PROJECT REPORT No. 313

IMPROVEMENT OF OILSEED RAPE ESTABLISHMENT BY SEED SELECTION OR SEED TREATMENT

AUGUST 2003

Price £9.75

PROJECT REPORT No. 313

IMPROVEMENT OF OILSEED RAPE ESTABLISHMENT BY SEED SELECTION OR SEED TREATMENT

by

G. D. LUNN[1], M. BULLARD[2], S. HOLME[1] AND R. BLUNT[1]

[1]Division of Agricultural Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD

[2]ADAS Arthur Rickwood, Mepal, Near Ely, Cambridgeshire, HR1 3PG

CONTENTS

ABSTRACT

It is difficult to establish oilseed rape successfully. Many interacting factors of seed, physical or biotic environment may cause failure at any point. The aim of this project was to increase the probability that a viable population of seedlings will establish through manipulation of the seed parent (mother crop) or harvested seed using a range of techniques including seed grading, florescence sorting, heat treatment and priming. Field experiments and controlled environment studies were carried out to demonstrate principles at a range of scales and levels of artificial climate control. The study formed part of a LINK project 0905 in collaboration with CPB-Twyford Ltd and Germain's UK.

Mother crop manipulation

Mother crops were successfully managed to produce seed with greater TSW and/or modified oil/protein contents and ratios. Subsequently, in controlled environment studies, these seed were shown to impart advantages in terms of germination and emergence. Large seed size in particular conferred advantages over a range of stress conditions, e.g. deep sowing, dry or waterlogged conditions. There were no effects of mother crop husbandry in the field but simulation of the effect of mother crop husbandry, through selection for larger seed, did exhibit a measurable effect. Assessment of several seed lots showed that denser, well-fertilised crops produced a greater proportion of large seed than sparser, less intensively-fertilised crops. Thus, we propose that mother crop husbandry is modified. A potential problem with such crops increased lodging risk, although this would not necessarily have an effect on seed quality. PGR use had no effect on seed germination, so these chemicals could be used to control lodging.

During this work it became apparent that improvements could be made in seed testing and the ability to discriminate poor seed lots. Current requirements (>85% germination in an ISTA test at 20°C) do not give a good indication of likely field performance. We propose that these refined tests could evaluate seedling performance at 10°C as well as 20°C, in drying and inundated moisture conditions. Propensity to secondary dormancy should also be evaluated for each seed batch. Improved establishment would almost certainly outweigh additional testing costs.

Post-harvest selection and treatment

The results show that there are relatively few realistic opportunities for enhancing seed quality by post-harvest selection and treatment. Gravity separation increased thousand seed weight slightly and could be used to manipulate protein and oil content. However, the changes achieved were small compared to those possible by mother crop manipulation and the optimum quality seed fraction was only a small proportion of the whole, making the process uneconomic. Similarly, the fluorescence sorter only removed a small proportion of seeds and did not result in significant improvement in rapeseed quality. Although some tests indicated reduced time to radicle emergence in Germain's primed seed, there generally appeared to be no advantage to priming seed from the sand emergence and field studies. The more rapid radicle emergence from some Germain's priming treatments suggests that further attempts to refine the process are warranted. Overall, the greatest improvements in seed quality were achieved by the simplest, cheapest, easiest and most reproducible method - sieving. In controlled environment and field assessments, selecting seed > 2 mm in diameter generally gave the greatest final percentage emergence. However, there were indications that the very largest seeds could reduce emergence capacity. Selection of a different proportion of seed (say 1.7-2.2 mm) could give an even greater benefit. This requires more research.

A key hypothesis tested was that selection and grading could impart a neutral or beneficial effect on seedling performance under all circumstances. For seed size grading and mother crop manipulation that hypothesis can be accepted. For other techniques the hypothesis was not true or techniques require further evaluation.

1.   **SUMMARY**

Poor establishment of rape is a long-recognised problem, calculated to cost the industry in excess of £30 M in the worst years (McWilliam, 1998). The problem is not so much one of low population, since oilseed rape can compensate well for populations below 10 plants m^{-2} (Lunn *et al.*, 2001), but of patchiness and complete emergence failure. The problem is multi-faceted, with interaction of many biotic and abiotic factors over an extended time period in excess of 50% of the crop's life cycle. For optimum establishment, oilseed rape needs a good seed bed, availability of water and oxygen and a sufficient temperature. Compacted, dry, and water logged soils, frost heave, soil-borne fungi, insect and other pests can all reduce establishment. Combined with extraneous environmental factors, intrinsic factors such as seed size, vigour, age and chemical composition all have their effects.

The work reported here was funded by HGCA and LINK with contributions from CPB Twyford Ltd and Germain's UK. The aim of the work was to evaluate strategies of mother crop manipulation or post-harvest modification and selection of seed in order to increase the probability that a viable population of seedlings will establish within the seedbed environment. The aim of the work was further to identify strategies that held universal benefit with no detrimental effects under any field circumstances. The study approached the research from first principles and therefore the physiological basis of improvements in seed and seedling vigour were identified where possible.

1.1   **Seed quality characteristics – fundamental observations**

The strategy of this work was to investigate the seed quality factors that affect establishment and which could potentially be manipulated by mother crop husbandry and post-harvest selection and treatment. The working hypothesis was that a general improvement in seed quality would have benefits for establishment across a wide range of stress conditions. The main factors of relevance were identified as seed size, maturity, vigour, protein/oil balance, resistance to pests and diseases and propensity to enter secondary dormancy. Where possible, the effects of these factors on germination, emergence and establishment were investigated in variable environments (different water availability, temperatures and sowing depths/soil structures).

At the initial stage of imbibition, the effects of seed size and protein/oil balance were investigated in interaction with temperature and osmotic stress. Profiles of imbibition were similar for all seeds at all temperatures, although imbibition was faster at higher temperatures and thus a constant thermal time for imbibition of ~400°Ch could be postulated. However, significant differences were found in the percentage water content imbibed at the plateau phase, the minimum required for subsequent germination. McWilliam (1998) proposed a minimum figure of 40% moisture uptake was necessary for subsequent germination, whereas here significant differences in water content were found between

3

the plateau moisture content of large (40%), medium (50%) and small seeds (55%). However, the gravimetric amount (i.e. weight) of water imbibed by small seeds was still smaller than that of large seeds. Effects of seed size and protein content on imbibition at high water potential (pure water) were statistically significant, but small. Studies of imbibition and germination where osmotic stress was controlled by polyethylene glycol (PEG) solutions showed germination (radicle protrusion) occurred at water potentials lower than the -1.5 MPa defined as the critical potential for germination by Pekrun *et al.* (1997). Assessment of imbibition and germination of seeds of different sizes and protein content at water potentials of - 2.0, - 2.25 and - 2.50 MPa showed advantages for greater seed size and high protein content, with high protein content seeds able to maintain germination under a greater degree of water stress than low protein seed. In addition, there was a greater final percentage germination of large seed than small seed at all water potentials studied. It must be remembered that the water potentials tested represent very dry soils, where large seed size and high protein content could thus give advantages for imbibition and subsequent germination. However, in most situations with a more plentiful supply, there would probably be little difference in germination and emergence (indeed, large seed size slows germination and emergence, although final percentage emergence may be the same). Water uptake and drought stress are undoubtedly of fundamental importance for ensuring establishment of oilseed rape. However, it appears that factors of size and protein/oil balance screened for overall effects on establishment may have initial benefits at imbibition. The initial process of germination is undoubtedly an area where future research would be beneficial and the effects of many factors and processes, such as priming, fluid drilling, coating *etc.* could be studied in more detail than has been possible here and may have the potential to help improve establishment.

The processes of seedling growth preceding emergence (root growth, hypocotyl extension and penetration of the soil by these structures) were not studied in detail, with the only effects of seed quality characters on final emergence studied. Such detailed work of assessing the forces operating during this phase of emergence requires specialist equipment and observations such as have been completed for species such as carrot, onion and pea (Finch-Savage *et al.*, 1998, Whalley *et al.*, 1999) and would undoubtedly be useful for oilseed rape, to quantify the particular advantages of varying individual quality factors. During these studies, observations were made of horizontal growth towards light sources (possible crack growth) and sub-surface growth under barriers such as a sand crust (presumably involving a sensing, signalling and hormonal mechanism), illustrating interaction between soil structure and seedling growth and emergence. Again, further studies of these effects would be useful.

Studies of germination showed that small seed germinated more rapidly than large seed, although final percentage germination was usually unaffected. The interaction of germination with temperature

confirmed the observations of other authors. A base temperature of about 3°C was calculated, agreeing with Marshall and Squire (1996), with increase in the rate of germination at temperatures up to 25°C. As found by Stokes *et al.* (2000) and Basu (2003), there was a great variation in response to temperature according to provenance (site and season of growth and subsequent post-harvest storage). The mechanism of this response is not understood, but has important ramifications for emergence and establishment. Some seed lots showed acceptable germination at the standard ISTA test temperature of 20°C but markedly reduced germination at 15 and 10°C. These temperatures are closer to conditions prevailing at the time of sowing and demonstrate that seed lots that pass ISTA regulations could still be of low vigour, causing poor establishment. Thus it is evident that better test systems are needed, for example a cold germination test such as is used in North America for corn and soya. We also found that low vigour seed lots leached larger amounts of electrolytes and glucose during steeping, which could possibly be developed into a quality test. We propose that, since 'vigour' remains a poorly understood character that effects seed quality over and above the main quality characteristics studied in detail in this report, with little opportunity for manipulation by mother crop husbandry, seed selection or treatment, then improvement in vigour testing methodology provides the most promising avenue of research likely to yield the greatest benefit in the short term.

Previous controlled environment studies have shown that high protein seed can emerge more rapidly than low protein seed (Stokes *et al.*, 2000) and these studies were not repeated here, although additional evidence for this finding was accumulated. Analysis of seed size and sowing depth, however, showed benefits (for final percentage emergence) of larger seed at deeper sowing depths. At shallow sowing, there was little difference between emergence of seed with diameters differing from about 1.4 mm to 2.4 mm. However, as sowing depth increased, emergence of small seed was markedly reduced. There also seemed to be a reduction in emergence of large seed (> 2.2 mm in diameter) and there is anecdotal evidence of similar findings in France. This may indicate that further refinements of the > 2 mm cut off for seed quality in this research might be possible (e.g. grading from 1.8 – 2.2 mm), although it has not been possible to pursue that approach during this research.

Studies of emergence in dry and water logged conditions at conventional (2 mm) sowing depths, also seemed to show advantages for large seed, with greater final emergence of large seed in both dry and water-logged soil. The effect of water-logging was to delay emergence and reduce the final percentage of emerged seedlings compared to the dry soil. Protein content also interacted with water availability, with small high protein seed showing greater tolerance for the stressed water-logged environment, with greater final percentage emergence. However, we need to be careful about generalisation of beneficial seed quality characters, since they may not improve establishment success in all environments.

Investigations of dormancy showed that conditions of darkness, low temperature and water stress could induce dormancy in the oilseed rape cultivar Apex. Imbibition was required for appreciable dormancy to develop, as significant dormancy (~40%) was found only in seeds incubated in aqueous polyethylene glycol solutions and not in those recovered from dried silver sand burial. Dormancy developed rapidly, with appreciable levels developed within one week of the PEG treatment and it would be interesting to determine the minimum period of water stress required to induce dormancy, since this may have ramifications for priming. There appeared to be no effect of mother crop on dormancy development, although only crops differing in mother crop plant population were studied and due to the highly variable nature of dormancy, this is difficult to assess. The effects of protein/oil ratio, maturity and seed size are unknown and merit further study.

As many as 80% of seeds could enter dormancy, which demonstrates how important the phenomenon could be to establishment, potentially reducing the number of plants per square metre from 60-120 to 12-24. The studies also indicated a propensity for dormancy to increase with time of water stress, with increasing development of a cold temperature requirement as well as a light requirement as time of water stress increased.

Study of the effects of soil-borne fungi on the emergence of different size fractions, showed a marked difference between emergence from sterile and non-sterile soil, with a large reduction in emergence in non-sterile soil (34% compared to 85%) due to soil-borne fungi. However, there was no interaction with seed size: in general larger seed gave greater emergence in both sterile and non-sterile soils and the ranking of seed sizes was the same in both soil types. There were indications of reduced emergence from the very largest seed size fraction, as was found with the sowing depth experiment.

1.2   Effects of mother crop management on seed quality

The effects of mother crop husbandry on quality characteristics of the cultivar Apex were investigated with growth of specific crops in 2000 and 2001 and by analysis of crops stored from previous experiments from 1996-1999. In many cases, there was a relationship between plant population, thousand seed weight and seed size distribution. As the pod canopy of low-density crops was made up of more late-formed branches, the seed yield was made up of a greater proportion of smaller, more immature seeds. Thus, as plant population increased, thousand seed weight, seed maturity and the fraction of seed greater than 2 mm also usually increased. This effect was found both in the inbred cultivar Apex and in the male sterile parent of the hybrid Navajo. Nitrogen fertilisation also had an effect, with greater thousand seed weight and fraction of seed > 2 mm associated with greater N

fertilisation. Increased population and nitrogen fertilisation also decreased the amounts of immature orange or brown-coloured seed. Effects of mother crop management on specific weight and density were not clear.

Nitrogen fertilisation had a very strong effect on the balance between protein and oil content in the seed. Increasing nitrogen content increased protein content and decreased oil content, which were strongly related in a reciprocal fashion. Thus it is possible to manipulate important quality factors of seed diameter and protein/oil content by specific crop husbandry.

Contrary to previous assessments, where it was postulated that seed quality would be greater from more open canopies characteristic of low density 'canopy managed' rape crops due to a better microclimate during germination and consequently better germination, no difference in germination between seed from lodged and standing canopies was found. Indeed, experimental findings were opposite to the original benefits since the effects of high population and plentiful nitrogen fertilisation (characteristic of dense canopies) on the main seed quality parameters of size distribution, maturity and protein/oil balance appeared to outweigh any effects of canopy structure and microclimate on vigour.

1.3   Effects of seed selection and treatment

Seed size selection by sieving over a 2 mm screen always gave significantly greater thousand seed weights (TSW) in the > 2 mm fraction than in the < 2 mm fraction, and usually gave greater TSW in the > 2 mm fraction compared to the ungraded control. Final germination percentage was usually unaffected, although germination was slower in large than small seed. The greatest effects of seed size separation were seen in field establishment (see later) and were greatest at deeper sowing depths (see previous section). The graded fraction recovered ranged from about 30-70%, depending on provenance, with more large seed from dense well-fertilised canopies.

Gravity separation also slightly increased TSW in 'prime' fractions compared to ungraded controls, although the percentage increase was smaller than for sieving and less often significant. The proportion of the prime fraction separated by gravity separation was smaller than that possible by sieving. Gravity separation also allowed manipulation of protein/oil balance, although the differentiation possible was less than that due to manipulation of nitrogen fertiliser and so it appears that sieving would be the preferable bulk processing treatment, since it is more easily defined and controlled than gravity separation.

Assessment of fluorescence-sorting techniques demonstrated that immature seeds with higher levels of chlorophyll in their seed coat could be separated from the main sample of more mature seeds. However, in the oilseed rape samples analysed, the fraction of seed isolated was very small and comparison of graded and ungraded seed lots did not identify any significant difference in germination. This is an expensive technique and its main use is likely to remain with small quantities of highly valuable horticultural seeds rather than with bulk agricultural commodities.

Previous studies have demonstrated possible improvements in germination and emergence due to seed advancement or priming. In these studies, both hydropriming and an experimental priming method developed by Germain's UK were studied. While there were often improvements in the rate of germination and emergence at high temperatures due to priming, in some cases the rate of germination at lower temperatures was reduced. In many instances, the final percentage germination and emergence of primed or advanced seeds was reduced. Previous tests had shown relatively large advantages in priming poor quality seed, but from these studies it appears that priming can equally reduce the quality of better seed lots. Thus priming does not cause a constitutive improvement in all cases, at least using the protocols adopted for the current study, and might only be implemented on an individual seed lot basis. Results from dormancy studies indicate that the controlled priming could alter the dormancy properties of the seed. The more rapid radicle emergence from some of the later Germain's priming treatments (in one case almost halving the time to 50% emergence) suggests that further attempts to refine the process are warranted. However, most information gathered in this study suggests that for seed of relatively high quality, where germination and vigour is relatively good, these processes resulted in no significant improvement in germination and emergence. One must stress that these priming treatments were experimental and might be further refined. Other work has reported greater improvements in low vigour seed due to priming.

1.4   Field-scale evaluations

Field experiments were conducted at ADAS research centres in 1999/00, 2000/02 and 2001/02 in order to investigate the field performance of seed that had been grown under different mother crop husbandry regimes or had been selected and graded post-harvest. Effects of seed carry-over were also investigated. Within year and site in the second and third year of the field studies time of sowing were included as additional factors in order to vary the environmental conditions. Common to all experiments was the accurate characterisation of seedbed conditions prior to drilling, the manipulation of the seedbed to provide the most hostile environment possible to the seed and quantification of seedling emergence and growth thereafter.

Mother crop husbandry and priming seed failed to demonstrate tangible effects in the field in terms of crop emergence or seedling characteristics. Three year's field work demonstrated that only seed grading by size could provide a measurable improvement in establishment performance, and even here results were inconsistent. Never did selection of large seed act against establishment performance but often failed to demonstrate an improvement. The likely cause is clear – selection of large seed produced larger and more robust seedlings but it did not improve the germinability and likelihood of seedlings to emerge under the field conditions experienced. Consequently, whilst selecting large seed could not be discouraged as a commercial practice we have failed to demonstrate a compelling reason for undertake the expensive operation in the first instance.

A key component of the hypotheses under test were that selection and grading could be expected to impart a neutral or beneficial effect on seedling performance under all circumstances. For seed size that hypothesis can be accepted, although the relative value of the effect is questionable. In all years and sites where the crop was taken to yield there was no significant impact of seed treatment on final yield. This was true even where the seedbed had been produced to be sub-optimal on purpose to exaggerate any beneficial effects of seed grading.

1.5   Conclusion

Of the seed quality characteristics identified as having potentially the greatest effects on crop establishment (size, maturity, protein/oil content, vigour, propensity to enter secondary dormancy and pest and disease resistance), seed size, protein/oil balance and vigour appeared to have the greatest effects in controlled environment and field studies. Vigour, the general strength and fitness of the seedling for its purpose of propagating the next crop, remains a very poorly defined and poorly understood property depending on genetic, site and seasonal factors during development and maturation and storage.

Secondary dormancy is undoubtedly potentially a major limiting factor for establishment in particular circumstances, including sowing genetically susceptible cultivars into conditions promoting dormancy (darkness, water stress, low temperatures and low oxygen availability). The phenomenon is still poorly understood and more research to understand the process is needed, along with a programme to remove low vigour traits from breeding material.

Effects of diseases and pests on establishment can be very great. Experimental work showed establishment of undressed seed in sterilised soil was far greater than in unsterilised field soil, due to

the effects of soil-borne fungi. Effects of insect pests such as cabbage stem flea beetle and molluscs such as slugs are also well known. However, agrochemical controls of these phenomena are available. Whilst seed factors conferring resistance to fungi and pests could be further investigated, identifying and incorporating such traits into breeding material would involve an ambitious research programme entirely outside the scope of this work and the authors believe that this is an area for more industry input.

Of the main factors effecting crop establishment, seed vigour, had great impact on establishment, with poor establishment of low vigour seeds and good establishment of high vigour seeds. Unfortunately vigour is the least understood quality characteristic and the least controllable and open to manipulation by mother crop husbandry and post-harvest treatment. Vigour is affected by many factors including membrane integrity, DNA damage and repair, chemical composition, age and size. These factors are influenced by environmental conditions during seed development, ripening and harvest and by harvesting and storage procedures, with improper storage potentially one of the major factors associated with rapid reduction in vigour. Better understanding of the development of vigour during seed growth and the subsequent effect of storage requires further extensive research and is outside the scope of this work. Perhaps the easiest route to improving vigour would be by enhanced seed testing methods, since current germination tests fail to discriminate many low vigour seed lots that would show poor field emergence. Many different tests have been applied to other species such as maize and soya, so transfer of the technology to oilseed rape could potentially be rapid. Current requirements (>85% germination in an ISTA test at 20°C) do not give a good indication of likely field performance. We propose that as a starting point these refined tests could evaluate seedling performance at 10°C as well as 20°C, in drying and inundated moisture conditions. Propensity to secondary dormancy should also be evaluated for each seed batch. Whilst there would undoubtedly be additional costs incurred in this additional testing, improved establishment would almost certainly outweigh the cost.

Of the remaining factors, protein/oil balance has been shown to effect emergence more reliably in controlled environment studies, although it has been more difficult to demonstrate convincing advantages in the field. More rapid emergence of high-protein seed has been demonstrated in relatively good emergence conditions, which could be advantageous for production of more homogeneous crops, possibly in late sowing situations. On the other hand, there are also indications that high oil content may be more advantageous in stress conditions such as dry seed beds, deep sowing and compacted soils. Thus, the advantages of high protein and high oil content may be mutually exclusive, with environmental conditions determining which type of seed may give the greatest benefit. It is very difficult to predict seed bed conditions with any accuracy at the time of

seed purchase and processing and so the producer would be unsure of which type of seed to sow to gain the optimum advantage. Seed protein content can be easily manipulated by mother crop nitrogen fertilisation, with high levels of fertiliser resulting in increased percentage protein and diminished percentage oil. However, with the high nitrogen strategy there is the danger of encouraging excess canopy production, lodging and yield loss. Our work has also shown that it is possible to manipulate protein and oil balance by gravity separation. However, the differences are smaller than those induced by fertiliser amounts and the proportions of seed separated into extreme fractions were small. As no universally applicable protein/oil balance can be defined giving advantages in all conditions, manipulation of this factor is unlikely to be economically worthwhile.

The main factor, found to give advantages over a wide range of environmental conditions both in controlled environments and in the field, was seed size. In this work, selecting seed > 2 mm diameter usually gave advantages over < 2 mm diameter and ungraded seed, giving greater final emergence. Although there was little advantage of large seed at shallow sowings under optimal conditions (due to more rapid germination and emergence of small seed), large seed showed numerous advantages at deeper sowing depths as well as in dry and waterlogged conditions. There are also reports in the literature of large seed giving rise to larger more competitive seedlings with greater resistance to pests. This was demonstrated in the third field season of the current study, where large seed gave rise to larger seedlings with greater green leaf area. It therefore seems that the greatest opportunity for improving seed quality currently rests with increasing seed size, or selecting the largest seeds. Studies of mother crop husbandry showed that for both conventional inbred and male-sterile parental lines of hybrids, a greater proportion of seed > 2 mm was usually produced with increasing mother crop plant density. For inbred lines, it was also shown that increasing nitrogen fertilisation correlated with an increased fraction of large seed. The advantages of grading seed by gravity separation and fluorescence sorting were marginal. The most effective priming treatment reduced the time taken to 50% emergence in controlled environment experiments, but most of our studies suggested a neutral impact on emergence profile or even potentially reduced field emergence. Priming does however warrant some further study. Thus the main opportunity for increasing seed quality was by seed size selection, by sieving or by improved mother crop husbandry.

Mother crop husbandry and priming seed failed to demonstrate tangible effects in the field in terms of crop emergence or seedling characteristics (the latter potentially due to induced secondary dormancy). Three year's field work demonstrated that only seed grading by size could provide a measurable improvement in establishment performance, and even here results were inconsistent. Never did selection of large seed act against establishment performance but often failed to demonstrate an improvement. The likely cause is clear – while selection of large seed produced larger and more

robust seedlings it did not improve the germinability and likelihood of seedlings to emerge under the field conditions experienced – which perhaps were not sufficiently challenging. Consequently, whilst selecting large seed could not be discouraged as a commercial practice we have failed to demonstrate conclusively that the activity would be cost effective.

A key component of the hypotheses under test were that selection and grading could be expected to impart a neutral or beneficial effect on seedling performance under all circumstances. For mother crop manipulation and seed size that hypothesis can be accepted.

2.   GENERAL INTRODUCTION

Sustainable rape growing (maximising light harvesting efficiency for optimal final seed yield but minimising the influence of weeds, pests and diseases) depends on the achievement of uniform plant populations at optimum density. Oilseed rape (comprising winter and spring *Brassica napus* ssp *oleifera* and *Brassica rapa (*syn. *campestris)* ssp *oleifera*) is the third most important combinable crop in the UK, after wheat and barley, covering the third largest arable area. In the UK, mostly *B. napus* is grown, and for the purposes of this report 'oilseed rape' refers to this species. In 2000/2001, 451,000 ha were grown producing 1.16 Mt of rapeseed, worth approximately £151 M (DEFRA, 2002). In most years over 80% of the area is sown with the winter crop, although this can vary significantly depending on autumn establishment conditions. The amount of oilseed rape grown in the UK has risen explosively since the early 1970s: before joining the EU in 1973, less than 1,000 ha were grown annually (DEFRA, 2002). Much seed has been traditionally farm-saved, but rapeseed used for sowing is potentially worth £7 M per annum (Gooding *et al.*, 2000). Recent changes in regulations have removed the requirement to test levels of seed glucosinolates, so farm-saving of seed may increase markedly in the next few years. Since the crop is a relatively recent domesticate and has been widely cultivated in the UK only for the last 30 years, the species has had less breeding effort than cereals and retains some wild and weedy traits. Thus many agronomic problems remain to be solved.

One of the major problems associated with cultivation of the oilseed rape crop is that of poor establishment. This particularly the case for the 60% of the national crop sown into heavy clay soils where cereal residues have been incorporated. Data collated by Daniels *et al.* (1986) from experiments at Wye College during the period 1979-1984 showed variation in percentage establishment from 27-88%, with a mean of 62% and a large coefficient of variation of 27%. More recently, analyses by McWilliam (1998) showed emergence varied from 10-79%, with a mean of 30%, in experiments over three sites and two seasons, with six seed bed preparation methods. Although the crop can compensate well for low plant populations, provided those plants are evenly-spaced (Lunn *et al.*, 2001), in the case of patchy establishment the plants cannot fully compensate. The consequent yield loss from patchiness may be exacerbated by other problems such as weed ingress and greater grazing pressure from pigeons. McWilliam *et al.* (1995) estimated a cost of £120 ha^{-1} for re-drilling poorly established winter rape with spring rape. In this case there would also be a yield penalty of approximately 1 t ha^{-1}, worth about £140 ha^{-1} at current prices (Anon., 2002). Consequently, for each per cent of the national winter oilseed rape area suffering poor establishment, losses to the industry of £1.05 M per annum could accrue. In the worst years, McWilliam *et al.* (1995) postulated that 30% of crops could fail, causing losses to the industry of over £30 M. As well as costs and lost yield due to re-drilling, losses are also incurred from reduced yield in exceptionally sparse

crops that are not re-drilled and from use of greater agrochemical inputs (herbicides and pesticides). All of these considerations reduce the sustainability and profitability of the crop.

Poor establishment or patchy areas directly reduce the sustainability of rape growing by increasing the reliance on autumn herbicides. This leads to uneven plant development and less uniform crop growth causing less even ripening and, necessitating greater use of pre-harvest desiccants. In some circumstances larger applications of nitrogen fertiliser may be applied in the spring to try and hasten canopy expansion but this has its own associated environmental and economic costs.

Rapid and synchronous establishment generally reduces the need for broadcast molluscicides which are proven to have a detrimental effect on wildlife, and negative effects on food chain dynamics. Re-drilling incurs costs in terms of additional seeds, additional labour, tractor hours, fossil fuels and agro-chemicals expended in establishing a second crop, and may also disrupt beneficial soil-living invertebrates. In a re-drilled or patched crop it is likely that reliance on agro-chemicals will be higher still because of the greater pressure from pollen beetle and other insects migrating from earlier flowering crops to the later, less developed later sowings. At the other end of the spectrum from thin, patchy crops, excessive plant establishment resulting from use of high 'insurance' seed rates creates its own problems. Excessive interplant competition increases crop height and lodging leading to pod abortion and uneven ripening in the lower portions of pod canopies, which will increase reliance on desiccants.

The risk of failure during establishment is high because the seed is small (and thus has few energy reserves) and production of suitable seedbeds difficult, especially where straw has to be incorporated into relatively dry heavy clay soils typical of the conditions in which the majority of rape is grown. In field and laboratory conditions, Bullard *et al*., (1996) and McWilliam *et al*. (1998) have examined the physical and biotic forces which operate either singularly or in combination to impair establishment. Losses can occur in any or all of three phases - sowing to germination, germination to first emergence and from emergence through to the establishment of a plant that will survive to contribute to yield. Pressures exerted on the seed and seedling often result from a combination of forces because most rape is grown on unstable, heavy clay soils where the previous cereal crop has dried out the soil profile and cereals residues have to be incorporated. Rainfall can be so low and evaporative losses from the soil so high that the young seedling is extremely vulnerable to desiccation. These previous studies by the University of Nottingham/ADAS Centre for Agronomy have shown that mechanical solutions alone will not reliably overcome establishment deficiencies because the factors that effect germination and seedling emergence processes seldom act in isolation (Bullard *et al.*, 1996). These factors have been characterised in detail by McWilliam *et al*. (1998). From these studies which

defined the impact of seed size, seed depth, soil temperature, aggregate size and moisture availability on the seed/seedling it was concluded that the best way to achieve reliable seed establishment was to improve the quality of the seed itself. To this end we have developed a number of hypotheses related to seed performance which should allow a conceptual 'ideotype' seed to be developed. The scope for the improvement of establishment through seed selection and pre-treatment was first demonstrated by Stokes *et al* (1998). There were indications that the improvements possible from each individual step, i.e. seedlot selection, seed grading and seed advancement, might combine to give a large overall benefit in specific situations. Thus, we have preliminary observations which suggest that oilseed rape could be graded or selected following harvest to improve establishment success.

HGCA-sponsored work (Bullard *et al.*, 1996, McWilliam *et al.* 1995, McWilliam, 1998) has reviewed the possibilities for ensuring better establishment by specific cultivation methods. However, despite the provision of a decision support tree (Bullard *et al.*, 1996), the prospects of this approach are slight, and for winter oilseed rape are highly dependent on unpredictable autumn weather conditions. Other HGCA-sponsored work (McWilliam, 1998, Stokes *et al.*, 1998, 2000) has shown a greater variation in seed quality than was expected, which could have large effects on establishment. Intra-varietal differences, due to site and season of production, husbandry, storage, *etc.*, have often been greater than inter-varietal differences due to genetics. Consequently, it was theorised that a general improvement in seed quality would enable better establishment across a wide range of seed-bed and environmental conditions. The possible routes to improved seed quality were hypothesised to be through specific mother crop husbandry, seed selection and seed treatment. Combined with optimised seed-bed structure where possible, the best quality seed gives the optimum chance for uniform establishment of the desired plant population.

Improving the quality of the seed is an approach which is likely to produce significant benefits in almost all circumstances without any significant increase in risk. It therefore appears that the most valuable gains in establishment are likely to come from improving seed quality and then integrating these benefits with the improvements that can be gained from better seedbed preparation.

Also, although selecting and pre-treating seed appears, on the basis of laboratory tests, to significantly improve performance (Stokes *et al.* 1998), there is little time between harvest of the seed crop and drilling of the following commercial crop to implement these procedures. In Scotland, the time is so short that seed has to be carried over winter. At present, there is little indication that seed crops are managed similarly to those grown for food and industrial use. Analysis of commercial seedlots, sown by growers in 1996, has shown there is much variation from seed to seed in stage of maturity (as indicted by the colour of the seed coat), and that black seed shows improved germination and early seedling growth. In some seedlots, the black (most mature) seed accounts for about 70-80% of the

seedlots and therefore almost immediate benefits could accrue from growing crops to improve the uniformity of maturity and increase the proportion of black seed. Does maturity have an effect on subsequent performance, and if so is that related to the absolute oil/protein content of the seed or the ratio of these products? If so, does the optimum differ from normal ware crops?

The majority of the national rape crop is sown from seed from the recent harvest, whilst a smaller proportion, for sowing in Scotland and the north, has been carried forward from one year's harvest to sowing 12 months later. This is because seed for planting is required at the same time as seed crops in the north are being harvested. In practice, the production of hybrid seed involves a carry forward from harvest to the sowing year, so this offers more opportunity for post harvest treatments. Overwintering of seed is strategically prudent to ensure stability and timeliness of supply. Hybrid production involves greater risks, there is a longer lead time but higher potential returns. Hybrid mother crops tend to ripen late. For isolation, they are remote from the main production area, usually where crops are less forward but where yield potential is high. These late ripening influences may then be compounded if seed set is poor, for this leads to exaggerated indeterminacy and late greenness.

For all seed sown it may be possible for seed producers to improve the general quality of seed. It must be demonstrated that seed with improved performance can be reliably produced as a result of more specific management of the mother crop to ensure that the conditions for seed development and maturation are optimised.

Relevance to hybrids
The principles that apply to the manipulation of ripening patterns will apply equally to the production of hybrid seed. In fact, any factor that advances and improves uniformity of ripening e.g. manipulation of length of growing season, nutrition, fungicide application and direct growth controlling measures, including plant growth regulators, will be equally, if not more relevant. The difference is that manipulation of plant population is constrained by considerations of seed supply and access to rogue. This increases the challenge to manipulate with other measures. There may be an impression in some quarters that all hybrid seed is larger than normal seed, and thus establishment problems would be minimised. In fact, there is a complete range of seed size from hybrids, but conditions can conspire to give more seedlots with the distribution at the larger end of the range. The production of hybrids is in crops where strips of male sterile up to 12m wide are interspersed with narrower strips of pollinator. Two elements may result in largish seed. Because of constraints of seed supply and to facilitate roguing, the male sterile strips may be sown at lower seed rate. Moreover, particularly in weather unfavourable for pollen spread, seed set can be poorer than when

there is an intimate mixture of pollinator and male sterile plants. Any apparent increased vigour of hybrids in both emergence and post-emergence survival, may be due to generally larger seed, but there is also a component of heterosis. The crucial point is that even with the largest seed stocks and with heterosis manifest, there is still a critical need and much scope to improve seed performance by selection and pre-treatment.

Experimental aims and objectives

The aim of the work reported here was to increase the probability that a viable population of seedlings will establish within the seedbed environment through manipulation of the seed parent (mother crop) or post harvest manipulation of harvested seed.

In order to achieve this the following experimental objectives were followed:

- identify the characteristics of the seed which best correlate with reliable optimal performance (i.e. high likelihood of successful germination and emergence) in the field, before and after storage;

- identify how management of the mother crop can be improved to produce the highest proportion of reliable seed with required characteristics identified above ;

- for non-carried-over seed, identify which selection procedures can be implemented in the short time available to give worthwhile improvements in performance with no associated risk. This will include examination of the place for prophylactic seed dressings;

- for carried-over seed, to devise and evaluate, seed pre-treatment systems using our understanding of the physiological basis of the vigour related process. This will include examination of the place for prophylactic seed dressings;

- identify the overall benefit to establishment from integrating the improvements in seed quality with the benefits that can be achieved from improvement in seedbed production.

These objectives were examined in a suite of experiments conducted under controlled or field conditions. To aid clarity of reporting the work undertaken to meet these objectives is reported in the following four sections:

Section 3: Investigations of fundamental factors effecting seed quality, germination and emergence in controlled environment studies
Section 4: Effects of mother crop management on seed quality factors
Section 5: Effects of seed selection and treatment on germination and emergence in controlled environment studies
Section 6: Field scale evaluations of seed selection strategies and seed treatments

3. **INVESTIGATIONS OF FUNDAMENTAL FACTORS EFFECTING SEED QUALITY, GERMINATION AND EMERGENCE OF OILSEED RAPE IN CONTROLLED ENVIRONMENT STUDIES**

3.1. Introduction and literature review

This next section reviews the basic problems of poor establishment and assessment of seed quality in more detail, and gives background details of some areas of fundamental significance where studies were completed under the auspices of this project.

3.1.1. Factors contributing to poor establishment.

In winter (autumn-sown) oilseed rape, the 'establishment' period lasts for approximately six months, from sowing in August/September to March. Final establishment is defined as the point when the ultimate population of plants that will contribute to yield remains after winter (Bradbeer, 1988). After seedbed preparation, the establishment period encompasses the three phases from sowing to germination (lasting days-weeks), germination to emergence (weeks), and from emergence to survival of the established plant after winter (months). During all three phases, environmental and seed/seedling quality factors may interact to effect final establishment.

3.1.1.1. Seed-bed structure and aggregate size

As described previously, the ideal seed bed consists of a fine tilth (aggregate size < 17 mm, ideally ~ 2 mm) to allow good seed-soil contact, with adequate moisture reserves for imbibition and seedling emergence. McWilliam (1998) showed emergence in a 12% moisture soil composed of aggregates > 23 mm was about 5%, compared to 80% in a soil with aggregates < 17 mm, due to poor seed-soil contact and inadequate moisture transfer. Cloddy soils also make it difficult to control sowing at the ideal 15 – 20 mm depth. Rape seeds are unable to emerge from deeper than about 75 mm (Garrett and Orson, 1989) and may have to grow around large aggregates, or under compacted soil or caps, thus using up their stored food reserves before they can emerge. As this work is concentrating on improvement of seed quality there has not been opportunity to study further effects of seed-bed structure and aggregate size in detail, although this is undoubtedly an area where further research could be beneficial. However, field assessments were undertaken by ADAS in soils of differing aggregate size distribution (Section 6).

3.1.1.2. Factors needed for seed germination

In order to germinate, oilseed rape needs an adequate supply of moisture, a sufficient temperature and availability of oxygen. In addition, the seeds have to be viable and non-dormant. Other factors that may prevent germination include infection with fungal pathogens and consumption by pests such as slugs and barriers such as deep sowing and compacted soil.

3.1.1.2.1. Moisture imbibition

By convention, the water potential (ψ) of pure water is zero, and water flows from high to low water potential. Water potentials of seeds and soils are negative and are given by the formula $\psi = \psi_\pi + \psi_m + \psi_p$. The osmotic potential, ψ_π, is due to the dissolved solutes in a cell or soil and is negligible in dry seeds. The matric potential, ψ_m, is due to the colloidal properties of seed constituents such as proteins and cell walls or soil particles. Both ψ_π and ψ_m are negative. The pressure potential, ψ_p, is positive. In dry soils and seeds ψ_π dominates and water transfer between seeds and soil is largely due to differences in matric potential. Dry seeds have strongly negative water potentials (-150 to -350 MPa) due to the colloidal properties of their contents and in moist soils water thus moves from the higher water potential of the soil solution to the lower water potential in the seed. Osmotic potential may be important in saline soils and may be effected by seed bed fertilisers: ψ_π also becomes the main driving force behind water uptake in developing roots. Water uptake in germinating seeds is triphasic. After the rapid initial uptake of water by imbibition, water content remains constant in a 'lag phase', before water uptake increases rapidly again in the tertiary phase of root and shoot elongation.

The first phase of water entry into the seed, imbibition, is a physical process, dependent on the colloidal properties of the seed contents. This may be limited by non-permeable seed coats in some species (termed 'hard-seeded'), although this is not thought to be the case in rapeseed where initial water uptake is rapid. Water molecules are attracted to the negatively-charged parts of polymers and hydration occurs in viable and dead seeds alike (Mayer and Poljakoff-Mayber, 1982). The main hydrophilic colloids driving imbibition in seeds are proteins, although mucilages (polysaccharides, mucoproteins *etc.*), cellulose and pectic substances of the cell walls also play a role. Starch does not play a role initially and does not swell, although it may bind water due to hydrogen-bonding eventually. Oil does not play a part in imbibition, as it is hydrophobic, due to long aliphatic side chains in fatty acids.

Although there has been little recent work, microscopical studies (Edwards, 1968) have identified large mucilaginous deposits in seed coat epidermis cells in the related species charlock (*Sinapis arvensis*), and variation in these deposits could effect imbibition properties, as could variation in other cell constituents (protein/cellulose). The swelling of seeds due to hydration of colloids causes a strong

'imbibition pressure'. This seed ψ_p balances the osmotic and matric potentials during the 'plateau' phase of imbibition and may split the seed coat, which is possibly softened by cell wall degrading enzymes such as cellulases and polygalacturonases (J.A. Roberts, pers. comm.), allowing radicle emergence. This process may thus be effected by the strength of the seed coat, activity of cell-wall degrading enzymes and any mechanical damage. With the removal of the positive ψ_p, the water potential again becomes negative due to ψ_π of the root cells, allowing water uptake to continue by osmosis.

McWilliam (1998) showed that rape seeds needed to imbibe about 40% by weight of water before germination could be completed (as defined by radicle protrusion). The metabolic processes of germination (e.g. DNA repair, DNA, RNA and protein synthesis, and respiration) appear to commence at lag phase moisture contents, but germination is not completed by root growth unless the water uptake continues above 40%. The final part of the triphasic water uptake curve does not occur in dead seeds. Seeds can be maintained in the lag phase for relatively long periods and they appear to undergo self-repair processes. These are however not thought to occur at lower moisture contents (e.g. 15-30%), leading to gradual vigour loss. Seed can survive desiccation from lag phase moisture content with relatively few ill effects and it is understanding this process that underpins seed advancement and priming technologies. After the tertiary phase of moisture uptake and radicle extension has begun, desiccation is usually lethal (McWilliam, 1998).

One potential problem is that with increasingly early drilling, oilseed rape seeds may be sown into conditions where the soil matrix potential, ψ_m, is below the critical value, postulated at approximately -1.5 MPa (Lutman *et al.*, 1994, Pekrun *et al.*, 1997, McWilliam, 1998) needed for germination. Different soils need different gravimetric water contents to provide the same amount of available water (measured as ψ_m), due to the different strengths of attraction between soil particles and water molecules. Clay soils require higher gravimetric moisture contents (about 20%) than sandy soils (8%) to provide the same ψ_m, due to the greater attraction of clay particles for water and McWilliam (1998) derived an equation to define the soil critical moisture content for germination based on its clay content and assuming a critical ψ_m of -1.5 MPa, below which germination cannot proceed. This information could be used to aid drilling decisions based on gravimetric moisture content measurements or simple moisture probes and knowledge of soil clay content, although validation of the approach on different soil types would be necessary. However, McWilliam (1998) also showed that large aggregate sizes reduced the efficiency of moisture transfer, thus altering ψ_m. In exceptionally dry soils, imbibition would not occur and seeds would remain in a resting state until rainfall increased the soil water potential. Problems are possible where ψ_m is sufficient to allow some water uptake, but insufficient to allow seeds to take up the required 40% by mass. In this situation,

seed vigour may decline and seeds may also enter secondary dormancy, which is discussed in a later section.

Different seed lots could have various imbibition properties due to genetic or environmental effects on seed composition and quality, such as variation in seed size, bulk protein and oil content, protein composition or levels of mucilages and other colloids in the seed coat. Damage to the seed coat (e.g. during combine harvest and subsequent seed processing) may also effect imbibition properties. There is also evidence from other species such as pea and soya that rapid imbibition can cause damage to rehydrating cells. These effects have not been studied in detail in oilseed rape, but differences between seed lots at the imbibition phase could effect later processes.

Stokes *et al.* (1998, 2000) showed more rapid and even emergence of seeds with high nitrogen (protein) contents from sand and soil, possibly due to more rapid water uptake by seeds with a greater hydrophilic protein content than seeds with more hydrophobic oil content. Smaller seeds also imbibed more rapidly than large seeds (McWilliam, 1998), probably due to a more favourable smaller surface area to volume ratio for water uptake, although there was no final effect on germination percentage. Consequently, advantages for rapid and synchronous germination (hence emergence) can be postulated for small, high protein content seeds in ideal fine-tilthed seed-beds with good moisture transfer properties. In these conditions, slower imbibition in large seeds could theoretically make them more susceptible to drying out and also increase heterogeneity of emergence. However, in less favourable conditions, advantages can be postulated for larger seeds with greater reserves of oil for growth, although imbibition and emergence could be delayed. These could emerge from greater depths and could be drilled deeper to take advantage of moisture reserves deeper in the soil profile, as shown by Stokes *et al.* (1998, 2000).
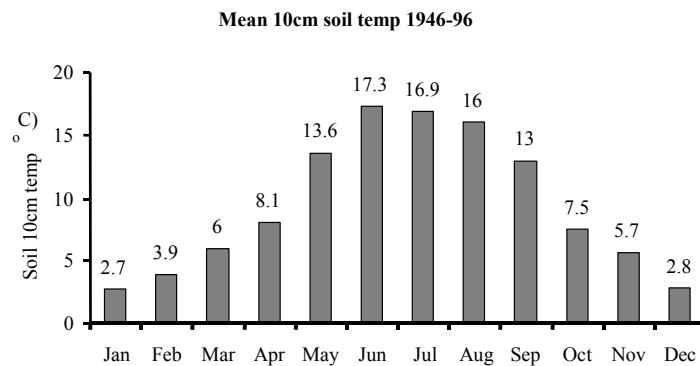
3.1.1.2.2. Temperature

The dry seeds of many species are able to tolerate quite wide temperature ranges, from -20°C (provided supercooling occurs and ice crystal formation is prevented) to about 90°C. Prolonged heating above 90°C generally reduces seed viability, but ultra-drying at 80°C may be useful for treating fungal infections. Hydrated, germinating seeds are however sensitive to a much narrower range of temperatures. Oilseed rape does not germinate below about 3°C (Marshall and Squire, 1996; Stokes *et al.*, 1998) and germinates slowly and often poorly below 10°C. The speed of germination increases from 10°C to 25°C and then diminishes above about 35°C, in the classical manner (Mayer and Poljakoff-Maber, 1982). Thus, linear relationships of germination rate (defined as 1/time to 50% or some other percentile of germination) to temperature can often be plotted to define the base

temperature (T_b) below which no germination occurs. Accumulated temperature in degree-days above T_b can then be used to predict germination. Marshall and Squire (1996), however, found significant diversion from linearity for early and late germinating percentiles of oilseed rape, giving a greater spread of germination than would be expected from a linear model (faster for early-germinators and slower for late germinators).

International Seed Testing Association (ISTA) regulations specify a temperature of 20-30°C for germination assessments to define quality of rapeseed (Anon., 1996). Figure 3.1 shows the long-term mean soil temperatures for Sutton Bonington in the UK. Even in July and August, the mean soil temperature is below the 20°C used in the standard test and, for later sowings, may be less than 10°C. For spring oilseed rape, soil temperatures may be even lower. In December and January, mean soil temperatures are below the 3°C necessary for germination.

Figure 3.1: Long term mean soil temperatures (10 cm) for Sutton Bonington

Stokes *et al.* (1998) and Basu (2003) showed that different varieties and different seed lots responded differently to temperature: some maintained high levels of final germination across a wide range of temperatures from 10-25°C; others showed good final germination at 20-25°C which was markedly reduced at 10°C. This could explain some of the variation in field emergence of seed lots meeting the ISTA criteria of >85% germination at 20°C and suggests that a cold temperature germination test analogous to that used with maize and soya in North America may be useful for rapeseed, since maintained germinability at low temperatures is a desirable trait, especially for later sowings.

Differences in cultivar, mother crop husbandry and post-harvest processing and storage may all effect the response of germination to temperature (Stokes *et al.*, 1998, Basu 2003), although it is unknown if factors such as seed size, protein content *etc.* also effect the response. Clearly for high quality seed, lots that germinate well across a wide range of temperatures are desirable. For UK conditions, good germination in the range 10-15°C is probably more important than germination at 20°C.

3.1.1.2.3. Oxygen supply

The resting seed shows very low levels of metabolic activity and needs little oxygen, but as germination progresses respiration increases rapidly to provide energy for growth and thus an adequate supply of oxygen is required. Oxygen availability is limited when the soil moisture is at field capacity (FC) and all soil pores are filled with water (water logging). Oxygen supply may also be limited in the soil if it is utilised by microflora, for example those growing on decomposing straw. Lack of oxygen may interact with other factors (*e.g.* darkness and low temperatures) to cause dormancy (Lutman *et al.*, 1994). However, in practice it has proven difficult to induce significant oxygen deficiency (Whalley and Finch-Savage, pers. comm.) and this factor will not be investigated in detail in this report.

3.1.1.2.4. Dormancy

Although oilseed rape lacks primary (endogenous) dormancy, adverse environmental conditions can cause secondary (induced) dormancy to develop (Lutman *et al.*, 1994). This secondary dormancy is 'photodormancy' and requires a stimulus of light to break it, although a very short pulse is usually sufficient (Lutman, pers. comm.), indicating the involvement of the phytochrome system. The main factors inducing secondary dormancy are low water potentials in darkness, such as might be experienced in a relatively deep dry August sowing (Pekrun *et al.*, 1997). As well as these factors, low temperatures and anaerobic conditions (water logging) may also cause development of dormancy.

Lutman *et al.* (1994) screened a large selection of varieties and showed there was a large variation in genetic propensity to enter secondary dormancy. Apex (the predominant cultivar of second half of the 1990s) was one of the most susceptible cultivars. Little is known of the propensity of more recent cultivars to enter dormancy, or how factors such as size and protein content might effect the process. There is anecdotal evidence of effects of harvest date and drying conditions on dormancy of spring rape (R. Weightman, P. Werner: pers. comm.) although little work has been done on winter rape.

3.1.1.2.5. Diseases, pests and toxins

Seeds may be destroyed by diseases and pests before they complete germination. Fungi such as *Alternaria brassicae* and *brassicicola*, *Peronospora parasitica*, *Pyrenopeziza brassicae* and *Leptosphaeria maculans* may be seed-borne (Hardwick *et al.*, 1991), but these are usually controlled by fungicide application to the mother crop and seed dressings such as Rovral, thiram and iprodione. They are thought to have little impact on germination, with effects usually becoming evident at the

seedling stage due to air-borne seed infection. However, parasitism of some seeds by some of these fungi can often be observed in germination tests. Soil-borne fungi such as *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani* may cause pre-emergence decay and 'damping off' and may form complexes (e.g. *Fusarium* and *Rhizoctonia*). These diseases are also usually adequately controlled at germination by fungicidal seed dressings, e.g. thiram, iprodione and fenpropimorph, but these do not confer extended protection post-emergence. However, work in wheat (Cockerel, 2002) suggests that effects of soil-borne fungi may be overestimated. Seed dressings themselves may have phytotoxic effects, which may slow or reduce germination, as shown by Stokes (1998) and may consequently be disadvantageous where there is low fungal inoculum. It is unknown whether seed size, oil/protein contents, maturity, advancement procedures *etc.* effect the susceptibility of oilseed rape to soil-borne pathogens or the phytotoxicity of seed dressings.

Slugs (*eg. Deroceras reticulum*) may be responsible for damage to emerging oilseed rape, although it is not known whether they eat seeds and seedlings in the same manner as wheat (Glen *et al.*, 1991) since 'glucosinolates' are repellent and deter generalist herbivores. The molluscicides methiocarb and metaldehyde can be applied in the form of pellets, and usage has increased. However, there is little evidence that slug pellets have a significant impact on slug populations or grazing (J. Oakley, pers. comm.). Resistance to slugs possibly could be improved by high seedling (not seed) glucosinolate levels, large seeds and seedlings, new chemicals or biocontrols such as nematodes but slug control *per se* is outside the remit of the project and has been investigated by other researchers (Green *et al.*, 2001).

Decomposing straw may produce toxins such as butyric and propionic acids which may effect germination, but Marshall *et al.* (1984) showed their concentration and efficacy declined exponentially from source and Stokes *et al.* (1999) found limited effects of decomposing straw leachates on germination in petri dish tests. Large levels of decomposing straw could also cause oxygen deficiency due to the large biological oxygen demand of the saprophytic microflora. The effects of allelochemicals (e.g. via glucosinolates) are well known and self-allelopathy could be possible during emergence, as could effects of residues being grown in close rotation. However, these topics are also outside the general remit of this work, although an HGCA-funded bursary student has examined some effects of allelochemistry.

3.1.1.3. Factors required for emergence

The main factors required for emergence are similar to those required for germination: an adequate supply of moisture, warm temperature and supply of oxygen. In addition, the drilling depth and soil structure (aggregate size, soil bulk density above and below the seed) have major impacts on

emergence. Toxins and nutrient shortage may also play a role in limiting establishment. 'Damping off' diseases, downy mildew (*Peronospora parasitica*), early phoma and light leaf spot infections, seed-borne *Alternaria* or root rot (*Phytophthora megasperma*) can all cause losses immediately post-emergence, but are usually controlled by seed dressings or early post-emergence sprays. Pests of importance at this stage include slugs (control discussed previously) and flea beetles (*Psilloides chrysocephala*). A chemical seed dressing is available to control flea beetles (Chinook, imidacloprid + beta-cyfluthrin) and although some researchers have showed greater resistance of large seeds and seedlings to flea beetles, studies of these pests is outside the remit of this research.

3.1.1.3.1. Moisture

After germination, continuous uptake of water is required to increase tissue moisture content to about 85%, expanding the cells of the hypocotyls and promoting emergence of the shoot and roots. McWilliam (1998) showed that after the protrusion of the radicle at the end of germination, desiccation of rapeseed usually resulted in seedling death, so drying conditions (e.g. in early August sowings) could be a significant cause of seedling death and poor establishment. Thus there may be advantages for emergence in drying conditions from more rapid root development to acquire deeper moisture reserves or in tolerance to desiccation. Faster germination is possible from small, high protein seed (Basu, 2003) but there is evidence in the literature that root development may be faster and better from large seeds. On the other hand, excess water causing anaerobic conditions could cause seedling death immediately pre- or post- emergence. It is not known whether variation in seed size, protein or oil content offers any advantage under conditions of water logging.

3.1.1.3.2. Temperature

As discussed in the previous section, low temperatures may slow hypocotyl extension and reduce the speed and uniformity of emergence, with the converse for warm temperatures. Freezing events may kill seedlings from late-sown crops (McWilliam, 1998).

Studies (Leterme, 1988, McWilliam, 1998) have shown the thermal time for emergence to be about 140-160°C days, *i.e.* about 8 days in August, 10 days in September and 18 days in October (using mean temperatures in Figure 3.1). Thus in late drillings the uniformity and final emergence may be adversely effected by low temperatures, especially in susceptible seed lots.

3.1.1.3.3. Oxygen

As with germination, water logging or microfloral respiration activity may limit oxygen supply to the roots, although after emergence the aerial parts can assimilate oxygen. Thus established plants may be less susceptible to water logging and there is evidence that oilseed rape plants can survive for extended periods, over 40 days, in water logged soils. Under such conditions they may show signs of stress such as purple coloration of the leaves, and there is less information of the effects of water logging at germination, emergence and seedling phases.

3.1.1.3.4. Sowing depth and soil structure

The seed of oilseed rape is very small (about 1.4 – 2.5 mm in inbred varieties) and contains limited food reserves (about 2000 µg oil and 800 µg protein per seed), although many of those reserves are stored as energy-dense oil bodies, which may be 40-45% of seed weight. Consequently, several workers (Garrett and Orson, 1989, McWilliam, 1998) have shown that oilseed rape is unable to emerge from below sowing depths of about 75 mm. Thus, in cloddy soils where seed may trickle to such depths, or in the case of too-deep sowing, poor establishment could result. Additionally, in poor soils with clods and stones, the rape seedling may need to grow around the obstacles and might not have sufficient food reserves for the longer path length required. Research has also shown that compaction/capping may cause subsurface growth if the seedling is unable to exert enough force to penetrate the soil of high bulk density, with changes hormonal activity altering the pattern of growth. This sub-surface growth has been observed in sand emergence tests where relatively weak crusts develop during drying (M. Smith, pers. comm.). A poorly structured sub-root zone may cause poor root growth and poor emergence. In these situations, large seed with greater food reserves may be advantageous.

3.1.1.3.5. Nutrient availability

Lack of nutrient availability (primarily N, P, K, S and micronutrients such Mn and Mg) could effect establishment, but in most cases should not be limiting, except in very wet seasons where nutrients may be leached and root development may be limited. In most soils there should be sufficient soil mineral nitrogen to provide for early establishment (30-50 kg N ha^{-1} needed for one unit of green area index) and up to 40 kg ha^{-1} seedbed nitrogen may be added at sowing. This is considered to aid establishment by some sources, although there is little evidence for an impact on establishment and yield. Work has shown that limited soil phosphate can effect establishment and growth (Major, pers. comm.) but that levels are rarely limiting enough to effect yield. It is now more widely known that

many crops and soils are sulphur-deficient due to reduced emissions from power stations but recommendations for sulphur fertilisation have been widely publicised. Consequently, assessment of interactions of seed quality parameters with soil nutrient availability will not be considered in this report.

3.1.1.3.6. Slugs

Slug grazing may be very damaging to emerging crops, and is particularly considered to be a problem in direct-drilled and broadcast crops where straw residues may make environmental conditions more conducive to slug activity. However, there are questions regarding palatability of seedlings and seeds to slugs. It is not known if slugs eat ungerminated oilseed rape seeds as they do cereals. Additionally, slugs apparently find glucosinolates (which tend to repel generalist herbivores) unpalatable. This may offer a route to increasing resistance to slug damage, although genetic approaches are required which are outside the remit of this project.

3.1.1.4. Factors effecting survival to final establishment

3.1.1.4.1. Winter kill (temperature)

Ice formation in tissues and ice heave in soils may kill plants during the winter and thus effect final establishment (McWilliam, 1998). This is probably dependent on plant size and winter hardiness, with larger, early-established plants with more well-developed root systems being more resistant to frost damage. Although plant size may be effected by seed quality characters (with larger plants expected from larger seed and from early, uniform emergers), plant size and winter hardiness is probably far more dependent on time of sowing than on seed quality parameters and this will not be studied in this report.

3.1.1.4.2. Moisture

Drought is unlikely to effect survival to final establishment frequently, although for early sowings it may limit germination and for exceptionally early-sowings (late July/early August) crops that have emerged utilising residual soil moisture may experience dry conditions in August/September and thus become desiccated. Later in the season, from October – March, desiccation is highly unlikely. A more likely scenario is that water logging may effect survival post-emergence, especially if climate warming (UKCIP, 2002) leads to wetter autumn and winter conditions. Water logging may exert its effects by restricting oxygen supply to the roots, causing anaerobiosis and by altering soil structure,

e.g. by slumping, which may restrict root or shoot penetration. The effect of water logging on emergence and post-emergence survival will be assessed in controlled environment work.

3.1.1.4.3. Disease

The main diseases effecting post-emergence survival include downy mildew *(Peronospora parasitica),* phoma *(Leptosphaeria maculans/Phoma lingam)* and light leaf spot *(Pyrenopeziza brassicae/Cylindrosporium concentricum),* mainly spread by airborne or rain splash dispersal. Soil-borne fungal complexes such as *Fusarium, Pythium* and *Rhizoctonia* may continue to exert an effect and root girdling rots may be caused by species such as *Phytophthora megasperma*. Light leaf spot can potentially cause severe over winter losses if early epidemics develop. As the effects of soil-borne fungi on emergence and damping off are being studied under the earlier section and as fungicide sprays are available to protect against the airborne diseases, this topic will not be considered further in this report. This topic was reviewed by Lunn & Holme (2001).

3.1.1.4.4. Pests

Although slugs may continue to graze larger plants post-emergence, the large plants are more able to sustain damage and recover. Cabbage stem flea and flea beetles (*Psilliodes chrysocephala, Phyllotreta spp.*) may lay eggs, which hatch into larvae that feed on the stem causing damage, which can kill plants. Pigeons may cause serious problems with grazing in February, but these are difficult to control and strategies for reducing pigeon damage have been covered in other work and will not be considered in this work, which focuses on earlier events (emergence and immediately post-emergence).

3.1.2. Seed quality factors contributing to establishment success.

3.1.2.1. The concept of 'seed quality'

Seed quality is a numinous idea, difficult to define categorically. The concept means different things to different sectors of the industry, ranging from genetic purity, through visual quality, to seed viability and vigour and field establishment. Terms are often used interchangeably, which can be confusing. Seed viability defines the number of living (viable) seeds and thus defines the maximum possible emergence under optimal field conditions, although this is almost never achieved. Viability is normally tested by standardised germination tests after treatments to relieve any dormancy (which

may prevent viable seeds from germinating) or by biochemical measurements such as the tetrazolium test, which distinguishes between living and dead tissues. 'Vigour' describes the ability of seeds to germinate and emerge under more stressful conditions than a standard germination test, e.g. from depth, at cold temperatures, at restricted water activity or with a pathogen load. A viable seed may have low vigour and a low viability seed lot may retain a fraction of vigorous seeds; likewise a high viability seed lot may be composed of low vigour seeds. Thus for high seed quality and good field performance, a seed lot needs both high viability (few dead/dormant seeds) and high vigour (ability to cope with a wide range of stress conditions).

Heydecker (1972) described a number of factors that could interact to effect seed quality;

(i)   genetic (species/variety factors),

(ii)   (ii) physiological, e.g. seed size, biochemical composition, maturity effects, *etc.*, studies of which compose the main bulk of this study

(iii)   (iii) pathological (fungal disease) effects, which will be considered here to a lesser extent, and

(iv)   (iv) mechanical effects, which may cause reductions in seed quality due to harvest, post-harvest processing *etc.* which will also be briefly considered.

3.1.2.2. Physiological factors effecting seed quality

(i)   Seed size

There are many indications that large seed size may be advantageous for establishment of a range of crops, both from studies of crop and non-crop species. Black (1956) summarised early work and with experiments concluded that there were advantages of large seed for emergence of *Trifolium subterraneum*. Furthermore, he generalised that for epigeal species such as oilseed rape (where the cotyledons emerge above the soil surface), seed size was important in limiting maximum hypocotyl expansion and in determining cotyledon size immediately post-emergence. Scott (1961) demonstrated the importance of a large endosperm for growth of wheat and later Scott *et al.* (1974) identified the benefits of larger seed size for growth and yield of monogerm sugar beet (*Beta vulgaris*).

In *Brassica* species, the benefits of greater seed size have been demonstrated by a number of workers. Lang and Holmes (1963) showed that leaf area in the early stages of growth was proportional to seed size for swede crops (*Brassica napus* ssp *napobrassica*). Ahmed and Zuberi (1973) found greater yields and larger seeds were produced by crops grown from heavier seeds of turnip rape (*Brassica rapa* syn *campestris*). Major (1977) found that seed size was correlated with seedling vigour for *Brassica rapa* in Canada, but was not related to final yield, due to rape's capacity for compensatory growth, and therefore concluded that seed grading would be of little value to the farmer. Shipway

(1981) and Mendham *et al.* (1981) described the effects of seed size (>2.25 mm compared to 1.75-2.00 mm) on crop growth of winter oilseed rape (*Brassica napus* ssp. *oleifera*); whilst they did not study establishment, they found that effects of seed size on plant size (dry weight per plant and leaf area) persisted long after emergence and concluded that large seed size would be most advantageous for the generally mores stressed conditions of late sowings. More recently, McWilliam (1998) showed numerically greater but non-significant differences in emergence of large seed (> 2 mm diameter) compared to small seed (< 2 mm diameter) over a range of soil bulk densities. Stokes *et al.* (1998, 2000) also showed greater emergence and greater seedling size with seeds selected to be > 2 mm, compared to seed < 2 mm. Heather and Sieczka (1981) studied emergence of three grades of broccoli (*Brassica oleracea* ssp. *italica*); 1.4-1.6 mm, 1.7-1.9 mm, and 2.0-2.2 mm in diameter and showed that emergence, dry weight and final yield increased as seed size increased, as did the capacity to emerge from crusted soils. Elliot and Rakow (1999) showed improved establishment, dry weight and final yield with increased seed size from 1.4-1.6 mm diameter, 1.6-1.8 and 1.8-2.0 mm diameter and also found that the larger seedlings emerged from large seed were less susceptible to feeding damage from crucifer flea beetles *Phyllotreta cruciferae*.

Work from wild and weed species also indicates that greater seed size may have advantages in a range of environments. In a survey of Californian plant species (Baker, 1972), a correlation was found between seed size and habitat aridity, indicating that larger seed reserves and an ability to develop a strong rooting system could also be advantageous for arable crops. Similar effects have been seen in species from semi-arid environments in Australia (Jurado and Westoby, 1992; Leishman and Westoby, 1994).

(ii)   Seed maturity

Stokes *et al.* (1999, 2000) showed that immature, red/orange coloured seeds germinated less well than mature, dark-coloured seeds, with slower germination and reduced final percentage germination. Leterme (1988) found faster radicle emergence from black seed than from red seed. Advantages have been found by colour-sorting cabbage seed due to chlorophyll fluorescence (Jalink *et al.,* 1998), since immature low vigour seeds retained more chlorophyll in their testas. Effects of seed maturity on membrane properties, mucilage content, water uptake, forces exerted by the root and shoot and propensity to enter dormancy are unknown.

(iii)   Protein/oil content
Stokes *et al.* (1998, 2000) found effects of seed protein/oil balance on establishment. Emergence was faster from high protein seed than from low protein (high oil) seed.

(a) Endogenous seed-borne pathogens

A number of species such as *Alternaria* and phoma may be seed borne, although transmission is usually by air-borne spores. Such seed borne infection may reduce establishment due to parasitism of the germinating seed or young seedling, although fungicide use in the mother crop and systemic fungicide seed treatments usually adequately control the seed-borne phase of the disease and so these diseases are not considered to have an important impact on oilseed rape establishment.

(b) Soil-borne pathogens and post-emergence seedling rots and blights

Soil-borne damping off diseases such as *Pythium spp.* and *Rhizoctonia solani* may rot ungerminated seed or newly germinated seedlings. As with seed-borne diseases, these fungi are usually controlled with protectant fungicides such as thiram, which is commonly applied as a seed dressing with Rovral. Therefore, these diseases are often not important and their effects are rarely obvious except in low seed rate crops drilled under adverse conditions.

Club root (*Plasmodiophora brassicae*) may effect oilseed rape, although its effects are more severe on transplanted Brassicas such as *B. oleracea* and resistant varieties (e.g. Mendel) are becoming available. The symptoms are manifested as stunted plants with deformed roots showing galls rather than by seedling death.

(c) Damping off, root rots, *etc.*

Downy mildew (*Peronospora parasitica*) is a widespread disease that may attack between the cotyledon and third-leaf growth stages, which may lead to shrivelling and seedling death. All plants may be effected in a wet autumn although the disease is most common during flowering and is considered relatively unimportant. Protectant fungicides such as chlorothalonil, maneb and mancozeb are available. Powdery mildew (*Erysiphe crucifearum*) infection may also start early, in thick heavily-fertilised crops although it is not thought important enough to warrant control.

A number of poorly characterised 'seedling blights' and 'root rots' may effect seedlings but assigning a cause to these is complex. Species such as *Phytophthora megasperma*, *Fusarium spp.*, may interact with some of the species above (e.g. *Rhizoctonia, Alternaria, Phoma*) and with root-feeding insects. Symptoms are manifested soon after emergence, shown by seedling collapse after infection at root

level, shown by dark discoloration and shrivelling of the root collar. Seed treatments may alleviate these effects, but due to the ability of oilseed rape to compensate for wide variations in plant population, yield is rarely effected by these diseases.

3.1.2.4. Mechanical effects

Seed coat damage, for example due to harvest of over-dry seeds with poorly set combines, post-harvest processing, *etc.*, may effect seed vigour and storability. For example, seed coat cracks may allow easier ingress of fungi and bacteria and uncontrolled gas exchange and imbibition, which may cause more rapid membrane damage than in intact seeds. In other species, such as pea and soya (Schlub and Schmitthenner, 1978), seed coat cracks have been associated with reduced vigour and increased electrolyte leakage. However, during this research programme it was not possible to assess the effects of mechanical damage.

3.1.3. Assessing seed quality

As described previously, seed quality or 'vigour' is a difficult concept to define rigorously (Heydecker, 1972) and may mean different things in different situations. Seed vigour tests are outlined and approved by the International Seed Testing Association (ISTA) (Anon., 1995). In the UK, the only tests available to assess rapeseed quality are the standard germination test and the tetrazolium (TZ) viability test. The requirement for all traded rapeseed is that samples should exceed 85% germination at 20°C in excess water (Anon., 1996), relatively benign conditions that many seedlots are able to pass even though they may produce poor establishment in real field situations. The TZ test assesses the proportion of viable (living) cells according to red staining by the compound tetrazolium. However, this is a difficult and subjective test, particularly on a small-seeded species such as oilseed rape.

Other vigour tests are available (Anon., 1995), and some are in use for other species such as maize and soya and in other areas such as North America. These tests generally include harsher stresses than in the standard ISTA germination test, including cold temperatures, use of an actual or simulated soil substrate or barrier (with or without pathogen load) and accelerated ageing or controlled deterioration to reduce seed quality and to assess resistance to such conditions (Tesnier *et al.*, 2002). Protocols are also available to assess seed health and infection with pathogens.

A number of biochemical tests exist which can be used to assess seed quality, these include assessment of electrolyte leakage by electrical conductivity, which is used to assess pea quality and has also been assessed on oilseed rape (Larsen *et al.*, 1998, Thornton *et al.*, 1990, Hill *et al.*, 1988).

3.2. Materials and Methods

3.2.1. Moisture uptake

3.2.1.1. Imbibition in pure water

Seed lots of contrasting protein content were chosen from the mother crops produced in 1999/2000. The low population/low nitrogen treatment (10 plants m^{-2}/40 kg ha^{-1} N) gave seeds of low protein content (20.1%) whereas the high population/high nitrogen (80 plants m^{-2}/200 kg ha^{-1} N) gave seeds of higher (22.1%) protein content. The 300 kg ha^{-1} N treatment was not used as this seed had been processed for ADAS field experiments.

Seeds were graded through Endecott's square mesh sieves to give seed grades of 1.18-1.70 mm, 1.70-2.00 mm, 2.00-2.36 mm and > 2.36 mm (small, medium, large and very large seed). The 1.40 mm sieve was not used as not enough seed were isolated in the 1.18-1.40 mm fraction. Seed from the very large fraction of the low protein seed lot were used in a profiling study to assess the timescale of imbibition for future experimentation. Replicates of 50 seeds were counted with a Pfeiffer automatic seed counter and weighed. Moisture content was determined from the fresh and dry mass of replicate samples dried in a fan-assisted oven at 85°C for 48 h. The replicates were placed onto two layers of Whatman No. 1 filter paper in a Petri dish that had previously been dampened with 5 ml distilled water. Excess water was drained by inverting the Petri dishes and seeds were spread out with a mounted needle to avoid seed-seed contact and clumping. The Petri dishes were lidded and incubated in the dark at 10°C. At regular intervals, seeds were removed from the filter papers into a separate tared Petri dish. Surface water was removed from the seed by tapping the dish and was absorbed with rolled dry Whatman No. 1 filter papers and the seed was weighed. Moisture content was calculated on a wet weight basis [(FW-DW)*100/FW] and the amount of water imbibed was calculated as the difference from the starting weight. The combination of large seed, low protein content and low temperature was anticipated to give the slowest profile of imbibition.

In a subsequent experiment, the effects of seed size, protein content and temperature on imbibition were investigated. Replicates of the large, medium and small seed from both the low and high protein

seed lots were incubated in Petri dishes as described above in the dark in incubators set at 10, 15 and 20°C.

3.2.1.2. Imbibition under water stress

In order to assess susceptibility to water stress, water potential was controlled with different concentrations of polyethylene glycol (PEG) 8000 in water. In the initial experiment, the effect of PEG concentration was studied. Seeds sieved to be 2-2.36 mm diameter from the low protein (low N/low population) treatment from 1999/2000 were used, as for the previous study. Replicates of 50 seeds were incubated at 15°C on two layers of Whatman No. 1 filter paper in Petri dishes, soaked with PEG 8000 of different concentrations. Water potentials from 0 (pure water) to -2.5 MPa (0.45 g PEG/g H_2O) were made according to the results of Michel (1983). The amount of water taken up by seeds was recorded at 48 and 120 h by weighing and calculating the difference from the initial weight. The number of germinated seeds (radicle > 2 mm) was also counted.

The results of this initial experiment were used to design a PEG 'water stress' test; samples were screened at -2.0 MPa at 15°C to assess ability of seed lots to germinate under water stress. Imbibition was recorded at 48 h and 120 h and germination was assessed at 120 h. Subsequently, water potentials of -2.25 and -2.5 MPa were included. The usefulness of the test was assessed on seed lots varying in size (1.18-1.70, 1.70-2.00, 2.00-2.36 and > 2.36 mm diameter) and protein/oil balance (22.1% protein and 18.1% protein).

3.2.2. Effects of temperature

Three replicates of 50 seeds of each seed lot were incubated on two layers of Whatman No. 1 filter paper with 5 ml water added. Petri dishes were incubated in the dark in incubators at 10, 15, 20 or 25°C. Dishes were assessed daily and germinated seeds (radicle protruding more than 1 mm) were counted and removed.

3.2.3. Dormancy analyses

Initially, conditions for induction of dormancy were screened. Pekrun *et al.* (1997) Lutman *et al.* (1998) showed that dormancy could be induced by conditions of water stress in darkness, at low temperature. Six replicates of 25 seeds were subjected to water stress by incubation in the dark at 10°C in Petri dishes on filter papers soaked in polyethylene glycol solution (-1.5 MPa, Michel, 1983) or buried in completely dry silver sand for 1 – 6 weeks. Dishes were stacked in trays and were

wrapped in black plastic dustbin bags to exclude light. At each time point (1, 2, 4, 5 and 6 weeks incubation), seeds were taken from the incubating medium and were placed in Petri dishes on two layers of Whatman No. 1 filter paper soaked in pure water, with all operations taking place under a green safe-light. Half of the replicates were then incubated at 20°C in the light and half at 20°C in the dark with daily assessment of germination (under green safe light). After eight days, remaining ungerminated seeds were incubated in the light at 4°C in a cold room for 48 hours. Viability of remaining ungerminated seeds was assessed using the tetrazolium test where red-staining indicated the seed was alive and no staining indicated the seed was dead. Dormancy values were calculated by the difference between light and dark germination before temperature shock, and the difference in germination after temperature shock and before.

3.2.4. Pathology analyses

Seeds from the 2001 mother crop production (see section 4 for details; medium population receiving 100 kg ha^{-1} N) were graded into fractions of <1.40 mm, 1.40-1.70 mm, 1.70-2.00 mm, 2.00-2.36 mm and > 2.36 mm, using the appropriate grade Endecott's sieves. Five replicates of 20 seeds were sown 2 cm deep in unsterilised or sterilised (autoclaved) soil in small modules. A split plot randomised block design was used, with sterile/non-sterile soil used as the main plot (i.e. soil types separated to avoid cross-infection), with seed diameter as the randomised sub-plot. Emergence was assessed on a daily basis and each module received 100 ml water. The number of seedlings attacked by fungal damping off diseases after emergence was assessed.

3.2.5. Seed size analyses

Seeds from mother crops produced in 2000 (see section 4; from a low population that had received no fertiliser nitrogen) were hand-graded using digital callipers into size grades of 1.45-1.55 mm (nominally '1.5' mm diameter), 1.55-1.65, 1.65-1.75, 1.75-1.85, 1.85-1.95, 1.95-2.05, 2.05-2.15, 2.15-2.25, 2.25-2.35, 2.35-2.45 and 2.45-2.55 mm diameter. There were insufficient seeds greater than 2.55 mm in diameter for any larger grades.

A randomised block design with three replicate blocks and an 11*3 factorial design of seed lot diameter (as above) x sowing depth (2, 4 or 6 cm) was set up in a controlled environment room. Seeds were sown in plastic plant pots (25 seeds per pot) in sterile horticultural silver sand (15% moisture w/w) and were incubated at 18°C/15°C on a day night cycle with an 8 h photoperiod. Water loss from blank pots was monitored gravimetrically and was replaced as appropriate. Emergence was monitored daily and fully emerged seedlings(cotyledons unfolded) were removed.

3.2.6. Water logging analyses

Seeds from the mother crop 2000 production with the greatest extremes of protein and oil content were selected for this experiment. Seeds from plot 14 had 21.50% protein and 41.20% oil (high protein/low oil) and seeds from plot 27 had 18.03% protein and 43.60% oil (low protein/high oil). Seeds were fractionated into large (2.00-2.36 mm) and small (<1.70 mm) diameter grades using the appropriate size Endecott's sieves. Two moisture content regimes were used, a 'dry' treatment at 10% (w/w) water and a waterlogged treatment of 17% (w/w) water, compared to the usual moisture content of 15% used in sand emergence tests. No additional water was added. The experiment was set up as a randomised block design with three replicate blocks and the 2*2*2 factorial treatment of protein content*size grade*water content as the randomised plots. The test was carried out in a controlled environment room set at 20°C in the day and 15°C in the night, with an 8 hour photoperiod.

3.2.7. Vigour testing assessments

Germination testing in related work (Smith, 2001), according to the standard ISTA protocol described previously, identified seed lots within the University of Nottingham collection (1996-2001) with variable vigour. In addition, accelerated ageing was used to reduce seed vigour: seed lots were placed at 100% relative humidity (in a sealed desiccator chamber over pure water) at 40°C and were incubated for different times.

Leakage of electrolytes from seeds of different vigour levels was assessed with a Mettler-Toledo M90 electrical conductivity meter. Initial tests showed that reproducible results could be obtained from incubation of 300 seeds in 200 ml distilled water at 20°C for 24 h. A blank reading was taken from the water control before all analysis and results were presented as $\mu S \ cm^{-1} \ g^{-1}$. The profile of chemicals leached into the steeping medium was assessed by preliminary liquid chromatography-mass spectrometry (LCMS) analysis.

3.3. Results

3.3.1. Imbibition profiles

The imbibition profile of very large, low protein rapeseeds at 10°C followed the classic triphasic pattern expected which could be well explained by a cubic equation (Figure 3.2). It was difficult to gain accurate measurements of imbibition at <2 h since much free water became associated with the

seeds before uptake, resulting in large errors. For even large seed and at low temperatures, imbibition was rapid in the presence of free water ($\psi = 0$). By three hours water content had doubled to about 20% and by eight hours it was about 30%. By 24 hours, water content reached about 40% (wet weight basis), with each seed having swollen and taken up about 2.9 mg water. This supports the evidence for a requirement of uptake of water to a moisture content of about 40% required for germination reported by McWilliam (1998). The plateau phase, where seeds remained swollen and turgid, but took up no additional water, lasted from about 24 to 72 hours. After this time water content again rose, ultimately to about 70-80%, with seed coat rupture and radicle emergence completing germination.

Figure 3.2: Profile of imbibition in large (>2.36 mm diameter), low protein (20.1)% seeds grown in 1999/2000
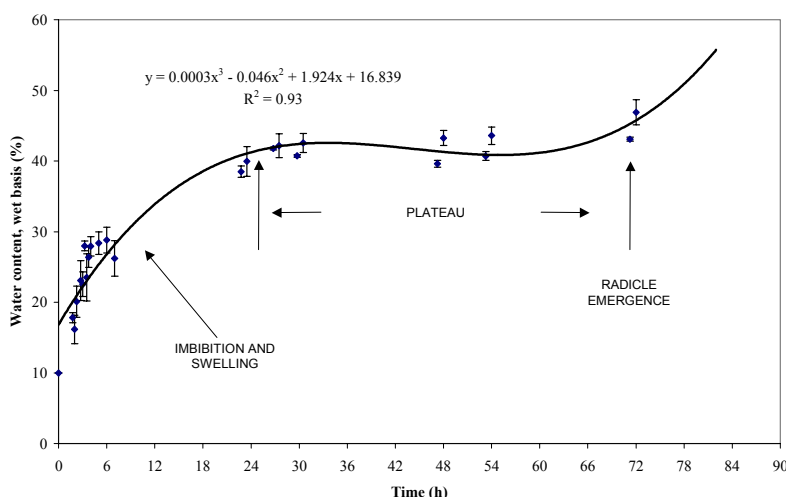
3.3.2. Effects of temperature, seed size and protein content on imbibition.

In this experiment, the general patterns of imbibition were similar to the triphasic curve observed in the profiling experiment on the large, low protein seeds at 10°C, although there were obvious differences in the patterns between treatments. The rate of imbibition was increased by increased temperature, with the plateau phase and radicle emergence occurring successively earlier as temperatures moved from 10, to 15 to 20°C. However, when plotted on a thermal time basis, there was no significant difference between the 10, 15 or 20°C curves as shown for large, low protein seed in Figure 3.3. Initial imbibition was very rapid, with increase in seed moisture content from about 10% to 35% within the first 100°C hours (i.e. 10 hours at 10°C and 5 hours at 20°C). Imbibition then slowed as the seed entered the plateau phase, which lasted from approximately 200°C hours to 800°C hours, when germination commenced by radicle protrusion (i.e. germination started in 3.3 days at 20°C or 1.67 days at 20°C).

Figure 3.3: Imbibition of large low protein seed at 10, 15 and 20°C with thermal time
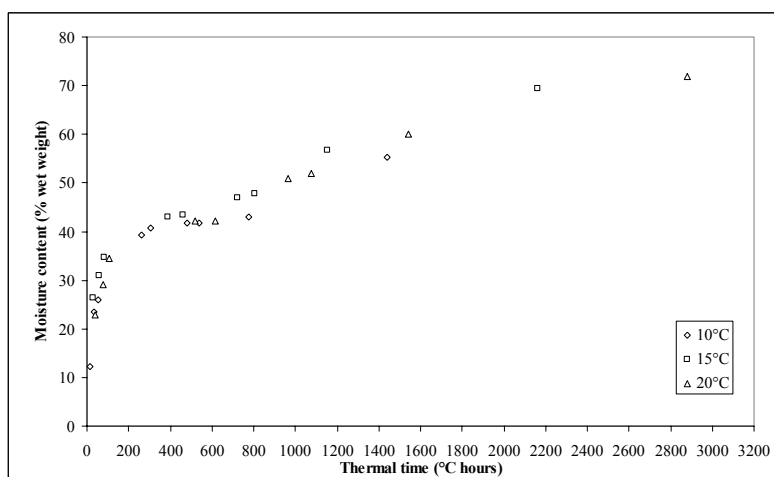
Seed size did not significantly effect the initial rate of imbibition on a percentage moisture basis. This was unexpected, since small seeds were hypothesised to imbibe more rapidly due to their greater surface area:volume ratio. However, in large seeds (2.00-2.36 mm), imbibition slowed sooner and the moisture content reached a plateau at about 40%. In medium-sized seeds (1.70-2.00 mm diameter), moisture content reached a plateau at about 45% and in the smallest seeds (1.18-1.70 mm diameter) the moisture content reached a plateau at 55%. In the small seeds, the tertiary phase of moisture uptake also appeared to be delayed by about 200°C h compared to the large and medium seed. The attainment of a higher moisture content during the plateau phase than the critical 40% proposed by McWilliam and a higher percentage moisture content than large seeds was unexpected. The total amount of water imbibed by small seeds was still, however, lower than medium or large seeds. Effects on moisture content were assessed by an ANOVA on the 48 h results (angular transformation of data, Table I.1) when all seed lots were in the plateau phase and germination had not begun.

In terms of percentage moisture (wet weight basis) temperature, seed size and nitrogen content all had a significant ($P<0.001$) effect (Table 3.1). Increasing temperature increased seed moisture content, whereas increasing seed size and nitrogen content both reduced seed moisture content. The reduced seed moisture content at higher protein content was unexpected, since high protein seeds have been hypothethised to imbibe faster due to greater amounts of colloidal protein molecules. Consequently, it is possible that formation of high protein content diverts metabolites from formation of other more important colloids. However, the effect was small.

Table 3.1: Effects of protein content, size and temperature on percentage moisture content after 48 h imbibition.

Protein	Size	Temperature (°C)				
		10		15		20
High	Large	39.00	40.48	42.31		
	Medium	43.63		42.99	44.94	
	Small	44.26		48.54	51.96	
Low	Large	40.25	43.28	45.51		
	Medium	44.13		44.66	48.73	
	Small	48.56		49.90	49.25	

Although the ANOVA showed significant effects of seed protein content on imbibed water content at 48 h, the percentage water content differences due to protein were generally quite small and less than the effects due to seed size. Protein content appeared to have no effect on imbibition properties of the seed when the imbibition profiles were compared (Figure 3.4), with profiles from the high and low protein content seed showing identical patterns in thermal time. This is shown in Figure 3.4 for large seed; the same effect was seen in medium and small seed, although they had different imbibition patterns (higher plateau moisture content) than the large seed.

Figure 3.4: Imbibition with thermal time of high and low protein large seed
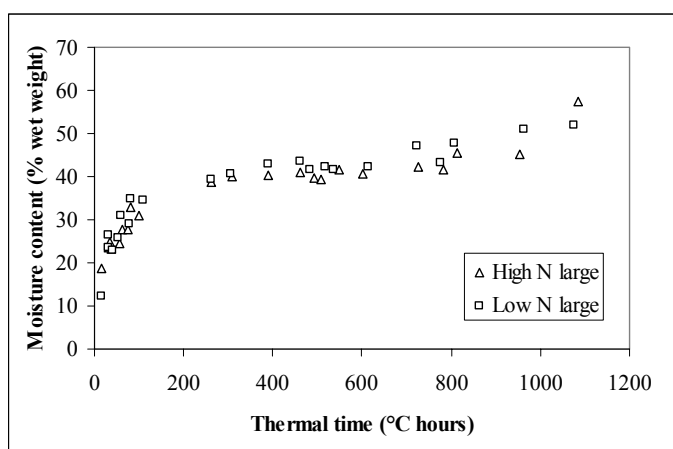
On a gravimetric basis (i.e. weight of water imbibed), small and large seeds imbibed very similar amounts of water (2.8-3.0 mg), thus explaining their different plateau moisture contents.

3.3.1.3. Effects of water potential on imbibition and germination

Increasingly negative water potential decreased imbibition, as shown in Figure 3.5 which shows the amount of water imbibed by 50 seeds (same sample as used for the initial imbibition profile) after 48 h and 120 h.

Figure 3.5: Weight of water (g) imbibed by 50 seeds in 48 h (circles) and 120 h (squares) with increasingly negative water potential due to polyethylene glycol.
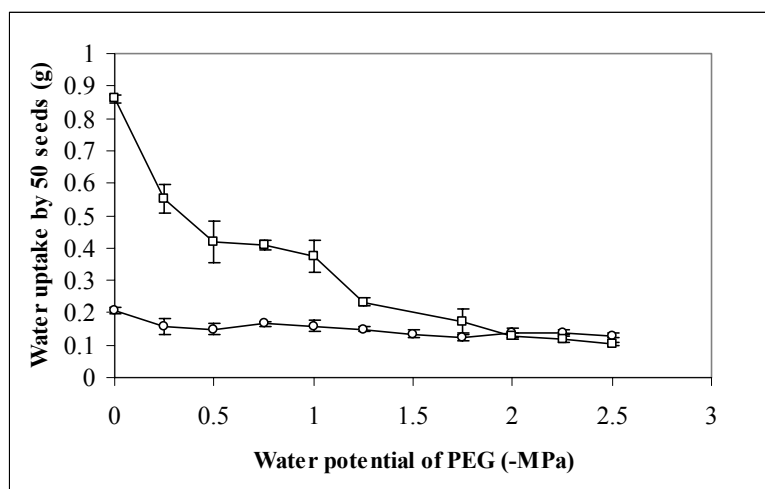
At 48 h, imbibition was significantly greater in pure water (0.21 g water imbibed) than in any of the samples with negative water potentials due to dissolved PEG (<0.17 g water imbibed). At 120 h greater differences were apparent. At ψ of -1.25 MPa and less, imbibition had continued, although the amount of water imbibed was far less in the osmotic solutions than in pure water (0.9 g). At ψ of -1.75 MPa and greater, the amount of water imbibed was not significantly greater than at 48 h. When germination was considered (Figure 3.6), after 48 h there was no germination at water potentials greater than -1.25 MPa. At 120 h, full germination was possible at water potentials below -1 MPa. However, at greater water potentials, germination was reduced to virtually zero at -2.5 MPa. These results did not agree with the critical value for germination of -1.5 MPa proposed by Lutman *et al.* (1994) and Pekrun *et al.* (1997) and used by McWilliam. With significant germination occurring at -1.5 to -2.25 MPa. This agrees with McWilliam, who found germination below -1.5 MPa. The results

were used to design a screening test for germination under water stress, with incubation in −2.00 MPa PEG for 120 h anticipated to give a severe water stress, but not restricting germination completely. This it was hoped that seed quality differences causing changes in tolerance to water stress might be picked up.

Figure 3.6: Percentage germination at 15°C in increasingly negative water potential solutions. Circles show germination (radicle > 2 mm) at 48 h and squares at 120 h. Error bars show ± SEMs
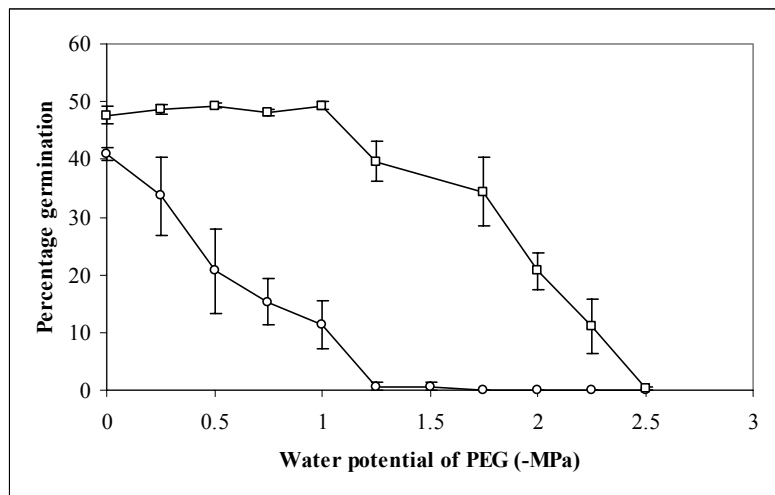
In -2.0 MPa PEG, imbibition of seed lots with different protein/oil contents showed no significant differences in water uptake after 48 h (data not shown). Unlike in the screening experiment, after 120 h imbibition had continued, although there were still no significant differences in water uptake due to protein content. There was no germination at 48 h, although there was significant germination at 120 h. There was no significant difference in the ability of seed lots to imbibe water and germinate under the PEG water stress test, although it may be that the range of protein contents was insufficient or that −2.0 MPa PEG was not a sufficient stress. A final assessment showed that, although −2.0 MPa PEG did not prevent germination as defined by emergence of the radicle, this water potential did prevent emergence of the cotyledons from the seed coat.

3.3.1.4. Effects of seed size and protein content on imbibition

A second experiment was conducted to examine the effect of water potentials of −2.0, -2.25 and −2.5 MPa to see if greater water potentials would discriminate more between different seed vigours. Two seed lots, with greater extremes of protein content (18.03 and 21.50%) than used in the previous experiment were selected from the SAPPIO 2000 mother crops. The seeds were size graded to

produce seeds of 1.18-1.70 mm, 1.70-2.00, 2.00-2.36 and > 2.36 mm diameter and three replicates of 50 seeds were incubated in the dark at 15°C as described previously.

This experiment showed greater discrimination between seed lots than the previous one (Figure 3.7). Analysis of variance showed significant ($P<0.001$) effects of seed protein content, seed size and water potential with a significant protein x size x water potential interaction ($P=0.013$). After 120 h, there was significantly greater germination in the high protein than the low protein seed lot. There was also significantly greater germination in >2.36 and 2.00-2.36 mm diameter seeds than in 1.70-2.00 mm seeds which in turn showed greater germination than the 1.18-1.70 mm seeds. In the low protein seed lot, germination was significantly reduced as water potential became more negative, indicating that –2.50 MPa PEG could be a better substrate than –2.00 MPa PEG for testing seed differences in susceptibility to osmotic stress. However, the high protein seed lot appeared more resistant to moisture stress, with germination maintained in –2.50 MPa PEG.

Figure 3.7: Germination of different size grade x protein content seeds in –2. –2.25 and –2.5 MPa PEG
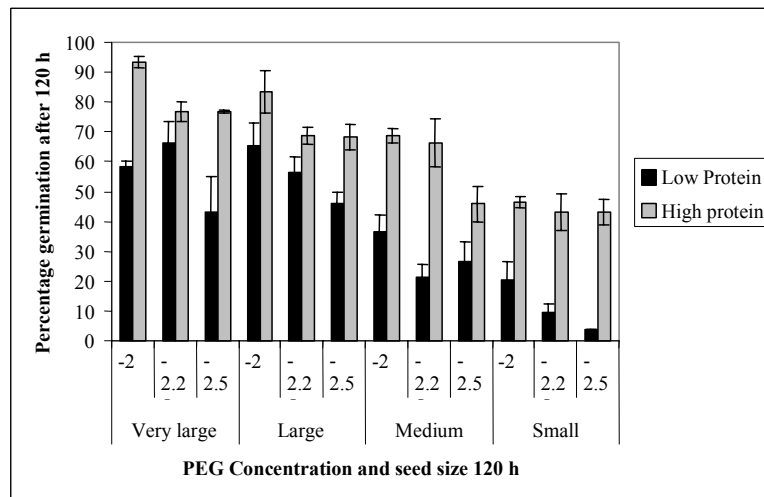
3.3.2. Effects of temperature

Imbibition studies showed that increased temperature increased the rate of imbibition and radicle emergence, and that a thermal time scale could be used (base 0°C) to plot imbibition at different temperatures on the same thermal scale. Initial imbibition was completed in about 200°C hours (8.3 °C days) and radicle emergence commenced after about 800 °C hours (33.2 °C days).

Germination profiles could be considered in a similar way. Initial studies showed a base temperature, T_b, of about 3.5-4°C for oilseed rape germination, which agrees well with the work of Marshall and Squire (1996). The thermal time (°Ch or °Cd > T_b) for 50% germination was 370°Ch or 15°Cd above T_b and for 90% germination was 605°Ch or 26°Cd above T_b. Thus for high temperatures, the time between mean and almost full germination is short, with both occurring on the same day at mean temperatures above 15°C. However, as the temperature is reduced below 10°C, the time between mean and late germinators increases markedly (to 7.3 days at 5°C), resulting in greater heterogeneity of emergence.

### 3.3.3. Effects of protein/oil ratio

Effects of protein/oil ratio on emergence, where rate of emergence was increased but final percentage emergence was rarely effected, were demonstrated in earlier work (Stokes *et al.*, 2000) and so there was less emphasis on controlled environment studies of this character during this work. Instead there was more concentrated effort on assessing manipulation of oil/protein ratio by mother crop husbandry (described in Section 4). However, in other experiments where a contrast in protein/oil ratio was included, the advantages of high protein content shown in other work were confirmed.

In the experiment described below, high protein content enhanced emergence under stress conditions (both relatively dry and water logged sand) and particularly allowed small seed to maintain better emergence under stress conditions.

3.3.4. Effects of water logging

An experiment designed to compare emergence in dry and water logged conditions showed significant effects of water logging, seed protein/oil balance and seed size. As shown in Figure 3.8, in general water logging (sowing in horticultural silver sand at 'field capacity' of about 18% moisture) reduced the rate of emergence and the final percentage emergence compared to drier silver sand (about 5% emergence). However, if the experiment had been continued without further irrigation, emerged seedlings in the drier treatment may have died off sooner than in the waterlogged treatment where adequate moisture supplies would still have been available. Large seed (from both high and low protein mother crops) gave better emergence than small seed in both dry and wet conditions. The high protein seed had greater emergence than the low protein seed and this was particularly marked with the small seed fraction, with the small high protein seed giving much greater emergence than the small low protein fraction.

Figure 3.8: Effects of water logging on emergence profile from seed of differing oil/protein ratios and seed size,
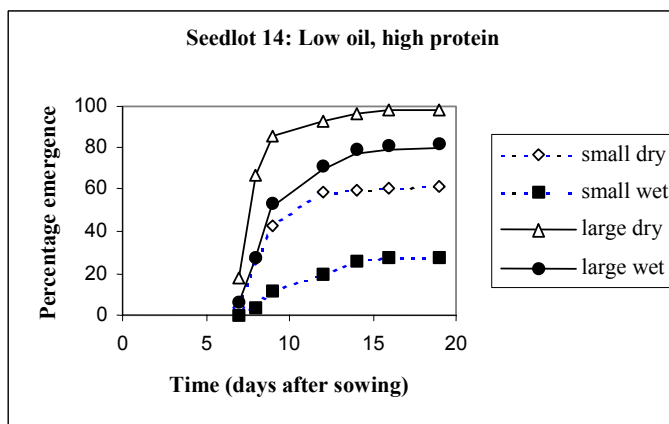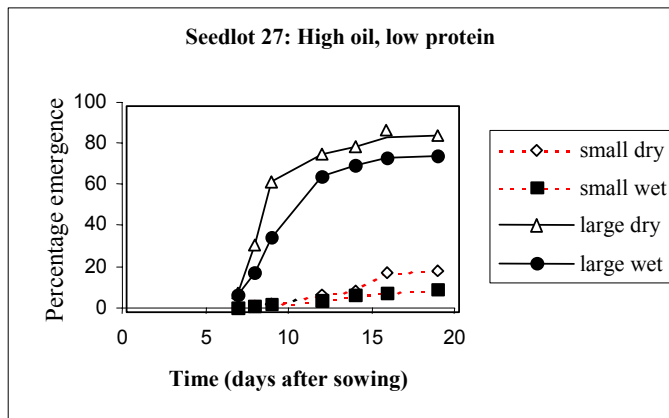
3.3.5. Effects of seed size and sowing depth on emergence

Assessment of emergence of seed separated into categories differing by 0.05 mm in diameter showed distinct effects of seed diameter at 2, 4 and 6 cm sowing depths. The trends were less marked in the 2 cm sowing depth than at the deeper sowings (Figure 3.9), but in all cases, emergence generally increased with increase in diameter from 1.5 mm to a maximum at 2.1 mm diameter. For example, at 6 cm sowing, emergence increased from about 40% to about 100% for this change in diameter. Optimum seed diameter seemed to be about 2.1 mm, and there were indications of decrease in emergence compared to 2.1 mm, at diameters up to 2.5 mm. as well as final emergence from horticultural silver (Figure 3.10). At the 2 cm sowing depth, 1.5 mm diameter seed emerged more rapidly than 2.4 mm diameter seed, although there was no significant effect of seed diameter on final percentage emergence from this shallow sowing depth. At the deeper 4 cm and 6 cm sowing depths,

44

emergence of 2.4 mm diameter seed was more rapid than 1.5 mm seed Examination of selected contrasts shows effects of seed size and sowing depth on rate of emergence

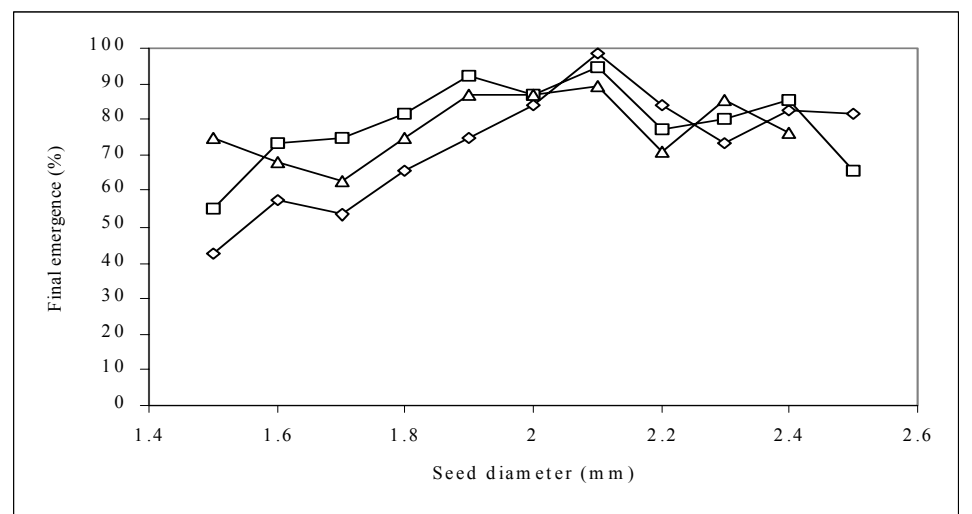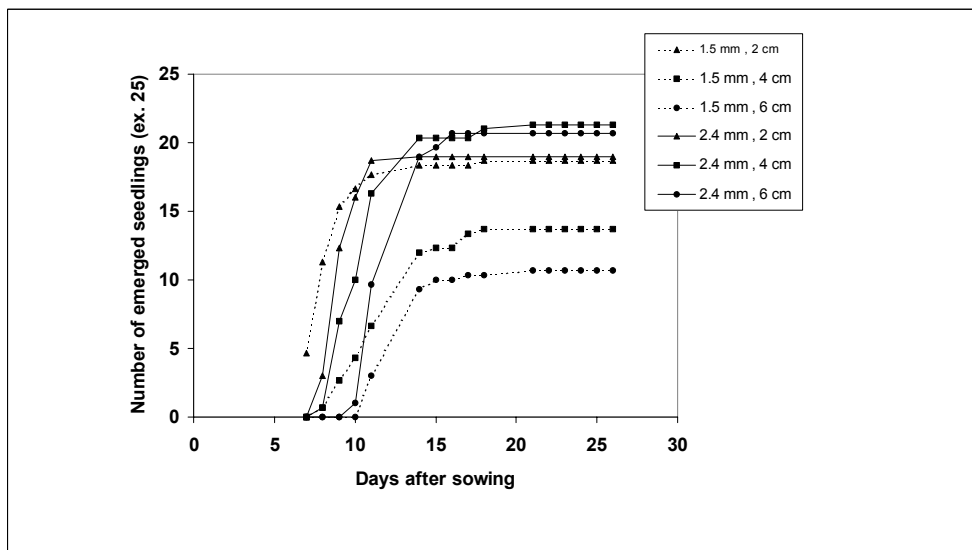Figure 3.9: Effect of sowing depth and seed diameter on emergence.

Figure 3.10: Effect of seed diameter and sowing depth on emergence profiles

3.3.6. Dormancy

From the literature, development of dormancy after various stresses was expected to be shown by development of photosensitivity of germination, i.e. a requirement for light, with less germination of light-requiring dormant seeds in the dark than in the light. This was indeed shown in many situations, represented by Figure 3.11. which shows the difference in germination of light- and dark-incubated seeds of the unadvanced/ungraded high population/300 kg ha^{-1} N mother crop produced in 2000.

Figure 3.11: Germination of seeds of unadvanced/ungraded high 300 mother crop incubated for one week in PEG.(Unbroken line = light, broken line = dark)
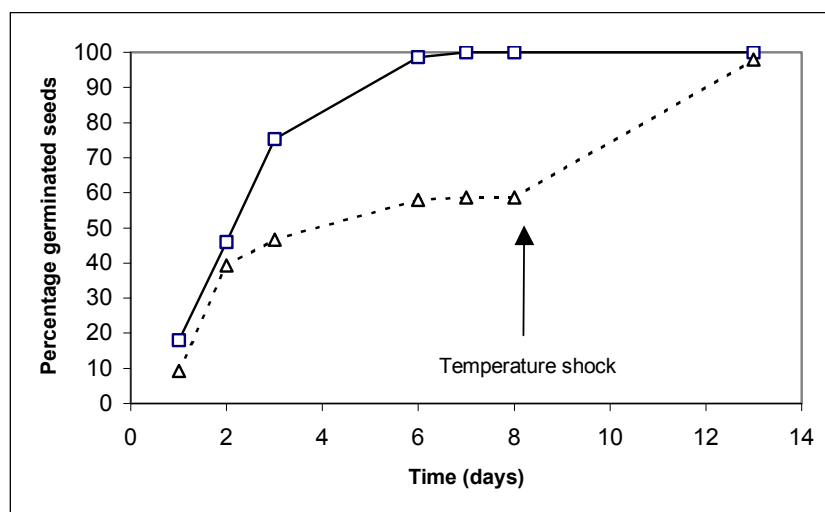
In this case, a clear difference in germination was seen between light and dark germination treatments, with about 45% non-germinating seeds in the dark. The fact that these seeds subsequently germinated in the light after a cold shock demonstrated that they were dormant. However, there were also large numbers of cases (Fig 3.12) where germination profiles in light and dark conditions were similar and where both light and dark treatments had greater final germination after temperature shocks. Thus, both the light/dark and post/pre-temperature shock differences in germination are discussed.

Figure 3.13 shows the levels of photodormancy developed in (a) dry silver sand and (b) −1.5 MPa PEG. The dry sand treatments gave very low levels of dormancy, below 10%, whereas the PEG treatments gave levels of dormancy up to about 40%. Thus it can be seen that some level of imbibition is necessary for development of dormancy, which does not develop in cold, dark

conditions in the dry state. However, the potential availability of moisture for limited imbibition even in very dry soils, demonstrates that induction of photodormancy could be a significant problem in oilseed rape, especially since the recent trend to early drilling can lead to sowing in dry soils in August.

Figure 3.12: Germination profiles without significant photodormancy, but with significant cold shock requirement.
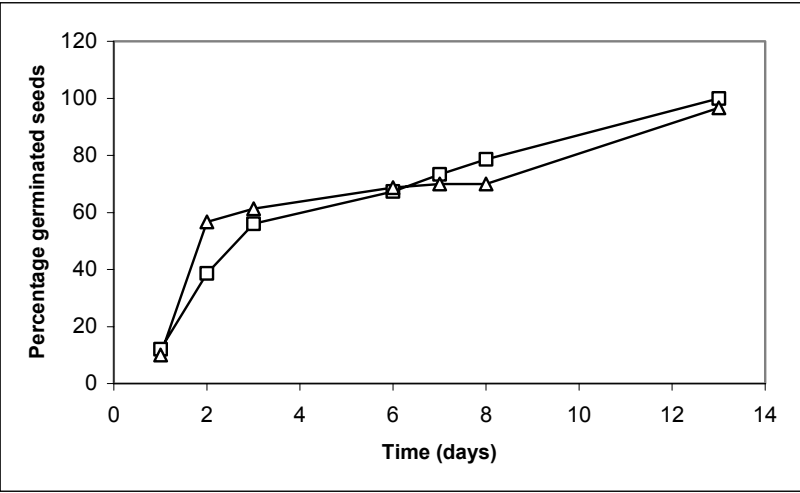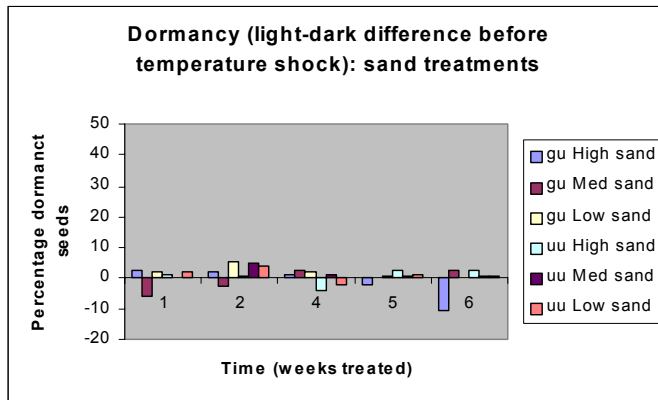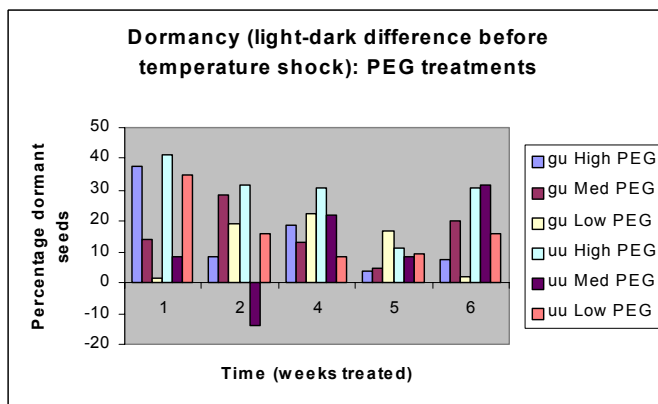

47

Figure 3.13: Development of photodormancy in (a) sand and (b) PEG treatments

(a)

(b)

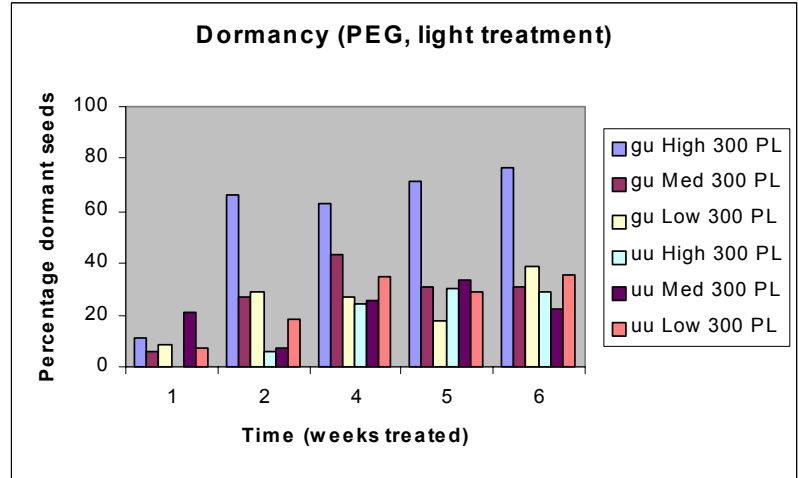Key: gu = Germain's priming ungraded, uu = unprimed, ungraded

Comparison of incubation in aqueous polyethylene glycol (water potential -1.5 MPa) with dried, sterile horticultural silver sand (all free water removed by drying at 85°C for 48 h), showed development of significant dormancy in the partially imbibed seeds treated with PEG (range from 0-80%, mean 33%), but not in the non-imbibed seeds in the silver sand (range 0-6%). Thus partial imbibition would appear to be a requirement for development of dormancy. Thus, seed stored well in dark and cool conditions after harvest should not be in danger of developing dormancy unless they become imbibed. However, if poor storage conditions allowed moisture imbibition by seed, then dormancy could develop in cold, dark conditions.

Figure 3.13 showed that there appeared to be no evidence of an effect of mother crop on dormancy development. The extent of photodormancy developed from week to week was highly variable and no pattern could be discerned to illustrate and effect of mother crop plant population or priming treatment.
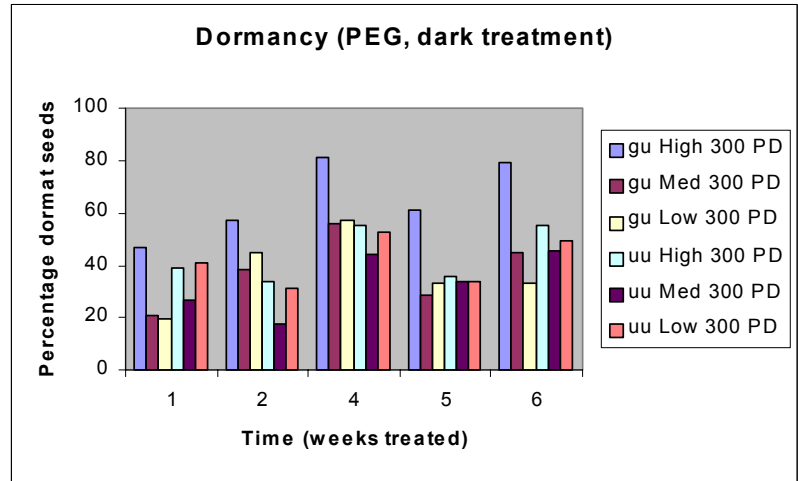
Assessment of the duration of dormancy-inducing stress showed significant dormancy induction could occur after one week (the shortest period investigated). For longer storage times, the degree of dormancy developed was variable and there was no evidence for increased dormancy with increased treatment time.

Figure 3.14: Development of dormancy requiring low temperature shock to break in a) light, b) darkness.

a)

b)

Key: gu = Germain's priming ungraded, uu = unprimed, ungraded

Assessment of the increased germination after temperature shock treatment showed very little dormancy in dark or light-germinated seeds after the dried silver sand treatment (~ 0-5%, data not shown). However, in the PEG treated seeds, a requirement for a low temperature shock also

developed in the seeds germinated in the light at 20°C (Figure 3.14). When the average temperature shock-broken dormancy developed was plotted against time, there was evidence that this propensity increased with time of stress treatment (Fig. 3.15).

Figure 3.15: Development of dormancy requiring temperature shock in light and dark conditions
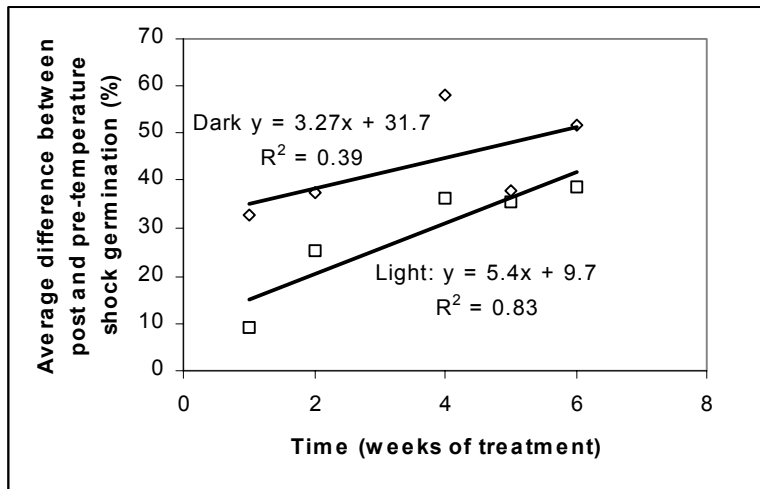
There was also weaker evidence that the propensity for pure photodormancy decreased with time of stress (as the cold shock temperature requirement was increased – Fig. 3.16).

Figure 3.16: Change in percentage of seeds demonstrating photodormancy with time
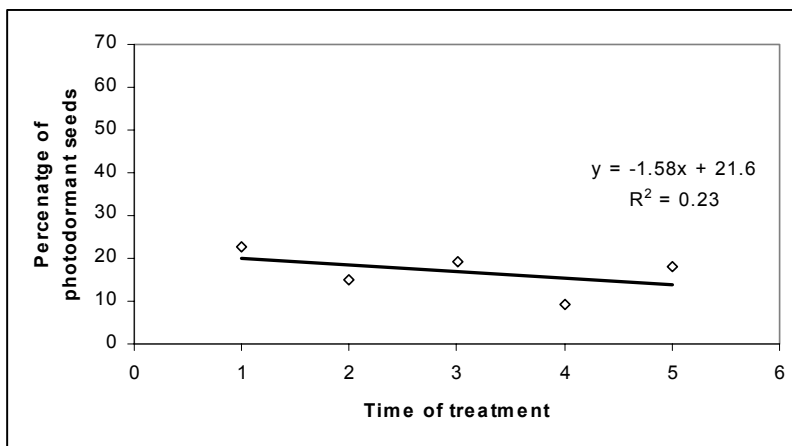
Thus it can be seen that high levels of dormancy can develop within a short period (one week of cold water stress), but that some water availability and imbibition is necessary for the phenomenon to

develop. The minimum period of water stress and its interaction with temperature is unknown and further research is necessary to determine the impact of drilling in dry soils and dormancy on establishment. The evidence suggests that as well as a light requirement developing early in moisture stress, as time in stress increased a cold temperature requirement also developed and propensity to enter dormancy could increase as time of stress increased. These observations have important ramifications for establishment.

There is also anecdotal evidence of differences in propensity to enter dormancy due to time of harvest from the mother crop and possibly due to post-harvest drying and treatment methods. Further research is needed.

3.3.7. Pathology

Comparison of emergence of non-dressed rapeseed in sterile, autoclaved soil and non-sterile soil (i.e. soil collected from the field containing the normal range of soil-borne fungal pathogens) showed a significant effect of the soil-borne fungal flora on emergence. On average, final emergence in non-sterile soil was 35%, whereas in sterile soil, average final percentage emergence was more than double at 84%. This is shown in Figure 3.17, where emergence from sterile soil is shown in red and from non-sterile soil in blue.

Figure 3.17: Emergence of different size grades of oilseed rape from sterile and non-sterile soil.
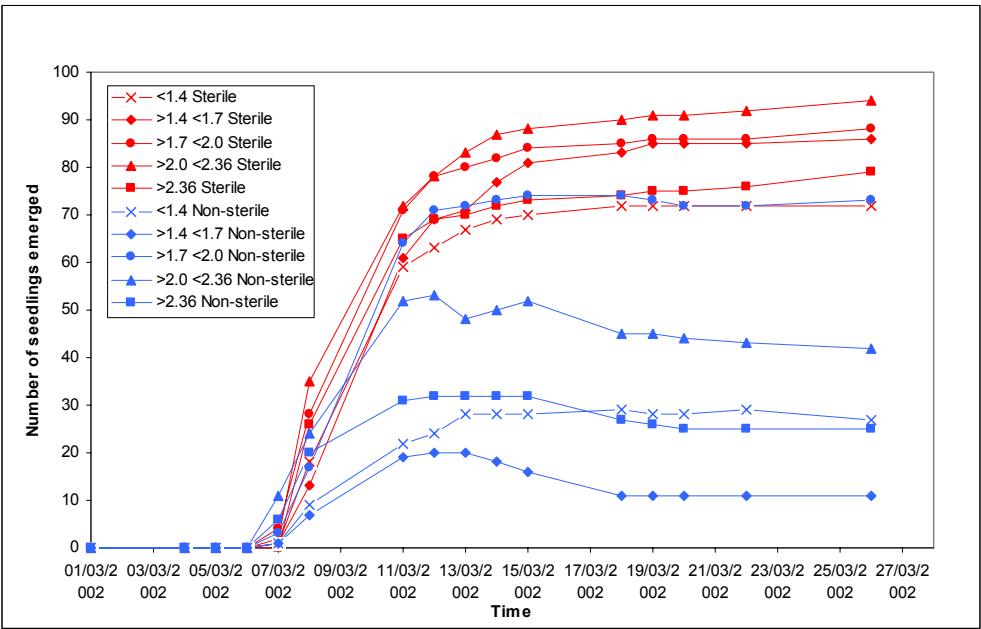
The results also showed interesting differences between size grades. In sterile conditions, the greatest percentage emergence was from the 2.00-2.36 mm diameter size grade, closely followed by the 1.70-2.00 and 1.40-1.70 size grades. Surprisingly, the >2.36 mm size grade had relatively low final percentage emergence, just above the smallest <1.40 mm size grade. This evidence corroborates the findings in the previous size grade x sowing experiment, where emergence of the largest seed sizes was slightly compromised and indicates that in seed size selection of a proportion of seed around the mean seed diameter, excluding the smallest and the largest seed, may be preferable from the approach of just selecting seed from above a particular cut-off (e.g. 2 mm). However, more work is required to investigate this further.

In the non-sterile environment, emergence was greatest in the 1.70-2.00 mm diameter seed lot, with levels comparable to emergence of the worst (smallest) seed lot in non-sterile soil. The next best seed fraction, 2.00-2.36 mm, however, experienced greatly reduced emergence in the sterile soil, less than 50% of that in non-sterile soil; the next worst seed lots were the < 1.4 mm and the > 2.36 mm diameter seed, the smallest and largest seed lots, which gave the lowest emergence in sterile conditions. However, the worst emergence came from the 1.40-1.70 mm diameter seed lot in sterile conditions.

Reduction in final percentage emergence was due to both failure to emerge and due to post-emergence death due to damping off (damped off seedlings are not included in final percentage emergence in Figure 3.17). Figure 3.18 shows a comparison of total emergence (including damped-off seedlings) in sterile and non-sterile soils. This shows that non-emergence was the greatest contributory factor to the difference in the <1.40 and 1.40-1.70 and > 2.36 mm seed diameter grades, and was less important in the 1.70-2.00 and 2.00-2.36 mm size grades. Analysis of damping off showed there to be slightly less in the 1.70-2.00 mm size grade than in the other size grades, which already had less emergence (potentially due to pre-emergence fungal parasitism).

From this work, it would appear that the prime size grade for emergence in both sterile and non-sterile environments would be the 1.70-2.36 mm size grade, since emergence was poorer in sterile conditions from smaller and larger seed diameter grades and these grades also seemed to be more susceptible to fungal parasitism, reducing emergence still further. This work supports the conclusions of the seed size x sowing depth experiments, but further work is required to prove these concepts and fully define the ideal size range for seed selection.

Figure 3.18: Comparison of total emergence (including damped-off seedlings) in sterile and non-sterile environments.
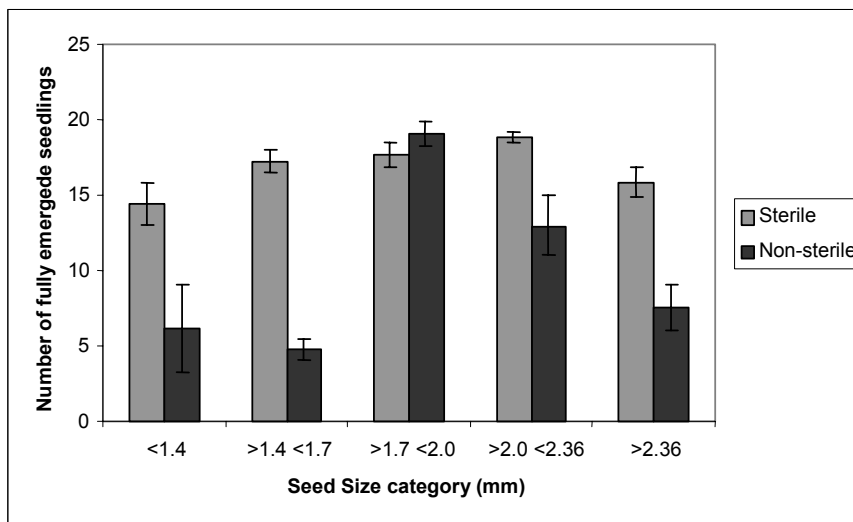
Figure 3.19: Percentage of damped off and healthy seedlings in the non-sterile environment (no damping off was observed in the sterile soil environment).
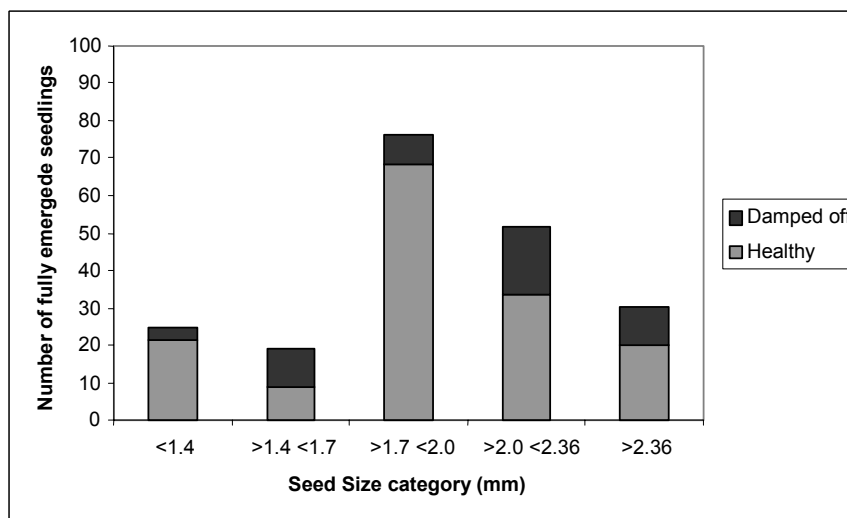
3.3.8. Vigour

The extreme variation in vigour and germination profile is shown in Figure 3.20, where the germination data at 10°C show good germination in the fresh mother crop seed produced in 2000, compared to very poor germination in seed stored (since 1997), where germination rate was slowed and final germination percentage was reduced.

Figure 3.20: Germination of low and high vigour seed from 1997 and 2000 respectively.
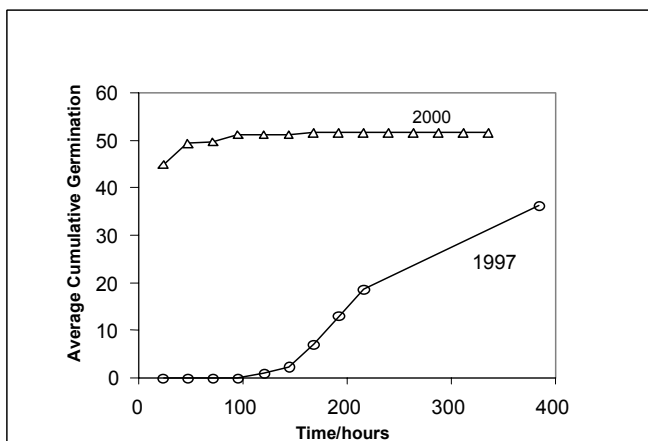
Assessment of the leakage of electrolytes from the two seed types showed important differences. Although both types lost electrolytes during steeping, producing higher electrical conductivities than the distilled water controls, the low vigour 1997 seedlot produced a significantly higher electrical conductivity than the high vigour 2000 seedlot (Figure 3.21).

Figure 3.21: Electrical conductivity (after 24 h steeping in water at 20°C) of 1997 and 2000 seed
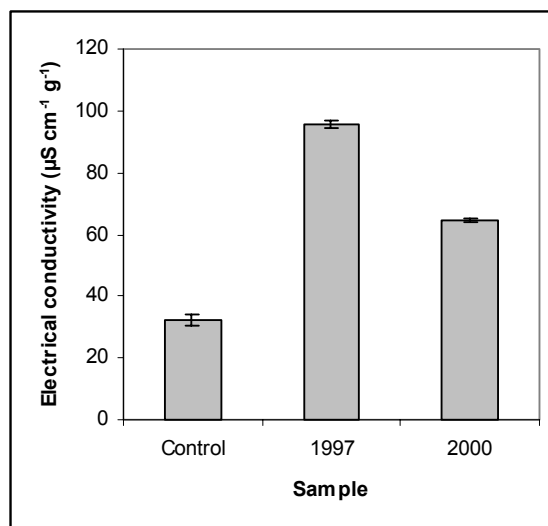
In sand emergence tests (data not shown), 1997 seed produced very low levels of emergence (<10%) whereas 2000 seed produced very good emergence (>75%). The electrical conductivity test has been used successfully for other species and is used to assess pea quality. However, further research is needed to assess whether the differences are large enough to detect smaller vigour differences than the one presented above.

LCMS analysis (data not shown) showed that the leachate contained many molecular species, with differences between the mass spectra of low- and high-vigour seedlots. It is thus possible that a specific molecular species, such as a specific amino acid or sugar, could leak more from low-vigour than high vigour seed, offering the prospect of a biochemical seed quality test, possibly utilising existing technology. However, more research is needed in this area.

3.4. Conclusion

The literature reviewed in this section and the fundamental experimental work completed demonstrates the complexity of establishment, a multi-phasic process which is effected by many different biotic and abiotic factors. Establishment requires successful seed dispersal, germination, emergence and survival through a range of potentially detrimental weather conditions and pest activity. Any number of interacting factors of the seed, physical environment, or the biotic environment may cause failure at any point.

Although quality factors such as large seed size, high oil content and high protein content, uniform maturity and high vigour may be identified as potential characters giving advantages for establishment, different factors may give different advantages in particular environmental conditions, which may not be advantageous in other conditions. For example, high protein content may give more rapid and uniform emergence in optimum conditions with a fine, moist seed bed. However, the relationship between oil and protein content is reciprocal, and high oil content may give advantages for emergence from deeper sowings in less ideal seed-beds. Large seeds appear also to have advantages in conditions of water stress, but where water is not limiting, germination and emergence may be more rapid in small seed. As it is difficult to predict conditions at emergence it will always be difficult to tailor exactly the appropriate quality of seed for optimal emergence in each situation.

This work has identified some factors, such as seed size and protein/oil balance, which may be manipulated by simple husbandry approaches and which may confer advantages in given situations (described in later sections). Large seed size in particular seems to confer advantages over a range of stress conditions, e.g. deep sowing, dry or waterlogged conditions.

A major problem touched on in this work is the effect of seasonal and storage conditions on 'vigour'. Although storage conditions may be improved it is almost impossible with current knowledge to predict the effects of weather conditions during development on seed vigour. However, an area where improvements could be made is in seed testing and the ability to discriminate poor seed lots. Current requirements (>85% germination in an ISTA test at 20°C) do not give a good indication of likely field

performance and definition of a better test is an important area for future research. We propose that as a starting point these refined tests could evaluate seedling performance at 10°C as well as 20°C, in drying and inundated moisture conditions. Propensity to secondary dormancy should also be evaluated for each seed batch. Whilst there would undoubtedly be additional costs incurred in this additional testing, improved establishment would almost certainly outweigh the cost.

4. EFFECTS OF MOTHER CROP MANAGEMENT ON SEED QUALITY

4.1. Introduction & literature review

As described in Section 3, poor establishment in oilseed rape is a major agronomic problem caused by interaction of abiotic (soil and weather) and biotic (seed, pest and disease) factors. The ideal seed-bed structure is known (Håkansson and van Polgár, 1984), although in the UK on the heavy clay soils most usually used to grow oilseed rape this structure is difficult to achieve reliably. We have hypothesised that improved seed quality will improve establishment over a range of sub-optimal seed-bed conditions (Stokes, 1998; Stokes *et al.*, 2000). However, it must be remembered that 'seed quality' is a combination of many different factors and different (possibly antagonistic) qualities may be appropriate for survival and optimum emergence from different environmental conditions (*e.g.* drying or waterlogged seed-beds, different temperature responses for early or late sowings). For oilseed rape, there are no specific guidelines for seed production compared to ware crop production (Kelly & George, 1998) and little research has been undertaken to investigate the factors that effect seed quality. However, there are indications from other species of the factors that may effect quality during development on the mother plant.

4.1.1. Environmental/seasonal conditions

It is well known that production environment influences seed quality in many species and seed quality tends to be greater when ripening occurs in warm, dry conditions than in cool, wet conditions. Stress (*e.g.* high or low temperatures, frost, drought, limited solar radiation) during flowering and the beginning of seed development may limit seed set and yield, but there is little evidence for effects of stress at this timing on seed quality, if the stress is subsequently relieved. Effects of environmental conditions and stress during grain development are better understood. The main factors that operate include temperature, water stress, light availability/photoperiod and position on the mother plant. Drought can limit germination ability and vigour due to production of light and shrivelled seeds, shown for soya and wheat (MacBeth, 1996). High air temperatures have also caused reduced germination and vigour for soya and beans (Siddique and Goodwin, 1980). Conditions preceding physiological maturity may be acceptable for production of well-filled grains of good appearance, but may be followed by conditions that reduce grain quality. Wetting and drying cycles can cause reduction of quality in soya and wheat. Excessively hot, dry conditions after physiological maturity reduced soya vigour. Wet conditions after ripening but before harvest can cause 'post-maturity' or 'pre-harvest' sprouting, with death of the precociously germinated seedling resulting from subsequent drying. The photoperiod during seed development has been shown to mediate the propensity of seeds to enter dormancy in some species. In fat hen (*Chenopodium album*), seeds grown in long days were more dormant than seeds grown in short days, indicating a role for phytochrome in mediating

57

subsequent germination. The effect of photoperiod on oilseed rape germination is not known. Although there is anectdotal evidence from spring rape of effects of time of harvest on dormancy (Weightman, pers. comm.), this could equally be a maturity or drying effect. For most oilseed rape crops in the UK, seed development would occur in conditions of similar day length, although significantly shorter days during maturation are possible for late-sown and spring crops and crops grown in Scotland compared to England. Position on the parent plant is also known to strongly effect dormancy of some seeds, *e.g.* the wild wheat relative *Aegilops* (Gutterman, 1980) and celery, *Apium graveolens* (Thomas *et al.*, 1979), although this would be difficult to study in oilseed rape due to the plasticity in canopy structure. Health of the mother plant is also important, as severe infections with viruses (*e.g.* beet western yellows, cauliflower and turnip mosaic viruses) and fungi (mainly alternaria, phoma and light leaf spot) may be perpetuated in the seed.

These factors have been little studied in oilseed rape, but may explain part of the well-known differences in seed lots of the same variety due to provenance (site and season), which have been found to be greater than inter-variety differences in some cases (Stokes *et al.*, 1998). However, they are largely uncontrollable effects, difficult to forecast due to the long-term unpredictability of the weather, and as such they will not form part of this study, which has been focused on how pre- and post-harvest management decisions may be used to optimise quality in any given season. Although a better understanding of the effects on seed quality of weather conditions/stress during ripening would be highly desirable, the options for dealing with such stresses are however largely confined to screening for genetic material that retains greater seed quality under stress conditions which is outside the remit of the project .

4.1.2. Husbandry factors

Agronomic factors may also exert large effects on seed quality and may also be controlled by the producer to optimise quality. Sowing date may exert large effects on canopy structure (Lunn *et al.*, 2001) effecting plant development and ultimately seed size distribution, thousand seed weight and ripening environment (temperature, water availability, photoperiod). Later sowing causes later flowering, thus later seed development and ripening, under altered prevailing environmental and photoperiod conditions compared to early-sown crops. However, the effect of sowing date depends on the particular season and on the conditions prevailing during ripening, which may be optimal for early sowing one year and late sowing another year. There is little current understanding of the conditions effecting rapeseed quality development and weather forecasting is insufficiently accurate to allow this to be predicted in advance in order to modify sowing date decisions. Thus sowing date decisions have not been studied as part of this research. Much work has been done to analyse sowing date (*e.g.* Mendham *et al.*, 1981, Lunn *et al.*, 2001) and the optimum sowing date for winter oilseed rape for

maximum yield in the UK Midlands is known to be in the first half of September. Earlier and later sowings potentially (but not necessarily) limit yield due to over- or under- growth. In recent years, however, the trend for oilseed rape drilling has been for increasingly earlier sowing.

Seed rate and the resultant plant population also exerts an effect on seed quality. Canopy structure is radically altered by different plant populations (Lunn *et al.*, 2001). In very sparse populations, due to compensatory growth the yield is made up from more late-formed branches (author observation), so seed size distribution and homogeneity of maturity may be effected. In large, dense, high population canopies, lodging may occur during flowering or seed filling and cause an unsuitable environment for ripening. Stokes *et al.* (1998) showed reduced germination from plots that had lodged due to high nitrogen applications and proposed that better quality seed would be produced from more open and erect canopies characteristic of 'canopy management' produced by later sowing, lower nitrogen fertilisation and smaller plant populations (Lunn *et al.*, 2001). However, this approach could also be counter-productive in terms of altered seed size and nitrogen content.

Weed control effects seed quality, due to reduced yield and contamination with foreign seed from weedy crops, which is undesirable and which causes downgrading or rejection of seed lots, although well-grown oilseed rape crops are highly competitive against weeds. Herbicides (*e.g.* glyphosate, diquat) may have effects on germination when applied late as desiccants, but are unlikely to have effects from their earlier weed control timings. These factors will not be considered in this study.

Fungal diseases such as *Alternaria* and *Phoma* may be seed-borne and diseased seed is generally of lower quality, with good fungicidal control required in the mother crop to prevent carry over in the seed. However, some modern fungicides (*e.g.* tebuconazole and metconazole) also have anti-gibberellin plant growth regulating activity and may also be used as plant growth regulators to control crop height and canopy size (Lunn *et al*. 2003). Theoretically, PGRs could effect gibberellin levels and germination capacity of seeds. This has not been investigated previously (Rademacher, pers. comm.). Seed dressings (*e.g.* Rovral and Thiram) may also be used to prevent seed and soil-borne fungal attack, but may have some deleterious phytotoxic effects in the absence of disease.

Insect attack (*e.g.* pod midge, seed weevil) may reduce seed quality and should be controlled with insecticides, which are unlikely to have major side-effects on seed quality. There are few storage pests of oilseed rape, but mite infestation (species) of improperly stored lots can impact on quality. Again, detailed study of these aspects are outside the scope of this report given that good control can usually be attained with insecticides and proper dry storage.

Crop nutrition status also potentially effects seed quality. Nitrogen nutrition effects the protein/oil balance of seeds, with more N fertilisation resulting in higher protein content at the expense of oil (Chalmers *et al.*, 1992; Stokes *et al.*, 2000). Other nutrients (*e.g.* P, K, S) and micronutrients (eg. Mo, Mn, B) may be important. Recently, work has focussed on the enhanced potential for sulphur deficiency in oilseed rape, due to reduced emissions from power stations. Sulphur deficiency is known to effect yield, and may also interact with nitrogen fertilisation but its effects on quality for sowing have not been tested. However, the phenomenon has been well publicised, S fertiliser is cheap and its application to most rape crops is now recommended and so it will not be further studied in this report.

Harvesting strategy is also important. Swathing time may effect seed chlorophyll content, maturity, drying environment and consequently quality. Desiccants may effect seed viability. The length of time mature seed is on the mother plant before harvest (and environmental conditions) may effect its propensity to enter dormancy (Weightman, pers. comm.), as may the conditions in which seed is dried (Werner, pers. comm.), although these effects are poorly understood. Mechanical damage during combining and post-harvest processing may also be a significant factor reducing seed vigour.

In this project the main agronomic effects tested were those of seed rate (thus population) and nitrogen fertilisation, in 1999/2000 and 2000/2001. Effects of nitrogen fertilisation on seed quality were further assessed by review of seeds collected from previous HGCA-funded 'canopy management' experiments (Lunn *et al.*, 2001) Potential effects of differences in weather conditions between 1999/2000 and 2000/2001 were assessed. With considerable differences in lodging in 1999/2000, the effects described by Stokes *et al.* were assessed, but there was no significant lodging in 2000/2001. The effects of PGR fungicides on germination were tested opportunistically with seed collected from a parallel HGCA project (No. 2097) in 2000/2001. Effects of weed control, fungicide application and harvesting method were not tested, although seed lots were screened for seed-borne fungal infection in routine germination experiments and pathology studies (Section 3).

4.2. Materials and Methods

4.2.1. Historic seed lots

Historic seed lots (cv. Apex) collected from 'canopy management' trials completed at Sutton Bonington in 1996, 1997, 1998 and 1999, which received different amounts of nitrogen fertiliser (0-300 kg ha^{-1}), were assessed for key effects on seed quality. The husbandry and treatments applied to these crops are summarised in Lunn *et al.* (2001).

4.2.2. Mother crop husbandry and growth analyses

4.2.2.1. University of Nottingham 1999/2000

The trial area was prepared at Field 31, Nottingham University Farm, Sutton Bonington, a Fladbury series soil (stoneless clayey river alluvium with sandy sub-soil). The preceding crop was barley, straw was baled and removed and the seed-bed was prepared by discing, subsoiling, rolling, power harrowing and Cambridge rolling. Before drilling, the trial area received an application of 5.6 kg ha^{-1} metaldehyde slug pellets, 3 l ha^{-1} of the herbicide Gallant (haloxyfop) on 18/8/99 and 1 l ha^{-1} each of Reglone (diquat dibromide) and Gramoxone (paraquat dichloride) on 3/09/99 for pre-emergence grass and broad-leaved weed control, including volunteer barley and oilseed rape. Seed of the inbred cultivar Apex (1999 production) dressed with Rovral (iprodione) and Hydraguard (gamma HCH + thiram) to protect against soil-borne fungal disease and cabbage stem flea beetle was sown with a Nordsten (2.5 m width) drill on 8/9/99. The seed rates were 240, 130 and 60 seeds m^{-2}, with additional 60 seeds m^{-2} plots drilled to allow thinning to lower plant populations in spring. Sufficient replicate plots of each seed rate were included to allow five different levels of nitrogen application in the spring. Plot size was 7.5 m x 22 m, to coincide with farm tramlines and allow application of farm sprays. A randomised block design with four replicates and factorial combination of plant population*nitrogen application within each block was used. The trial was sprayed with 1.25 l ha^{-1} ButisanS (500 g l^{-1} metazachlor) and 0.25 l ha^{-1} cypermethrin on 9/9/99 for additional grass weed and flea beetle control. Metaldehyde slug pellets were applied at 15 kg ha^{-1} on 23/9/99 and 6/10/99. On 24/9, emergence was judged to be relatively patchy and 33 kg ha^{-1} nitrogen was applied as ammonium nitrate prills on 19/10/99. This has been proposed to aid establishment by some researchers and advisors. However, samples taken just before drilling for soil mineral nitrogen determination later showed residues of 143 kg ha^{-1} N in the top 90 cm of soil, which should have been sufficient for establishment. On 4/11/99 the field was sprayed with 0.4 l ha^{-1} Falcon (butroxydim) and 1.4 l ha^{-1} Judo (propyzamide), post-emergence grass herbicides. Sprays of 0.3 l ha^{-1} of the fungicide Punch C (flusilazole + MBC) for control of phoma (*Leptosphaeria maculans*) leaf spots and canker and light leaf spot (*Pyrenopeziza brassicae*) and 0.25 l ha^{-1} of the insecticide cypermethrin (10%) for flea beetle control were applied on the same date. Spring nitrogen was applied as a single application of ammonium nitrate prills on 20/03/00, at 0, 77, 177 and 277 kg ha^{-1}. There were no further applications of fungicide or insecticide as pest and disease pressure was low. The crop was desiccated with 1 l ha^{-1} Reglone and was harvested with a Sampo plot combine harvester.

In March 2000, some of the plots drilled at 60 seeds m^{-2} were manually thinned. For all thinning treatments, alternate rows were removed with a mechanical rotavator. For the lowest desired

61

populations, alternate plants within the remaining rows were pulled up or cut off at the base with a knife. Growth analyses were carried out at regular intervals during the season (12/12/99, 10/4/00, 8/5/00, 5/6/00, 19/6/00, 11/7/00, 18/7/00). All the plants within a randomly placed 0.72 m^2 quadrat were pulled up or cut off at the base. Samples were removed to the laboratory as soon as possible and were stored at 4°C in a cold room. Soil was washed from roots and leaves where necessary and the plants were dried with paper towelling. The number of plants in the sample was counted by placing into five piles and the total fresh weight was determined. One 20% subsample (SS1) was cut up and dried for 48 h at 85°C for determination of dry weight. Another subsample (SS2) was divided into different organs: stems, leaves and where appropriate, buds, flowers and pods. The fresh and dry weight of each organ fraction was recorded and the projected green area of leaves, stems, and pods was recorded with a Licor planimeter. Fresh and dry biomass in t ha^{-1} was calculated with a correction factor calculated from the total sample fresh weight and SS1 fresh and dry weights and the area of the quadrat. Green area indices (area of green material per square metre of ground) were calculated by division by the quadrat area. Projected areas of stems and pods were multiplied by /2 (~1.57), since half the surface area of cylindrical structures is theoretically considered to contribute to GAI (Bilsborrow, 1985).

Throughout the season, regular measurements of crop height were taken, with ten assessments per plot recorded with a randomly placed metre rule or graduated 2 m pole. Immediately pre-harvest, a visual assessment of lodging was made, with a subjective recording of the percentage of the plot leaning or lodged. Grab samples of the crop were made approximately 10 days before harvest. Samples from randomly-placed 0.72 m^2 quadrats were cut off at the base of the stem and placed in large plastic bags. These were stored in a glasshouse to maintain dryness before processing. Pods were removed from stems by hand and the pod and seed was mechanically threshed to separate pod and seed. The fresh weight of stem, seed and chaff was determined and stem and chaff was dried for 48 h at 85°C to determine dry weight. A sub-sample of seed was dried to determine seed dry weight and moisture content, to allow calculation of the yield in t ha^{-1} at 9% moisture. Fifty pods were reconstituted (two pod walls, plus one placenta and peduncle) and were weighed, to calculate the number of pods from the dry weight of chaff.

4.2.2.2. University of Nottingham 2000/2001

In 2000, the trial area was prepared in Dewsbury's field, University of Nottingham farm, Sutton Bonington. The soil series was Dunnington Heath (slightly stony light to medium sandy clay loam over Keuper Marl) and the previous crop was barley, the straw baled and removed. The seed bed was prepared by subsoiling, power harrowing, rolling and power harrowing. Seed from the same lot of

Apex as drilled in 1999, that had been stored over winter at ambient temperature conditions and dressed with Chinook (imidacloprid + beta-cyfluthrin) and Rovral/Thiram before sowing was drilled on 12/09/2000 and was rolled after drilling. The plot size was 7.5 x 22 m, the design was a 3*3 factorial of seed rate (60, 120, 240 seeds m^{-2}) by nitrogen fertilisation (0, 100 and 300 kg ha^{-1}) in a randomised block design with four replicate blocks. The trial was sprayed with 1.4 kg ha^{-1} of the herbicide Kerb (propyzamide), 0.4 l ha^{-1} of the fungicide Punch C (flusilazole + carbendazim) and 0.25 l ha^{-1} of the insecticide cypermethrin on 14/12/00. Nitrogen was applied at appropriate rates as a 50/50 split dose on 06/03/01 and 27/04/01. The insecticide Fastac (-cypermethrin) was applied at 0.1 l ha^{-1} on 08/05/01 for pollen beetle, and 1.0 l ha^{-1} of the fungicide Bavistin (500 g l^{-1} carbendazim) was applied on 20/05/01. Plots were direct combined on 10/08/2001 with a Sampo plot combine harvester except for high nitrogen treatments, which were harvested on 22/08/01 as crops were still green and at high moisture content on 10/08/01. As moisture contents were too high for storage, seeds were dried at 50°C for 48 h in a fan assisted oven and were cleaned by hand sieving or with a threshing machine.

4.2.2.3. CPB Twyford hybrid seed production 1999/2000 and 2000/2001

In 1999/2000, some small plots of male sterile hybrid lines were hand-thinned and received different nitrogen applications, but due to problems with weeds and insufficient pod set did not produce enough seed for detailed analysis.

In 2000/2001, two experiments were initiated, one looking at the effects of different populations at a single N treatment and one with different N treatments on a single plant population. Due to restrictions caused by the Foot & Mouth epidemic, neither crop could be monitored so no crop assessments were made. Only the population experiment could be harvested for study at Sutton Bonington. This was examined according to the same protocol as the SB crops were.

4.2.2.4. Effects of plant growth regulators on germination

As an adjunct to a parallel experiment investigating remediation of canopy architecture with plant growth regulators (Lunn *et al.*, 2002), areas of a 2000/2001 University of Nottingham farm crop of Apex were sprayed with the triazole fungicides tebuconazole (Folicur) and metconazole (Caramba) at various rates and timings (Lloyd, 2002). Seed collected in this experiment were assessed for germination using the standard protocols, to assess potential effects of PGR chemicals (Rademacher, pers. comm.).

4.2.3. Seed Quality Assessments

4.2.3.1. Thousand seed weight

Batches of 1000 seed (cleaned to remove pod wall and other detritus, broken seeds and weed seeds such as cleavers, *Galium aparine*) were counted with a Pfeuffer automatic seed counter and weighed. Mean values were calculated from at least three replicates.

4.2.3.2. Specific weight (hectolitre weight)

Samples of seed, cleaned to remove broken pod and weed seeds, were poured into a specific weight vessel (volume 570 ml) designed for determination of wheat specific weight. The seeds were poured from a fixed height to attempt uniform packing. The seed was decanted into a beaker and weighed, with hectolitre weight (kg/hl) calculated as (weight in g/570)*1000.

4.2.3.3. Seed size distribution

A 250 g sample of seed (cleaned to remove pod wall and large weed seeds such as cleavers) was placed on a tower of Endecotts sieves (mesh size 2.80, 2.36, 2.00, 1.70, 1.40, 1.18, collecting pan) on an automatic sieve shaker and were sieved for five minutes. The weight of seed retained by each sieve was recorded and calculated as a percentage of the whole seed lot. At least three replicates per plot were measured. Previous experimentation had determined the sample size and sieving time to be optimum.

4.2.3.4. Maturity

Replicate random sub-samples of 100 seeds were taken from each sample and the number of immature (non-black, i.e. brown, 'red' or 'orange') seeds was counted.

4.2.3.5. Protein and oil content

Protein and oil content was determined by near infra-red spectrometry (NIR) by CPB Twyford.

4.2.3.6. Germination

Fifty seeds were randomly selected from each sample and were evenly spread out on two layers of Whatman No. 1 filter paper in a Petri dish, wetted with 5 ml distilled water. Previous experimentation showed that this volume of water was sufficient to wet the filter papers for the duration of the tests and did not cause water sensitivity. Germination tests were incubated at the recommended ISTA temperature of 20°C and also at 10, 15 and 25°C for 7-10 days in the dark in incubators. Germinated seeds (radicle protrusion > 1 mm), were removed at assessments taken at 9 am, 12 am and 4 pm. Numbers of fungus-attacked seeds were recorded. At the end of the test, seeds were incubated at 4°C in the light for 48 h to break dormancy and then incubated at 20°C to assess seed viability. Seeds that germinated after this treatment were viable but dormant.

4.3. Results and Discussion

4.3.1. Historic crops – effects of N on thousand seed weight and seed size distribution

Seed from 1996 showed effects of nitrogen application on thousand seed weight and seed size distribution. Thousand seed weight varied from about 4.55-4.90 g, and increased with increasing nitrogen fertilisation. Overall, there was a relatively weak regression relationship of TSW to N application, since different strategies (early, late and split) were included in the experiment. However, the relationship in samples that received a single application of N (from $50 – 250$ kg ha^{-1}) showed a strong relationship between nitrogen and TSW (Figure 4.1). Increasing nitrogen application also significantly increased the fraction of seed greater than 2 mm in diameter (Figure 4.2), from 30% at zero N to 60% at the highest level of N fertilisation (250 kg ha^{-1}). A similar relationship was maintained when the samples were grouped by N application strategy. Hectolitre weight, however, showed no trend with nitrogen application (data not shown).

Figure 4.1: Relationship between TSW and nitrogen application rate (single N application strategy), for 1996 seed.
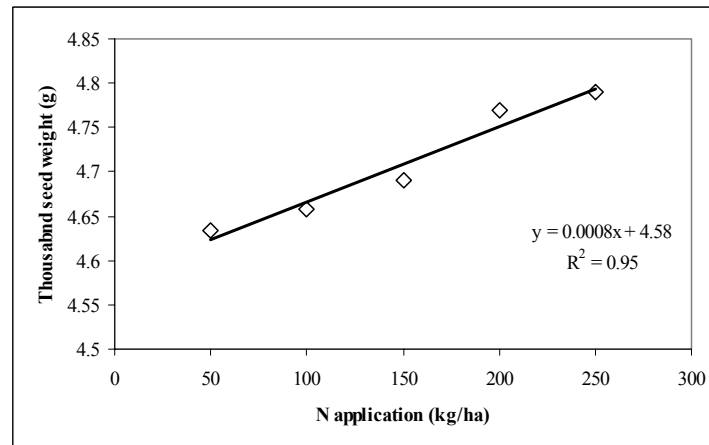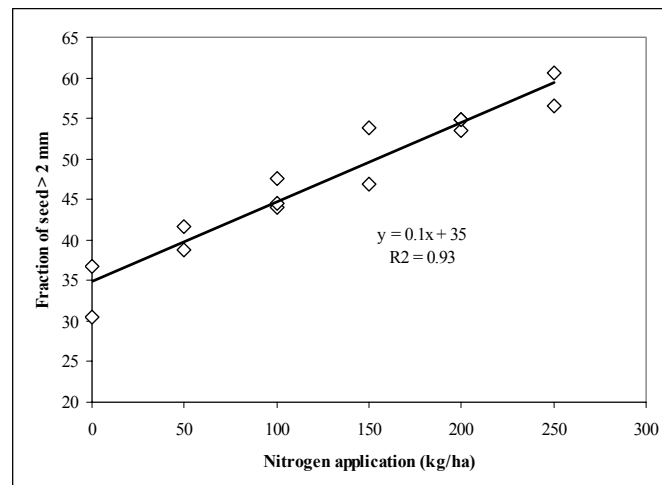

Figure 4.2: Effect of nitrogen fertilisation (kg ha^{-1}) on fraction of seed greater than 2 mm diameter (for 1996 seed).

1997 seed showed a similar trend to 1996: thousand seed weight again increased from about 4.60 to 4.85 g, and increased with increasing N fertilisation. The overall relationship was weak, as again there were various N management strategies in the experiment (single, split and with and without seedbed N). However, the N/TSW relationship was considerably stronger in the samples that received a single dose of N (data not shown). The fraction of seed above 2 mm diameter also increased with increasing N fertiliser, from about 30% at zero N to 47% at 200-400 kg ha^{-1} N, although the difference was less marked than for 1997 seed. The overall relationship was weaker that that seen in 1996, although when the seed bed nitrogen treatment was excluded, strong regression lines could be fitted to the single and

split applications (data not shown). Hectolitre weight also increased with increasing N fertilisation, from 67.5 to 69.5 kg hl^{-1} (Figure 4.3).

Figure 4.3. Effects of nitrogen application on hectolitre weight (kg hl^{-1}) for 1997 seed.
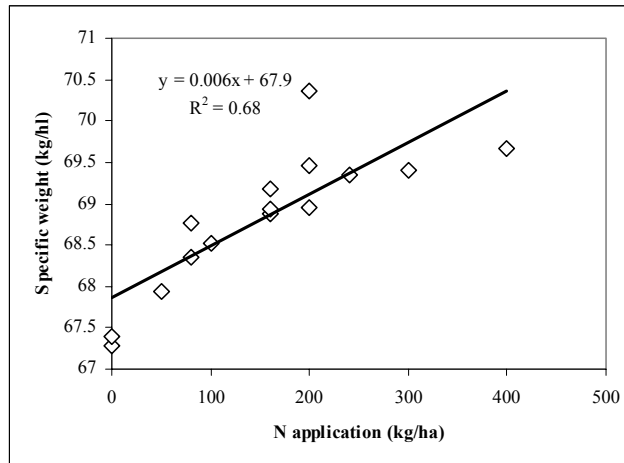
Analysis of 1998 seed lots did not show the exactly the same trend as 1997, since thousand seed weight was randomly distributed. However, increased N fertilisation was again significantly related to an increased fraction of seed greater than 2 mm diameter, with the fraction of seed above 2 mm increasing from 48 to 58% with N fertilisation increasing from 0 to 400 kg ha^{-1}. However, in 1998 there was no relationship between N fertilisation and hectolitre weight, which was randomly distributed.

Seed from 1999 did not show any significant trends for increased TSW, hectolitre weight or fraction of seed > 2 mm and there were slight numerical indications of reductions in TSW and fraction of seed > 2 mm at high levels of nitrogen fertilisation.

4.3.2. Mother crops – effects of N and population on crop growth, thousand seed weight and seed size distribution – 1999/2000

4.3.2.1. Crop growth

Detailed measurements of crop growth were taken throughout development on 0 and 100 kg N ha^{-1} treatments. The analyses showed the great compensatory ability of oilseed rape. Despite the large difference in plant populations (Table 4.1), there were no significant differences in crop green area, biomass, numbers of pods per square metre *etc.* (P>0.5, data not shown). However, the plants

composing the crops were of radically different structure, with lower plant populations producing shorter, more-highly branched plants with greater green area per plant and thicker stems (data not shown).

Yield, analysed by ANOVA (Table 4.1), did not differ significantly due to N or population and there was no significant interaction (P>0.2). The difference in plant structure led to differences in lodging (Table 4.2), presumably due to the differences in lodging susceptibility caused by plant structure (height, stem thickness and leverage of the pod canopy).

Table 4.1: Effects of plant population and N fertilisation on combine harvest yield (t ha^{-1} at 91% dry matter)

Seed rate and thinning	Plants m^{-2*}	Nitrogen (kg ha^{-1})				
		40	*100*	*200*	*300*	*Mean (Pop)*
60 seeds m^{-2} Inter and intra-row thin	10	2.97	3.27	3.44	3.00	**3.25**
60 seeds m^{-2} Inter-row thin	16	3.14	3.02	2.90	3.41	**3.12**
60 seeds m^{-2}	40	3.02	3.26	3.32	3.27	**3.22**
120 seeds m^{-2}	65	3.19	3.37	3.18	3.20	**3.24**
240 seeds m^{-2}	81	3.21	3.08	3.22	3.44	**3.24**
Mean (N)		**3.10**	**3.20**	**3.21**	**3.32**	3.21[b]

	P	SED (57df)
Population	0.814	0.12
Nitrogen	0.255	0.11
Pop*N	0.635	0.24

[b] Grand mean * Mean of four nitrogen treatments

4.3.2.3. Thousand seed weight, seed size distribution and hectolitre weight

Thousand seed weight varied significantly (*P*<0.001) due to plant population and nitrogen application, with a significant population x nitrogen interaction (*P*<0.001). The main nitrogen effect was similar to that seen in the 1996 and 1997 seed lots (data not shown). Thousand seed weight increased with increased plant density and increased nitrogen fertilisation, ranging from 4.55 g (10 plants m^{-2}, 40 kg ha^{-1} N) to 6.08 g (80 plants m^{-2}, 300 kg ha^{-1} N). At greater levels of N fertilisation, the effect of plant population on TSW was increased, with increases in plant population raising TSW at a greater rate. The fraction of seed greater than 2 mm in diameter increased significantly with both increasing N fertilisation (from 44 – 57% seed >2 mm from 40 – 300 kg ha^{-1} N), and also with

increasing plant population (40 – 62% from 10 – 80 plants m^{-2}). The main effect of population is shown in Figure 4.4. Hectolitre weight and seed density were both significantly effected by population and nitrogen fertilisation (P<0.001), although there were significant interactions and the data did not show simple trends. The percentage of immature seed (coloured brown or orange) decreased with increasing population and also with increasing nitrogen fertilisation (Table 4.2).

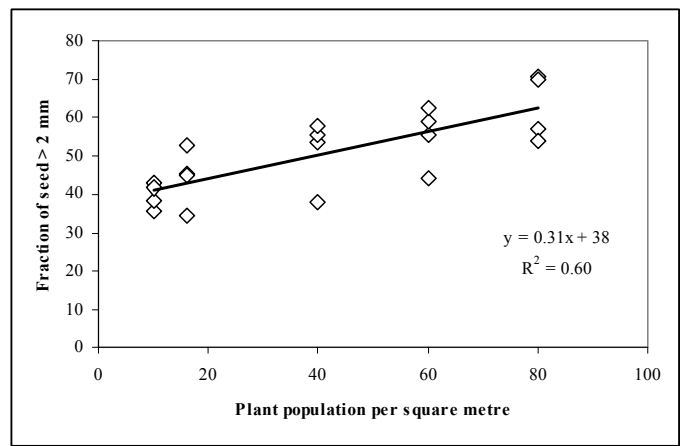Figure 4.4. Effect of N fertilisation on fraction of seed > 2 mm, 2000.

Table 4.2. Percentage of immature seeds with population and nitrogen (standard angular transformation of data, untransformed data in brackets)

Seed rate and thinning		Nitrogen (kg ha^{-1})				
		40	100	200	300	Mean (Pop)
60 seeds m^{-2}		30.8	30.9	23.4	25.6	27.7
Inter and intra-row thin	(26.6)	(26.3)	(15.9)	(18.8)	(21.9)	
60 seeds m^{-2}		26.4	29.8	30.2	24.9	27.8
Inter-row thin		(19.9)	(25.0)	(25.4)	(18.3)	(22.2)
60 seeds m^{-2}		25.0	26.4	27.2	23.4	25.5
		(18.1)	(20.2)	(21.0)	(15.8)	(18.8)
120 seeds m^{-2}		19.8	29.4	20.6	20.0	22.4
		(11.6)	(24.3)	(12.7)	(11.6)	(15.1)
240 seeds m^{-2}		22.2	20.3	20.8	19.1	20.6
		(14.4)	(12.0)	(12.8)	(10.6)	(12.5)
Mean (N)		24.9	27.4	24.5	22.6	24.8[b]
		(18.1)	(21.6)	(17.8)	(15.2)	(18.1)

	P	SED
Population	<0.001	1.26
Nitrogen	<0.001	1.13
Pop*N	0.046	2.52

47df

4.3.2.4. Germination

4.3.2.4.1. Comparison of germination from lodged and unlodged plots

In previous work (Stokes *et al.* 2000), it was hypothesised that seed produced from more open and erect canopies would be of better quality, giving better germination than seed from dense, lodged canopies. When this was tested with mother crops produced in 2000, however, germination of lodged crops was slightly more rapid and gave slightly greater final percentage germination than from unlodged crops, although the differences were not significant (Figure 4.5). However, lodging occurred relatively late in the seed development period and earlier lodging could possible effect seed germination more deleteriously.

Figure 4.5: Germination from lodged and unlodged plots
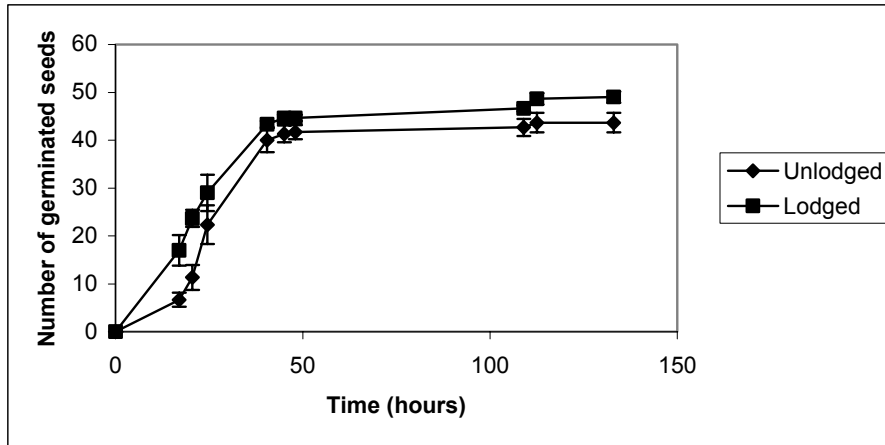
4.3.2.4.2. Effects of temperature

As discussed in Section 3, the effects of temperature generally followed previously-observed trends, with a base temperature of about 3.6°C. The rate of germination increased over the range of temperatures studied (10-25°C). However, the different mother crop management regimes used in 1999/2000 did not produce seed lots with significantly different responses to temperature (data not shown). This implies that the large intravarietal seedlot differences observed by Stokes (1998) and Basu (2003) were due to differences in site and seasonal provenance factors (mainly environmental conditions), rather than factors such as plant population, nitrogen managemement and lodging.

4.3.2.4.3. Protein/oil balance

Figure 4.6 shows the strong reciprocal relationship found between protein and oil content in rapeseed in 2000. As protein content increased, so oil content decreased. Thus as additional nitrogen fertiliser increased percentage protein content in the seed, so oil content was reduced. Thus protein/oil balance could be readily manipulated by the amount of nitrogen fertiliser added to the mother crop.

Figure 4.6: Relationship between rapeseed oil and protein contents (determined by NIR spectroscopy)

y = -1.1271x + 67.249
$R^2 = 0.8133$
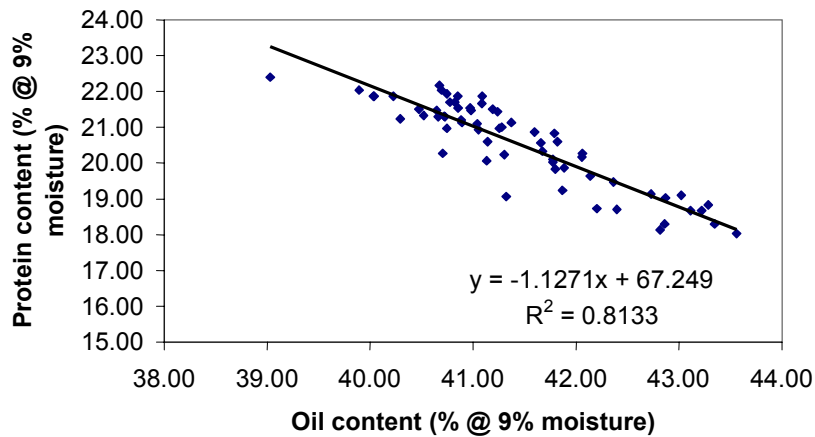
4.3.3. 2001 seed lots.

4.3.3.1. Crop Growth

The establishment period was very problematical in 2001. Between drilling in September and December, rainfall was approximately 314 mm (60% more than the previous year). This resulted in a period of prolonged water-logging on a low-lying flood-prone alluvial soil type, resulting in visible signs of stress in many cases (purple coloration of leaves) and plant death. Additionally, water logging caused slumping of the soil surface which had probably also been compacted during the extensive seed bed preparation and this resulted in poor and patchy emergence, with significant over-winter loss of some small plants. This lead to bare patches in many plots sparse areas in others. Over-winter rainfall and soil conditions did not allow re-establishment of the crop. Although the decision was made to maintain the plots and collect the seed for 2000/2001 field evaluations, only rudimentary assessments of crop growth were made compared to 1999/2000 (data not shown)

4.3.3.2. Thousand seed weight, hectolitre weight, seed size distribution and seed maturity

Seed lots from 2001 did not show the same trends as those grown in 2000. The effect of population on thousand seed weight was just significant ($P=0.051$) and nitrogen treatment also had a significant ($P<0.001$) effect. However, there was no trend in the data, since medium populations had significantly lower TSW than the high and low populations and the 100 kg ha^{-1} N treatment had significantly lower TSW than the 0 or 300 kg ha^{-1} N treatment. Grand mean TSW was lower in 2001 than 2000 (4.46 cf. 4.96 g) and the range of values was also lower (3.89 – 4.78 g cf. 4.37 – 6.16 g).

This effect was probably due to the poor crop establishment and development in 2000/2001. Heavy rain immediately after drilling and throughout the winter resulted in soil slumping, water logging and reduced emergence. Crops were very patchy, with large differences in population across the plots. Thus the plots were not representative of 'good crops' and the treatments were not uniform.

There was no significant difference in the number of immature (red/orange-coated seeds) in 2001 due to population or nitrogen treatment. Analysis of seed size distribution also did not show the relationship between plant population, nitrogen application and the fraction of seed > 2 mm observed in 2000. Population had no significant effect ($P=0.467$) on the fraction of seed above 2 mm in diameter. Nitrogen had a significant effect ($P<0.001$), with a smaller fraction of seed > 2 mm in the 100 kg ha^{-1} N treatment, but there was no significant population x nitrogen interaction ($P=0.599$). Thus, results from 2001 cannot be used to support the trends seen in 2000. However, as stated previously, this was probably due to the unusual conditions and poor establishment in 2001. Other evidence from historic crops supported the trends seen in 2000.

4.3.3.3. Germination and response to temperature

In 2001, due to the patchiness of the crops there was considerable inhomogeneity in development and thus many seedlots had high moisture contents (>25%) at harvest. Consequently, these seeds needed to be dried before storage, to prevent microbial spoilage. Unfortunately, the drying process lead to abnormal germination (death of the radicle) in many cases, so it was not possible to compare germination responses to temperature across the whole range of 2001 seed lots. However, in those seed lots where drying did not damage the radicle, there was no evidence of a differential response to temperature (data not shown).

4.3.3.4. Protein/oil balance

Although the same seed size effects were not seen in 2001 as in 2000, the clear inverse relationship between protein content and oil content was again seen in this year, with high protein seed having lower oil content, and higher protein content resulting from a greater level of N fertilisation (Figure 4.7)

Figure 4.7: Relationship between protein content and oil content, 2001.
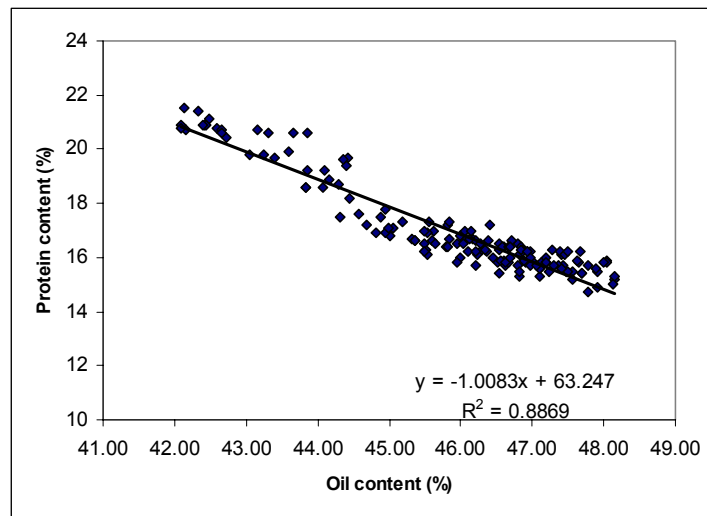
4.3.4. Hybrid seed production from CPB Twyford

The effects of population on TSW and seed size distribution in a crop of the male sterile parent of the Navajo hybrid were assessed in 2001. The effects were similar to those observed in historic seedlots and in the SAPPIO 2000 mother crop production, with an increased TSW and increased fraction of seed > 2 mm with increased population (Figure 4.8).

Figure 4.8: Effect of plant population density on the proportion of seed above 2 mm diameter in the male sterile parent of Navajo.
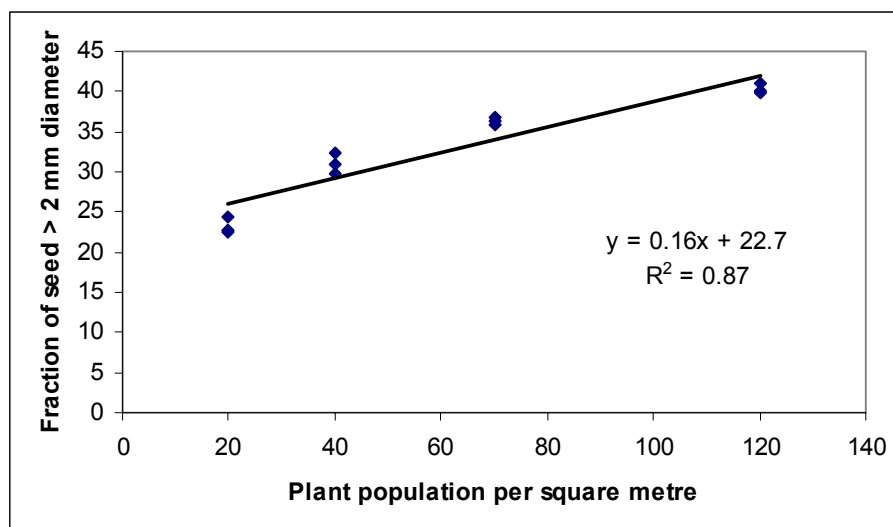
4.3.5. Effects of PGRs

Seed collected from trial plots treated with the plant-growth regulating fungicides tebuconazole (Folicur) and metconazole (Caramba) were assessed for their germination capacity, according to the standard ISTA protocol. Th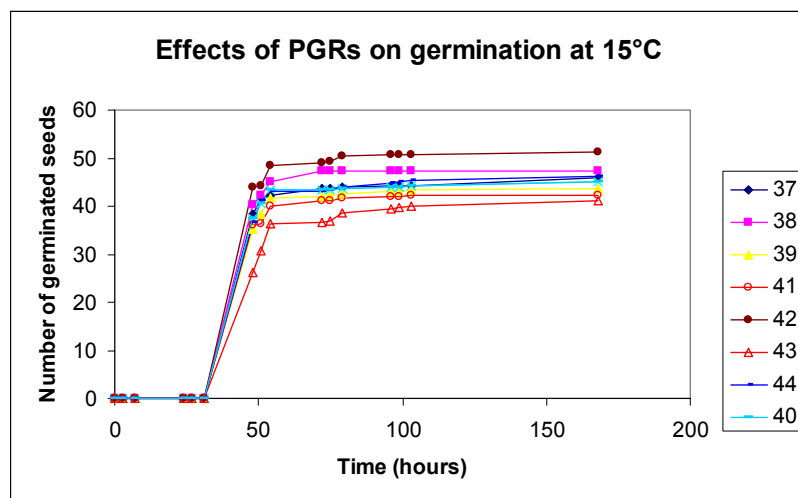eoretically, seed from plants treated with the gibberellin-biosynthesis inhibitors tebuconazole and metconazole could have reduced levels of seed GA and hence reduced germination. However, in this experiment, no significant differences were found (Figure 4.9).

Figure 4.9: Effects of PGR application on germination

4.4. Conclusion

The work reported in this Section has shown that it is possible to manipulate important seed quality factors by husbandry of the mother crop. Interim studies proposed that quality advantages might be gained from the open non-lodged canopies characteristic of 'canopy management'. However, in this work little difference has been detected in seed quality due to lodging; large seed size has been shown to be the most consistently important quality attribute associated with improved emergence performance and assessment of several seed lots has shown that a greater proportion of large seed is produced by denser, well-fertilised crops than by the sparser, less intensively-fertilised crops of canopy management. This is because the yield of sparser crops is made up of more seed produced from later-formed branches, which is usually smaller.

The work has also shown that the protein/oil balance of seed can be manipulated by nitrogen fertilisation, with greater N fertilisation producing higher protein seed. The higher the protein content

of seed produced, the lower the oil content. High protein content seed may be of value in some field situations, although there is less evidence of a benefit than with large seed size.

Thus, the evidence outlined in this Section suggests that optimum seed quality for re-sowing (i.e. the most large seed, to give advantages for emergence from depth and over a range of stress conditions reported in Section 3, with high protein content to give rapid, homogeneous emergence) could be gained from relatively dense, highly fertilised crops. Such crops would not be recommended for oil production, due to reductions in percentage oil content with greater inputs and potentially yield reductions. Another potential problem with such crops is an increased risk of lodging, although this would not necessarily effect seed quality. However, this work has also shown that there appears to be no impact of PGR use on seed germination, so these chemicals could be used to control lodging (Lunn *et al.*, 2003).

Although this approach may give the greatest likelihood of achieving high quality seed from a particular crop, site and seaonal effects, drying and storage can all have a large impact on seed quality, and so a large seed size/high protein seed lot may still be of low vigour and poor germination capacity.

5   EFFECTS OF PROCESSING AND TREATMENT ON SEED QUALITY.

5.1. Introduction

Many attempts have been made to improve the quality of seed post-harvest, before re-drilling, and there is a wealth of literature on the subject. However, most attempts have been made on seed lots of higher value than oilseed rape (*e.g.* sugar beet, vegetable brassicas, ornamentals) and although the principles are well-known there have been relatively few attempts to apply them to rapeseed. Additionally, many of the species that have benefited from seed selection and treatment have been spring crops, allowing a greater timescale for processing without the need for an excessively long storage period. For winter oilseed rape, the turnaround time between harvest and resowing is short. Overwintering of seed is undesirable due to the potentially rapid loss of vigour in storage.

Essentially the possibilities for quality improvement can be divided into grading processes, to separate out the best fraction of seed, seed treatments (advancement and priming) to improve the overall quality or vigour of entire seed lots and treatments (including sterilisation and chemical application) to reduce the effects of pests and pathogens.

5.1.1. Seed Grading

There is much evidence that large seeds have advantages in some situations, such as in deeper sowing, by way of their greater food reserves. Work on many species has demonstrated the value of large seeds (see in Section 3). In oilseed rape, Major (1977) demonstrated greater emergence from large seeds and advantages have also been shown by Mendham *et al.* (1981), McWilliam (1998) and Stokes *et al.* (1999, 2000). Seed cleaning and sorting is a usual part of seed processing and seeds may be separated according to differences in physical attributes such as size, shape, length, density, surface texture or buoyancy in air or liquids (Halmer, 2001). These procedures are normally utilised to separate undesirable contaminant weed species and are less often used for grading the purified seed lot into different size fractions. However, a wide range of commercial fractionating equipment exists which could also be used to grade purified seed lots. These include various sorts of size-grading machinery of spiral, disc, cylindrical or flat-bed structure with screens consisting of round or slotted holes or concave depressions. Other systems include air-classifiers, which use upward-moving air flows and gravity separators. Gravity separation is able to separate impurities (mud, stones), weed seeds and immature, deteriorated, mouldy, and insect-damaged seeds, which have defects that reduce their density. The principles of most gravity separators are similar (Copeland and McDonald, 2001). Seed is introduced to a porous metal or fabric vibrating deck, with an upward airflow. The combination of vibration and upward air pressure causes the seed lot to layer according to density,

with heavier components close to the deck surface and lighter components floating on the air cushion. The deck can be tilted in two directions to facilitate separation: heavy particles in contact with the deck are forced to 'walk' towards the top of the deck where they fall off and are separated. The lighter particles tend to float above the heavier ones, but toward the lower end of the deck where they too are separated. Seed in the medium density range ('middlings') are separated from the middle area of the deck.

A novel method of sorting is able to separate seeds based on the seed coat colour or composition. Jalink *et al.* (1998) have recently developed a system that can sort seeds based on the laser-induced fluorescence of chlorophyll in the testa. Seeds with greater chlorophyll fluorescence tend to be immature and after detection can be removed with an air-jet. The system has so far been applied with some success to tomato, pepper, leek, cucumber and cabbage.

5.1.2. Seed treatment

5.1.2.1. Removing dormancy
Many species may be dormant immediately post-harvest (primary dormancy). This can be due to an impermeable seed coat (hardseededness) which needs to be softened and made permeable to water and gases by mechanical scarification or acid treatment. Dormancy can also be caused by endogenous inhibitors that need to be leached away. Seed mucilages may also impair gaseous exchange and may need to be removed. Some species, e.g. barley, require an 'after-ripening' period of dry storage and other species (mainly trees and shrubs) require deep physiological dormancy to be removed by prolonged imbition at low temperatures in an aerated state (stratification). However, none of these factors are thought to be important in oilseed rape, which lacks primary dormancy.

5.1.2.2. Germination enhancement

A number of treatments are available for improving the rate, synchrony and degree of germination, often termed 'advancement' or 'priming' techniques, although the terminology can become confused.

5.1.2.2.1. Steeping, hydropriming and fluid drilling

Soaking in water, followed by re-drying, may be useful for leaching inhibitors or introducing seed treatments such as fungicide, for example in sugar beet (Durrant *et al.*, 1998). The process of imbition in water, allowing the first phases of germination may also be termed 'hydropriming' and may or may not be followed by a re-drying process. On-farm steeping may be useful for subsistence

production of some species such as rice and sorghum, but is difficult to arrange for intensive mechanised production. There has been some success with 'fluid drilling' of pre-germinated vegetable seed suspended in a viscous nutrient gel (Pill, 1991) by specialist horticultural growers of species such as tomato, celery and carrot, although treatment cost makes it uneconomic for an arable crop such as oilseed rape.

5.1.2.2.2. Priming

The process of priming, which exposes seeds to restricted water availability under controlled conditions, has been developed over the last 25 years and has found application in many species such as carrot, celery, leek, lettuce, onion, pepper, tomato, several flowers and herbs and some large-scale arable crops such as grasses and sugar beet. The treatment allows some of the initial phases of germination to be completed. McDonald (2001) defined three commercial priming techniques. A fourth technique (pre-germination) is still in its infancy and will not be considered further.

(i)   Hydropriming

This process involves soaking seeds in water or misting with specific volumes to minimise water uptake, with re-drying to prevent germination. The problems with this process are that the rate and degree of hydration may be difficult to control (possibly resulting in imbibition damage of some species) and seeds may not be evenly hydrated or activated. The process has been applied to close relatives of oilseed rape such as Brussels sprouts, cauliflower (Fujikura *et al.*, 1993) and radish. Preliminary studies (Noon, 1997, Stokes *et al.*, 1998, 2000, Basu, 2003) indicated that hydropriming might have some value for oilseed rape.

(ii)   Osmopriming

In osmopriming, seeds are incubated in aerated solutions of various chemicals with known osmotic potentials, such as polyethylene glycol, mannitol, KNO_3, KH_2PO_4 or other salts. Some of the osmotica may be absorbed and have toxic side effects (salts and mannitol) and the inert, high molecular weight PEG is the preferred compound, since the large molecular size (6-8 kDa) prevents transport across the cell membranes. Osmopriming has been successfully used on species such as carrot, celery and leek (Gray *et al*., 1991). During preliminary studies (Noon, 1997), no advantage of osmopriming over hydropriming was found at a laboratory scale, so this process did not form a major part of the research.

(iii)   Matriconditioning

A third approach to priming involves immersion of seeds in solid carriers with low matric potential, high water-holding capacity and large surface area:volume ratio such as peat moss, vermiculite and diatomaceous silica products. The matric priming process mimics imbibition of water by the seed from soil particles as the surface of the compounds create matric forces that bind water, which is gradually absorbed by the seed. The process has been used for species such as carrot and celery. However, the process is logistically unattractive for the large seed amounts needed for an arable crop such as oilseed rape and has not been studied in this research.

5.1.2.3. Chemical seed treatment

Dressing seeds with various chemicals has long been used to reduce damage due to seed and soil-borne fungi and pests. A few chemical treatments are also available that purport to enhance seed vigour. In oilseed rape, fungicide seed dressings such as Thiram and Rovral are applied to reduce infection of pathogens such as *Alternaria*, light leaf spot, phoma, *Rhizoctonia*, *etc.* There is some anecdotal evidence of phytotoxic effects of some of these chemicals, with slower emergence and establishment of dressed seed compared to undressed seed reported. Stokes (1998) reported slowed and reduced germination in one comparison but no effect in another. Analysis of this phenomenon has not been possible in detail during this study. During this study the main insecticide seed dressing for oilseed rape, lindane, was banned due to concerns over human health. Alternative dressings were tested by ADAS (Green *et al.*, 2002) and the replacement Chinook (imidacloprid + beta-cyfluthrin) was judged not to effect seedling vigour.

5.2. Materials and Methods

5.2.1. Seed Grading

5.2.1.1. Sieving

Samples of a range of selected seed lots were size separated by passing over Endecott's square-mesh sieves of various aperture sizes (2.36, 2.00, 1.70, 1.40 and 1.18 mm). The main purpose of grading was comparison of seed greater than 2 mm in diameter against ungraded or < 2 mm seed. The effects of sieving on thousand seed weight, germination, emergence, *etc.* were assessed as described previously.

In May 2000, samples of the spring rape cultivar Maskot were graded over a 2 mm sieve producing fractions >2 mm and < 2 mm for comparison with ungraded seed. In 2000, bulked seed from six mother crops (low, medium and high plant populations with 100 and 300 kg ha^{-1} N, see Section 4) were graded over 2 mm sieves for field scale analyses by ADAS, to compare with ungraded and gravity separated seed (<2mm seed was retained). In 2001, seed from medium populations receiving 100 kg ha^{-1} N from 2000 and 2001 were sieved to remove seed <1.4 mm, <1.7 mm and < 2 mm for comparison with ungraded seed in ADAS field experiments.

For some controlled environment assessments, individual seeds were divided manually into size grades varying by 0.05 mm using digital callipers to measure the seed diameter.

5.2.1.2. Gravity separation

Initial investigations of gravity separation were made with a Westrup LA-K laboratory scale gravity separator (Figure 5.1) at ADAS Arthur Rickwood in 1999. The table was set up according to the manufacturers instructions. The angle of the separation bed (in two dimensions) and the air flow was varied until, subjectively, there appeared to be some separation. Samples of Apex and Gemini seed to be used for 1999 drilling at ADAS were passed over the machine and the five fractions were kept separate. Specific weights were estimated by recording the volume of fixed weights of seed in a plastic measuring cylinder and thousand seed weights were measured using the standard protocol.

Figure 5.1: Laboratory scale gravity separator

For 2000 field analyses, seed were gravity separated at CPB Twyford, using an equivalent pilot scale gravity separation machine set up to CPB specifications (confidential). Seed from the six selected

mother crops were repeatedly passed over the gravity separator and the 'prime' (PGS) fraction was removed and pooled. The thousand seed weight and density of the PGS and remainder samples was determined and the weights of each sample were recorded. The weights of remainder and PGS seed were recorded.

In 2001, samples of overwinter-stored 2000 mother crops, of contrasting oil and protein content, were sent to CPB for gravity separation on the same pilot scale gravity separator. The samples were separated into five fractions and the protein and oil content was determined by NIR at CPB. The samples were returned to the University of Nottingham for assessment in controlled environment studies.

5.2.1.3. Fluorescence sorting

Samples from SAPPIO 2000 mother crop production (high, medium and low populations receiving 100 and 200 kg ha^{-1} N) were sent for fluorescence sorting on a pilot scale machine at Seed Processing Holland, developed from the work of Jalink *et al.* Germination was tested by Germain's in pleats at 10°C with 35 ml water, using 300 seeds.

5.2.2. Advancement/priming

5.2.2.1. Hydropriming

Preliminary studies at the University of Nottingham (Noon, 1997; Stokes, 1998; Stokes *et al.*, 2000; Basu, 2002) indicated the potential improvements in oilseed rape laboratory germination possible by small-scale hydropriming achieved by soaking at room temperature in Petri dishes followed by drying back in air at room temperature. However, the feasibility of bulk hydropriming was unknown.

In 2000, bulk samples of the six selected mother crops (~7 kg) were prepared for hydropriming. The germination profiles of samples of seed were recorded at 20°C using the standard method to assess priming time (i.e. to allow the process to be stopped before radicle emergence). Radicle emergence did not start until post 16-18 h. Seed lots of about 2 kg were placed in domestic buckets and an equivalent weight of tap water was added; the seed was stirred for one minute to ensure all seed came into contact with water. The buckets were then incubated in a dark controlled environment room overnight for 14 h at 20°C. The following day, seed was sieved and shaken to remove excess water. Due to the lack of a large drying facility, the seed were spread out onto large plastic sheets in a glasshouse for drying. Seed was turned regularly to promote drying.

In 2001, hydropriming was carried out by Germain's UK (methods subject to commercial confidentiality).

2.2.2. Germain's Priming

Samples of spring rape (drilled May 2001), mother crop seeds for September 2000 drilling and a second batch of 2000 harvest seed stored until 2001 were processed by Germain's UK using an in-house experimental priming method, which is subject to commercial confidentiality.

5.3. Results

5.3.1. Seed grading

5.3.1.1. Sieving

Separating seeds into fractions above and below 2 mm in diameter by sieving resulted in significantly greater thousand seed weight in the above 2mm fraction than in the below 2 mm, in all of the seed lots tested. Table 5.1 shows this effect for the mother crops from year 2000 production, prepared for re-sowing in autumn 2000. There were significant ($P<0.001$) effects of mother crop and sieving and a significant mother crop x sieving interaction. Thousand seed weights of > 2 mm seed were always greater than those of < 2 mm seed (with > 2mm thousand seed weight 164% of < 2 mm seed on average).

The difference between TSW of > 2 mm diameter and ungraded seed was small. Although the main effect was significant ($P<0.001$), overall TSW of > 2 mm seed was only 104% of ungraded seed. The significant interaction is explained by the Medium 300 and High 300 mother crops, where the ungraded TSW measurement was greater or equivalent to the > 2 mm seed. This could be due to differences in density in the different seed diameter fractions. In other mother crops, > 2 mm seed did have significantly greater TSW.

Table 5.1: Effects of grading by sieving on thousand seed weight

Mother crop	Thousand seed weight (g)			
	Ungraded	> 2 mm	< 2 mm	Mother crop mean
Low 100	4.97	5.27	3.39	4.54
Low 300	4.58	5.45	3.36	4.46
Med 100	5.30	5.59	3.20	4.70
Med 300	6.14	5.73	3.71	5.19
High 100	5.47	5.82	3.64	4.97
High 300	6.06	6.03	3.41	5.17
Sieving mean	5.42	5.65	3.45	**4.84**[a]
P (Crop, sieving, interaction)	<0.001, <0.001, <0.001			
SED (34 df) (Crop, sieving, interaction)	0.0996, 0.0704, 0.1725			

[a] Grand mean

In an experiment designed to allow study of the effects of seed size on imbibition (see Section 3) seed from two mother crops of contrasting protein content (20.1% and 22.1) were graded to be of 1.18-1.70, 1.70-2.00, 2.00-2.36 and > 2.36 mm diameter. The effect on TSW is shown in Figure 5.2; thousand seed weight more than doubled between nominal diameters of 1.45 and 2.40 mm. However, these two size grades composed a very small fraction of the seed, most of which was in the 1.7-2.0 and 2.0-2.4 size grades, and this explains why removal of seed below a certain diameter may or may not effect TSW significantly, depending on the seed size distribution. TSW still differed significantly between these two size grades, but the average was around 4.8-5.0 g. The TSW of the high protein seed lots appeared slightly higher for equivalent size grades. This can be explained by their greater composition of denser protein compared to the higher level of less dense oil in the low protein seed lot.

Figure 5.2: Effect of seed size grading on thousand seed weight.
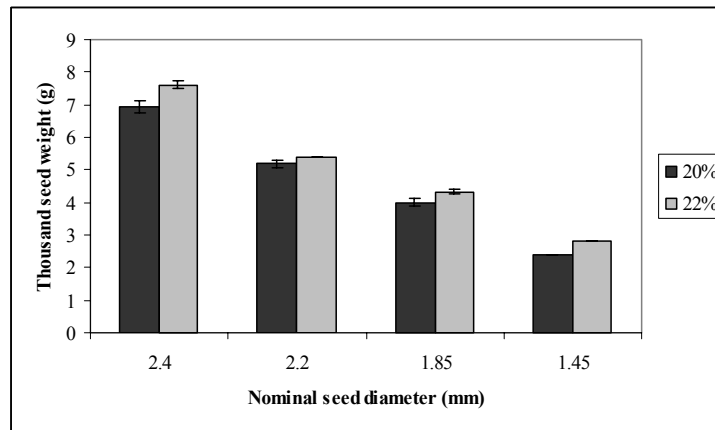
The effects of seed increased seed diameter on emergence from different depths were reported in Section 3, with particular advantages at deeper sowing. Large seed also appeared to have benefits for emergence in both dry and water logged conditions. These advantages were also observed in the field work reported in Section 6 and were generally greater than those due to manipulation of other factors, such as maturity and protein content.

5.3.1.2. Gravity separation

Initial experiments at ADAS Boxworth in 1999 did not produce large differences in thousand seed weight or density after gravity separation. ANOVA showed no significant difference in TSW of samples of Apex and Gemini separated into five different fractions (data not shown).

During processing of samples at CPB Twyford for autumn 2000 field assessments, TSW of 'prime gravity separated' and residue samples were compared. For all the samples processed, the 'PGS' sample TSW was greater than the remainder sample TSW. Insufficient replicate TSW measurements were taken during processing to allow an ANOVA, although a significant regression relationship could be fitted (Figure 5.3). This showed the TSW of the PGS fraction to be about 10% greater than the remainder fraction. This is a smaller difference than comparison of the above and below 2 mm sieved fractions.

Figure 5.3: Relationship between TSW of remainder and prime gravity separated (PGS) seed.
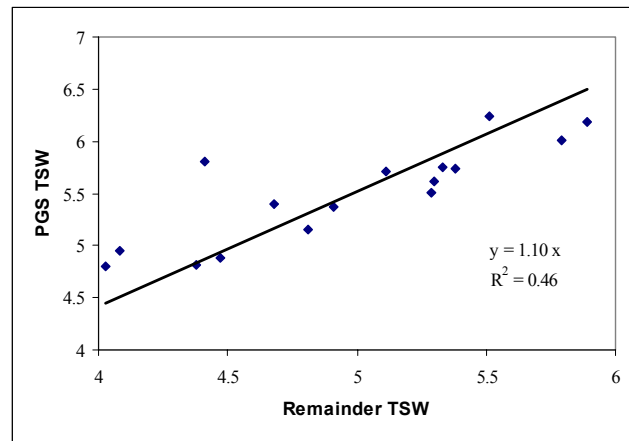
Calculation of the amount of seed retained in the PGS fraction showed an average of 33.7% (range 19.9-77.5%), i.e. separation of a generally smaller amount than that possible by sieving to collect seed > 2 mm diameter. In commercial production, especially of high value seed lots such as hybrids, separation of such small proportions of prime quality seed from the bulk would probably not be commercially feasible. However, gravity separation is a possibility for farm-saved seed where the remainders could be returned to the bulk ware crop destined for crushing.

In an experiment in 2001, over-wintered seed lots from 2000 production that differed in protein/oil content (low, medium and high plant populations that received 40 or 300 kg ha^{-1} N) were gravity separated by CPB as previously and the oil and protein content was determined by NIR. This work showed that protein and oil content could be manipulated by gravity separation; protein content increased from fractions 1 through 6 (Figure 5.4). This was seen in all population and nitrogen combinations, although only data for the medium populations with 40 kg ha^{-1} or 300 kg ha^{-1} is shown.

Figure 5.4: Effects of gravity separation on protein content (2 nitrogen regimes; solid line 40 kg/ha, dashed line 300 kg/ha)
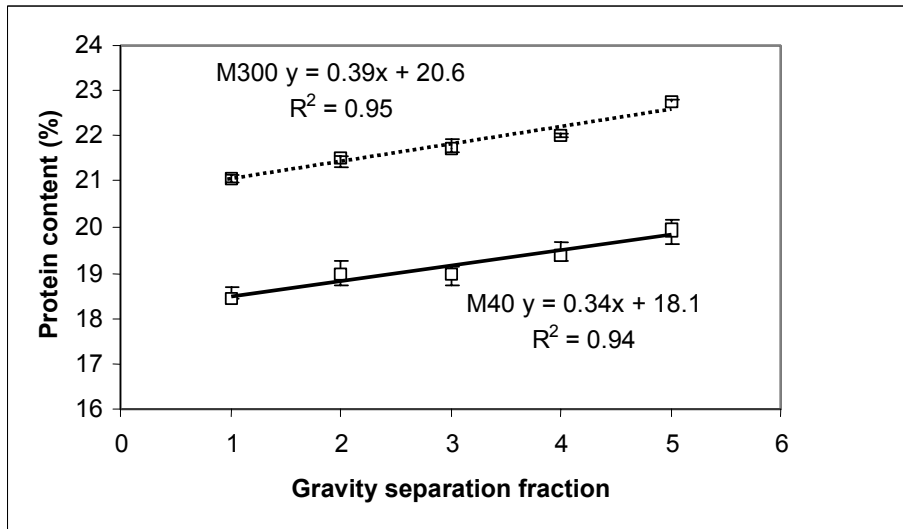
As well as the effect of increased protein content with increased gravity separation fraction number, the reciprocal relationship of oil and protein content caused reduced oil content with increased fraction number (Figure 5.5).

Figure 5.5: Effects of gravity separation on oil content (2 nitrogen regimes; solid line 40 kg/ha, dashed line 300 kg/ha)
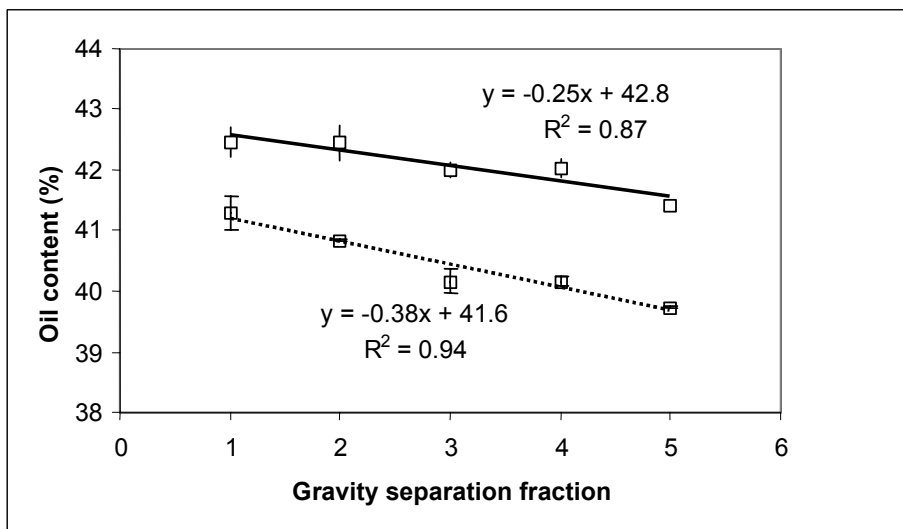
Thus, as well as manipulation of nitrogen fertilisation, gravity separation offers a mechanism for manipulation of protein and oil content. However, the differences due to gravity separation (in the order of 1%) were smaller than those possible by manipulation of nitrogen fertilisation (~2% by increase from 40 to 300 kg ha^{-1}). Also, as was found previously for manipulation of thousand seed weight by gravity separation, the prime fraction of isolated seed is small in comparison to the residue, making the process economically questionable.

5.3.1.3. Fluorescence sorting

Samples over wintered from 2000 harvest were selected for fluorescence sorting on a pilot machine in the Netherlands derived from the work of Jalink *et al* (1998). Samples were submitted by Germain's UK. Fluorescence sorting removed only a very small proportion of seed (Table 5.2), which resulted in no significant improvement in germination.

Table 5.2: Germination of oilseed rape sorted by chlorophyll content

Apex. 100kg N ha^{-1} @ 40 plants m^2

Chlorophyll	*Sample Proportion (%)*	*All germ 50hrs*	*Germn. (%)*	*Abn. (%)*	*G_{50}[1] (hrs)*	*(%)*
Unsorted	-	29.3	89.7	6.7	56.8	-
Low (L)	89.6	13.7	95.7	3.3	58.7	103.5
Medium Low (ML)	3.4	25.0	95.7	3.0	57.3	101.0
Medium (M)	1.8	31.7	93.3	4.3	56.2	98.9
Medium High (MH)	1.7	12.0	89.3	8.3	62.0	109.3
High (H)	1.8	12.2	80.0	12.2	65.8	116.0
Very High (VH)	1.7	4.7	70.3	14.7	78.6	138.4

Apex. 200kg N ha^{-1} @ 40 plants m^2

	Sample Proportion (%)	*All germ 50hrs*	*Germn. (%)*	*Abn. (%)*	*G_{50}[1] (hrs)*	*(%)*
Unsorted	-	12.3	88.7	8.3	61.4	-
Low	71.1	3.0	95.7	2.7	62.2	101.3
Medium Low	9.0	9.3	95.0	4.0	61.2	99.8
Medium	6.2	3.7	90.7	7.3	64.3	104.8
Medium High	5.2	10.7	92.0	5.3	62.3	101.5
High	2.1	5.7	86.3	9.7	65.4	106.7
Very High	6.4	0.0	71.0	14.0	85.2	138.8

[1]Hours to 50% of combined germination and abnormal counts.

88

The results show that only the high and very high chlorophyll content seeds had reduced germination, increased germination time and increased numbers of abnormal seedlings. However, these fractions were a very small component of the seed lot and their removal would not significantly effect overall germination.

Although the technology was proven, with separation of immature seed due to chlorophyll fluorescence, this is a slow process using apparatus currently available making it impractical for oilseed rape. The technology may have application in particular situations where, for example, early harvest or inappropriate swathing data may have caused a high proportion of immature seed, although it is unlikely that a large improvement in general oilseed rape seed technology would be possible by this method.

5.3.2. Priming

5.3.2.2. Hydropriming

(i) 2000, Sutton Bonington

In 2000, bulk hydropriming of about 42 kg of seed was possible, in 18 vessels of about 25 l each, contained in an area of approximately 10 m^2 in a controlled environment room. It should be noted that this is only sufficient seed to drill an area of about 10.5 ha at a relatively low seed rate of 4 kg ha^{-1}. Imbibition proceeded well, and most of the added water was imbibed by the end of 14 h, without any signs of radicle emergence. However, the drying process was more problematical, due to the lack of sufficient oven drying space. To achieve drying, seed was laid out in a thin layer on plastic sheets in a glasshouse, each seed lot taking about 2 m^2. Although the top layer of the thin layer dried, the bottom layer remained damp enough to allow germination to continue, resulting in radicle protrusion. This resulted in about 28.9% sprouted seeds in the final hydroprimed sample. In retrospect, an area of about 4 m^2 would have been preferable, enabling greater spread of seed and thus more uniform drying.

In 2001, some seeds stored over-winter from 2000 were bulk hydroprimed by Germain's UK. With their more extensive drying facilities, drying without radicle protrusion was possible.

5.3.2.3. Germain's priming

The first seed lots to be primed by Germain's were samples of cv. Maskot spring rape. Most work was undertaken on cv Apex. These were assessed for germination using standard ISTA protocols at 10, 15 and 20°C (Figure 5.6). At 20°C (Fig. 5.6 (a)), the standard temperature for the ISTA test, there was no significant difference in the germination profiles. At 15°C, however, germination of the primed seed lots was significantly slowed compared to the untreated seed lots (Figure 5.6 (b)). Although early germination was similar, and final percentage germination (FPG) was not significantly different, between approximately 30 and 60 h, germination was lower in the primed seeds. A similar pattern was seen at 10°C (Fig 5.6 (c)), with a reduced rate of germination. As the test ended at 70 h, there was also significantly greater germination in the untreated compared to the primed seeds. Thus the vigour of Maskot spring rape was reduced, with slower germination at 10-15°C (relevant to field conditions). However, this reduction in vigour was not noticeable at the standard ISTA germination test temperature. In the field 9 (Section 6), reduced emergence was observed from primed seeds.

Figure 5.6: Effects of Germain's priming process on germination at (a) 20°C, (b) 15°C and (c) 10°C

(a)   (b)

(c)
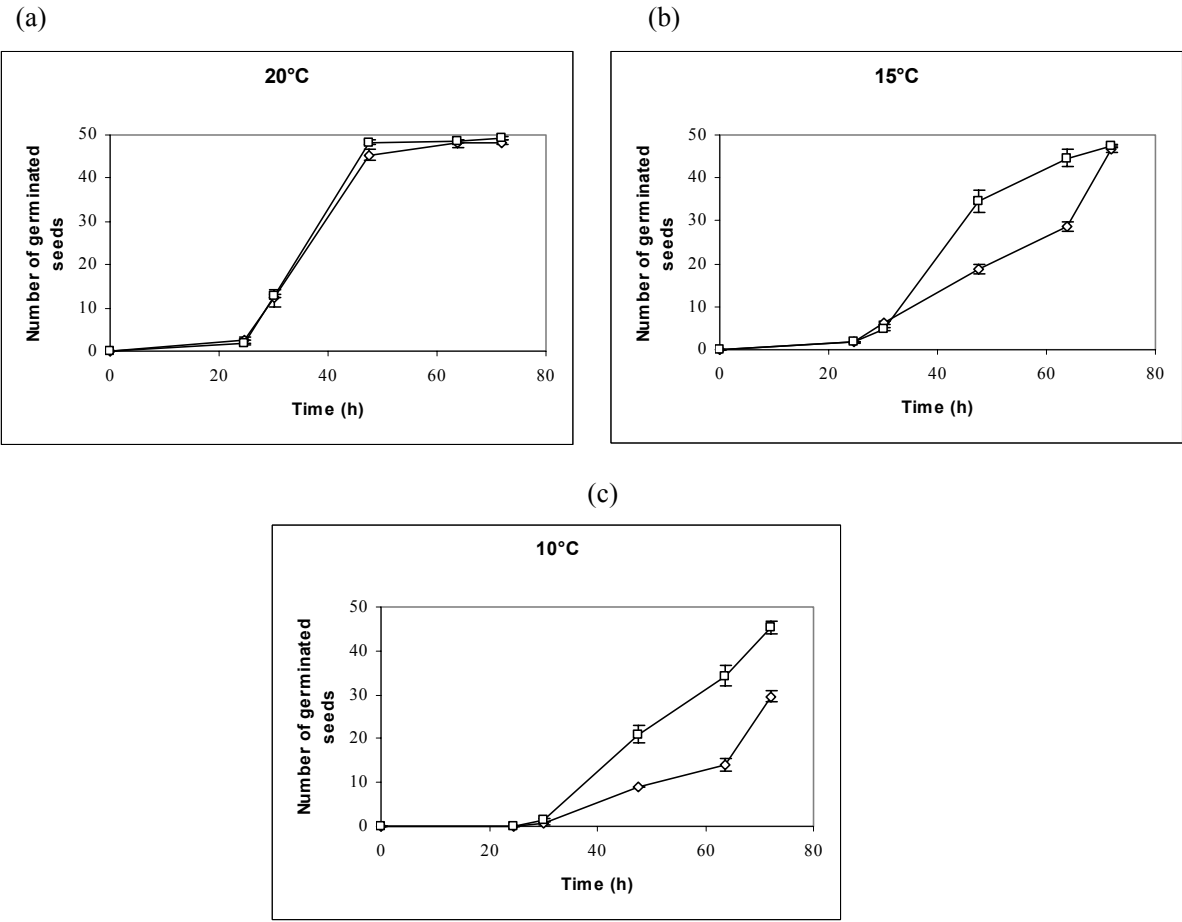
In the second Germain's priming treatment, seeds from the six mother crops for autumn 2000 sowing were primed with their in-house priming method. The details of this process remain confidential to

Germain's, but it involved a controlled moisture advancement over 7 days (with a 7 day pre-assessment period). Germination tests (Germain's data) of the 300 N mother crops (Table 5.3) showed a small degree of advancement in the high and low population seed lots, but not the medium. In the 100N crops, which were not used for field scale evaluations, the effect of advancement appeared to be greater, with more rapid germination of medium and high population crops, but not in the low population crops. The effects on the time to 50% germination (G_{50}) are shown in Table 5.3. In some case the time to G_{50} was about 70% of the unadvanced control, in other cases no difference was found. This compares to improvements in G_{50} to about 60% of control in other species (Halmer, personal communication). In 2001 the assessments were repeated. Although there was no significant advantages over untreated seeds in terms of percentage emergence, Germain's priming (and to some extent hydropriming) did reduce the time taken to achieve 50% emergence and also reduced the number of abnormal seedlings that developed (Table 5.4). This suggests that additional refinement of the Germain's Priming techniques might provide substantial benefits in terms of timeliness to emergence.

Table 5.3: Effects of Germain's priming procedure on germination rate, percentage abnormal seedlings and time to 50% germination (G_{50}) at 10°C (cv. Maskot).

Mother crop	Treatment	Early Count 53.5 hrs	Germination (%)	Abnormals. (%)	G_{50} (Hrs)	(%)
100N Low	Control	2.7	93.3	4.0	70.2	-
	Primed	8.0	94.7	2.3	65.9	93.9
100N Medium	Control	14.3	92.7	4.0	66.7	
	Primed	50.7	97.0	2.0	52.9	79.3
100N High	Control	0.0	97.0	2.0	94.1	
	Primed	11.0	94.0	3.3	67.2	71.4
300N Low	Control	7.0	94.7	3.0	66.8	
	Primed	55.0	97.0	1.0	52.4	78.4
300N Medium	Control	6.7	96.7	2.0	66.4	
	Primed	15.0	95.0	3.0	67.6	101.8
300N High	Control	15.3	91.7	4.7	62.6	
	Primed	41.0	93.7	4.3	56.8	90.7

Table 5.4: Effects of Germain's priming and hydropriming procedure on germination rate, percentage abnormal seedlings and time to 50% germination (G_{50}) at 10°C (cv. Apex).

	Germination	Abnormals	G_{50}	
	(%)	(%)	(hrs)	(% of unprimed)
100kg N ha^{-1}, 10 plants m^2				
Unprimed	94.3	4.0	63.2	-
Germain's priming	93.3	1.0	52.6	83.2
Hydroprimed	94.3	2.7	59.5	94.2
100kg N ha^{-1}, 80 plants				
Unprimed	87.3	7.7	73.1	-
Germain's priming	95.0	2.3	57.3	78.4
Hydroprimed	92.3	4.7	65.7	89.8
200kg N ha^{-1}, 10 plants m^2				
Unprimed	92.7	4.7	72.2	-
Germain's priming	96.7	1.3	42.8	59.3
Hydroprimed	93.0	4.3	62.1	86.0
200kg N ha^{-1}, 80 plants m^2				
Unprimed	89.0	7.3	70.7	-
Germain's priming	93.3	4.0	38.4	54.3
Hydroprimed	89.3	7.0	60.1	85.0

Assessments of emergence in sand (Lunn and Bullard, 2001) showed no significant advantage to priming over control treatment, or reductions in emergence (Figure 5.7). Although there were some concerns about validity of the sand emergence experiments (some replicates experience transient waterlogging), these results were also borne out in the field experiments (Section 6) where both primed and hydroprimed seedlots gave lower final percentage emergence than untreated seeds.

Figure 5.7. Emergence from sand of 2000 mother crop post-harvest treated seed.
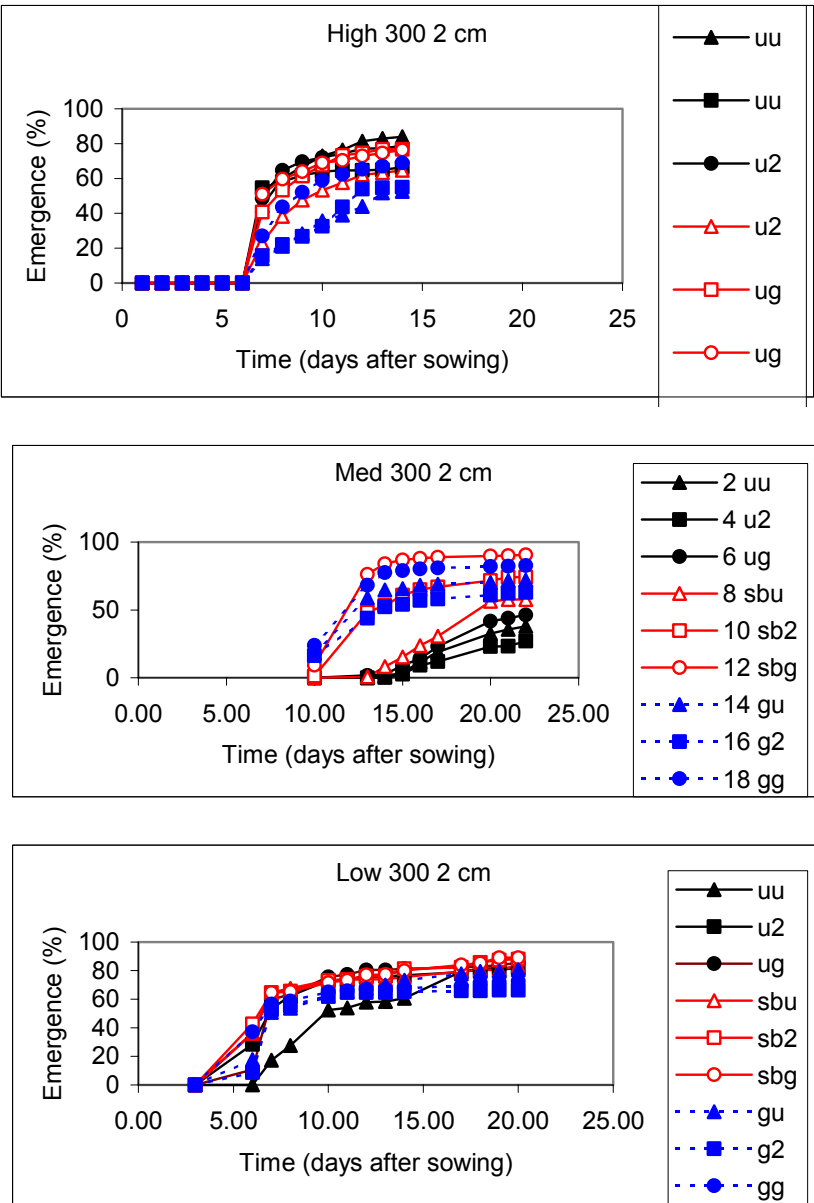


key: uu = unprimed, ungraded; u2 = unprimed, <2mm seed; ug= ungraded, > 2mm seed; sbu = hydroprimed, ungraded; sb2 = hydroprimed, <2mm seed; sbg= hydroprimed, > 2mm seed; gu = Germain's primed, ungraded; g2 = Germain's primed, <2mm seed; gg= Germain's primed, > 2mm seed.

In this first experiment there were complicated interactions between seed lot, priming and grading. In the Low 300 seed lot, the ungraded untreated seedlot was significantly slower to emerge than the other seedlots. At the final percentage emergence there was no significant difference between the hydroprimed (water advanced) and unprimed seedlots and the Germain's primed > 2 mm seedlot. However, there was evidence of lower emergence from the ungraded and gravity separated primed seedlot.

In the Medium 300, hydroprimed and Germain's primed seedlots gave faster and greater final emergence than the unprimed seedlots, although on average hydropriming was as effective as priming. In the High 300 seed lot, both hydropriming and Germain's priming gave a reduced rate and final percentage emergence compared to unprimed seeds. Thus in these experiments, priming treatments only gave an advantage in around 33% of cases, with the interaction with seedlot making a application of a single guaranteed method unlikely.

The 2000 sand emergence tests may have been compromised due to waterlogging so the tests were conducted for the hydroprimed and Germain's primed seedlots produced in 2001 (Table 5.4).. Four seed lots, from low populations (10 plants m^{-2}) having received 100 or 200 kg ha^{-1} N (i.e. with higher protein content in the 200 kg ha^{-1} N treatment) and from high populations (80 plants m^{-2}) receiving 100 or 200 kg ha^{-1} N were studied (Figure 5.9).

Figure 5.8: Sand emergence of 2001 harvested Apex seed using Germain's Priming, Hydropriming or control.
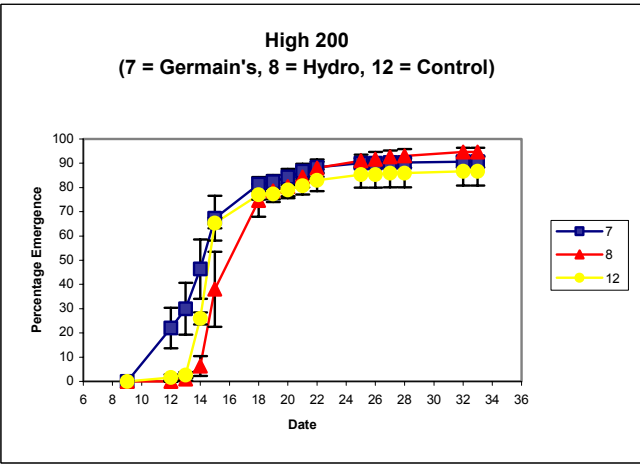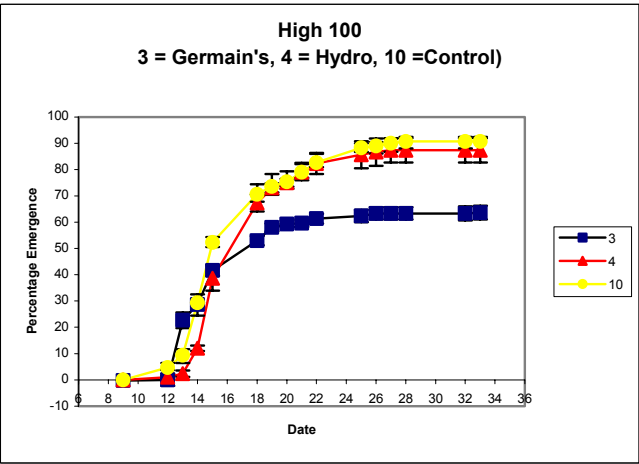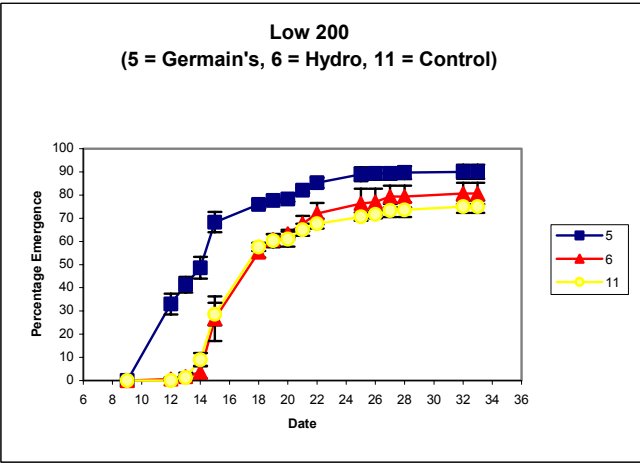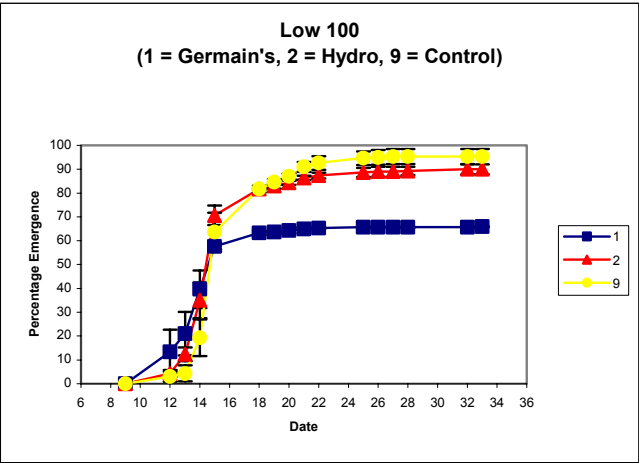
The results were broadly similar to those found previously, showing an interaction between seedlot and priming, which would probably prevent introduction of priming as a useful technology for rapeseed. However, the hydropriming and Germain's priming techniques used in this study were experimental and further refinement might lead to additional advances in rate of emergence and percentage seedling survival. There appeared to be no significant difference in emergence between mother crops, and no significant improvement in emergence due to hydropriming, which slowed emergence slightly in the early stages, but which led to equivalent final percentage emergence. In the 100 kg ha^{-1} N application (low protein) seed lots, from both the low and high populations, there was a significant reduction in final percentage emergence due to priming. In the 200 kg ha^{-1} N application seed lots, priming led to a significant increase in rate and final percentage emergence in the low plant population. In the high population seedlot, whilst the rate of emergence in the early stages was increased, there was no significant increase in the final percentage emergence compared to untreated or hydroprimed seedlots. Thus priming reduced percentage emergence in 50% of cases, had no significant effect in 25% and improved emergence in 25% of cases. Hydropriming did not lead to significant improvement in emergence in any case.

5.4. Conclusion.

The results from these studies show that there are relatively few realistic opportunities for enhancing seed quality by post-harvest selection and treatment. Although some tests indicated a reduction in time to radicle emergence in Germain's primed seed, there generally appeared to be no advantage to priming (hydropriming or Germain's priming) seed from the sand emergence and field studies undertaken. Possibly the maximum benefit would be seen in more stressful conditions than examined in this study. The more rapid radicle emergence from some of the Germain's priming treatments suggests that further attempts to refine the process are warranted. However, most information gathered in this study suggests that for seed of relatively high quality, where germination and vigour is relatively good, these processes resulted in no significant improvement in germination and emergence, and most importantly even acted to impede establishment. One must stress that these priming treatments were experimental and might be further refined. Other work has reported greater improvements in low vigour seed due to priming.

Gravity separation increased thousand seed weight slightly and could be used to manipulate protein and oil content. However, the changes achieved by this method were small compared to those possible by mother crop manipulation (Section 4) and the optimum quality seed fraction was only a small proportion of the whole, making the process uneconomic. Similarly, the fluorescence sorter

96

only removed a small proportion of seeds which did not result in a significant improvement in rapeseed quality.

Overall, the greatest improvements in seed quality were achieved by the simplest, cheapest, easiest and most reproducible method, sieving. In both controlled environment and field assessments, selecting seed > 2 mm in diameter generally gave the greatest final percentage emergence (although emergence could be slower than from smaller seed). However, there were indications from other experiments that the very largest seeds could have reduced emergence capacity, so selection of a different proportion of seed (say 1.7-2.2 mm) could give an even greater benefit. This requires more research.

6 FIELD SCALE EVALUATIONS OF MOTHER CROP MANAGEMENT, SEED SELECTION AND SEED TREATMENT

6.1. Introduction

As described previously, there are no general guidelines for specific management of commercial seed crops (Kelly and George, 1998), or areas of crop to be used for home-saved seed, as opposed to ware crops destined for crushing for oil. Optimising seed quality consequently often receives limited attention from producers. Husbandry recommendations to produce optimal seed quality (e.g. plant population, nitrogen fertilisation) may be different to those for maximum yield and oil production (see Chapter 4).

Consequently, the main objective of the work described in this Chapter was to test some of the seed quality characteristics and mother crop manipulation, seed selection and seed treatment procedures identified in the preceding work on a field scale, to identify if any of the theoretical quality factors and processing strategies had practical applications.

Literature review and preliminary research had already identified a number of characteristics (Stokes *et al.*, 1998, 2000) potentially effecting seed quality, including seed size, protein and oil content, seed age (harvest date), canopy structure, position of the seed within the canopy and mother crop 'provenance' (due to site/season and weather). The effects of mother crop management and seed processing/treatment on many of these factors are described in Chapters 4 and 5. Of these factors, only seed size appears to have been evaluated in the field to any extent in *Brassica napus*, although many of the other factors have been evaluated in other species, for example seed priming in sugar beet (Durrant and Mash, 1990, Durrant *et al.*, 1993) which is used commercially. Major (1977), found greater emergence from large seeds but did not find an effect on final yield. Mendham *et al.* (1981) did not study emergence but found effects on crop size and suggested that large seed would be beneficial in late sowings. The other characteristics identified by Stokes *et al.* (1998, 2000) had only received preliminary study in controlled environments.

It was decided that the majority of the field assessments should be carried out on fully characterised mother crops grown at Sutton Bonington in 1999/2000 and 2000/2001, at various ADAS field sites in 2000/2001 and 2001/2002. However, in order to evaluate some of the characteristics for study in ADAS field experiments, preliminary studies were conducted at the University of Nottingham in 1999/2000, during the period of production of mother crops for subsequent field studies.

In June 1999, an experiment was conducted to study field emergence of farm-saved Apex seed collected from 1997 'Canopy Management' experiments (Lunn *et al.*, 2001). The effects of harvest date, seed protein/oil balance and position of the seed in the canopy (top or bottom half) were studied. In September 1999, the effects of seed size selection and seed protein/oil balance on emergence and establishment were studied in contrasting seed beds, again using farm-saved Apex seed from 1997 canopy management experiments. In May 2000, the effects of seed size, Germain's priming, hydropriming and heat treatment were analysed in the spring rape cultivar Maskot.

6.2. Materials and Methods

6.2.1. 1999/2000

6.2.1.1. Effects of harvest date, pod canopy position and nitrogen content on emergence and establishment

Seeds of the cultivar Apex were collected from 1997 'Canopy Management' experiments (Lunn *et al.*, 2001) at various dates in July and August and were stored in sealed plastic containers at ambient temperature (c. 18-25°C). Seed protein and oil contents of the seed were determined by near infra-red reflectance (NIR) spectrophotometry by ADAS Wolverhampton laboratories. On 23 June 1999, undressed seed collected on 21st July or 1st August 1997 from the upper or lower half of the pod canopy of plots receiving zero N, 100 or 300 kg ha^{-1} N at the normal timing and 80 or 160 kg ha^{-1} N later than usual at flowering were drilled in a 2*2*5 factorial design with four replicate blocks. Plots of 1.6 x 10 m were sown at 200 seeds m^{-2} with an Oyjard drill into Dunnington Heath series soil. Three 0.72 m^2 quadrats were randomly placed in each plot for emergence assessment and growth analysis sampling. The number of emerged seedlings in each plot was assessed on 13/7/99, 20 days after sowing. A growth analysis was completed according to the standard protocol (see Appendix II) 23 DAS on 16/7/99 (21st July harvest date, both canopy positions and zero, 300 or 160 kg ha^{-1} N only). The experiment was terminated on 4/8/99 due to the start of cultivations for the next field season.

6.2.2. Effects of seed nitrogen content, seed size and seed bed structure on emergence and establishment

The initial experiment showed no significant effects of maturity or pod position on emergence so subsequently seed harvested from the top half of the canopy on 21st July 1997 was used. Three

nitrogen treatments expected to have caused differences in seed nitrogen content (zero N, 300 kg ha^{-1} N at normal timing and 160 kg ha^{-1} N at flowering, with protein contents of 15.6, 25.1 and 25.8 % respectively) were sieved over a 2 mm mesh Endecott's sieve to produce two size grades of seed, > 2 mm diameter and < 2 mm diameter. These were sown into two different seed beds: the 'good' seed bed had been prepared for a trial that was not drilled, had been fallow for a year, and had a relatively fine tilth with little straw. The adjacent 'bad' seed bed followed a crop of barley, with the stubble ploughed in and was more cobbly with larger aggregates and much straw. The design was split plot (with seed bed quality as the main plot), with a 2*3 factorial of seed size x nitrogen content as the randomised sub-plots and four replicate blocks. Undressed seed was drilled with an Oyjard drill (1.6 x 10 m) at 200 seeds m^{-2} at 2 cm depth on 8th September 1999. Four 0.72 m^2 quadrats were placed randomly in each plot for emergence studies and destructive growth analyses. Before drilling, seed germination was assessed with standard germination test according to ISTA protocols (see Chapter 3) at 10, 15, 20 and 25°C. Shortly after drilling, five samples of approximately 2.5 l soil were taken from random positions within each seed bed to assess if true differences in aggregate size distribution existed. The samples were left to dry naturally in the laboratory at ambient temperature for one month. Stones and straw were removed from the sample and weighed and the soil was then sieved over square mesh sieves of aperture size 47, 23, 17, 11 and 4 mm. The proportion of each fraction was calculated by weight. Soil moisture analysis was also carried out to identify periods when water may have been limiting. Samples of approximately 250 ml were taken from the top 5 cm of soil where seed had been drilled and the fresh weight (FW) of the soil was determined. The samples were then dried for 48 h at 85°C and the dry weight (DW) was determined. Moisture was calculated as (FW-DW)*100/FW. Emergence assessments were made by marking with coloured cocktail sticks as described previously, with the first assessment 9 DAS and maximum emergence reached at 17 DAS. A growth analysis was completed in December 1999 as described previously and plant losses were determined.

6.2.3. Effects of seed size and treatment on emergence and establishment of spring rape cv Maskot

An experiment was carried out at the University of Nottingham in Spring 2000 to assess seed size and advancement effects on spring oilseed rape. Undressed Maskot spring rape seed was passed over an Endecott's 2 mm aperture sieve to give large (> 2mm), small (< 2 mm) and ungraded seed lots. These samples were divided into four sub-samples for seed treatment. Treatment one was a priming process similar to commercial techniques and carried out by Germain's UK (exact details subject to commercial confidentiality). The second treatment was hydropriming (Noon, 1997); seeds were imbibed on Whatman No. 1 filter papers dampened with distilled water for 18 h at room temperature (c. 18-25°C) and were subsequently removed from the filter papers and dried at room temperature.

Treatment three was an ultra-drying treatment, with dry seeds incubated at 85°C for 24 h in an oven in paper bags. The final subsample was the untreated control.

The seed lots were drilled on 8/5/00 in a 3*4 factorial design with four replicate blocks and 1.6 x 10 m plots in Field 31, University of Nottingham Farm (Fladbury series soil) with an Oyjard drill at a rate of 200 seeds m^{-2}. Four 0.72 m^2 quadrats were randomly placed in each plot and emergence was monitored on different days by marking seedlings with differently coloured cocktail sticks. For growth analysis, all above-ground material was collected from a quadrat and returned to the laboratory in plastic bags where the sample was stored at 4° C. Plant numbers and total fresh mass was determined. Where appropriate, the sample was divided into stems, leaves, buds and flowers and the fresh and dry mass of each organ fraction was determined. Green areas of leaves and stems were determined as described previously and green area indices determined. Germination was assessed according to standard protocols and controlled environment emergence studies were also undertaken.

6.3. 2000/2001: Effects of seed nitrogen content (mother crop), grading (size selection and gravity separation) and seed treatment (Germain's priming and hydropriming) on emergence and establishment

6.3.1. Seed preparation

Seed produced at the University of Nottingham in 1999/2000 from replicate plots of mother crops of 60, 40 and 15 plants m^{-2} (high, medium and low), receiving 100 or 300 kg ha^{-1} N was pooled to give six resource mother crops, and was cleaned with a laboratory seed cleaning machine. The seed was then divided into three equal parts of 7.5-8.5 kg each. The first part was the untreated control, the second part was water advanced by (hydroprimed) at Sutton Bonington (see Chapter 5 on seed treatment) and the third part was sent for Germain's priming by their confidential methods. After advancement, each seed lot was again sub-divided into three parts. One part was ungraded, the second part was sieved manually over a 2 mm Endecott's sieve (the seed > 2 mm was retained) and the third part was gravity separated at CPB Twyford (see Appendix III), with the 'prime' gravity separated sample retained. Thus a matrix of 54 samples was available for field studies. Due to limitations of experimental size and design, it was decided to compare plant population at the 300 kg ha^{-1} N treatment rather than to compare N treatments, since there was high residual SMN at the mother crop production site and relatively little variation in seed protein content due to nitrogen fertilisation (see Chapter 4). The seeds for field studies were dressed with an experimental seed dressing containing 100 g l^{-1} imidacloprid and 100 g l^{-1} beta-cyfluthrin (later approved and marketed as Chinook) against flea beetles and also the fungicidal seed dressings Rovral and Thiram.

6.3.2. Field experimentation

Seed of Apex, produced from University of Nottingham crops in 2000 were drilled in Factorial combinations of 3 mother crops * 3 advancement techniques * 3 seed selection techniques were drilled in 3 replicate blocks at ADAS Boxworth. Paired plots were drilled in order to allow differential drilling dates, aimed at altering seed bed conditions, giving 162 plots in total. Some additional plots were drilled with semi-dwarf seed supplied by CPB at the boundary of the experiment in order to quantify volunteer rape. Each plot measured 24m x 2.05m (Oyjord drill width). 12m of each plot was retained for final harvest with a Sampo combine. The experiment included two drilling dates.

Cultivation treatments prior to drilling were kept to a minimum as the objective was to produce a cloddy seedbed with poorly incorporated straw. The land was disced and then power-harrowed once immediately prior to drilling. The 8 treatments were drilled at 200 seeds per m^2, with 4 replicate plots per treatment. There was no further cultivation of the late-drilled plots prior to drilling.

Mother crop treatments were based on N fertiliser application rates to the mother crops;
Seed selection treatments were; no selection, graded by sieving to select > 2mm seed; gravity selection to select heaviest seed;
Priming technique; no priming, hydropriming, Germain's priming technique

6.3.3   Assessments

Immediately after drilling, a visual assessment and description was made of seed bed conditions. Samples were taken from the discard area of each replicate, two 50 x 20 x 5cm deep quantities of soil were carefully removed and placed into a tray. The next 5cm profile was removed in the same manner. The samples were dried at ambient temperature for a period of at least 2 weeks, until dry, and graded through round hole sieves of the following aperture sizes; 53.0mm, 26.5mm, 9.5mm, 4.75mm and 2.00mm. The fractions on each sieve and in the receiver were weighed and each expressed as a percentage of the total weight.

Assessment of gravimetric seed bed moisture was done immediately after sowing and approximately every four days, or on any day following rainfall. Six soil-samples were taken from the top 20 cm of soil, in 5cm strata, from the discard areas of each block.

Seedling emergence was monitored in a 1 m^2 fixed quadrat placed in the 12m destructive harvest zone of each subplot. The number of emerged seedlings in each fixed quadrat was recorded every

day until maximum emergence (*i.e.* no further increase in emergence for four consecutive days). Emerged seedlings were removed from the quadrat area. After maximum emergence plant number was assessed at weekly intervals until November. Crop growth stage was recorded weekly from emergence until the end of seedling emergence for each plot.

6.3.4   Harvest

Although it was intended to differentially harvest the plots when each drilling date was ripe harvesting the early drilled plots was delayed by desiccation of the surrounding crop and wet weather. All plots were therefore harvested on 24 July when both early and late drilled plots were ripe. There were no apparent signs of seed shedding on plots of either drilling date. Yields were expressed as 91% dry matter.

6.3.5   Statistical analysis

All data were subjected to analysis of variance. Treatment means were separated using SEDs where the variance ratio was significant (p $\leq$0.05).

6.4 2001/2002: Effects of size grading and overwinter storage on emergence and establishment at multiple sites

In early 2001, the SAPPIO Project Management Committee decided that the emphasis on field experimentation should be reduced in favour of more fundamental studies. Consequently, factors such as mother crop provenance, seed nitrogen content, seed advancement and gravity separation, which had shown little or no advantage in the previous field studies, were removed from the analysis. The subsequent field studies concentrated on continued assessment of seed size grading, the characteristic which caused the greatest differences in fundamental laboratory and controlled environment studies and the only factor to have shown consistent advantages in the field.

6.4.1. Seed preparation

Mother crops grown at a medium plant population and receiving 100 kg ha^{-1} N grown in 1999/2000 and 2000/2001 were compared to allow assessment of effects of overwinter storage. Samples were cleaned with a laboratory seed cleaning machine and replicate plots were pooled. Samples were manually sieved with Endecott's sieves to remove seeds <1.4 mm, < 1.7 mm and < 2.0 mm with one

sample was left ungraded. As with the previous trial, seeds were dressed with the experimental Chinook seed dressing by Bayer to protect against flea beetle damage and with Rovral and Thiram to protect against fungal disease.

6.4.2. Field experimentation

In order to test the hypothesis that increased seed size would impart a consistently regardless of soil and time of sowing an experiment was established at three ADAS sites; High Mowthorpe, Terrington and Boxworth.

The experiment design was 3-way full factorial, with four replicate blocks. Additional plots of a semi-dwarf variety were drilled around the surrounding area in order to quantify any volunteer rape growing in the experiment. Each plot measured 24m x 2.05m (Oyjord drill width). 12m of each plot was retained for final harvest with a Sampo combine. The experiment included two drilling dates.

Two seed batches cv. Apex, one from harvest 2000 and the other from harvest 2001 grown at Sutton Bonington were sieved and divided into four sub-batches, on the basis of size; ungraded, > 1.4mm, > 1.7mm and > 2.0mm.

Cultivation treatments prior to drilling were kept to a minimum as the objective was to produce a cloddy seedbed with poorly incorporated straw. The land was disced and then power-harrowed once immediately prior to drilling. The 8 treatments were drilled at 200 seeds per m^2, with 4 replicate plots per treatment. There was no further cultivation of the late-drilled plots prior to drilling.

6.4.3   Drilling dates

At Terrington drilling took place on 7 September and also 28 September. Additional plots of semi-dwarf cv. Lutin were drilled on 7 September at approximately 130 seeds per m^2. At High Mowthorpe drilling took place on 6 September and also 19 September. At Boxworth drilling took place on 8 September and also 28 September.

6.4.5   Pest and disease control

At Draza pellets at 5.5 kg/ha were mixed with the seed for slug control.
All other crop treatments were as per farm practice.

Treatments

Treatment	Drilling date	Seed year	Seed size
T1	Early	2000	Ungraded
T2	Early		>1.4mm
T3	Early		>1.7mm
T4	Early		>2.0mm
T5	Early	2001	Ungraded
T6	Early		>1.4mm
T7	Early		>1.7mm
T8	Early		>2.0mm
T9	Late	2000	Ungraded
T10	Late		>1.4mm
T11	Late		>1.7mm
T12	Late		>2.0mm
T13	Late	2001	Ungraded
T14	Late		>1.4mm
T15	Late		>1.7mm
T16	Late		>2.0mm

6.4.6   Assessments

Immediately after drilling, a visual assessment and description was made of seed bed conditions. Samples were taken from the discard area of each replicate, two 50 x 20 x 5cm deep quantities of soil were carefully removed and placed into a tray. The next 5cm profile was removed in the same manner. The samples were dried at ambient temperature for a period of at least 2 weeks, until dry, and graded through round hole sieves of the following aperture sizes; 53.0mm, 26.5mm, 9.5mm, 4.75mm and 2.00mm.   The fractions on each sieve and in the receiver were weighed and each expressed as a percentage of the total weight.

Assessment of gravimetric seed bed moisture was done immediately after sowing and approximately every four days, or on any day following rainfall. Six soil samples were taken from the top 20 cm of soil, in 5cm strata, from the discard areas of each block.

Seedling emergence was monitored in a 1 m^2 fixed quadrat placed in the 12m destructive harvest zone of each subplot. The number of emerged seedlings in each fixed quadrat was recorded every day until maximum emergence (*i.e.* no further increase in emergence for four consecutive days). Emerged seedlings were removed from the quadrat area. After maximum emergence plant number

was assessed at weekly intervals until November. Crop growth stage was recorded weekly from emergence until the end of seedling emergence for each plot.

6.4.7 Harvest

Crops were not taken to commercial harvest.

6.4.8 Statistical analysis

All data were subjected to analysis of variance. Treatment means were separated using SEDs where the variance ratio was significant (p ≤0.05). NS = Not significant.

6.5. Results

6.5.1. 1999/2000

6.5.1.2. Effects of seed maturity, pod canopy position and nitrogen content on emergence and establishment (University of Nottingham)

The hypotheses under test in this experiment were that more mature seed would have better emergence, since previously impaired germination of immature orange/red/brown coated seed had been observed (Stokes *et al.*, 1998, 2000). Additionally, more rapid emergence was expected from high protein content seed (from high levels of nitrogen fertilisation), as high seed protein content had been observed to increase rate of emergence in controlled environment emergence tests in horticultural silver sand (Stokes *et al.*, 2000) and these seed were hypothesised to imbibe water and germinate more rapidly. However, it was also postulated that final percentage emergence could be reduced in high nitrogen seed, since there is a strong reciprocal relationship between nitrogen and oil content (Stokes *et al.*, 2000), the seed's main energy reserve. Thus seed with high protein content may be expected to have less energy to devote to emergence. Differences could also expected due to canopy position. Literature in other species shows an effect of seed position on dormancy (e.g. *Aegilops* and celery) and seed from the top half of the canopy had been shown to have greater thousand seed weight and greater oil content than seed from the bottom half of the canopy (Stokes *et al.*, 2000) and so was also expected to show better emergence and establishment, unless canopy position effected the propensity to enter secondary dormancy.

The first assessment of emergence on 13/7/99 by analysis of variance showed no significant differences (*P*>0.67, data not shown) in emergence due to harvest date, canopy position or seed protein content, and no significant interactions (*P*>0.18). Grand mean emergence at 20 DAS was

43%, close to the maximum achieved (grand mean 49%). The lack of an effect due to seed maturity may have been due to the relatively close timings of the samples (11 days) and relatively late stage during crop development when most of the seeds were black and mature, so there was no significant difference in immature seed numbers between the July and August harvest dates. Consequently, all subsequent analyses on the 21st July harvest date only. ANOVA also did not show any significant differences due to pod position or nitrogen treatment. This may have been because seed bed conditions were relatively unstressed (fine tilth, high moisture content, high temperatures) and drilling was relatively shallow, so size and composition differences due to pod position showed little effect. However, the data did not support the hypothesis that pod position could effect propensity to enter secondary dormancy. The main effects of pod position were on seed size and protein/oil balance (Stokes *et al.*, 2000). As these factors could be manipulated more reliably by other means (sieving, N fertilisation), study of pod position was discontinued in field trials. Insufficient data points during emergence were taken to assess effects of seed protein content on rate of emergence.

The destructive growth analysis data taken on 16/7/99 (only plots of zero N, 300 kg ha^{-1} N and 160 kg ha^{-1} N from top and bottom of canopy harvested on 21st July 1997 analysed) also showed no significant differences in emergence by ANOVA (P>0.1). Regression analysis, however, showed some trends in the data. Percentage emergence was negatively correlated with protein content (Figure 6.1), although the differences were not large. This supports the hypothesis that oil reserves may be more important than protein reserves for final emergence *per se*, although other work has shown increased protein content may increase the rate of emergence. However, there were quite strong correlations between protein content and leaf dry weight per m^2 and dry weight per plant (Figure 6.2), indicating that protein reserves could be more important for post-emergence competitiveness, producing fewer but larger plants. There was also a weaker positive association between seed protein content and green area index. As would be expected from the negative correlation between seed protein and oil content, greater establishment was positively correlated with high oil content. As with protein content, there was no significant relationship to overall crop dry weight m^{-2} or GAI, but there were negative correlations between leaf dry weight m^{-2} and dry weight per plant. Thus, high protein seed produced fewer but bigger seedlings whereas high oil seed produced more but smaller seedlings. However, since frequent emergence counts could not be taken due to the labour-intensive nature of the procedure, assessment of the effects of protein content on the rate of emergence could not be made.

Figure 6.1: Effect of protein content on percentage emergence on 16/7/99
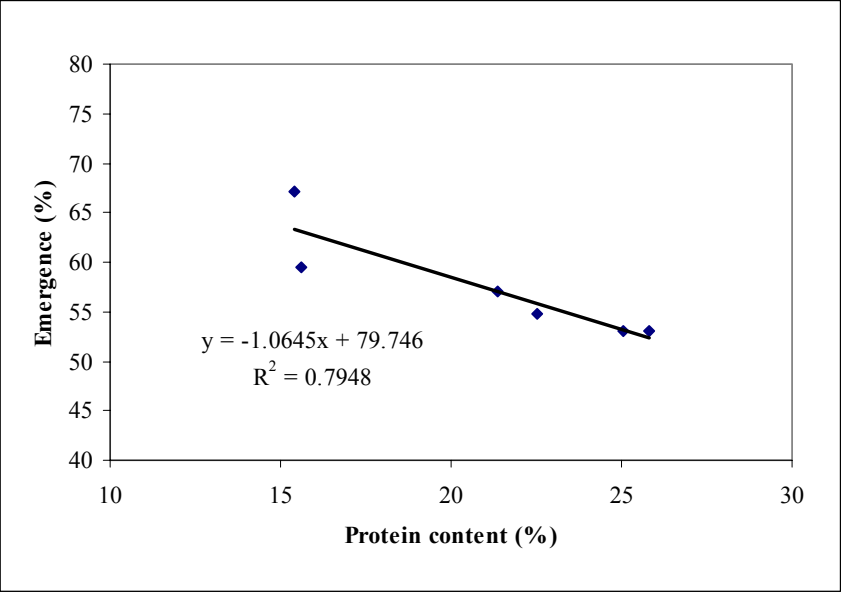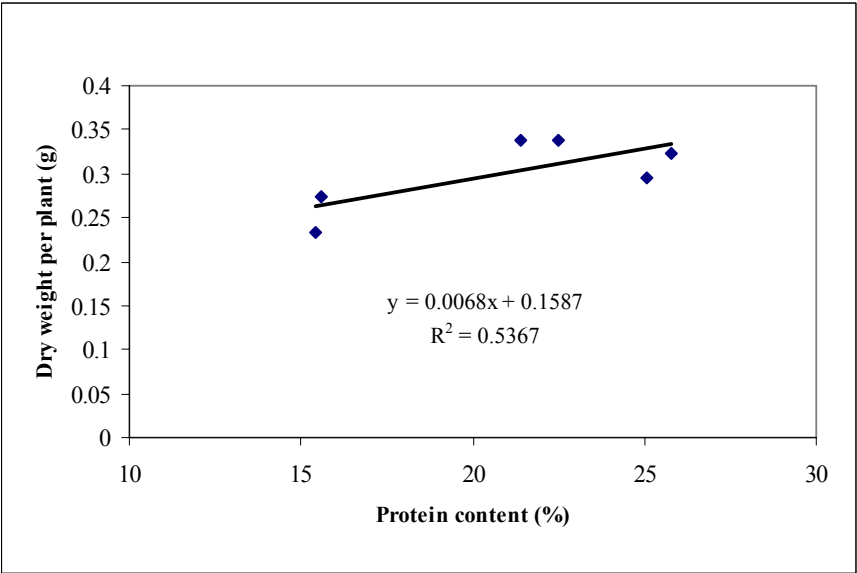

$$y = -1.0645x + 79.746$$
$$R^2 = 0.7948$$

Figure 6.2: Effect of seed protein content on individual plant dry weight on 16/7/99


$$y = 0.0068x + 0.1587$$
$$R^2 = 0.5367$$

6.5.3. Effects of seed bed, seed size and protein content

As no significant effects of harvest date and pod canopy position on field emergence were found in the preceding experiment, the autumn experiment focussed on the effects of seed size grading and protein content in contrasting seed beds. Subsequent analyses of these factors were discontinued since it was thought that they provided little scope for practical seed quality manipulation.

Analysis of variance of peak emergence 17 days after sowing on 25th September 1999 showed no significant differences in maximum emergence due to seed bed, seed size or seed protein content. This was presumably because seed bed conditions were good (even in the presumed 'bad' seed bed) and weather conditions were favourable, so differences in establishment vigour were not expressed, as maximum emergence was high (grand mean 76%, compared to 49% in the June sowing).

However, the emergence profiles did show some indications of differences. Figure 6.3 shows that, surprisingly, emergence was more rapid from the presumed 'bad' seed bed than the presumed 'good' seed bed, although final emergence was similar. This may have been due to an initially higher moisture content in the bad seed bed (data not shown).

Figure 6.3: Percentage emergence with time from bad (closed diamonds) and good (closed squares) seed beds
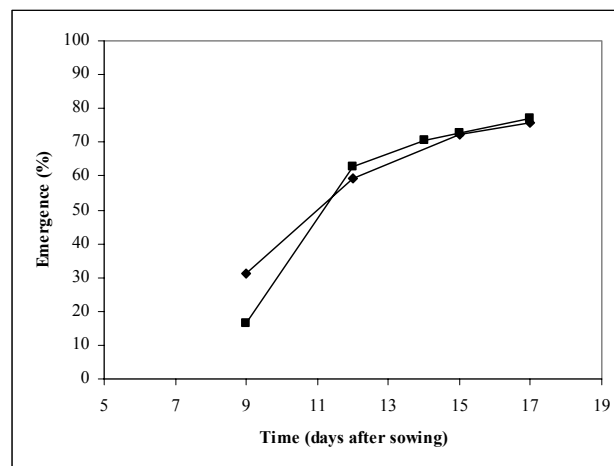

The effect of seed size is shown in Figure 6.4. No differences in rate of emergence could be detected, although the > 2 mm seed did give numerically greater emergence by 17 DAS than < 2 mm seed. However, emergence was quite similar, again presumably due to the relatively good conditions.

Figure 6.4. Percentage emergence with time from small (closed diamonds) and large (closed squares) seeds
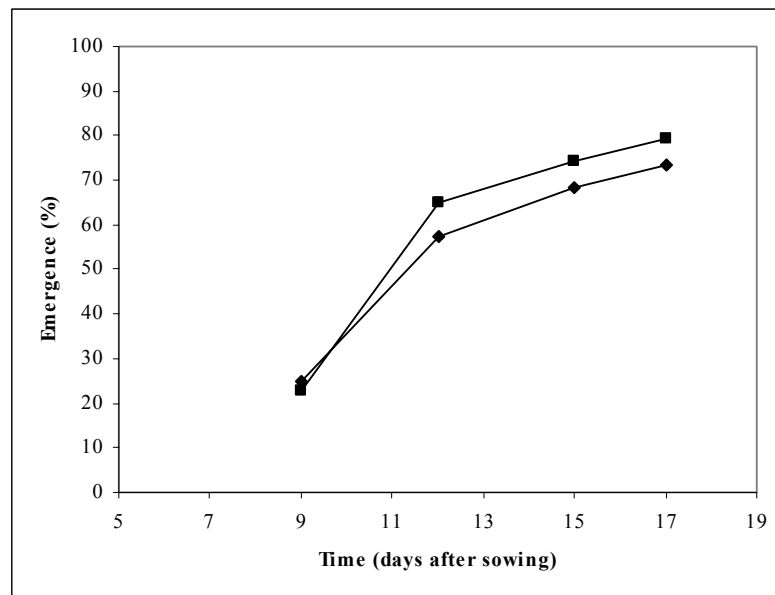
The effects of protein content on emergence are shown in Figure 6.5. As with seed size, it was not possible to measure the expected differences in rate of emergence, probably due to too few assessment time points. Again the differences in final emergence were small, although the low protein seed (nil N treatment) gave numerically the greatest emergence, with the higher N seed (from 300 kg ha^{-1} N at normal timing and 300 kg ha^{-1} N at flowering) giving similar but lower levels of emergence.

Figure 6.5 Percentage emergence with time for low protein (closed squares), medium protein (closed diamonds) and high protein (closed triangles) seed
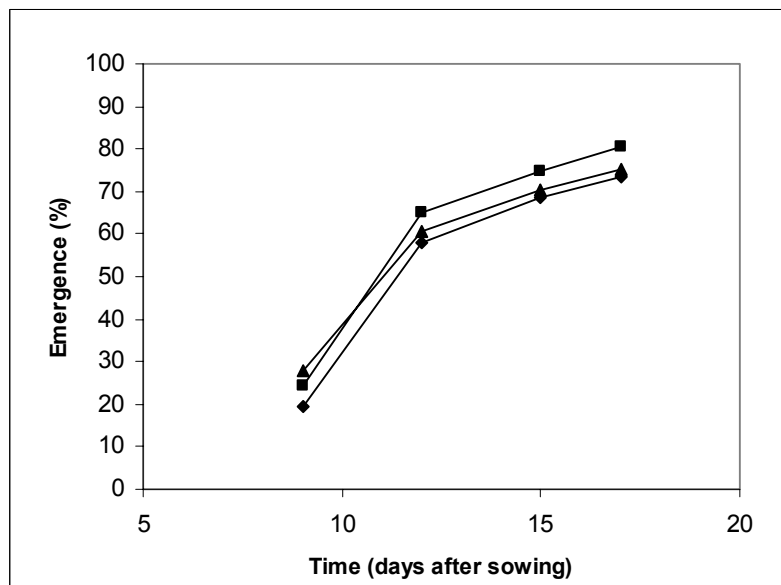
These results support the hypothesis that high oil reserves are more important for maximal emergence than high protein reserves (which reduce oil reserves, Stokes *et al.*, 2000). Similar results, with a trend for lower emergence from high protein seed, were found in the June sowing. The possible rate advantages of high protein seed and larger cotyledons may give advantages in some situations, although preliminary evidence appears to indicate that seed size and oil reserves are more important for determining initial populations.

6.5.4 Effects of seed treatment and seed size on emergence of spring rape

Percentage establishment was low, with a grand mean of 40% and a range from 30.3% to 62.3%. Analysis of variance of final plant numbers per quadrat at maximum emergence showed significant effects of seed size, (P=0.046) and seed treatment (P=0.049). On average, large seed gave significantly greater emergence than ungraded seed and water advancement gave significantly greater emergence than heat treatment. However, there was a significant size x treatment interaction. Figure 6.6 shows the number of emerged seedlings in the different treatments. In the control, large seed gave numerically greater emergence, although this was not significant. The Germain's priming treatment showed no significant difference in emergence to the ungraded, untreated control. In the hydroprimed treatment, large seeds gave significantly greater emergence than all the other treatments. Heat treatment caused non-significant numerical reductions in emergence compared to the ungraded, untreated control. A similar pattern was seen with leaf area (data not shown).

Figure 6.6: Number of emerged seedlings per 0.72 m^2 quadrat (ex. 144) with seed treatment (control, Germain's priming, Hydropriming, Heat treated)
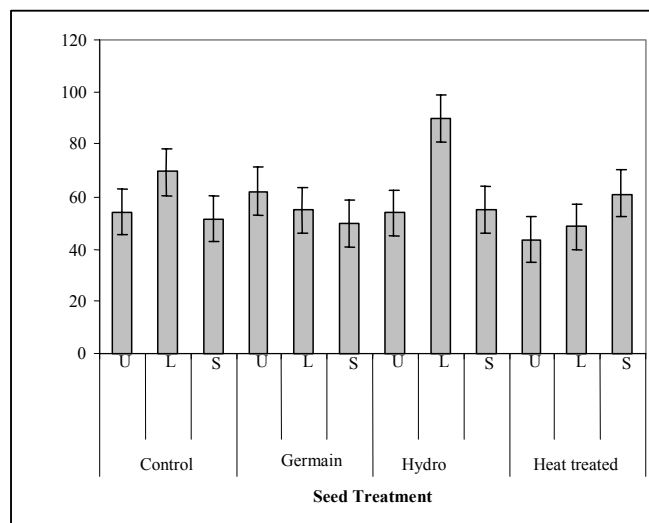
KEY: U = ungraded, L = large seed (>2mm), S = small seed (<2mm)

111

The results from this experiment confirm the potential benefits of large seed size (> 2 mm) for establishment, compared to ungraded or small seed, supporting the work completed in Autumn 1999 and other reports in the literature. Assessments of hydropriming and heat treatment appeared to show little general value for these procedures, with no significant increase in final establishment or leaf area and with numerical decreases in some cases. This may be explained for the Germain's primed seed due to the slowing of germination at low temperatures. Water advancement appeared to offer the best potential improvements for oilseed rape, although only final emergence and leaf area of large seeds were increased by water advancement. Assessment of heat treatment in other studies (Basu, 2003) showed little applicability of this method, so only hydro- and hydropriming studies were continued.

6.5.5. Field experiment 2000/2001

Seed drilling date was seen to exert a significant effect on seedling emergence, with early drilled seed (112 seedlings/m^2) producing higher emergence than late drilled seedlings (88 seedlings/m^2). Selection and priming were seen to have a significant effect on the emergence of seedlings (Table 6.1). Since there were no significant interaction effects between drilling date and any other main factor, focus of all following analysis and interpretation is on the early drilling date.   Grading either by gravity or by size resulted in significantly higher levels of plant emergence (P>0.001) and priming using the Germain's technique gave rise to significantly poorer emergence than either hydropriming or the control.

Table 6.1 The influence of seed treatment on seedling emergence (plants/m^2)

Priming Treatment	Control	Sieving	Gravity	Means
Control	92	140	131	121
Germain's	87	94	108	96
Hydropriming	116	111	127	118
Mean	98	115	122	
		SED =4.1		

Similar trends were apparent for green leaf area at full emergence (grading P=0.015, advancement P<0.001) and dry weight (P<0.001 for both factors). Interaction effects, where present, were due to the variable performance of the priming treatments and were not an artefact of other main factors.

By the time of assessment of biomass characteristics at green bud stage there were no significant differences in individual plant performance due to any treatment factors. On a plot basis the only significant differences, consistently, were that the Germain's advancement treatment was significantly poorer that the other advancement treatments, which did not differ between themselves.

6. 5.6. Multi-site experiment 2001/2002

Since the response of seedlings and mature crop was anticipated to differ depending upon the site location encountered, it was though appropriate to examine the sites separately on a statistical basis. Due to slug and pigeon grazing at High Mowthorpe the site was felt not to have produced robust data and the site data are not presented.

6.5.6.1 Boxworth.

Final plant emergence
There were significant differences due to date of sowing (P>0.001) and seed size (P=0.016) only. Early sown plots achieved significantly higher levels of emergence (127 plants m^{-2}) than late sown plots (99; SED=5.99). Seed size followed the trend;
Control=small seed<< medium seed = large seed (Table 6.2). There were no interaction effects.

Table 6.2. Effect of treatments on final plant population (plants m^{-2}), measured at full emergence, at Boxworth

Drilling date	Seed year	Seed size				Means Seed year	Means Drilling date
		Control	>1.4mm	>1.7mm	>2.0mm		
Early	2000	114	116	122	145	124	127
	2001	114	123	143	142	130	
Late	2000	100	106	107	117	130	99
	2001	75	79	106	101	90	
Means Seed size		101	106	119	126		

Individual plant weight and component green area characteristics
The individual seedling weight showed significant sowing date (P=0.002) and seed age effects (P=0.001) but no seed size effects. Unsurprisingly, early drilled seedlings (2.4 g vs 1.8 g, sed 0.17 g) were larger than late drilled seedlings. Seedlings from 2000 were larger than seedlings from 2001 (2.4g vs 1.8g, sed 0.17 g). There was a significant interaction between sowing date and seed size. Only ungraded and medium graded seed demonstrated a response to drilling date.

Significant sowing date (P<0.001) and seed age effects (P<0.001) were noted on emergence levels but no seed size effects or interactions. Unsurprisingly, early sowing (5358 mm^2) virtually doubled the green area of plants compared with the late sowing (2480 mm^2). The green leaf area of seed from 2000 (4597 mm^2) was significantly greater than that of 2001 (3241 mm^2).

Total plot weight.
There were no significant differences attributed to any of the treatments.

6.5.6.2 Terrington

Final plant emergence
Complete crop emergence was achieved 21 days after drilling for both early and late drilled treatments. There was a decrease in final plant population as a result of late drilling, consistent with the results seen at Boxworth. Emergence rates were considerably higher at Terrington than at Boxworth (grand means of 151 plants m^{-2} and 113 plants m^{-2}, respectively). However there were no

effects attributable to seed age or seed size. There were no significant interactions in the data (Table 6.3).

Table 6.3. Effect of treatments on final plant population, measured at full emergence, at Terrington

Drilling date	Seed year	Seed size				Mean Seed year	Mean Drilling date
		Control	>1.4mm	>1.7mm	>2.0mm		
Early	2000	161.0	164.3	152.0	184.8	165.5	164.4
	2001	159.3	155.3	164.5	174.0	163.3	
Late	2000	152.5	155.3	119.2	152.8	144.9	137.2
	2001	138.3	129.0	121.2	129.5	129.5	
Mean Seed size		152.8	150.9	139.3	160.3		

.

6.6. Discussion

The first experiment showed little difference in emergence from early-harvested rapeseed samples and seed collected at the normal harvest time for oilseed rape in Sutton Bonington. However, even the early-harvested seed was black and mature. Severe problems with seed immaturity would most probably arise with inappropriate swathing date (causing too many green seeds). Crops showing inhomogeneous development and extended flowering due to late sowings, patchy emergence and/or extremely low plant populations could also have a larger immature seed population. As optimum swathing date can be judged by assessing seed maturity and problem immature patches can be identifies and could be combined separately and removed from any lots used for re-sowing, study of the different harvest dates in field trials was discontinued. Likewise, seeds from different pod positions showed no difference in emergence. The major effects of pod position in the study by Stokes *et al.* (2000) were to alter seed size distribution and protein oil balance. As harvesting from different parts of the canopy would be impractical and because these seed characteristics can be more reliably manipulated by other means, analysis of this factor was also discontinued.

Indications of effects of seed size and protein content were found throughout the preliminary work. Greater emergence was found from large seed > 2 mm in diameter compared to the < 2 mm fraction. High protein content seed also reduced total emergence, presumably due to the smaller reserves of oil,

although the greater individual seedling size from high protein seed may have advantages in some situations. Hydroprimed seed showed some advantages in 1999, although there seemed to be little effect of Germain's priming and heat treatment. Study of Germain's priming was retained as a comparison to hydropriming (and to enable further refinement), but study of heat treatment was discontinued. The main focus of the subsequent work field was on the effects of size grading and sowing large seed.

Mother crop husbandry and priming seed failed to demonstrate tangible effects in the field in terms of crop emergence or seedling characteristics. However, Germain's and hydropriming treatments were experimental and it is possible that further refinement could be achieved.

Three year's field work demonstrated that only seed grading by size could provide a measurable improvement in establishment performance, and even here results were inconsistent. Never did selection of large seed act against establishment performance but often failed to demonstrate an improvement. The likely cause is clear – whilst selection of large seed produced larger and more robust seedlings it did not improve the germinability and likelihood of seedlings to emerge under the field conditions experienced. Consequently, whilst selecting large seed could not be discouraged as a commercial practice we have failed to demonstrate a compelling reason for undertake the expensive operation in the first instance.

A key component of the hypotheses under test were that selection and grading could be expected to impart a neutral or beneficial effect on seedling performance under all circumstances. For seed size that hypothesis can be accepted, although the relative value of the effect is questionable. In all years and sites where the crop was taken to yield there was no significant impact of seed treatment on final yield. This even where the seedbed had been produced to be sub-optimal on purpose to exaggerate any beneficial effects of seed grading.

7.   REFERENCES

Ahmed, S.V. and Zuberi, M.I. (1973): Effects of seed size on yield and some of its components in rape seed, *Brassica campestris* L. var Toria. *Crop Science*, **13**, 119-120.

Anonymous (1995): Handbook of Vigour Test Methods, 3rd Edition. Eds. J.G. Hampton and D.M. TeKrony, ISTA, Zurich, Switzerland.

Anonymous (1996): International Rules for Seed Testing, ISTA, Zurich, Switzerland

Anonymous (2002): Establishing oilseed rape using Autocast. Topic Sheet No. 59. Home-Grown Cereals Authority, London.

Anonymous (2002): Commodity prices, Farmer's Weekly

Baker, H. G. (1972): Seed weight in relation to environmental conditions in California. *Ecology*, **53**, 997-1010.

Basu, K.R. (2003): Improving establishment of oilseed rape, PhD thesis, University of Nottingham.

Bilsborrow, P.E. (1985): Physiology of yield of oilseed rape. PhD thesis, University of Nottingham

Black, J.N. (1956): The influence of seed size and depth of sowing on pre-emergence and early vegetative growth of subterranean clover (*Trifolium subterraneum* L.). *Australian Journal of Agricultural Research*, **7**, 98.

Bradbeer, J.W. (1988). Seed dormancy and germination. Blackie and Sons Ltd., London.

Bullard, M.J., Heath, M.C., Clare, R.W., McWilliam, S.C., Stokes, D.T., Scott, R.K. and Davies, D.B. (1996): Optimising establishment of oilseed rape on clays. Research
Review No. OS10, Home-Grown Cereals Authority, London.

Chalmers, A.G., Darby, R.J., Evans, E.J., Sinclair, A.H. and Withers, P.J.A. (1992): Crop nutrition and fertiliser requirements for the double low oilseed rape crop. HGCA Oilseeds Research Review No. OS5, Home Grown Cereals Authority, London.

Cockerel, V., Mulholland, V., McEwan, M, Paveley, N.D., Clark, W.S., Anthony, S., Thomas, J.E., Bates, J., Kenyon, D.M. and Taylor, E.J.A. (2002): Control of seed and soil-borne diseases: importance of seed health. Agronomic Intelligence: The Basis for ProfitableProduction. R&D Conference, Home-Grown Cereals Authority, London. pp 4.1 – 4.11

Copeland, L.O and McDonald, M.B. (2001): Seed Science and Technology, 4th Edition. Kluwer Academic Publishers, the Netherlands.

DEFRA (2002): Oilseed rape production statistics, http://www.defra.gov.uk

Daniels, R.W., Scarisbrick, D.H. and Smith, L.J. (1986): *Oilseed rape physiology*. Ch. 3 in 'Oilseed Rape', Eds. D.H. Scarisbrick and R.W.Daniels, Collins, London.

Durrant, M.J. and Mash, S.J. (1990): Sugarbeet seed treatments and early sowing. *Seed Science and Technology*, 18: 839-850.

Durrant, M.J., Mash, S.J. and Jaggard, K.W. (1993): Effects of seed advancement and sowing date on establishment, bolting and yield of sugarbeet. *Journal of Agricultural Science*, 121: 333-341.

Edwards, M.M. (1968): Dormancy in seeds of charlock. I. Developmental anatomy of the seed. *Journal of Experimental Botany*, **19**, 575-582.

Elliot, R.H. and Rakow, G.F.W. (1999): Influence of seed size on the agronomic performance of oilseed rape. Proceedings of the 9th International Rapeseed Congress, Canberra, 1999.

Finch-Savage, W.E., Steckel, J.R.A. and Phelps, K. (1998): Germination and post-germination growth to carrot seedling emergence: predictive threshold models and sources of variation between sowing occasions. *New Phytologist*, **139**, 505-516.

Garrett, H.J. and Orson, J.H. (1989): Depth and date of emergence of volunteer rape (*Brassica napus*, L.) and its control with herbicides used in peas, beans, potatoes and sugar beet. *Proceedings of the Brighton Crop protection Conference – Weeds*, 3, 811-816.

Glen, D.M. Wiltshire, C.W. Butler, R.C. (1991): Slug population changes following molluscicide treatment in relation to distance from edge of treated area. *Crop Protection,* **10**, 408-412.

Gooding, M.J., Murdoch, A.J. and Ellis, R.H. (2000): *The value of seeds*. Chapter 1 in 'Seed technology and its Biological Basis', Eds. M. Black and J.D. Bewley, Sheffield Academic Press, UK. pp 3-42.

Green, D.B, Walters, K. and Oakley, J.N. (2000): Seed treatments for pest control in winter oilseed rape. Project report No. OS 43, Home-Grown Cereals Authority, London.

Gutterman, Y. (1978): Germination of seeds as a function of the maternal environments. *Acta Horticulturae*, **83**, 49-55

Håkansson, I. and van Polgár, J. (1984). Experiments on the effects of seedbed characteristics on seedling emergence in a dry weather situation. *Soil and Tillage Research*, **4**, 115-135.

Halmer, P. (2000): Seed processing technology. Chapter in 'Seed technology and its Biological Basis', Eds. M. Black and J.D. Bewley, Sheffield Academic Press, UK. pp 3-42.

Hardwick, N.V., Fitt, B.D.L., Wale, S.J. and Sweet, J.B. (1991): Oilseed rape diseases. Oilseeds Research review No. OS4, Home-Grown Cereals Authority. London.

Heather, D.W. and Sieczka, J.B. (1981): Effect of seed size and cultivar on emergence and stand establishment of broccoli in crusted soil. *Journal of the American Society of Horticultural Science*, **116**, 946-949.

Heydecker, W. (1972): In Viability of Seeds, p. 209 (Ed. E.H. Roberts), Chapman and Hall, London.

Hill, W.J., Taylor, A.G., and Huang, X.L. (1988): Seed viability determinations in cabbage utilizing sinapine leakage and electrical conductivity measurements. *Journal of Experimental Botany*, **39**, 1439-1447.

Holme, S. and Lunn, G. (2000): Effects of soil- and seed-borne fungal diseases on emergence of oilseed rape (*Brassica napus*) and potential interaction with seed selection and treatment techniques. In MAFF final report AR0707, 2000.

Jalink, H., van der Schoor, R., Frandas, A., van Pijlen, J.G. and Bino, R.J. (1998): Chlorophyll fluorescence of *Brassica oleracea* seeds as a non-destructive marker for seed maturity and seed performance. *Seed Science Research*, **8**, 437-443.

Jalink, H., van der Schoor, R., Frandas, A., van Pijlen, J.G. and Bino, R.J. (1998): Chlorophyll fluorescence of *Brassica oleracea* seeds as a non-destructive marker for seed maturity and seed performance. *Seed Science Research*, **8**, 437-443.

Jurado, E. and Westoby, M. (1992): Seedling growth in relation to seed size among species of arid Australia. *Journal of Ecology*, **80**, 407-416.

Kelly, A.F. and George, R.A.T. (1998): Encyclopaedia of Seed Production of World Crops. Wiley & Sons Ltd., London.

Lang, R.W. and Holmes, J.C. (1964): The growth of the swede crop in relation to seed size. *Journal of Agricultural Science (Cambridge)*, 63, 221-227

Larsen, S.U., Povlsen, F.V., Eriksen, E.N. and Pedersen, H.C. (1998): The influence of seed vigour on field performance and the evaluation of the applicability of the controlled deterioration vigour test in oilseed rape (*Brassica napus*) and pea (*Pisum sativum*). *Seed Science and Technology*, **26**, 627-641.

Leishman, M.R., Westoby, M. (1994): The role of seed size in seedling establishment in dry soil conditions – experimental evidence from semi-arid species. *Journal of Ecology* (1994), **82**, 249-258.

Leterme, P. (1988): Croissance et dévelopment du colza d'hiver: les principales étapes (Growth and development of winter rapeseed: the main stages). In: *Colza: Physiologie et Elaboration du Rendement*. CETIOM, Paris, 23-33.

Lloyd, E. (2002): Effects of plant growth regulators on the canopy structure of winter oilseed rape. BSc thesis, University of Nottingham.

Lunn, G.D., Spink, J., Stokes, D.T. and Clare, R.W. (2001). Canopy management in winter oilseed rape. HGCA Final Report OS 42, Home-Grown Cereals Authority, London.

Lunn, G.D. and Bullard, M. (2001). SAPPIO Link project LK0905, Effects of seed vigour on establishment of oilseed rape, *Brassica napus*. Annual Interim Report

Lutman, P.J.W., Pekrun, C. and Albertinin, A. (1998): Dormancy and persistence of volunteer oilseed rape. Project Report No. OS32, Home-Grown Cereals Authority, London.

Macbeth, J.E. (1996). The prediction of mature grain weight in winter wheat (Triticum aestivum, L.) Harper Adams University College, UK. 267 pp.

Major, D.J. (1977): Influence of seed size and yield components of rape. *Agronomy Journal*, **69**, 541-543

Marshall, B. and Squire, G.R. (1996): Non-linearity in rate-temperature relations of germination in oilseed rape. *Journal of Experimental Botany*, **47**, 1369-1375.

Marshall, A.H. Naylor, R.E.L. (1984). The effect of leachates from grass trash on establishment of ryegrass. *Annals of Applied Biology,* **105**, 75-86.

Mayer, A.M. and Poljakoff-Mayber, A. (1982). 'The Germination of Seeds', Pergamon Press, Oxford.

McWilliam, S.C. (1998): Plant establishment, canopy structure and yield formation in oilseed rape. PhD thesis, university of Nottingham.

Mendham, N.J., Shipway, P. and Scott, R.K. (1981): The effects of seed size, autumn nitrogen and plant population density on the response to delayed sowing in winter oilseed rape (*Brassica napus*). *Journal of Agricultural Science (Cambridge)*, **96**, 417-428.

McWilliam, S.C., Stokes, D.T., Scott, R.K., Norton, D.G., Sylvester-Bradley, R. and Davies, D.B. (1995): The relationship between establishment and yield of autumn-sown oilseed rape. Project Report No. OS13, Home-Grown cereals Authority, London.

McWilliam, S.C., Stokes, D.T., and Scott, R.K. (1998). Establishment of oilseed rape: the influence of physical characteristics of seedbeds and weather on germination, emergence and seedling survival. Project Report No. OS31, Home-Grown cereals Authority, London.

Mendham, N.J., Shipway, P. and Scott, R.K. (1981): The effects of seed size, autumn nitrogen and plant population density on the response to delayed sowing in winter oilseed rape (*Brassica napus*). *Journal of Agricultural Science (Cambridge)*, **96**, 417-428.

Michel, E.B. (1983). Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiology*, **72**, 66-70.

Noon, M.M. (1997): The potential for seed advancement to improve the establishment of winter oilseed rape. MSc thesis, University of Nottingham.

Pekrun, C., Lutman, P.J.W. and Baeumer, K. (1997): Induction of secondary dormancy in rape seeds (Bassica napus L.) by prolonged imbibition under consitions of water stress or oxygen deficiency in darkness. *European Journal of Agronomy*, **6**, 245-255.

Scott, R.K. (1961): The significance of seed size and associated factors in the early growth of winter wheat. B.Sc. dissertation, University of Nottingham.
Scott, R.K., Harper, F., Wood, D.W. and Jahhard, K.W. (1974): Effects of seed size on growth, development and yield of monogerm sugar beet. *Journal of Agricultural Science (Cambridge)*, **84**, 517-530.

Siddique, M.A. and Godwin, P.B. (1980): Seed vigour in bean (Phaseolus vulgaris L. cv. Apollo) as influenced by temperature and water regime during development and maturation. *Journal of Experimental Botany*, **31**: 313-323.

Smith, M. (2001): Effects of allelopathy, seed quality and advancement on establishment of oilseed rape (*Brassica napus*, L). HGCA Undergraduate Bursary report, Home-Grown Cereals Authority, London..

Stokes, D.T., Bullard, M.J., Scott, R.K. and Clare, R.W. (1998). An analysis of the potential for improving seed quality in oilseed rape as a basis for optimising establishment. Project Report No. OS29, Home-Grown Cereals Authority, London.

Stokes, D.T., Bullard, M.J., Lunn, G.D., Basu, K.R., Clare, R.W. and Scott, R.K. (2000). Establishment of oilseed rape: seed crop management effects on seed quality and performance. Project Report No. OS42, Home-Grown Cereals Authority, London.

Tesnier, K., Strookman-Donkers, H.M., van Pijlen, J.G., van der Geest, A.H.M, Bino, R.J. and Groot, S.P. C. (2002): A controlled deterioration test for *Arabidopsis thaliana* reveals genetic variation in seed quality.

Thomas, T., Biddington, N.L. and O'Toole, B.F. (1979). Relationship between position on the parent plant an dormancy characters of seeds of three cultivars of celery (*Apium graveolens*). *Physiologia Plantarum*, **45**, 492-499.

Thornton, J.M., Powell, A.A., Matthews, S. (1990): Investigation of the relationship between seed leachate conductivity and the germination of *Brassica* seed. *Annals of Applied Biology*, **117**, 129-135.

Whalley, W.R., Finch-Savage, W.E., Cope, R.E., Rowse, H.R. and Bird, N.R.A. (1999): The response of carrot (*Dauca carota* L) and onion (*Allium cepa* L) seedlings to mechanical impedance and water stress at sub-optimal temperatures. *Plant, Cell and Environment*, **22**, 229-242.