the plants are male-sterile. Studies of a limited number of varieties showed that all carried the *Ltp1* gene on 5A (Chapman & Miller, 1981). Since then, no further studies have been undertaken so that the constitutions of present day varieties and varieties at the time of Moulin are completely unknown. A temperature of 15°C is relatively high and temperatures below this are likely to be experienced by most wheats in the UK around about meiosis, certainly at some time during their development, so it would seem unlikely that such genes are present in current varieties. However, they could still be involved through interactions with other genes or if *Ltp* alleles with lower temperature limits occur.

6.1.8 Incompatibility genes.

Three loci, *Kr1*, *Kr2* and *Kr3*, are known to affect the crossability of wheat with rye or barley (Lein, 1943; Riley and Chapman, 1967; Krolow, 1970). These genes appear to be non-specific since lines giving poor crossability with rye behave in a similar way when crossed with *Hordeum bulbosum* (Snape *et al*, 1978). As yet no-one has considered the possibility that such incompatibility systems may also be operating at the varietal level. Self-incompatibility is present in rye, so it is conceivable that related genes may be present amongst wheat varieties, partially suppressed perhaps because of homoeoalleles on other chromosomes. Even if such systems were less potent than with inter-specific crosses, they could still be sufficient to hinder self-pollination and increase the level of sterility, particularly under adverse environmental conditions. It should be mentioned that the 1B/1R translocation, originating from rye, is prevalent in many of today's wheats. Although there is no direct evidence that it is implicated in the fertility failures observed at Aberdeen, a contributory cause cannot be ruled out entirely.

6.2 Environmental

6.2.1 Temperature

Throughout the reports of male-sterility occurring in wheat, temperature seems to be high on the list as one of the prime causes. Several studies have been made to investigate the effects of temperature on plant growth, particularly during the stages that are likely to affect pollen viability. High temperatures, 30°C or more, have been cited as causing male-sterility. Often the effects of high temperature have been recorded as reduced seed set rather than a specific effect on pollen, although it is very likely that pollen is involved (Saini and Aspinall, 1982; Saini *et al*, 1983). Low temperatures around about the time of ear development are also widely thought to be a major factor in causing male sterility. In this regard, work with low temperature pairing genes is of interest. Because asynapsis is induced at a temperature of 15°C but not at 20°C it has been possible by switching sensitive genotypes between the two temperature environments to pin-point the sensitive stage of development with some degree of accuracy. This study of low temperature pairing genes showed that the sensitive stage occurred at about 39 hours before metaphase of meiosis. Since the total time taken for meiosis is only 24 hours at the higher temperature, the stage must occur before meiosis is initiated and probably around the time that the last pre-meiotic mitosis takes place.

As already mentioned, the work on low temperature pairing appears to be the only one to establish the cause of subsequent pollen failure, i.e., asynapsis. Most studies in wheat appear to have confined themselves to examining just pollen viability by staining techniques and have not examined cytologically earlier stages to see if they have been affected. This seems to be a recurring weakness of most of the studies.

In Sorghum, low temperatures at night (25°C day/ 10°C night)for a period of five nights gave male-sterility in which non-viable starchless pollen grains were produced. The most sensitive stage of development was pre-leptotene of meiosis. Pollen development proceeded in an apparently normal manner following low night temperatures, but was arrested just prior to maturation at the vacuolate two-celled microspore stage (Brooking, 1976). Similar effects of low night temperatures have been reported in rice and mango (Satake *et al*., 1969; Issarakraisila and Considine, 1994).

6.2.2 Light - intensity and duration

Numerous shading experiments have been conducted in which shades have been applied for different periods at different stages of wheat development. Shading can reduce the amount of light by anything up to two thirds in the field and by much larger proportions in controlled environment chambers. The greatest effects on grain numbers, the parameter used most frequently in these studies, occurred at about 15 days before anthesis. Shading periods ending 45 days before anthesis or starting at anthesis affected grain numbers hardly at all (Evans, 1978; Fischer, 1985). In these studies, no attempt was made to link the effects on grain number to pollen fertility or to accurately define the most sensitive developmental stage, particularly in relation to the onset of meiosis.

Demotes-Mainard *et al* (1995) treated the varieties Moulin and Pernel to low temperatures and low light intensity for 7 days commencing at meiosis (This was defined as being when the oldest floret in the main ear had reached first meiotic division). They found that reduced light rather than low temperatures in both controlled environment conditions and the field affected pollen viability. In further experiments, they showed that under low light and chilled conditions seed set was reduced due to poor fertilization. Pollen viability was decreased and anthers were frequently small, curved or shrivelled, and did not dehisce. Female fertility was unaffected (Demotes-Mainard *et al*, 1996). The stress conditions used in the experiments of Demotes-Mainard *et al.*, are probably very similar if not identical to the test that PBIC use to screen their advanced lines for the Moulin effect. They place plants just before meiosis of the main tiller into controlled environment cabinets for five days at temperatures reduced by 6°C and light intensity which mimics heavy cloud cover (P.I. Payne, personal communication).

Both these stress environments are effective at a developmental stage which is probably just prior to meiosis and could be possibly identical to the sensitive stage for low temperature treatment cited above.

6.2.3 Drought

Water deficit about the time of meiosis has been reported to cause male sterility leading to reduced seed set. Bingham (1966) carried out experiments in the glasshouse and, based upon meiotic screening of control plants, suggested that the sensitive stage was just before meiosis. Saini and Aspinall (1981) subjected wheat plants to water deficit during various stages of floral development, including those immediately before meiosis and all stages until just after anthesis. They found that seed set was reduced when water deficit occurred during and immediately after meiosis rather than before. They established that the anthers in water stressed plants were often small and shrivelled, contained abnormal pollen and did not dehisce properly. Both these studies demonstrated that it was male rather than female sterility which was responsible for the reduced seed set.

The induction of male sterility by water stress has been attributed to an increase in the concentration of abscisic acid resulting from the stress (Morgan, 1980). It is also thought that the effect of water stress on male sterility cannot be attributed to desiccation of the sporogenous tissues because the water status of such tissues is conserved even though leaves lose water. Consequently, pollen sterility is probably an indirect effect resulting from a deficit in other tissues. In stressed plants, meiotic divisions proceed normally, but development stops at the onset of pollen mitosis. In normal pollen, at this stage, starch granules begin to appear but not so in stressed plants. This suggests that reduced carbohydrate availability may play a key role in drought induced male sterility. Further studies have however shown that this is probably not the case and it is possibly an inability to metabolise incoming sucrose rather than carbohydrate starvation which may be involved (Dorion, *et al.*, 1996).

6.2.4 Boron

Reduced levels of boron have been shown to be a prime cause of sterility in wheats grown in Nepal, Thailand, Bangladesh, and Southern China. In some years and at some sites sterility leading to disastrous yield failures has been reported. The sterility appears initially as poorly formed anthers and aborted pollen. A later sign of this sterility is gaping glumes where the palea and lemma remain open after anthesis for several days. This is a very close to the description of the symptoms that Moulin displayed during the 1987 season.

Boron is required by the generative organs of the plant for normal development although wheat has a lower requirement than other plants. The reason for boron being required is not clearly understood. There is some evidence that boron is involved in RNA metabolism and synthesis so that it could affect many plant processes (Mengel and Kirkby, 1982). In pot grown experiments with wheat, the complete absence of boron in the nutrient water supply gives total male-sterility. By transferring plants at different developmental stages between adequate and zero levels of boron in culture media, the sensitive stage for sterilization was found to be relatively short (7 days) and occurred about the time of meiosis (Rawson, 1996). Claims that boron starvation is always responsible for male sterility in Bangladesh and Nepal, in particular, have been questioned and low temperatures, again around about the time of meiosis, have been suggested as being the critical factor (Saifuzzaman and Meisner, 1996; Subedi *et al,* 1996). Cloudy dull weather has also been suggested as a contributory factor.

Rawson (1996) has suggested that these conflicting views can be reconciled if boron availability is considered as the primary factor and other environmental variables impinge upon this availability. This could happen if the transpiration flow carrying boron from the root to other tissues in the plant was restricted. Low radiation, associated with cloud cover, will close stomata and thereby reduce transpiration and since boron moves within plants almost exclusively in the transpiration stream, the effect will be to cause boron starvation. Other variables which might affect transpiration include drought, waterlogging and high temperatures, all of which can lead to stomatal closure in wheat. Conditions such as high humidity combined with low light and high temperatures, calculated to reduce transpiration by 88 percent, have been shown to produce sterility even when adequate levels of boron are available (Rawson *et al*., 1996). Enclosing whole wheat plants in plastic bags to reduce transpiration rates during the conjectured critical period also gave sterility even with sufficient boron provided in sub-irrigated gravel culture (Rawson, 1996).

Varieties differ in their response to low boron (Subedi *et al.*, 1997). A total of 41 varieties from a number of Asian countries but including several from CIMMYT were assessed in plots known to cause sterility due to low boron. About one third of all the varieties had less than 25 percent sterility whereas 16 of them suffered more than 75 percent sterility. Of interest, added boron significantly reduced the sterility of the more sensitive varieties.

Genetic variation in boron tolerance is of prime interest to plant breeders in many parts of the world and some think that the exploitation of this variation is the answer to the problems of environmentally induced sterility (Joshi and Sthapit, 1996; Rerkasem & Jamjod, 1997). Rawson (1996) has suggested that the reason for certain varieties being more tolerant is that they carry a reserve of tissue boron that can be mobilised when external boron becomes limiting. Evidence for this comes from experiments with varieties that are either tolerant to boron deficiency (e.g. Fang 60) or are very sensitive (e.g. SW 141). When these varieties are grown initially on a medium with boron and are then removed to conditions with zero boron, sensitive varieties respond quickly to the lack of boron whereas tolerant varieties take much longer for sterility symptoms to appear, suggesting that they have reserves of boron which can be mobilised.

In the UK, boron deficient soils are confined mainly to the SW of England, most parts of Wales, parts of East Anglia and large areas of Yorkshire. Although some of these are wheat growing areas, reports of wheat showing symptoms of boron deficiency have not been reported. Where crops known to be sensitive to lack of boron, e.g., sugar beet, deficiency symptoms appear to be more frequent in dry summers following on from a wet spring. It is thought that this is due to leaching of available boron during wet weather succeeded by a period of rapid growth when the plant requirement is greatest. The wet conditions experienced during the most severe Moulin year, 1987, should therefore be borne in mind when considering boron as a factor.

6.2.5 Copper

Copper deficiency reduces the level of grain set in wheat. The primary cause of this failure is the inhibition of anther formation, and the production of fewer, non-viable and starchless pollen grains per anther. The development of viable egg cells is unaffected. The critical sensitive period is meiosis but whether this is just before or during meiosis is not clear (Graham, 1975). Addition of copper to the soil or as a foliar spray overcomes the pollen sterility to varying degrees.

6.2.6 Growth regulators

Many growth regulators owe their efficacy to the production of ethylene in plant tissues. Ethylene affects many plant characters of which one is to induce male-sterility. Indeed, this is one of the reasons why growth regulators are used as gametocides for hybrid wheat production. Another effect is to inhibit stem growth and it is this effect which is exploited most widely in wheat growing. However, because of the potentially disastrous effects on fertility, the use of growth regulators is very carefully prescribed by manufacturers so that the time of application and the concentration of the treatment is appropriate for inhibiting stem growth but not pollen fertility. Some reduction in seed set may occur but usually this is so low as to be easily off-set by increases in grain size so that yields are not affected adversely.

In the Moulin years, some effect of the growth regulator on Moulin fertility was acknowledged by the manufacturer and a warning was given on future sales of the regulator, informing customers of the potential dangers of using it on Moulin. However, examination of untreated and treated NIAB trial data for the Moulin years 1985 and 1987 shows that both are equally affected by sterility problems. Growth regulators cannot therefore be considered as being the prime instigator of subsequent failures to set seed in this variety. A similar argument also applies to the trial at Aberdeen in 1997.

6.2.7 Herbicides and fungicides

The same case may be used to rule out fungicides as a possible major cause of sterility because these would also have been applied to the treated trial but not to the untreated. Herbicides however would have been given to both trials, untreated and treated, so a possible interaction involving herbicide usage with climatic variables cannot be ruled out and should be considered in future studies.

6.2.8 Orange-blossom midge

Attacks by midge (*Sitodiplosis mosellana*) can affect the level of seed set in wheat. The adult fly emerges in early June and lays eggs in the outer spikelets of wheat ears. These hatch to produce larvae that feed on the developing grain. In severe cases the grain is so badly damaged that it appears as if no grain has in the affected florets. As might be expected, varieties with open flowering habits appear to be more susceptible to infection. Asynchrony leading to gaping ears may be a contributory factor. In 1993, conditions, i.e., temperatures above 15°C and low wind speeds at about the time of flowering were such as to favour the insect and damage caused by the midge was widespread in the south and east of England but not elsewhere. Damage assessment revealed that varieties differed in their susceptibility to the midge. The varieties appearing to show the greatest effect were Haven, Lynx and Beaver. However, this did not seem to adversely affect overall yield, presumably because some compensation had taken place.

No major attacks have been reported since, although it is possible that some of the spasmodic instances of sterility reported by breeders from time to time may have midge damage as a partial cause. Certainly the major failures of fertility in 1985 and 1987 with Moulin and the 1997 result in Aberdeen cannot be ascribed to midge damage. For the purposes of this review into sterility in winter wheat attacks by the orange blossom midge would seem to be only a minor contributory factor.

7. Discussion

7.1 Sub-clinical sterility

The dramatic reductions in yield with Moulin in 1985 and 1987 and with other varieties and lines at the Aberdeen site in 1997 were all due to major reductions in seed setting arising most probably from male sterility.

A worrying claim advanced by a number of breeders interviewed during the course of the review was that varieties and lines will on occasions display high levels of sterility. This can occur at just one site or in one season only and appears just as an isolated instance. Although NIAB, in their trials, routinely check negative residuals to see whether these can be explained by evident effects such as disease or lodging, not all negative residuals are eliminated by this means. Some of these could therefore be due to failures in sterility. This is a feature unlikely to be noticed unless the trial crop is subjected to close scrutiny throughout its development.

One view is that this type of sporadic event may be just an extreme manifestation of a more widely occurring phenomenon of sterility in wheat. Most if not all varieties of wheat show some failures to set seed even in good high yielding years which may be due to a low level of sterility. Such sub-clinical levels of sterility are usually ignored because it is argued that wheat compensates for this by increasing seed size, as a consequence of less competition for assimilate, to a degree which effectively minimises the effect on overall grain yield. It is only when the level of seed setting falls below an as yet undefinable lower limit that yield levels become adversely affected.

There are of course other factors which influence numbers of grains produced in an ear. The number of spikelets and florets within each spikelet defines the limit to the potential grain numbers in each ear. These are determined by the length of time devoted to spikelet and floret production during ear development which is strongly influenced by genes controlling its onset and duration as well as by the environment. Thus, studies have shown that the ratio of solar radiation to temperature during the period preceding anthesis can be a good predictor of final grain numbers (Fischer, 1985), whereas allelic substitutions of genes for vernalization and photoperiodism are well documented ways for producing changes in floret numbers (Scarth, *et al.*, 1985).

The efficiency of pollination can also be influenced by a number of pollen characteristics. These include the size and number of pollen grains produced as well as the size of the anther itself, all of which are known to vary between varieties of wheat (De Vries, 1974), and all of which are thought to affect the degree of fertility (Hoshikawa, 1960; Rajki & Rajki, 1966). The longevity of pollen may also be a factor in fertility, particularly if there is some degree of asynchrony.

There are therefore many influences determining the numbers of grains developed within an ear of wheat, ranging from the number of potential sites to their ability to set seed through the control of pollen or egg fertility. Because of this complexity, most breeders tend to ignore the detailed and laborious procedures required if one of these component characters e.g., male fertility, was to be used as a selection criterion. Rather they have concentrated upon eye-ball assessments such as "plump, well-filled ears" and leave any quantification to final yields in their selection procedures. This is more than understandable given the scale that wheat breeders operate under. However, it is perhaps worth emphasising that this nevertheless skims over and ignores a range of yield component characters, several of which are known to be environmentally sensitive, and each of which could surface at any time as an important and perhaps detrimental influence on final yields. Indeed, the story of Moulin is a useful lesson in this respect—even though the threat to commercial cropping was not fully understood at the time.

The work undertaken at SAC highlighted not only the strong correlation between percent fertility and yield at Aberdeen, the badly affected site, but also at Kelso which gave yields similar to those obtained at other UK sites. This would suggest that percent fertility is not just the explanation for some dramatic failures in yield but is an important influence on yield levels generally. If the Kelso fertility spectrum were to be found occurring at other sites, then it surely would indicate that the different fertilities between varieties are inherent, and that the Aberdeen result was an extreme manifestation of this, triggered perhaps by rather adverse weather conditions. Such sub-clinical levels of sterility may therefore be worthy of a closer scrutiny by breeders than hitherto and certainly should be considered more seriously as a research topic in the near future.

7.2 The critical sensitive stage

A period during meiosis or possibly just before is commonly cited as being the most likely sensitive stage.

However, the errors in making these observations are almost certainly very large so that it is impossible to be certain that only one critical stage is implicated even though the majority of the observations point to a stage prior to meiosis. The complexity of the genetical basis of fertility control suggests that several sensitive stages may exist, each arising from the activities of different genes and these could be operating within a relatively small period of development.

The effects on pollen sterility, as opposed to the point where activities affecting pollen development are disrupted, have usually been noted during the later stages of pollen development, after meiosis has been completed. This suggests that the majority of examples of induced sterility are to do with pollen nutrition, possibly relating to mis-functioning of the tapetal cells (Mascarenhas, 1990). In only one instance has male-sterility been associated with a failure of chromosome pairing at meiosis itself. Taken on its own, this could be interpreted to mean that there are at least two critical sensitive stages, one resulting in asynapsis, and the other later at pollen development. However, the majority of studies are glaringly defective in their descriptions of the early stages of pollen meiosis, many failing to note whether such stages were examined at all, so it is not possible to be clear about this point. This is a weakness that ought to be rectified in future studies.

7.3 Comparison between the events of 1985/87 and 1997

As discussed in Section 4, the prime difference between these two events is the widespread collapse in the fertility of one variety, Moulin, across all trial sites in England and Scotland, whereas in 1997 the effects were confined to just the one site in

Scotland and affected more than one variety. As noted, weather patterns in 1985 and 1987 were different from preceding years. During late May and early June, the time in which anthesis commonly takes place for all winter wheat varieties in the UK, temperatures and levels of sunshine were below average. If anything temperatures were even more extreme at Aberdeen and most likely levels of sunshine were also low because rainfall was high during both May and June. It would appear therefore that conditions were similar for both situations. Why then did many varieties and lines appear susceptible at Aberdeen in 1997, but only Moulin in 1985/87, albeit at many sites? One possible explanation is that Moulin genes were more widespread in other varieties in 1997 than in the previous years. But this would only apply if the genes responsible for the Moulin effect were also responsible for the effects at Aberdeen.

Is Moulin therefore connected with the anomalous 1997 results in Aberdeen? This question was considered in Sections 4.1 and 4.3, and it was concluded on the basis of parentage and evidence provided by PBIC that, apart from a few lines, the full Moulin syndrome was unlikely to have been transferred to the majority of the affected lines and varieties tested at Aberdeen. The most likely explanation is therefore that different genes are involved in the two environmental interactions. The only rider to this is that if several genes were involved in the Moulin effect then some of these genes could have found their way into the lines being tested at Aberdeen and these may have contributed partially but not entirely to their anomalous behaviour in 1997.

7.4 Likely environmental causes

Of the environmental variables reviewed in Section 6.2, low temperatures and light around about meiosis seem to provide the strongest case for inducing the male-sterility observed in winter wheats in the UK. This applies to both the 1985/87 and 1997 episodes of sterility. Although the exact details of the tests certain UK breeders use are not known, they are believed to involve lowered temperatures and reduced lighting. In some instances, screens are used in the field to reduce light levels at anthesis. Presumably in such tests low temperatures are unlikely to be achieved, so that this would suggest that light is the major factor. This has support from the work of Demotes-Mainard *et al* (1995) in France who showed that in a factorial experiment, light rather than temperature was the influencing factor. Nor did they detect any interaction between the two. However, in a later published experiment, Demotes-Mainard *et al* (1996) used a treatment to induce sterility in Moulin which combined both low light and low temperature. Low temperatures also seem to be cited as a frequent causative agent in cases of sterility in other parts of the world (see sections 5.3, 5.5 and 6.2.1). There therefore seems to be some basis for considering that two separate systems are involved, one for low temperature and the other for low light, each with their own separate gene control.

The intriguing connection of these weather variables with boron levels suggested by Rawson and colleagues is one that should be considered seriously with respect to UK conditions. Rawson proposes that the primary factor in causing sterility is boron (as well as possibly copper?) and that these other environmental variables, whilst perhaps still having a direct effect on sterility, have a major indirect effect on the movement of boron into the anthers during meiosis and pollen development because they reduce the rate of transpiration. This effectively starves the developing anther of boron during the time that the critical stages are thought to happen. This can occur even when the soil is not deficient in boron: the situation for most UK soils.

The ACIAR Proceedings (Australian Centre for International Agricultural Research, Proceedings of a workshop, Sept., 1995, Lumle Agricultural Centre, Pokharra, Nepal: Eds. H.M. Rawson & K.D. Subedi) are full of descriptions of low light caused by cloudy, misty conditions combined with low temperatures during the period from flag leaf emergence and anthesis as being responsible for sterility in wheat. These are exactly the conditions cited in the Moulin years and also at Aberdeen in 1997. In Bangladesh and Nepal, soil boron levels are often low and these adverse climatic conditions seem to acerbate the level of sterility. However, wheat grown on soils with adequate levels of boron, given the conditions cited, can also produce sterility.

This proposed key role for boron needs to be tested for UK wheats, a start being made perhaps with Moulin itself, to see whether it is particularly sensitive to low boron levels. Rawson's view that varieties differ in their ability to store boron and then mobilise it and that this is responsible for some varieties being tolerant and others susceptible to climatically induced sterility also requires close examination.

One possible check of whether the critical years 1985 and 1987 in the UK and 1997 at Aberdeen were in fact boron deficient years due to the effect of the climatic variables on boron uptake would be to examine the experience with a sensitive crop plant such as sugar beet. Were these years noted for a higher incidence of boron deficiency in sugar beet?

7.5 Likely genetical causes - few genes or many ?

Without further studies it is impossible to pin-point the most likely genetical cause of the interaction with the environment leading to male-sterility. Although the semi-dwarfing genes affect grain setting in the ear and this is under the influence of environmental variables possibly associated with stress, many varieties carrying such genes have never shown any sign of reduced fertility and have always behaved in a stable manner so far as this character is concerned. If these genes are involved then they can only be so as one component and other genes have to be invoked.

Because low temperatures have been implicated, the involvement of *Ltp* genes for low-temperature pairing cannot be ruled out entirely even though it would seem to be unlikely. The *Ltp* genes form part of a duplicate gene system which requires that both loci should be homozygous recessive for asynapsis and sterility to appear. All the varieties tested so far were homozygous for at least one of these loci and possibly also at the second. This is perhaps not surprising since there would have been strong selection against any recessive loci producing sterility at temperatures at 15°C or just below. However, different alleles with different temperature thresholds would get around this argument so that these genes cannot be dismissed as not having a role.

It should also be emphasised that this is the only effect which definitely pin-points a failure of chromosome pairing during meiosis as the cause of sterility. Whenever the site of failure has been properly studied, the effects manifest themselves much later at pollen grain formation. However, many other studies have never examined meiosis thoroughly so it is not possible to rule out an *Ltp* involvement on these grounds.

Male-sterile cytoplasms are very unlikely to be implicated. The vast majority of the varieties screened by breeders and by NIAB through its trialling system must, because of their predominantly maternal inheritance and the absence of any means of generating new variation through recombination, carry a nearly constant *T. aestivum* cytoplasm. The only source of variation is through spontaneous mutation, whose contribution, because of the time scales involved, is unlikely to be large. This is not the case though where "alien" cytoplasms have crept in because of the need to introduce a beneficial gene not found in wheat itself and where the initial cross has had to be made using the "alien" species as a female. The "alien" species used in a wide cross may have been separated from wheat by many thousands of generations so that mutations will have accumulated leading to significant diversion

The prime example of this is of course the variety Rendezvous whose parentage involves *Aegilops ventricosa* used as female in the initial cross to produce VPM, a breeders line used in developing Rendezvous. In the 1997 trials only one variety, Beaufort, could claim to have been derived from Rendezvous used as a female parent, and this variety gave normal yields and therefore no marked reduction in male-sterility at all sites including Aberdeen. This would seem therefore to rule out cytoplasmic variation as a possible causative agent for environmentally induced male-sterility.

The question as to how many genes are involved is also not easy to answer. Clearly more than one gene is implicated, otherwise how could these effects appear in Aberdeen in 1997 amongst lines which have be screened for the Moulin syndrome? Some breeders think that many genes could be involved and these interact with a range of environmental variables. From aneuploid studies, there are certainly a large number of genes which could potentially be implicated. It is also most likely, given the complexity of the environmental agents which have been identified, that several genes are involved. If Rawson's boron model is correct then it is easy to envisage several separate genes controlling transpiration and therefore the movement of boron as well as its storage in response to environmental changes.

7.6 The influence of "exotic" germplasm

As has been pointed out in Section 3.1, the parentage of Moulin includes two CIMMYT derived varieties, Yecora and Ciano 67. This would be regarded as a fairly wide cross, if not quite involving exotic germplasm. Doussinault *et al*, (1988) mentions that sterility problems occurred amongst semi-dwarf lines carrying the Norin 10 dwarfing genes and originating from America (CIMMYT ?). He also found similar effects in material derived in France from *Aegilops ventricosa*. Breeders in North America have also cited CIMMYT germplasm as being prone to such bouts of sterility (R. Metzger, personal communication). CIMMYT wheats have been used extensively in tropical Asia but there does not appear to be increased sensitivity of these varieties over others, although several were found to be sensitive.

These observations raise the possibility that the genetical source of the environmentally induced male-sterility is through the use of unadapted and exotic germplasm. CIMMYT wheats for example would have been bred under conditions where low temperatures and low light at the time of anthesis would be very unlikely to occur so that selection of genotypes adapted to anthese under these conditions would not have taken place. Another example of a wide cross using unadapted germplasm is the variety Pentium which succumbed rather dramatically at Aberdeen. This variety resulted from the cross of Rendezvous - a VPM derivative - with Talon which has Yugoslav material in its parentage.

7.7 Development - varietal differences

An undoubted influence on whether a variety succumbs to environmentally induced male-sterility is the timing of critical developmental stages. This differs between varieties. It is thus easy to see how a variety can miss being hit by an adverse environment in one season but not in another because the critical development stage occurred earlier or later in the first season but not in the second. Developmental rates and events are determined through interactions between genes and environment so this provides another instance whereby genes can influence environmentally induced male-sterility. At least one breeding company tries to allow for this by routinely growing trials with different sowing dates.

The tendency of present day farming practice to sow winter wheats earlier and earlier in an effort to maximise the length of the growing season could also be making environmentally induced sterility problems more likely—especially in day length sensitive wheat. By advancing development in this way, the chance of a sensitive stage coinciding with a late spring period of low temperature and sunlight will be increased.

7.8 Are the trial systems used by NIAB adequate to prevent another Moulin?

This may not be the right question. The trial system in place during the Moulin period was smaller than it is to-day but, despite what can now be recognised as warning signs, the problem was not effectively dealt with until it was too late, and a disaster took place in 1987. This was not due to the trials being too small but was because of a lack of a full understanding of what was happening to Moulin. This lead to the variety winning an appeal against registration because of uniformity problems on the grounds that it was susceptible to out-crossing. The variety behaved this way simply because of fertility problems. The size of the trial system to day is probably more than adequate but what is lacking is any organised scheme for monitoring behaviour of varieties in trial.

This highlights the total lack of any understanding as to how much outcrossing goes on in wheat. Breeders have varying opinions about its level, and at least one breeder has the view that wheat is really an out-crossing species! The degree of outcrossing is also important because as the Moulin story demonstrates it is a good indicator of male-sterility problems. It may however be difficult to estimate because cross-pollination may mask the true level of male-sterility. However, where trialling involves small scale plots which allow opportunities for cross-pollination between different varieties in adjacent plots then the amount of cross-pollinating can readily be identified by screening grain electrophoretically. There is therefore a need for further studies of outcrossing, and possibly the instigation of routine electrophoretic screening of grain from varieties undergoing trial should be considered (see Recommendations).

7.9 Is there likely to be another Moulin scare in the UK?

The majority of breeders when questioned on this matter were optimistic. Having gone through the Moulin episode and having some awareness of the environmental causes, they felt that they were unlikely to see another incident like it in the future. As mentioned earlier, several, routinely, subject their advanced breeding material to low light intensities, some such as PBIC also include lowered temperatures. Some however, have confidence that the trialling systems in place are sufficient to provide an early warning. A few breeders are however not so sure, and cite instances of fertility failures or near failures which occur sporadically and seem to be associated with some adverse weather conditions around about anthesis time. The evidence collected in this review provides some support for all of these views. On the matter of a re-occurrence of a Moulin episode, however, the evidence suggests that a catastrophic and widespread failure of a single variety such as Moulin is unlikely to occur in the UK in the foreseeable future. The results of NIAB's trialling since 1987 show no evidence of a variety emerging with such a weakness even though conditions similar to 1985 and 1987 had been repeated in later years. A dramatic change in weather patterns such as to increase the incidence of days with adverse climatic conditions might of course change this. In this respect, global warming may act as a trigger if one of its outcomes was to move the UK into a colder and wetter weather pattern. Likewise a sudden infusion of unadapted germplasm could have a similar effect. In both instances though, the diligent use of environmental screening should alert both breeders and those responsible for trialling to a developing problem and allow them the time to adjust their breeding material.

7.10 The significance of the Aberdeen results

The majority of breeders dismissed the Aberdeen results as an anomaly and a "one off". There is some logic in this because a similar episode has not been recognised before or has occurred since. However, the correlation between the fertility levels at Aberdeen with those at Kelso suggest that although the result is extreme and may be a "one-off" it is nevertheless connected to the possibly more normal fertility responses observed at Kelso. There is also the fact that the weather conditions at Aberdeen were very close to those responsible for the earlier Moulin failures and that at least three of the lines sown at Aberdeen probably carried the full Moulin syndrome. For these reasons it should not be dismissed. It may well be an example of what can happen to sub-clinical variation in fertility when it is exposed to adverse weather conditions similar to those that triggered the Moulin catastrophe further south.

8. Conclusions

8.1 The dramatic reductions in yield with Moulin in the 1980's and with several varieties and lines at the Aberdeen site in 1997 are due to environmentally induced sterility. The environmental conditions responsible for this induction are not clearly defined but they almost certainly involve low temperatures and low light intensities at or about the time of meiosis.

8.2 The actual stage which is sensitive to these environmental effects is not known clearly, some investigations pin-pointing a stage before meiosis and others during meiosis. It is possible that there may be more than one sensitive stage involved.

8.3 A lack of trace elements, particularly boron, may be the prime cause of the male-sterility. The critical climatic variables, low light and temperature, may operate by restricting the uptake of boron through directly affecting transpiration and thereby the translocation of boron from the soil to reproductive tissues at a crucial stage of their development. This could occur even though UK soils are not generally low in available boron.

8.4 The difference between varieties prone to give sterility in response to the above named climatic conditions and those which are tolerant and are fertile could be their ability to store a reserve of boron. This would enable them to tide over occasions when a climatically induced starvation of boron occurred.

8.5 The genetical basis of the responses involves more than one gene and probably encompasses several. The genes responsible for the Moulin effect are probably different from those responsible for the majority of the sensitive varieties and lines at Aberdeen, although if several genes are implicated then there may be some overlap.

8.6 It is possible that two sets of genes may be operating, one reacting to low temperatures, the other to low light. Genes affecting the rate of development and the timing of different growth stages, will inevitably be implicated and could be a major reason for the differences in response between varieties. The semi-dwarfing genes because of their influence on seed setting may be involved in the response but only indirectly.

8.7 The origin of the genes responsible is most likely from the use of germplasm, unadapted to UK conditions, in varietal crosses.

8.8 Sub-clinical levels of fertility arising from partial male sterility may be widespread and may be an inherent feature of varieties. If this is the case then breeders need to be more aware of this character in their selection procedures.

8.9 If breeders are diligent and institute regular surveys of their breeding material involving the screening for adverse reactions to extreme environments, and if routine trialling is strengthened by increased monitoring, then it is unlikely that another catastrophe of the scale of Moulin will happen.

9. Recommendations

Although one of the conclusions indicates that it is unlikely that UK Agriculture will suffer another Moulin episode, it is nevertheless a rather unsatisfactory position since there is still no clear understanding of the conditions that were responsible, nor of the genes involved. A similar point can also be made about the different conditions and genes implicated at Aberdeen in 1997. Indeed, the most striking aspect of this review of sterility in winter wheat is the almost total absence of a body of established facts about its causes in the UK. The most important recommendation therefore is the need to instigate further research studies. In order of priority I would list these as follows:-

9.1 Identify the genes responsible for the "Moulin effect" and clarify the environmental components involved in the interaction. Although several genes are probably implicated, some genes are very likely to stand out as being of particular significance and should be identifiable. The material for making these studies is available in research Institutes such as the JIC so there is no expectation that this would require a long protracted period of development before detailed analysis could take place. It is imperative though that such research should include environmental variables, particularly low light levels and low temperatures, but also boron which may be central to inducing this type of environmental sterility. It is essential to include these variables because some of the genes could be specific to particular environments, but also there is a need to examine the Rawson hypothesis that the other environmental variables act by influencing boron levels.

9.2 This research should also include a parallel study of at least one of the vulnerable varieties shown up at Aberdeen. Pentium, because of its very different parentage from Moulin, is probably a good candidate for this.

9.3 One consequence of a successful outcome to this research should be the characterisation of the major genes involved in the sterility, their modes and times of action and how they are influenced by the environment. More importantly for future breeding, the expectation would be that these genes could be marked by molecular probes and these would give the breeder the opportunity to screen for their presence in his wheat breeding programme. Although this is a long term answer, it is nevertheless the one which is likely to be the most effective in overcoming any problems in the future.

9.4 The pattern of weather conditions responsible for inducing sterility in wheat in the UK should be defined more precisely. This is a difficult task but if an accurate description were available it might alert growers to potential problems of sterility in their crop—although this might come too late.

9.5 If as a result of the investigations described above, boron was found to be the important intermediary then the use of boron sprays administered at an appropriate time may be a way of alleviating the problem. Indeed, the routine application of boron could prove to be a useful way of reducing fertility problems in wheat, irrespective of weather conditions, and might even be a way of removing varietal differences in sub-clinical levels of sterility. Some research into this aspect is recommended but the role that boron plays in preventing sterility needs to be established first before such work should be started.

9.6 The on-going research into sub-clinical levels of fertility/sterility amongst varieties needs to be continued to find out if there are consistent differences between varieties. If this should turn out to be the case then the genetical basis of these differences needs to be established since it could have far reaching consequences on the development of future varieties. This should be research that industry should be eager to support.

9.7 The effectiveness of the NIAB trialling systems in detecting fertility disasters of the Moulin kind was a continuing theme of discussions with breeders and in assembling the review. The trialling system adopted by NIAB and associated organisations is extensive and it is fair to say that it covers the majority of the conditions likely to be met by wheat within the UK. It has been argued that the Aberdeen result was a "one-off" due to an extreme and unrepresentative site. However, what may seem an extreme result in one year may be more widespread the next year or the year after. This is indeed one of the strengths of the system since it samples the environment widely, and arguably one of its virtues is that it covers the extremes.

However, irrespective of the number of sites, is the monitoring at sites adequate? One of the deficiencies noted in this review was the absence of any organised scheme for "eye-ball" monitoring the fertility of varieties in trial. Could trial officers be trained and encouraged to observe sterility/fertility levels in this way as part of their routine recording of trials? Would the inclusion of sensitive "marker" varieties such as Moulin and Pentium in trials help? Both these questions are worth considering by the testing authorities. However, if "marker" varieties were to be used, then provision should be made to screen electrophoretically seed from these varieties to establish levels of outcrossing since final seed set may be misleading and could obscure the true level of sterility occurring.

9.8 The existing and extensive trialling system is sufficient for detecting environmental sensitivities and it is unnecessary for NIAB and others involved in the testing system to create expensive to run controlled environment facilities to pre-test lines submitted for trialling. At this stage of understanding such a detailed study of sensitivities is more properly the role of research institutes or breeding companies.

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