IMPROVING SEED QUALITY IN WINTER OILSEED RAPE

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ABSTRACT

The majority of UK oilseed rape (*Brassica napus* L.) is September-sown on heavy clay soils where straw has been incorporated following the cereal harvest in August. A series of germination, emergence and field experiments was conducted to assess the effects of variation in seed quality on establishment and to evaluate the improvements possible by mother crop nitrogen management and pre-sowing seed treatments on commercial and farm-saved seedlots.

In germination experiments designed to examine the performance of commercial seedlots at temperatures ranging between 5 and 25°C significant differences were recorded in the speed and uniformity of germination, particularly at 10 and 15°C, which are comparable to UK field temperatures in late-August to September. The analysis of Apex variety seeds grown from nitrogen-managed mother crops in 1996 and 1998 showed a negative correlation between their nitrogen and oil percentage, which was significantly affected by both the amount (kg ha⁻¹) and timing (vegetative growth period or flowering period) of nitrogen application.

The highest nitrogen seeds were produced from mother crops that had received medium (160 kg ha^{-1}) amounts of nitrogen fertiliser during the flowering period. Seeds that were harvested from pods taken from the lower (< 1.5 m) section of the crop canopy also had a significantly higher nitrogen and significantly lower oil percentage than those taken from the upper (> 1.5 m) section of the canopy. In germination and emergence experiments the highest nitrogen (3.46 to 3.61%) seeds germinated significantly faster than the seeds of lowest nitrogen (2.30 to 2.95%) content but they did not emerge as well as low nitrogen seeds from depth.

Selecting small (< 2 mm diameter) seeds over large (> 2 mm diameter) seeds significantly improved the rate of germination and emergence and the final percentage emergence at 10 mm sowing depth although the final percentage emergence at 20 mm sowing depth was significantly greater from the large seeds. Hydrating seeds in water for 18 hours at 15°C before drying them back in the laboratory at 20°C significantly improved the speed of germination and the speed and final percentage emergence at 10 mm sowing depth compared with control treatments provided that the radicle had not emerged before drying back; small seeds derived the most benefit from hydration. Seed heat treatment at temperatures of 80°C significantly delayed the onset of germination and emergence but significantly hastened field establishment.

The effects of seed nitrogen percentage, seed size and heat treatment on seedling emergence and subsequent plant growth and development were examined in the field between October 1999 and July 2000. Growth analyses, which were performed in February (growth phase), May (flowering) and July (pre-harvest), showed that the high nitrogen, large seeded and heat-treated populations had a significantly lower rate of plant loss than the low nitrogen, small seeded and control populations.

Under field conditions, the higher growth rates and growth parameters of the seedlings produced from the high nitrogen and/ or large seeds were not always significant nor were they consistently maintained until harvest. Large seed size and heat treatment significantly increased the number of established plants per m^2 and significantly increased the initial plant size. The final yield was not significantly affected by seed nitrogen percentage, seed size or seed heat treatment.

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	accelerated ageing
Abs	absorbance
ADAS	Agricultural Development Advisory Service
ANOVA	Analysis of Variance
AOSA	Association of Official Seed Analysts
Chl _(a)	chlorophyll a
Chl _(b)	chlorophyll b
$Chl_{(a+b)}$	total chlorophyll content
°C	degrees Celsius
cm	centimetres
СРВ	Centre for Plant Breeding
d	days
DEFRA	Department for the Environment, Food and Rural Affairs
DF	degrees of freedom
DNA	Deoxyribonucleic acid
DWT	dry weight
Ε	emergence
EEC	European Economic Community
F	flowering phase
FAO	Food and Agricultural Organisation
FP	final percentage
FPE	final percentage emergence
FPF	final percentage field emergence
FPG	final percentage germination

FWT	fresh weight
g	grams
G	germination
G	growth phase
GAI	green area index
GC	gradient coil
h	hours
ha	hectare
HEAR	high erucic acid rape
HGCA	Home Grown Cereals Authority
"	inch
ISTA	International Seed Testing Association
kg	kilograms
L.	Linnaean system of classification
LEAR	low erucic acid rape
ln	natural logarithm
log	logarithm (to the base 10 unless otherwise specified)
MAFF	Ministry of Agriculture, Fisheries and Food
m	metres
μg	microgrammes
mm	millimetres
MT	million tonnes
NIAB	National Institute of Agricultural Botany
<i>N</i> ₂	nitrogen
nm	nanometres
No.	number

Nos.	numbers
NO_x	nitrous oxides
NMR	Nuclear Magnetic Resonance
Р	probability
PEG	polyethyleneglycol
%	per cent
рН	negative log of the hydrogen ion concentration
П	pi
ррт	parts per million
R	rate
RF	resonant frequency
RMAV	Repeated Measurements Analysis of Variance
RNA	Ribonucleic acid
SD	Standard Deviation
SEM (a)	Standard error of the means of different processes at the same
	timepoint
SEM (b)	Standard error of the means of the same process at different
	timepoints
SED	standard error of deviation
SMP	solid matrix priming
syn	synonym
<i>T</i> ₁₀	time to 10%
T ₅₀	time to 50%
TSW	thousand seed weight
UK	United Kingdom

PUBLICATIONS

Data from this thesis have appeared in the following publications:

Stokes D.T., Bullard M.J., Scott R.K. and Clare R.W. (1998). HGCA Project Report No. OS29: An Analysis of the Potential for Improving Seed Quality in Oilseed Rape as a Basis for Optimising Establishment. (HGCA: London).

Stokes D.T., Bullard M.J., Lunn G.D., <u>Basu K.R.</u>, Clare R.W. and Scott R.K. (2000). HGCA Project Report No. OS42: Establishment of Oilseed Rape: Seed Crop Management Effects of Seed Quality and Seedling Performance. (HGCA: London).

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CHAPTER I INTRODUCTION TO OILSEED RAPE

1.1 The History and Production of Oilseed Rape

Brassicas constitute the main oilseed crop in the temperate climates of Europe and North America and parts of Asia and Africa (Desai *et al.*, 1997) and *Brassica napus*, oilseed rape, is grown in a wide variety of climates from the near Arctic (Finland and Norway) to the subtropical or tropical (Bangladesh and India) (Murphy *et al.*, 1989).

Until the Middle Ages, rapeseed was gathered for its oil, which was used for illumination and soap making (Bell, 1982) and field-scale cultivation became common in Europe in the thirteenth century. Rape was grown extensively in Eastern England between the seventeenth and nineteenth centuries both as a pioneer reclamation and as an oil crop (Gardner, 1984). Since the 1950s rape has been used in rotations (Theo, 1981) and it is the most predominant break crop from cereals (Davies *et al.*, 1994).

The total world production of edible oils and fats is about 100.3 MT, of which 79.8% is from plant species and the remainder is from animal sources. The main uses of oils and fats are for food (81%), animal feed (5%) and the industrial chemical sector (14%) (Palmieri, 2000, personal communication). The world production of rape oil has doubled from about 7% of the total production of vegetable oils and fats in 1970 to nearly 14% in 2000 and the UK has seen the largest increase in production from 12% to 77% over the same period (FAO, 2001).

In 1999, oilseed rape covered 500,000 ha in the UK (Nix, 1997) occupying the third largest arable area in the country after wheat and barley (Burns *et al.*, 1984) but only about 10% of the UK crop is spring sown (Kings, 1999). The highest density of

cropping in the UK is in Eastern England, the East Midlands and Eastern Scotland and within these regions, more than half the crop is grown on clay soils (McWilliam *et al.*, 1995).

1.2 The Uses of Oilseed Rape

The largest cultivation of oilseed rape today is for edible oil production (Downey and Rimmer, 1993), industrial applications, animal feeds (Schjoerring *et al.*, 1995), fertilisers (Ward *et al.*, 1985) and transport fuel (Price *et al.*, 1996).

1.3 Oilseed Rape Research and Development

Until about 1971, when the United Kingdom joined the European Economic Community (EEC) and the strategic importance of an indigenous oil crop became clear, oilseed rape was almost completely ignored by plant breeders in Britain. However, by 1976, much of the EEC research was focused on the production of double low varieties, that is, varieties having low-erucic acid (< 2%) and low-glucosinolate (< 30 ppm in the meal) contents (Christmas and Hawkins, 1992).

Initial hybrid success was achieved using a low-erucic acid Canadian variety '*Oro*' and hybrid cultivars of rapeseed now account for about 20% of UK seed. Low-erucic acid ('LEAR') varieties typically contain less than 0.5% erucic acid (Kimber, 1981) while in contrast, high erucic acid (HEAR) varieties are grown specifically for their high (50 to 60%) erucic acid content, which is used to produce erucamide for polythene (Merrien, 1989).

1.4 Botany of Oilseed Rape

The family *Cruciferae* comprises some 300 genera and 3000 species (Griffiths *et al.*, 1998). *B. napus* and *B. rapa* (syn. *B. campestris*) constitute oilseed rape (Downey and Rimmer, 1993) thus the terms "rapeseed" and "rapeseed oil" can mean the seed and oils of *B. napus* (rape) or *B. rapa* (turnip rape or yellow sarson) or a mixture of both. *B. napus* is an allotetraploid derived from *B. rapa* and *B. oleracea* (Kryzmanski and Downey, 1969; Bell, 1982; Kimber and McGregor, 1995) as shown in figure 1.4.1.

Oilseed rape has a long taproot and dark green compound leaves with leaflets displayed on a central stalk (Allaby, 1992). The rape inflorescence is an elongated terminally borne raceme on the main stem and on the branches (Desai *et al.*, 1997). Flowering is indeterminate starting on the lowest bud of the main raceme (Noon, 1997) and the crop takes 90 to 100 days to mature (Kimber and McGregor, 1995).

Both *B. napus* and *B. rapa* have an annual (spring) variety and a biennial (winter) variety (Ward *et al.*, 1985). In cool climates, the winter (*biennis*) forms of *B. napus* are the most productive and most of the area cultivated to oilseed *Brassicas* in Europe and China is sown to winter oilseed rape. However, in warmer climates, the winter form of *B. napus* is often supplanted by the spring (*annua*) form of *B. napus* (Downey and Rimmer, 1993). LEAR winter rape (*B. napus* subsp. *Oleifera* var. *biennis*), which gives the highest seed and oil yields is the most popular rape seed grown in most of the UK and northwestern Europe. If the crop is well established by the onset of winter, it can withstand very cold weather (Ward *et al.*, 1985) with the growth period in northern Europe being about 324 days (Holmes, 1980).

Figure 1.4.1 The genomic relationship of the *Brassica* species (Kimber and McGregor, 1995)



n = number of chromosomes

1.5 Development of Oilseed Rape

The development and growth of rape can be divided into four stages: (a) vegetative, (b) plant framework formation and flowering, (c) pod development and (d) seed development as shown in table 1.5.1. Potential pod and seed numbers are determined at the end of the first stage and pod and seed mortality are high during the second stage due to competition for assimilates as leaf photosynthesis declines. The maximum pod area and weights are obtained during the third stage and seed oil and protein deposition are dependent upon assimilates from pods and stems during stage four (Bilsborrow and Norton, 1984).

Table 1.5.1Overview of the development and growth of oilseed rape. Codesrefer to the main stem (Sylvester-Bradley and Makepeace, 1984).

\wedge	Development	Code	Growth
	Sowing	0.0	-
	Emergence	0.8	Expansion of cotyledons, growth of
			taproot.
Vegetative	Leaf production	1.00	Establishment of root system and leaf
development	(as rosette)		expansion.
		\downarrow	
		1.20	Interception of solar radiation,
			photosynthesis, increased leaf dry
\checkmark			weight.
$\mathbf{\Lambda}$	Inflorescence initiation		
	(vernalisation and	-	-
	photoperiod responses)		
	Stem elongation	2.00	Stem dry weight increases, stem
		\checkmark	photosynthesis commences, reserves
Plant	(photoperiod responses)	2.20	laid down.
framework extension	Flower bud development	3.0	
1		\mathbf{V}	-
	Ovule numbers determined	3.9	
	Flowering (pollination,	4.1	Leaf area and root extension close to
	seed set)	\downarrow	maximum. Flowers shade leaves and
		4.9	young pods.
Ť	Pod development (pod	5.1	Pod and stem photosynthesis replaces
Pod	and seed abortion, final	\checkmark	declining leaf area as leaves senesce.
development	numbers determined)	5.9	Pod walls reach maximum size and
\downarrow			seed growth commences.
٨	Seed development	6.1	Seed growth with assimilates from
Seed development	(formation of embryo and	\checkmark	leaves, stems and pods. Oil and
\bigvee	storage cells)	6.9	protein storage and synthesis.

1.6 Agronomy of Oilseed Rape

1.6.1 Seedbed cultivation and drilling method

Rapeseed is small (about 1.4 to 2 mm in diameter), so a firm yet fine seedbed is required for even establishment. Minimal cultivation methods and/ or direct drilling, which do not disturb the fine tilth that can develop in clay soils due to self-mulching at the soil surface, help the seedbed to retain sufficient moisture for germination (Hakansson and Von Polgar, 1984).

Rapeseed is often direct drilled because it tends to follow cereal crops in rotation cycles but sowing rapeseed has become more problematic since the straw burning ban in 1992. The ban prohibited farmers from burning the cereal straw that was left in the field following the cereal harvest so it is necessary to find a method to establish winter oilseed rape in the presence of the remaining cereal straw. The generally accepted methods of establishing rape are by direct seeding into the cereal stubble or by drilling after minimal cultivation on heavy soils but a late cereal harvest can leave little time for seedbed preparation (Ward and Orson, 1981).

Direct drilling is generally likely to ensure good germination but the seedlings can be slow to establish so an alternative is to broadcast the seed either with slug pellets or mixed with fertiliser (Bearman, 1981). Although broadcast populations are generally smaller than direct-drilled populations, the yields can be larger and there are generally fewer winter losses (Darby and Yeoman, 1994). The optimal drilling depth for rapeseed is 15 mm but it is not possible to constantly achieve this depth in the field (Lutman, 1994).

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1.6.2 Sowing date

During the 1970s, greater yielding winter cultivars led to a change in the drilling time from spring to winter (Gardner, 1984). The sowing date can determine the yield of winter rape varieties (Jasiñska *et al.*, 1987) and the optimum date for winter oilseed rape in the United Kingdom is generally between mid-August and the first week in September (Mendham and Scott, 1975; HGCA, 1998). Early sowing can advance the crop too far by the onset of winter and if premature flowering occurs, frost damage can be severe. Furthermore, because the height and weight of plants is greater, there can be greater lodging and thus mechanical damage as well as increased pest damage since warmer conditions increase pest activity and favour the wind dispersal of fungal spores (Anon 1, 2000). In contrast, late-drilled crops can be too poorly established or too small to survive a hard winter (Archer, 1981; Ward *et al.*, 1985) and their subsequent growth can be limited by lower pod production (Mendham *et al.*, 1990).

1.6.3 Seed rate

Within wide limits, the planting density has relatively little influence on oilseed rape yield (Ward *et al.*, 1985; Andersson and Bengtsson, 1989; Davies *et al.*, 1994). Variability of performance is a feature of oilseed rape (Jenkins, 1990) and under field conditions of between 30 and 100 plants m^{-2} , the final yields obtained can be quite similar as a result of the compensatory nature of rape growth (Davies *et al.*, 1994).

Adequate yields can be achieved over a wide range of planting densities because lowdensity rape crops compensate by producing a greater leaf area, more branches and a greater pod number per plant. The greatest effect of increasing the planting density is to alter the pod distribution within the canopy profile: at high densities a far greater proportion of the fertile pods are present on the terminal and uppermost branch than at low densities. This shortens the duration of pod development and seed production, hastens maturation and results in earlier and more uniform crops at harvest as well as more predictable and uniform seed quality. However, although the harvest can also be more accurately timed if the seeds mature at the same time, it can lead to serious seed loss due to mass pod shatter (Leach *et al.*, 1999).

Although there is no specific seedrate for oilseed rape and establishment varies with variety, season, soil type, seedbed quality, location, and environmental conditions, the average seed rate for UK winter oilseed rape is 6 to 7 kg ha⁻¹ (Leach *et al.*, 1999). Winter UK rape crops usually achieve 80 to 100 plants m⁻² through sowing at these rates and under normal conditions about 24 plants m⁻² will be lost due to weather effects and pigeon damage (Scarisbrick and Daniels, 1986).

1.6.4 Crop nutrition

<u>Nitrogen</u>

Nitrogen is needed for protein production and high nitrogen fertilisation can increase plant height and inflorescence branching (Holmes, 1980) as well the number of flowers, pods and the seed dry matter (Perkin, 1981; Darby and Yeoman, 1994) but excessive nitrogen can depress the yield.

Nitrogen deficiency causes stunting, leaf chlorosis (Harrington, 1960) and early leaf drop (Perkin, 1981) but seedbed nitrogen has little effect on germination (Harrington, 1960), mainly due to substantial losses through ammonia volatilisation, denitrification (Gabrielle *et al.*, 1998b) and nitrate leaching from the root zone (Vos *et al.*, 1994). The

recommended solution, therefore, is to apply nitrogen as a split application during growth of up to 190 kg ha⁻¹ per application (MAFF, 2000).

<u>Sulphur</u>

Sulphur, which is taken up in the form of sulphate (Blake-Kalff *et al.*, 1998), is needed for protein production (Zhao and Withers, 1997) and is essential for photosynthesis. High nitrogen fertilisation can induce sulphur deficiencies, which can depress protein synthesis in the seeds (Zhao *et al.*, 1993). Oilseed rape is particularly sensitive to sulphur deficiency (Holmes, 1980) and the developing leaves are the first to show the deficiency symptoms of stunting and chlorosis while in contrast, sulphur-rich soils can produce high levels of glucosinolates in the seeds and foliage (Merrien, 1989).

Since the clean air act of 1960, sulphur deficiency has become a problem in some areas of the UK (Pinkerton, 1991). Large cost-effective (McGrath and Zhao, 1996) increases in seed yields can be obtained through applying 40 to 66 kg ha⁻¹ of sodium sulphate at seeding (Nuttall and Ukrainetz, 1991).

Phosphorus

Phosphorus is important to the development of a large plant and seed yields are related to its concentration in young plants (Pinkerton, 1991). Although phosphorus restriction impedes plant growth, it does not affect either the oil content or the fatty acid composition of the seed. Its deficiency is expressed by stunting and purpling of the midribs of the leaves (Harrington, 1960) and about 50 kg phosphate ha⁻¹ is advisable (Ward *et al.*, 1985) as monoammonium phosphate and polyphosphate (Grant and Bailey, 1993).

<u>Potassium</u>

Potassium is essential for plant enzyme systems, photosynthesis, carbohydrate and nitrogen metabolism as well as for disease, frost and drought tolerance (Grant and Bailey, 1993). Potassium deficiency symptoms include wilting (Ward *et al.*, 1985) and necrotic spots (Harrington, 1960) and up to 40 kg ha⁻¹ of potash fertiliser (Ward *et al.*, 1985) can be needed on sandy soils (Archer 1981).

Micronutrients

Zinc has a profound effect on root growth and seed yield and there is considerable genotypic variation for zinc efficiency in oilseed rape. Where subsoils are zinc-deficient, ameliorative action may be taken through subsoil fertilisation or by growing oilseed rape genotypes with improved zinc efficiency (Grewal *et al.*, 1997). Magnesium is an essential component of chlorophyll and is involved in enzyme systems (Pouzet, 1995) thus its deficiency can inhibit protein synthesis (Holmes, 1980); about 140 kg ha⁻¹ magnesium sulphate is recommended to avoid plant damage (Pouzet, 1995).

Calcium affects membrane permeability (Holmes, 1980) and its deficiency can cause the death of growing points (Harrington, 1960) while manganese deficiencies cause yellowing and chlorosis and can reduce the seed yield and seed oil content. Boron deficiency is relatively rare in the UK (Archer, 1981), but deficiencies can lead to plant stunting, leaf rolling and chlorosis (Ward *et al.*, 1985).

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Oilseed rape harbours many insect species and pest incidence levels tend to increase with increased growing area and intensity (Ward *et al.*, 1985). The use of insecticides or pesticides such as α -cypermethrin can help to protect the oilseed rape crop against pollen beetles and stem weevils while phosalone can protect against seed weevils; flea beetles can be controlled by imidacloprid and β -cyflutinin (Green, 2002). Natural biological control parasites have also greatly reduced seed weevil (*Ceutorhyncus assimilis*) and club midge (*Dasineura brassicae*) larvae damage in the south of England. Cabbage root fly can also cause extensive plant damage (Ward *et al.*, 1985) and during flowering, pollen beetles can seriously affect seed set (George and Kelly, 1998).

Oilseed rape is also susceptible to a number of soil-, stubble- or air-borne diseases. Although some varieties such as 'Jetneuf' and 'Rafal' have reasonable resistance to phoma spot and stem canker (Leptosphaeria maculans/ Phoma lingam) (Ward et al., 1985), both phoma canker and light leaf spot (Pyrenopeziza brassicae/ Cylindrosporium concentricum) still cause losses of up to £40 million per year in the United Kingdom (HGCA, 1997).

Dark leaf and pod spot (*Alternaria*) causes premature ripening and seed shedding while light leaf spot causes pod losses (Anon 1, 2000). Spraying with triazole fungicides (O'Brien, 2001) can control white leaf spot (*Pseudocercosporella capsellae*) and grey mould (*Botrytis cinerea*) (Downey and Rimmer, 1993) while powdery mildew (*Erisyphe cruciferarum*), sclerotinia stem rust (*Sclerotinia sclerotiorum*), and club root (*Plasmodiophora brassicae*) can be reduced through crop rotation. The incidence of downy mildew (*Peronospora parasitica*) is increased if the crop is poorly established
and the problem is then exacerbated as small plants are less able to compete with weeds (Evans and Gladders, 1981).

Aphid-transmitted beet western yellows virus, turnip mosaic virus and cauliflower mosaic virus also attack oilseed rape and it is important to apply aphicides (Ward *et al.*, 1985). Bees are strongly attracted to the crop in flower and every effort should be made to avoid harming them: crops should not be sprayed in flower and only low toxicity insecticides or pesticides such as phosalone should be sprayed in the late evening or early morning (Graham, 1981).

Grey field slugs (*Deroceras reticulum*) are a major pest problem on oilseed rape (Glen, 1989) and leaf shredding can be fatal to young seedlings (Glen *et al.*, 1990). Slugs are common on heavy soils (Glen *et al.*, 1989) and since they move through soil fissures, cobbly seedbeds or open drill slits promote their movement. A high proportion of small aggregates in the seedbed reduces the risk of slug damage as does deep (to 40 mm) drilling, which renders the seed inaccessible (Glen *et al.*, 1989). Large populations may develop (Graham, 1981) especially in the presence of straw, which forms a damp mulch (McWilliam, 1998) and acts as a secondary food source (Glen *et al.*, 1989).

Wood pigeons (*Columba palumbus*) are also a major pest of oilseed rape, eating away the leaves from the midrib (Anon 2, 2000) and in extreme cases removing the growing tips of young seedlings and plants (Inglis *et al.*, 1989). Pigeons choose to land on bare ground and grazing progresses outwards from poorly established patches, reducing overwinter survival (McWilliam, 1998).

Black grass (*Alopecurus myosuroides*), wild oats (*Avena fatua*), couch grass (*Elymus repens*), rye-grass (*Lolium multiflorum*) and volunteer barley (*Hordeum vulgaris*) all of which can severely reduce rape yields (Anon 1, 2000), can be controlled through graminicides such as Falcon or herbicides such as Fortrol (HGCA, 2000). Chickweed (*Stellaria media*), mayweeds (*Tripleurospermum maritimum, Matricaria recutita* and *M. matricarioides*), groundsel (*Senecio vulgaris*), red dead nettle (*Lamium purpureum*) and speedwells (*Veronica persica* and *V. avensis*) germinate at the times at which rape is establishing and compete with rape in its early stages. Furthermore, since all of these weeds germinate near the soil surface and are encouraged by minimal cultivation techniques (Ward and Orson, 1981), their damage might be minimised by deep cultivation.

Cleavers (*Galium aparine*), which increases lodging and reduces harvestability by increasing moisture levels at the bottom of the crop and preventing the stems drying out (Anon 1, 2000), can be controlled by benazolin (Galtrak) herbicides (HGCA, 2000). The identification of charlock (*Sinapis arvensis*), black mustard (*B. nigra*), white mustard (*Sinapis alba*) and wild radish (*Raphanus raphanistrum*) is difficult because their seed size is similar to that of oilseed rape. However, since these weeds mainly germinate in the spring, their removal should occur before flowering to ensure genetic purity of the seed crop (Ward and Orson, 1981). Trifluralin (pre-sowing), Butisan-S (pre-emergence) and Benazalox (post-emergence) herbicides may also be used to control these weeds (Anon 1, 2000).

1.7 Environmental Conditions for Oilseed Rape Growth

1.7.1 Light and temperature

Within wide temperature ranges (5 to 35°C), the overall percentage germination of oilseed rape has been reported to be similar although the rate of germination varies (Wilson *et al.*, 1992). Rape is quite tolerant to high temperature during its vegetative phase of development but heat and drought together can cause reductions in the seed size and oil content (Ward *et al.*, 1985). Low temperatures constrain plant productivity (Paul *et al.*, 1990), affect nitrate uptake and reduce xylem nitrogen translocation (Lainé *et al.*, 1994).

Rape is also considered to be frost hardy (Single and Marcellos, 1981) since metabolic changes convert starch into sugars and form water-soluble proteins, which confer cold-tolerance (Kacperska-Palacz and Wclinska, 1972; Rapacz, 1998). Survival following freezing is associated with regrowth from young unexpanded leaves or from the crown meristem (Andrews and Morrison, 1992) although some plants may be killed by frost heave (McWilliam, 1998).

1.7.2 Water

A moisture content of about 40% is needed for germination to commence (McWilliam, 1998). Imbibed seeds can tolerate drying to below 40% moisture prior to germination although drying after the onset of germination usually results in seedling loss (Lutman, 1994).

Since most of its dry matter production takes place before the soil dries in the late summer, most winter rape tends to avoid the worst effects of drought but rape yields on sands, loamy sands and shallow or stony soil types in the south and east of England can be reduced (Archer, 1981). Water stress after flowering can reduce the seed yield and oil content and double the glucosinolate content (Mailer and Cornish, 1987), which may be related to the uptake and translocation of sulphur within the plants (Mailer and Pratley, 1990). *Brassica* species tend to avoid drought stress by restricting water loss through sensitive stomatal responses and fast leaf abscission (Jensen *et al.* 1996).

In contrast, oilseed rape is quite susceptible to waterlogging (Gutiérrez *et al.*, 1996) and the yield can fall by up to 20% (Zhou and Lin, 1995). Excess soil sodium can reduce seedling emergence due to soil crust development in areas of high rainfall (Gutiérrez *et al.*, 1996).

1.7.3 Oxygen supply, pH and toxins

A sufficient supply of oxygen must be available to meet the respiratory requirements of the seed but its supply can be limited by ponded water in the seedbed (Lutman, 1994). The soil pH for winter rape should be greater than 5.6, although some acidity symptoms such as chlorosis, wilting, distorted leaves and/ or pink-purple coloration may be seen below pH 6.0 (Archer, 1981). Acid soils should be limed to a pH of between 6.0 (Ward *et al.* 1985) and 6.5 (Archer, 1981).

1.8 Crop Yield

B. napus cultivars are potentially high yielding (Degenhart and Kondra, 1984) and the theoretical potential yield of winter oilseed rape in Great Britain is approximately 7.56 t ha⁻¹ at 9% moisture (Daniels *et al.*, 1986). However, since the yield can vary widely both with season and location, a mean yield of about 3.25 t ha⁻¹ is achieved in the UK

(Nix, 2000) and in commercial crops, yields in excess of 4 t ha⁻¹ are rarely obtained (Jenkins and Leitch, 1986). The distribution of carbon and nitrogen assimilates is also an important determinant of yield (Thorne, 1985) and growth studies have shown rape yield to be strongly influenced by assimilate availability, pod angle and pod clustering (Gabrielle *et al.*, 1998a), which affect canopy light distribution (Habekotté, 1997).

1.9 Rapeseed

1.9.1 Seed and structure

B. napus seeds are spherical, black or reddish-black in colour and are about 2 mm in diameter (Vaughan, 1970) although the size varies with variety: samples can contain seeds between 1.4 and 2.5 mm in diameter (Ward *et al.*, 1985). The thousand seed weight (TSW) also varies with season and variety but it is usually between 4 and 6 g (Desai *et al.*, 1997). Rape seeds, which contain about 45 to 47% oil and 28 to 30% protein, are carried in pods each containing between 10 and 40 seeds; due to its high oil content, the seed moisture level of oilseed rape is usually 6 to 8% (Appelqvist, 1971).

The seed coat, which is relatively low in protein (15%) and oil (15%) and high in polysaccharides and crude fibre (30%), comprises 15 to 20% of the total seed weight. The seed contains two cotyledons (seed leaves) (Anon 3, 2000) and a hypocotyl (Appelqvist, 1971), which lies between the cotyledons and the root system (Anon 3, 2000).

The cotyledons absorb food reserves from the endosperm during embryo development so that by maturity, the endosperm is almost totally degraded (Anon 4, 2000). Oilseed rape cotyledons contain protein grains as well as storage lipids (Vaughan, 1970) in the oleosomes (Stymne and Stobart, 1987; Lee *et al.*, 1991).

1.9.2 Seed pigments

The cotyledons of developing *Brassica* embryos are rich in chlorophyll until they undergo chlorophyll loss after mid-maturation (Johnson-Flanagan *et al.*, 1991). Although mature seeds only contain small amounts (5 to 10 ppm in the oil) of chlorophyll and related pigments, these pigments are extracted with the oil, darkening it and requiring it to be bleached (Ward *et al.*, 1994).

1.9.3 Seed harvesting

Since rapeseed is distributed throughout the top half of the crop, seed ripening is variable and the optimum harvest time should be when about half of the pods are yellow with brown seeds (Nordgestaard, 1965; Ward *et al.*, 1985). At harvest, the moisture content can be as high as 25% (Desai *et al.*, 1997) but once dried to 8 to 9% moisture (Ward *et al.*, 1985), rapeseed may be safely stored.

1.9.4 Seed oil

About 90% of rapeseed oil consists of about 16 fatty acids including eicosenoic, erucic, linoleic, linolenic, oleic, palmitic, and stearic (Kimber, 1981). Glucosinolates are bitter, inhibit thyroid gland function and inhibit iodine uptake but the defatted meal from low glucosinolate oils can be safely incorporated into livestock feed (Booth and Walker, 1990).

CHAPTER II BACKGROUND TO THE PROJECT

2.1 The Problems of Oilseed Rape Establishment

Over the past fifteen years the area sown to winter oilseed rape has increased tenfold and it now occupies the third largest arable area in the United Kingdom (Darby and Yeoman, 1994). Rape is usually sown during late August to early September and follows cereal crops such as wheat or barley (Baker *et al.*, 1991).

Possibly the most important management practice in crop production is the production of a satisfactory crop stand (Dubetz *et al.*, 1962) and 'establishment' is the point at which a stable plant population has been produced. The establishment period of winter oilseed rape covers the seven-month period between mid-August and March (McWilliam *et al.*, 1998) and it is the culmination of four sequentially linked phases: (a) sowing to germination, during which the seed imbibes water, the seed reserves are mobilised, the seed coat is shed and the hypocotyl and radicle emerge, (b) postgermination growth during which the seedling grows above the soil surface, the stem lengthens and leaves are produced and (c) emergence, during which the seedling cotyledons appear above the soil surface and (d) post-emergence growth during which the seedling develops into a plant that is capable of producing flowers, pods and new seeds.

However, even under ideal conditions about 30% of seeds fail to establish. In poor years establishment failure can cost the UK farming industry about £50 million per year through ploughing up and re-sowing the crops - most of the cost of which is borne directly by farmers (Nix, 1997). This failure to establish is a major problem for the

producer both in terms of manpower and economic costs since the ability of the crop seeds to keep pace with or outstrip the germination and emergence of vigorous weed seeds is perhaps the initial factor that determines crop establishment and the resulting yield for the farmer (Osborne, 1972). By improving seed germination and seedling emergence, it could be possible to increase plant establishment in the field, maximise the growth period before the onset of winter and provide the plants with a better chance of surviving frost damage between December and February.

Good establishment of oilseed rape, that is, the uniform distribution of a number of plants within an optimum range for yield formation, can be difficult to achieve (Stokes, 1999, personal communication). Between autumn and spring, several factors can reduce plant populations (McWilliam *et al.*, 1998) from initial seed germination through to emergence and establishment. Several studies (McWilliam *et al.*, 1995; Bullard *et al.*, 1996; McWilliam *et al.*, 1998) have examined the contributions made by different factors to seedling losses and plant failure in winter oilseed rape.

McWilliam *et al.* (1995) examined the effects of several soil physical characteristics on germination, emergence and survival post emergence. However, it was difficult to determine the individual effects of the soil characteristics because of interactions between extreme temperatures, water excesses or deficits, salinity effects or soil crusting and biological stresses, which can all adversely affect germination and seedling growth (Bradford, 1986).

It is important that rape follows an early maturing crop to allow sufficient time for seedbed preparation and weed control (Almond *et al.*, 1984; Darby and Yeoman, 1994) thus it is important to balance the desire to sow as early as possible to avoid late-sowing

losses, with sowing late enough to allow sufficient time for seedbed preparation. Yield losses can occur if sowing is delayed after the middle of September (Bunting, 1957; Scott *et al.*, 1973) but very early sowing does not necessarily ensure good crop yields since with warm spring conditions, late sown crops can outyield early sown crops (Mendham *et al.*, 1990). The time to full establishment, at which a plant contributes to the final yield, can be more important than the actual time of sowing (Almond *et al.*, 1984).

A firm seedbed helps to control drilling depth and promotes water transfer to the seed while a fine tilth improves seed-soil contact. A well-structured sub-zone provides good drainage and facilitates rapid root growth but sowing immediately after the cereal harvest allows little time for seedbed preparation (Almond *et al.*, 1984). The task of ensuring a friable soil for maximum seedling growth has further been compounded since the straw burning ban in 1992 since the cereal crop residues that remain need to be either removed from the soil or incorporated in such a way as not to adversely affect seedling emergence.

Cereal straw residues in the soil have been associated with poor establishment in oilseed rape (McWilliam, 1998). Incorporated cereal straw can block drills and/ or prevent drill penetration, culminating in poor seed placement (Naylor *et al.*, 1983; Lynch and Elliott, 1984). Cereal residues also have allelopathic effects on oilseed rape since phytotoxic compounds can be released from the decomposing residues (Lynch and Gunn, 1978; Wallace and Elliot, 1979. The decomposition of cereal straw, which has a high carbon: nitrogen ratio, also immobilises nitrogen (Russell, 1973) since the soil fungi and bacteria use it to create organic nitrogen compounds (Jensen, 1929).

Although the wetting and drying cycles that occur during the summer can produce a topsoil that is suitable for rape establishment, heavy cereal-harvesting machinery can compact the topsoil (Eriksson *et al.*, 1974), making it difficult for roots to grow downwards and for seedlings to emerge (Davies *et al.*, 1993). Unstable and very heavy clay soils can also be prone to waterlogging, which can exacerbate compaction problems whereas clay-loam soils can suffer from a lack of water as the soil profile can be dried by the cereal crop. In addition, rainfall during the autumn can be low while the evaporative demands are high, which makes the seed even more vulnerable to desiccation.

The soil moisture content controls seed germination and emergence by influencing the aeration and hydraulic conductivity of the soil as well as the area of seed-liquid contact (Collis-George and Hector, 1966; Ward and Shaykewich, 1972) and germination and emergence are both progressively delayed as the soil water content is lowered (Ashraf and Abu-Shakra ,1978).

2.1.1 Germination

Germination is initiated by hydrating the seed and ends in the formation of a plant with shoot and root systems, which is capable of using inorganic material, water and light energy for normal growth from its external environment (Abdul-Baki and Baker, 1973). Germination is a three-stage process (Hadas and Russo, 1974) by which a seed initiates the formation of a seedling from the embryo (Mayer and Poljakoff-Mayber, 1989): (a) imbibition, during which water absorption occurs, (b) development, during which enzymatic transformation and the initiation of meristematic activities takes place and (c) growth of the seedling, which begins with radicle elongation and emergence through the seed coat (Hadas and Russo, 1974). Germination is the period during which the seed

depends entirely upon its capacity to mobilise and utilise its own resource of stored substrates. From the time that the seed first takes up water until the moment that the seedling emerges above the soil the potential new plant must combat stresses of heat, cold, drought and attacks by pests and pathogens (Osborne, 1972).

Seed imbibition is due to the interactions between proteins, cellulose and mucilage colloids, thus differences in the contents of these components can alter the rate of imbibition. Seeds require an optimal range of moisture below or above which they will either not germinate or, if germination does occur, it will be very poor or slow (Rao and Gupta, 1976). The soil water content influences absorption during the pre-germination stages (Shaykewich and Williams, 1971) since as the soil dries out, less water is available to the plants (Rao and Gupta, 1976); if a germinating seed is slow to imbibe, both emergence and the final crop stand can be impaired.

The rates of water movement across the seed-soil interface and in the seed itself are thus particularly important during early imbibition when the rate of water uptake is highest (Hadas and Russo, 1974). The depletion of oxygen in the seedbed due to water logging results in anaerobic respiration (Brady and Weil, 1996) with the production of acetaldehyde, ethanol and lactate, which are toxic to germinating seedlings (Bradbeer, 1988). Events occurring within the seed as it proceeds from the dry to the fully hydrated state are not well understood (Armstrong and McDonald, 1992) but the seed coat, atmospheric conditions and moisture level can all affect germination (Hepburn *et al.*, 1986).

Variations in the rate of germination also have important implications for crop production in areas such as the semi-arid tropics where there can be prolonged exposure to high temperatures and increased risk of dehydration; clearly a rapid growth rate is advantageous in such environments (Mohamed *et al.*, 1988). Germination is important both from a physiological standpoint as well as from an economic perspective. If we can understand the biochemical and physical way in which seeds germinate and can describe the necessary environmental conditions for germination, it should be possible to manipulate the germination capacity of seeds. Furthermore, since seeds capable of extending a root do not necessarily have the vigour to establish a plant (Temu, 1994), it is also necessary to evaluate the potential of a seed to emerge and establish in the field.

2.1.2 Emergence

The time to emergence describes the post germinative growth and development of a seedling until, for oilseed rape, the cotyledons appear above the soil surface and the stage of autotrophism is reached (Harper, 1983). In order to achieve optimum plant densities in the field, it is necessary to have seeds that can emerge well and quickly under a range of environmental conditions. Early emergence maximises the growth period and a crop that emerges uniformly can be treated more effectively during crop growth and produces more even seed quality at harvest (Halmer and Bewley, 1984). Vigorous seedling emergence is thus important for optimum crop production (Sepaskhah and Ardekani, 1978).

Final seedling emergence is reduced by deep sowing and low moisture content (Sepaskhah and Ardekani, 1978), which could be due to differences in the seed composition, seed imibibition or energy reserve supply. Low soil temperatures during early seedling growth can also increase the probability of soil crusting, which can slow the rate of oilseed rape seedling emergence and can reduce total seedling emergence (Lutman, 1994) and increase seedling disease. The physiological and yield effects of waterlogging, which may occur on clay soils, vary with the different stages of oilseed rape growth with the seedling stage being most susceptible followed by the floral bud appearance and pod formation stages (Zhou and Lin, 1995).

Young oilseed rape seedlings may also be killed before or shortly after emergence by slugs but more typically the leaves are holed and shredded, which reduces the growth potential of the plant. Slugs can cause extensive damage in wet autumn especially on direct-drilled crops on heavier soils (Graham, 1981). Pigeons can cause also cause extensive damage to rape crops by eating freshly planted seeds and newly seedlings.

Rapid and uniform field emergence and the final percentage emergence are essential prerequisites to increase seed yield, crop quality and ultimately profits in annual crops (Wurr and Fellows, 1983).

2.1.3 Establishment

The post emergence phase of seedling growth covers the period from cotyledon expansion until plant establishment and an established seedling is one that has the expectation of reaching maturity (Bradbeer, 1988). In rape, it is considered desirable to have at least five true leaves and a good root system by early December since maximum cold tolerance occurs when the rape plant has achieved approximately eight leaves and a root collar diameter of 8 mm (Lutman, 1994). Uniform crop cover can buffer the crop against variable spring weather (Almond *et al.*, 1984) and may also be able to compensate for later problems that might occur, such as frost during the early flowering period (Bearman, 1981). Frost heave during winter can also kill plants (McWilliam *et al.*, 1998) since ice formation in the soil exerts an upward pressure on the seedling, pulling it out of the ground and/ or causing the stem to break (Miller, 1980).

The crop must also have a deep taproot and extensive lateral rooting, which together are capable of extracting large quantities of nutrients and water during spring drought conditions (Almond *et al.*, 1984). Rape exhibits dehydration avoidance through decreases in the leaf area, photosynthetic capacity and leaf conductance (Jensen *et al.*, 1996). Although water logging can reduce crop establishment (Perkin, 1981), rape plants at the flowering stage, can withstand waterlogging without significant alterations to the yield (Zhou and Lin, 1995).

Rape varieties that are low in glucosinolates are especially palatable to grazing pests such as pigeons and hares and are highly susceptible to attack (Kings, 1999). However, a crop that is well established by the beginning of November should be able to withstand attacks from pigeons and other pests in the winter (Bearman, 1981). Stem canker (*Phoma lingam*) infection (Anon 1, 2000), which may be stubble borne can often be derived from phoma leaf spot (Ward *et al.*, 1985) while blight (*Alternaria*) causes premature ripening, pod splitting and hence seed shedding before harvest (Anon 1, 2000).

Early sowing under suitable conditions allows the earlier utilisation of radiant energy and ensures that the oilseed rape crop achieves a suitable growth stage before the onset of winter (Lutman, 1994). All evidence thus suggests that rapid seedling establishment of oilseed rape is important for successful crop production.

2.2 Improving Seedling Establishment in Oilseed Rape

Successful rape growing depends upon producing uniform plant populations at optimal density. Establishment is a complex interaction of physical, chemical and biological factors but seedbed and seed factors are the main factors influencing establishment. Some yield improvements have been made through influencing weeds, pests and diseases or maximising light harvesting efficiency (Stokes, 1998, personal communication) thus improvements in establishment may be gained by considering the action of some of these factors independently (Stokes *et al.*, 2000). There is, therefore, a good chance that a series of steps can provide incremental gains in seed quality, which together can provide all growers with significantly improved chances of achieving satisfactory establishment (Stokes *et al.*, 1998).

2.2.1 Seedbed management

The seedbed can influence the incidence of pests and diseases but the most important seedbed factor affecting establishment is the physical seedbed from which the seeds will emerge. Seeds have a limited amount of stored energy for growth in the absence of sunlight and they need to emerge and expand their cotyledons before these reserves are exhausted. The presence of impenetrable objects in the soil profile such as stones, large aggregates or straw, which require the hypocotyl to grow around them, can seriously diminish the seed reserves. The seedbed should thus be as free as possible of stones and there should be a fine topsoil through which seedlings can emerge (Lutman, 1994). *Brassica napus* has been reported not to emerge from depths greater than 75 mm (McWilliam *et al.*, 1998) and although the usual planting depth is about 25 mm (Vigil *et al.*, 1997), depending on the seedbed structure, seeds may be placed at depths of up to 40 mm (Glen *et al.*, 1989).

Since the ideal sugar beet seedbed was characterised by Hakansson and Von Polgar (1984), management of this crop has drastically changed and establishment has been greatly improved. However, the establishment of rape in autumn has provided a greater challenge than the establishment of sugar beet in spring and experiments have indicated that a flexible approach to cultivation should be taken with respect to the natural seedbed at the time of cultivation, the stability of the soil, and the amount of cereal residue present (Bullard *et al.*, 1996). The rooting zone for rape may be 1 to 1.5 m below the soil surface so the sub-soil should also be friable, well fissured and well oxygenated with adequate moisture if optimal growth is to be achieved (Lutman, 1994). More specific recommendations for oilseed rape state that the seed must be placed in a firm, moist, warm, aerated and well structured seedbed for rapid germination and seedling growth (Pouzet, 1995).

The incorporation of cereal straw on heavy soils can prove difficult (Graham *et al.*, 1986) particularly if a period of wet weather coincides with the limited time between the cereal harvest and seed sowing. Conversely, dry conditions before sowing can lead to a failure of seeds to germinate in time, the seeds may be killed during cultivation and it can be difficult to obtain a sufficiently fine, firm seedbed, which can further delay germination. Cereal straw that is buried near the soil surface can produce puffy seedbeds with little moisture retention, resulting in poor rooting and patchy establishment (Darby and Yeoman, 1994) and can also temporarily immobilise the soil mineral nitrogen (Russell, 1973) with a consequent reduction in plant growth through the autumn. A possible alternative is to cut the cereal straw and spread it behind the combine so that it can act as a mulch and trap essential soil moisture but it can then directly interfere with the drilling process (Lynch and Elliot, 1984) and result in a loss of seeds.

Ploughing, which incorporates the cereal straw directly into the seedbed, has become an increasingly practised method of seedbed preparation but it can produce fissures down which the small seed can fall and cause excessive moisture loss from the soil. This problem of moisture loss is exacerbated in environments where water is a limiting factor in germination since the cereal straw itself can form a physical barrier between the seed and the soil moisture, preventing essential water uptake by the seed (Davies *et al.*, 1994). Growers often attempt to overcome the adverse effects of a dry soil surface by increasing the sowing depth but this can also reduce seedling emergence (Gul and Allan, 1976).

Most UK rape crops are grown on clay soils, which contain a minimum of 35% clay particles. The choice of cultivation method for autumn seedbed preparation is strongly influenced by the topsoil clay content since these soils are only friable over a narrow moisture range. Soil drying produces strong clods, which require strong cultivation before a satisfactory seedbed can be produced but most clay soils can develop a fine surface tilth during the summer months. Any soil compaction problems resulting from the cereal harvest can generally be alleviated by further ploughing just before seed sowing, which can produce a better established rape crop (Almond *et al.*, 1984).

When compared with discing or ploughing, which can produce very uneven establishment, minimal surface cultivation techniques combined with direct drilling can produce more even establishment and the seeds are able to extract water from greater depths (Almond *et al.*, 1984). Developing a successful method of establishing oilseed rape in the presence of cereal straw or stubble is thus important in maintaining winter rape as a viable break crop in heavy-land rotations (Darby and Yeoman, 1994).

Seedbed and crop management techniques are important factors in improving establishment (McWilliam, 1998) but even under ideal conditions such as those obtained in crop trials, a significant proportion of seedlings fail to establish (Shipway, 1981). Seedbed preparation and crop management techniques can thus solve only part of the problem of establishment and 'seed quality' in terms of specific seed characteristics, may be able to solve at least part of the remaining problem.

The successful completion of each of the three stages of oilseed rape growth is governed by a combination of inherent and environmental factors. Since the successful germination and emergence of *Brassica napus* seeds under ideal environmental conditions are still major obstacles to obtaining a suitable stand (Rao *et al.*, 1987), the seed provides a convenient starting point for improving establishment (Thomson, 1979). If the success of each of these stages in rape growth and development can be improved through targeted "seed management", that is, through pre-sowing treatment of the seed itself, it should be possible to improve establishment of the winter oilseed rape crop.

Defining and assessing seed quality

Seed germination has been defined as "the emergence and development from the seed embryo of those essential structures, which for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions" (Perry, 1978).

Germination tests, in which a sample of seeds drawn from the bulk seedlot is placed under optimum conditions for germination (Hepburn *et al.*, 1986), can be used to predict the potential field performance of seedlings. The speed of germination or germination rate is one the oldest concepts of seed vigour (Maguire, 1962) and the germination test has been widely accepted as a measure of seed quality (Fernandez and Johnston, 1995). Although the production of a visible radicle can sometimes be unsatisfactory as a measure of germination since the seedling, which subsequently develops may be abnormal in structure and incapable of establishing a normal plant in the field, this test remains popular (Perry, 1981).

The warm germination test reflects the stand producing potential of a seed lot under ideal planting conditions (Anon 6, 2001). The correlation coefficients between germination, vigour and seedling emergence following the warm germination test vary according to the species, vigour test method and the field sowing conditions (Wang *et al.*, 1996). The impairment of membrane function can retard enzyme synthesis, reduce respiration and biosynthetic capacity and culminate in a loss of seedlot uniformity. Deteriorated seeds also exhibit reduced field emergence and these changes in seed quality are eventually expressed by an increased incidence of abnormal seedlings, which is another component of the standard germination test (Anon 5, 2001).

However, although germination and hence seedlot viability may initially be high, seed viability declines during seed development and maturation on the parent plant (Bullard *et al.*, 1996; Shephard *et al.*, 1996). Optimal growth conditions are also rarely encountered in the field and upon storage seeds progressively deteriorate (Roberts, 1972a). In view of this, the Association of Official Seed Analysts (AOSA) Vigour Testing Committee proposed that "seed vigour comprises those seed properties, which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions" (McDonald, 1980).

Pollock and Roos (1972) divided seed and seedling vigour into genetic (differences between two or more genetic lines) and physiological (differences between seedlots within one genetic line) components. Perry (1972) also stated that although seed vigour is determined by the genotype, it is modified by the environment and vigour differences have been reported for many crops (Rowe and Gorwood, 1978; Agarwal, 1979; Wright, 1980).

The term "seed viability" thus refers to the germinative capacity of seeds, that is, a high viability seedlot will have a high percentage germination while a low viability seedlot will have a low percentage germination. In contrast, "seed vigour" describes both the rate of the process and the probability that a normal seedling will be produced. A high vigour seedlot thus has a high capacity to germinate rapidly and will produce many seedlings capable of producing a plant under a wide range of field conditions while low vigour seedlots may germinate more slowly to a lower percentage germination and may produce many malformed or damaged seedlings. It is also important to note, however, that not all viable seeds are vigorous: it is possible that although a seedlot may have low viability, that is, few seeds germinate, the few seeds that germinate may do so rapidly and thus their individual seed vigour is high (Mohapatra *et al.*, 1987).

Seed quality is thus affected by the seed viability and vigour (Barla-Szabo *et al.*, 1990; Makkawi *et al.*, 1999) as well as by the sowing date and growth environment of the crop and even the end-use of the oil or meal that is to be produced. The ability of developing seedlings to survive partially depends on the initial vigour of the embryo contained within the seed (Roberts, 1972b). The guide to the germination potential of winter oilseed rape is that more than 85% of four replicates of 100 seeds per replicate

will germinate at 20°C (International Seed Testing Association, 1993) under controlled conditions.

Vigour tests distinguish between seeds of different levels of vigour with seedlots that have a high germination capacity and rapid, uniform emergence being termed 'high vigour' and those that emerge poorly and slowly being termed 'low vigour' seeds (Alsadon *et al.*, 1995). Vigour testing procedures have employed two basic approaches to predict stand establishment potential: stress (direct) tests and parameter (indirect) tests (Wilson and McDonald, 1986). During direct (physiological) testing, environmental stresses that are expected in the field are reproduced in the laboratory and the percentage and rate of seedling germination or emergence are recorded. During indirect (biochemical) testing, characteristics of these seeds which have proved to be correlated with an aspect of field performance such as respiration rate, topographic tetrazolium test or seed conductivity are measured (Perry, 1978).

The objectives of seed vigour testing are to provide the grower with an estimate of the planting value of the seed (Perry, 1981) and it has become vital to quality control since both the seed producer and farmer need accurate portrayals of the potential field emergence. Vigour testing predicts the performance of the seeds and seedlings in the field but the type of test used depends on the seeds, the test conditions and the potential uses of the seeds. The most common tests are the cold germination, controlled deterioration and accelerated aging tests (stress tests) and the tetrazolium and electrical conductivity tests (biochemical tests), each of which evaluates different seed qualities (Anon 6, 2001).

The cold germination test is often used to measure the ability of maize seeds to germinate under high soil-moisture content and low soil temperature. This test simulates early season adverse field conditions and usually represents the lowest germination that would be expected from a seed lot planted under such conditions. The final percentage germination of the cold test often represents the lowest emergence that would be expected from a seedlot when planted under reasonably satisfactory field conditions whereas the warm germination test represents the highest emergence potential that could be expected (Anon 6, 2001).

During the controlled deterioration test seeds are allowed to imbibe to a specific water content, typically around 20% moisture. The seeds are sealed in foil overnight at 10°C after which they are incubated in water at 45°C before being germinated at 20°C. This test has been used to predict the field emergence turnip, swede, kale, cabbage, cauliflower, sprouts, sugar beet, carrots, lettuce and onion and is superior to the cold germination test in terms of its prediction of field emergence (Perry, 1981; Powell and Matthews, 1981b; 1984).

The accelerated ageing (AA) test is used to evaluate seed storability and predict field emergence since high vigour seedlots deteriorate at a slower rate than low vigour seedlots. The AA test records the percentage germination of normal seedlings after a period of storage under high temperature and seed moisture conditions. When compared with the standard germination test results of the same seedlots prior to ageing, the AA germination will be either similar to the standard germination if the seedlot is highvigour or less than the standard germination if the seedlot is low-vigour (Anon 7, 2001).

The tetrazolium test is based upon the reaction of tetrazolium with active dehydrogenase to produce red formazon. Colouration of a cell by tetrazolium is a definite indication of its viability because necrotic, non-respiring cells remain uncoloured (Lakon, 1948). This test gives an indication of viable, abnormal, and dead seeds in the seed lot but although it is highly reliable for determining viable seeds of maize, wheat, oats, barley and other grasses, it is limited in its ability to estimate seed vigour (Anon 6, 2001).

The bulk electrical conductivity test has been widely used to identify low vigour dwarf bean (Oliveira *et al.*, 1984) and pea (Powell and Matthews, 1981b) seedlots. Low vigour seedlots leak high levels of solutes, including electrolytes, the concentrations of which may then be used to predict the germination capacity of seeds (Pandey, 1988). Although this test has been accepted as the vigour test for peas (Matthews and Bradnock, 1968; Powell and Matthews, 1981b), measurements from individual seeds have sometimes been shown to be unrepresentative of whole seedlot germination and unable to differentiate between non-viable and viable seeds (Hepburn *et al.*, 1984).

Although there has been a demand for more realistic vigour testing to provide better indications of the planting value of seedlots (TeKrony and Egli, 1991) and seed vigour testing is recognised in the ISTA rules (Powell, 2003, personal communication), the germination test has remained popular (Abdalla and Roberts, 1968). The germination tests used in this research determined the germination potential at a range of temperatures between 5 and 25°C.

The question that we must now ask is whether we can improve the performance of seed in these initial stages of germination during which the embryo is living entirely at its own expense. Anything that can be done to hasten the first stage or to achieve stage one before the seed is sown will speed emergence of the seedling and increase the chances of establishing a good crop stand (Osborne, 1972).

2.3 Seed Treatment Methods to Improve Crop Establishment

2.3.1 Seed hydration treatments

One group of seed treatments aims to improve the rate and uniformity of germination and emergence (Heydecker *et al.*, 1975) through pre-soaking seeds in water. The main techniques, which have been developed to improve the rate and uniformity of emergence are (a) 'priming' or 'osmopriming' of seeds in low water potential solutions of organic and inorganic solutes (Cantliffe and Elballa, 1994) and (b) 'advancement' or 'hydropriming' seeds at low to moderate temperatures (Armstrong and McDonald, 1992).

Priming involves the controlled imbibition of seeds such that the seed water content can be adjusted to a level that permits the seeds to go through all the essential preparatory processes of germination but prevents cell elongation and hence radicle emergence (Cantliffe and Elballa, 1994; Duthoit, 1999). The treated seeds are then dried back and stored and when sown in a germination medium, their radicles emerge rapidly and almost simultaneously even at low temperatures (Heydecker *et al.*, 1975). Priming has been used to treat many vegetable and flower species (Parera and Cantliffe, 1994b) such as beet (Khan *et al.*, 1983), celery and onion (Brocklehurst and Dearman, 1983), pepper (Bradford *et al.*, 1990), tomato (Argerich *et al.*, 1990) and sugar beet (Durrant *et al.*, 1993). Salt solutions such as potassium nitrate (Argerich and Bradford, 1989) can be as effective as polyethyleneglycol (PEG) in seed priming but considerable volumes of solution are required and their cost is high. To reduce the volumes of solution required, solid matrix priming (SMP) was developed (Claypool *et al.*, 1996) to allow large amounts of seeds to be efficiently primed at one time (Parera and Cantliffe, 1994a) while enclosed in a gel matrix. Drum priming is a similar technique in which seeds are hydrated to the desired moisture level by using limited amounts of water and specific soaking durations rather than osmotic solutions (Warren and Bennett, 1997).

The major benefit of seed priming is the reduction of the mean germination time (Dell'Aquila and Tritto, 1991) although there is considerable discussion as to the reasons for the improvement of germination. Hydration treatments have commonly been assumed to allow the onset of some of the germination processes such that following hydration and drying back, the seeds are more "advanced" in terms of their stage of germination and hence they are able to germinate more promptly than non-hydrated (control) seeds. However, since the beneficial effects of hydration treatments are most clearly observed in low vigour seedlots, which are more deteriorated and damaged than high vigour seedlots, it is perhaps more likely that the period of hydration allows repair mechanisms to repair some of the damage to cell contents.

Some oilseed rape seedlots naturally have low percentage germination and poor seedling vigour and the effect of priming oilseed rape on both petri dish germination and sandy-loam emergence in the controlled environment was investigated by Zheng *et al.* (1994). They reported that priming enhanced both seed germination and seedling emergence from soil at 10°C. Seed priming techniques thus have the potential to improve oilseed rape seed germination and subsequent seedling emergence under low

soil temperature conditions (Zheng *et al.*, 1998). They may also reduce the risk of poor stand establishment in cold and moist soils and allow earlier planting of slow emerging *Brassica* cultivars to provide forage during the spring forage deficit period (Rao *et al.*, 1987).

The ideal seed hydration system would bring the seed to the desired seed moisture content using only water (Warren and Bennett, 1997), however, which is cheaper than the osmotica required for priming and does not present disposal problems. Seed hydration methods in which seeds are hydrated at 15 to 25°C for various times with a limited amount of water are particularly effective in improving the percentage germination of old seeds and have also been shown to impart drought tolerance (Henckel, 1964) and improve the storage life of seeds (Basu, 1976; Basu and Dhar, 1979).

Pre-soaking hydration treatments have been reported to improve the vigour of low quality pea seeds before ageing and treated seeds have been observed to maintain their viability and vigour for a longer time than control seeds (Savino *et al.*, 1979). However, the specific treatment conditions must be optimised by trial and error for each seedlot since the size, structure, biochemical constitution, position of seed, protecting layers and the soaking time influence solution penetration into the embryo (Parera and Cantliffe, 1994b). Furthermore, although both priming and seed hydration can reduce the time to 50% germination, the period of drying after treatment may adversely affect seed germination (Berrie and Drennan, 1971). For this research seed hydration in water was used since germination experiments with PEG did not offer any significant improvements over water (Noon, 1997).

It is generally assumed that seeds have sufficient nutrient reserves to carry them through germination and early seedling growth (Wood, 1990) yet within a seedlot there can be a wide distribution in the size of individual seeds. Several studies have investigated the effects of seed size selection on plant growth and development from germination through to harvest and differences in the germination rate and vigour of different sized seeds have been noted both among species and between varieties of the same species (Kneebone and Cremer, 1955; Austin and Longden, 1967; Salih and Salih, 1980; Shephard *et al.*, 1996).

Black (1956) showed that clover cotyledon weight could be varied by using seeds of different sizes as the larger the seed size, the greater the cotyledon area. Kneebone and Cremer (1955), hand separated several grass caryopses and evaluated the vigour of the seedlings that were produced by the various seed sizes in several plant media both in the greenhouse and in the field. In terms of the vigour score, speed of emergence, stand, height and forage production in the early stages of plant growth, they found that as the seed size increased, the seedlings became more vigorous, emerging faster and growing at a faster rate.

Seed size can affect the yield as well as other characteristics of several field-grown crops and there can be considerable benefits of selecting large seeds over mixed or small seed samples. Kiesselbach (1924) found that wheat and oat seedling vigour was related to seed size with the more vigorous seedlings producing more vigorous plants unless some environmental factor differentially affected the performance of the plants. Major (1977) similarly reported that large (> 2.22 mm in diameter) rape seeds produced more vigorous seedlings with larger leaves and larger plants than small seeds although

there was no effect on the final yield. However, although for the most part large, heavy seeds produce plants of greater size and higher yield than small seeds, in sweetcorn, for example, there is no distinct germination advantage in choosing large or small kernels (Hoffman, 1925). In wheat, selection for seedling vigour can thus be achieved by selecting for seed size, speed of emergence and rate of plant development (Lafond and Baker, 1986). The technique of seed size selection has long been practised on a wide variety of crops but relatively little work has been performed on the effect of seed size selection on the germination and emergence capacity of oilseed rape.

2.3.3 Heat treatment

Microorganisms and pests in the seed or in the environment can destroy seeds and seedlings resulting in a low plant stand and crop failure (Jeffs, 1986). Heat treatment methods were originally developed to ensure seedling emergence and subsequent plant establishment through reducing the level of pathogens either within the seeds or on the seed testa. Their original mode of action was to expose the seeds to temperatures that could kill pathogens without reducing seed viability and there were three main methods: (a) hot water, (b) steam-air mixture and (c) dry heat.

The method of heat treatment that can be successfully employed depends upon temperature and duration of the treatment that can be successfully resisted by the seed (Baker, 1969). Hot water treatment was developed by Jensen in 1883 to control blight (*Phytophthora infestans*) on potato seed tubers and loose smut fungi (*Ustilago avenae* and *Ustilago nuda*) on oat and barley seeds (Priestley, 1986). After further development by Bant and Storey (1951) to control *Septoria* on celery seeds, it was used commercially in the United Kingdom until replacement by the thiram soak method (Maude, 1964).

In America and Australia, hot water treatments were used extensively to control black rot *(Xanthomona campestris)* and black spot *(Alternaria brassicola)* infections in cauliflower and other *Brassica* seeds (Walker, 1923) but since all the seeds must be raised to the same temperature at the same time it was only possible to treat a small amount of seeds at any time. In addition, the germination of older or low-vigour seeds was reduced (Bant and Storey, 1951) and the length of the heating period varied with both the moisture content of the grain and the temperature, with moist seeds being more susceptible to thermal damage than dry seeds (Watson, 1970). Hot water treatments also released high levels of water-soluble materials, which ruptured the seed coat or rendered them sticky; accurate temperature control was easier to achieve with aerated steam.

Steam-air treatment was originally developed for soil sterilisation but its use was extended to seeds in particular, flower seeds, which were commonly subjected to temperatures of 56 to 67°C for 30 minutes (Baker, 1969). Aerated steam has been used to kill the eggs and larvae of the Mediterranean fruit fly, bulb flies and stem and bulb nematodes and to commercially treat citrus fruit, vegetables, strawberries, bulb crops and ornamentals (Smith, 1966). This method reduces leaching and since the seeds are cooled by evaporation, the problem of seed drying is avoided (Baker, 1969).

As with all the other forms of heat treatment, seed thermotherapy relies on the tolerance of bacteria, fungi, and parasites to heat as well as on the resistance of the plants or seeds to the temperatures being used (Baker, 1969). Baker (1972) also used thermotherapy for the control of leaf spot (*Phoma betae*) on beet seeds and blackleg (*Phoma lingam*) and black spot (*Alternaria brassicola*) on cabbage seeds and it has also been used to treat parsnip (Smith, 1966), sweet corn and tomato (Navaratnam *et al.*, 1980). Thermotherapy has not been widely used commercially (Baker, 1969; Navaratnam *et* al., 1980) but there has been renewed interest in this method particularly in Japan (Nakamura, 1975; Sorensen, 1995; Bell, 2002).

Hot water and steam-air methods of heat treatment allow contact between the seed and moisture whereas dry-heat methods have the opposite effect of drying the seed and the combination of temperature and reduction in seed moisture kills the pathogen. There has been much research into the effect on seed viability of high temperature storage but it is important to differentiate between heat treatment through seed storage at elevated temperatures from dry heat treatment methods followed by storage under cold (5°C) or ambient (20°C) conditions.

Dry-heat methods of treating seeds were originally developed to eliminate internal seed infections. and seeds were commonly immersed in a solvent such as carbon tetrachloride at a raised temperature for a given duration. In principle, the solvent allowed the passage of heat into the seeds without itself penetrating their tissues but because the moisture content of infected seeds was low, both the pathogens and the seeds were equally tolerant of dry heat. It was thus difficult to find a temperature that would eliminate the pathogen but not adversely affect the seeds and solvent-based or solely dry-heat treatments have had little practical use.

However, solvent-free dry-heat treatment of tomato seed for 70 days at 70°C, has been shown to free seeds from both externally and internally-borne tobacco mosaic virus (Broadbent, 1965; Rees, 1970). The effect of the same dry-heat treatment on the germination of Lucerne seed were investigated at the National Institute of Agricultural Botany (NIAB). Seed samples were dry-heated in an incubator at between 35 to 50°C for 4 to 6 days before being tested for germination, greenhouse emergence and field

establishment. The results indicated that the higher the temperature the shorter the duration of treatment required (Zalewski, 1957). Furthermore, for seeds in general, the thermal tolerance to heat treatment declines with age resulting in a reduction in the average seedling emergence; low vigour seeds are also less resistant to heat treatment techniques than high vigour seeds (Baker, 1969).

It has generally been assumed that the drying process itself has no harmful effect on the seeds but studies on beans (*Vicia faba*) have shown that bean seeds cannot be dried extensively without damaging the seeds. The resistance of seeds to extreme drying was probably first examined by Nobbe in 1897, who reduced the water content of rye to 1.2% by drying it at 80°C in ovens (Priestley, 1986). At temperatures that did not reduce the moisture content of the seeds below 5 to 6% there was very little effect upon subsequent germination but when higher temperatures were used, the moisture content fell considerably lower than this, and seed germination was often reduced.

Ewart (1908) dried wheat, maize, barley, peas, haricots, hemp, squash, rape and sunflower seeds in a vacuum desiccator at 37 to 38°C and concluded that it was impossible to reduce the water percentage held by even the most resistant seeds to lower than 2 to 3% of their dry weight without injuriously affecting their vitality. However, this was in contrast with observations by Harrington (1918) who dried two types of barley, grass and wheat and to 1% moisture or less without injury and in the case of Johnson grass seed, reduced the seed moisture to 0.1% without injury. Furthermore, although the germination of the control seedlots was more prompt than those of the dried seedlots, the differences were scarcely perceptible after the second day of the germination test, the delay probably being due to an increase in the time required for imbibition before germination could begin. None of the seedlings produced were kept

for further regrowth but Harrington saw no reason to suppose that the dried seeds would produce any less vigorous plants than those which were not dried. Rees (1970) later showed that tomato plants produced from heat-treated seeds appeared very similar to control plants in their growth and development.

Experimental work in which seeds in the air-dry condition have been submitted to high temperatures falls into three categories. In the first category, researchers considered the effect of high temperature treatments on germination, for example, in 1907 Atterberg showed that if poorly germinating barley seeds were exposed for a short time to relatively high temperatures they at once increased their germination capacity (Priestley, 1986). In the second category, heat treatment has been investigated as a simple method of sterilising seeds (Broadbent, 1965; Rees, 1970) and finally, the pre-determining effect upon growth and yield of exposing seeds to relatively high temperatures have been assessed (Wollny, 1879, 1885).

This research has combined the first and last of these categories by assessing the effects of exposure to high temperatures and extreme drying on germination, emergence and field establishment of winter oilseed rape.

2.3.4 Mother crop nitrogen management

Mother crop management to improve seed quality and hence subsequent establishment can include manipulating the sowing date, site, season, position, seedbed and rate of fertilisation. However, management techniques, which result in early nutrient uptake can affect seedling size and thus improve the ability of seedlings to survive stress and compete with weeds (Wood, 1990). Crop management techniques, can improve the nutrient use efficiency of seedlings but current agronomic practices tend to produce crops that are significantly larger than the optimum size through early sowing at high seed rates with plentiful nitrogen fertilisation.

Mother crop nitrogen management has been reported to increase the seed yield and protein content of several crops including sugarcane (*Saccharum officinarum*) (Kumar and Singh, 1996) and canarygrass (*Phalaris canariensis* L.) (Holt, 1988) while for crops such as creeping red fescue (*Festuca rubra* L. var. *rubra*) (Fairey and Lefkovitch, 2000), no effects have been reported. Nitrogen fertiliser has a dominant effect on the number of pods initiated and the size of the rape canopy but although it has been shown in some cases to increase the rape seed yield (Lammerink and Morice, 1970), this is not always the case (Lunn *et al.*, 2001).

Effects of mother crop nitrogen management (amount of nitrogen) on the seed protein and oil content have been reported for a number of crops including oilseed rape (*Brassica napus*) (Hocking *et al.*, 1997). Sugimoto *et al.* (1998) also observed that the timing of mother crop nitrogen application could significantly affect the contents of storage compounds in soybean (*Glycine max*) seeds: nitrogen application at flowering decreased the contents of total and some amino acids (glutamine and asparginine) in developing seeds except for at the early maturation stage and decreased the protein content of mature seeds.

The amount of nitrogen required by a rape crop usually varies from between 30 (Lutman, 1994) to 40 kg ha⁻¹ (Desai *et al.*, 1997) to as much as 240 kg ha⁻¹ (Ward *et al.*, 1985) although there may be sufficient soil mineral nitrogen (SMN) in some soils. Although nitrogen managed crops have many advantages in terms of improved competition against weeds and resilience to pigeon damage, the production of such

crops can reduce the profitability of oilseed rape due to the extra costs of unnecessary inputs (Lunn *et al.*, 2001).

Selecting and pre-treating seeds can significantly improve performance but there is often little time between harvest of the seed crop and drilling of the following commercial crop to implement these procedures. If it can be demonstrated that there are differences in seed quality within the crop canopy, however, then it may be possible to manipulate the mother crop to improve seed performance.

2.4 Aims, Objectives and Hypotheses

The establishment of oilseed rape is difficult and can account for up to 50% of the total cost of growing the crop. Establishment problems are often due to small seeds and poor seedbed conditions on the heavy clays on which 60% of the national crop is grown (Stokes *et al.*, 2000). The overall aim of this thesis was to assess the potential for improving the germination, emergence and field performance of winter oilseed rape.

The experimental research conducted for this thesis was designed to:

- Examine the inherent variation in germinative capacity between different varieties of winter oilseed rape and between different seedlots of the same variety of oilseed rape as measured by the T_{10} , T_{50} , FP and rate of germination.
- Examine the effects of seed hydration on germination and post-germinative taproot growth in the controlled (growth room) environment at 5 and 15°C and on germination at 20°C and emergence at 15°C from 10 mm sowing depth in the controlled environment.

- Examine the effects of seed size selection on germination in the controlled environment at 20°C, emergence from 10 mm sowing depth in the controlled environment and establishment and plant growth and development in the field.
- Examine the effects of seed heat treatment on germination in the controlled environment at 15 and 25°C, emergence from 20 mm sowing depth in the semi-controlled environment and establishment and plant growth and development in the field.
- Examine the effects of mother crop nitrogen management, pod position in the canopy and harvest date on the seed nitrogen and oil content, seed size, thousand seed weight and seed yield of the Apex variety of winter oilseed rape.
- Examine the effects of mother crop nitrogen management on germination in the controlled environment at 15°C, emergence from 20 and 40 mm sowing depth in the semi-controlled environment and establishment and plant growth and development in the field.

It was hypothesised that both inter- and intra-varietal differences in germination response would be observed, that hydrated, large, high nitrogen and heat-treated seeds would have superior germination and emergence responses than non-hydrated, small, low nitrogen and non-heat-treated seeds.

CHAPTER III MATERIALS AND METHODS

3.1 Experimental Methods

3.1.1 Seed material

The "1996 seedlots" were commercially available in 1996 and were supplied by CPB, Twyford. No details of the growing locations in which seed varieties or individual seedlots had been produced were provided.

For the "1997 harvest" and "1999 harvest" seedlots, differences in the seed nitrogen and oil percentage were produced by different mother crop nitrogen management regimes. Both the 1997 and 1999 harvest Apex seedlots received single nitrogen fertiliser applications either in February or in March or a split-application with half the nitrogen applied in February and the other half in March. The levels and timings of nitrogen application to the mother crop and their subsequent effects on the seed nitrogen and oil percentage are described in the germination results chapter.

3.1.2 Seed hydration

For seed hydration, each 3 g seedlot sample was placed in a different plastic beaker containing 100 ml distilled water. The seeds were soaked for a specified duration at 15°C after which time they were removed from the water and blotted dry with paper towels before being dried back in the laboratory environment at 20°C. The moisture content of the seeds was not tested after drying back.

Where the method of drying the seeds (drying-back) following seed hydration was investigated, the seeds were either placed in a convection oven at 20°C until dry (slow
drying) or in a wire mesh holder while a hairdryer was passed over them at 20°C until they were dry (rapid drying).

3.1.3 Seed size selection

Samples from each seedlot were hand-sieved through an Endecott's metal 2-mm diameter round-hole sieve. The seed fraction that did not pass through the sieve (> 2 mm diameter) was termed "large seed" and the fraction that passed through the sieve (< 2 mm diameter) was termed "small seed".

3.1.4 Seed heat treatment

For heat treatment, 3 g samples from each seedlot were placed in paper bags and were heat-treated in a Raven convection oven at 80°C for 48 h. After seed treatment each sample was stored in a paper bag in the laboratory before being used

3.1.5 Germination tests

The seeds, which were hand-counted for each replicate, were placed on two Whatman No. 1 filter papers in an 8.5 cm diameter triple-vented Sterilin Petri dish. The filter papers were standardised since previous experiments have shown that Lehmann lovegrass (*Eragrostis lehmanniana*) seeds germinated to a higher percentage on Whatman No. 2 filter papers than on Whatman Nos. 4 and 5 (Wright, 1973). Even different lots of Whatman No. 1 filter paper influenced the germination of mouse-ear cress (*Arabidopsis thaliana*) seeds (Rehwaldt, 1968): wide fluctuations in germination from 12% to 92% were observed, which coincided with changes in the filter paper used as the seed substratum.

At the start of the germination test 6 ml of distilled water was added to the seeds in each Petri dish. An initial experiment determined that under conditions of between 2 and 8 ml water per 25 seeds there was no significant effect on the final percentage germination that was achieved (data not shown). Although clumping is not generally a problem with small, round seeds (Baskin and Baskin, 1998), the seeds were spaced out on the filter to avoid increases (Linhart, 1976; Waite and Hutchings, 1978) or decreases (Bergelson and Perry, 1989) in the percentage germination.

The Petri dish lids were replaced and the dishes were moved from the laboratory to the growth room. The seeds were germinated in darkness (Kuraś *et al.*, 1999) with the first germination count after 12 to 14 hours followed by subsequent counts at regular intervals until either all the seeds had germinated or until 8 days after the start of the experiment. A seed was regarded as having germinated when the radicle had emerged from the testa and had grown more than 1 mm in length.

Heat treatment resulted in extensive cracking of the seed testa, which loosened quickly upon imbibition and fell off the seed, exposing the cotyledons. It was not possible to apply the standard germination definition to the majority of heat-treated seeds since the undeveloped radicle that was exposed once the testa had been discarded, was already approximately 1 mm in length. Germination for these seeds was thus defined as "growth of the radicle of more than 2 mm". The number of seeds for which this was necessary varied with a maximum of 63% split seeds observed in the high nitrogen, early harvested seedlots and 10% in the high nitrogen late harvested seedlots; the low nitrogen seeds suffered from an average of 61% split seeds.

After 8 days, three categories of seed germination were identified in accordance with Hepburn *et al.* (1984): (1) normal seeds or seeds with slight defects, (2) abnormal seeds with moderate abnormalities and (3) dead seeds, which either failed to germinate and rotted in the Petri dish or germinated with stunted and/ or discoloured radicles, which subsequently failed to grow. In this research only normal or dead (non-viable) seeds, which failed to germinate and rotted in the Petri dish were observed.

3.1.6 Emergence tests

The emergence experiments were performed in either the controlled growth room environment at 15 or 20°C or in a polythene tunnel during August 1998 and 1999, which allowed diurnal daylight and temperature variations and some pigeon damage but offered protection against wind and rain. The seeds were sown in 5" diameter plastic flowerpots and two Whatman No. 1 filter papers were placed in the bottom of each pot to ensure that the growing medium was not released from the bottom of the pot. The growing medium was then used to fill the pots to the desired sowing depth.

The sowing media were either horticultural grade silver sand or perlite. Horticultural silver sand was initially used as the growing medium but because the level of compaction could not easily be controlled, perlite, which was thoroughly wetted before use, was used in later experiments. Perlite is an inert volcanic product, which is less prone to water logging and compaction than either silver sand or soil although the bulk density was not measured. Although perlite is less comparable with field conditions than either sand or soil, previous experiments (data not presented) recorded low emergence from horticultural sand because of its high compaction and low aeration.

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For each replicate, the hand-counted seeds were placed on the surface of the growing medium before the plant pot was filled to the top with more of the same wetted medium. The seeds were watered each day so that the growing medium was at its water capacity to ensure that water was not a limiting factor in emergence. The number of seedlings was counted once each day until either constant emergence was achieved or until 17 to 20 days after the start of the experiment. A seedling was considered to have emerged when both of the cotyledons were visible, although not necessarily expanded, above the surface of the growing medium after watering.

Growth analyses consisting of the mean area and weight of the cotyledons were performed on the seedlings that were grown in the polytunnel using a Licor Li-3100 area meter, which was cleaned between each sample. In addition both the number of seeds that germinated but did not emerge and the number of seeds that had not germinated after 17 to 20 days were recorded. The 17 to 20 day emergence period allowed enough time for even the slowest germinating seedlings to emerge as well as enabling both the emergence test and the subsequent growth analysis to be completed in a three-week period in the middle of August to minimise temperature fluctuations.

3.1.7 Field tests

The field experiments took place on the University Farm in Sutton Bonington on a sandy-clay-loam field (Fladbury series soil) with residual soil mineral nitrogen of 165 kg ha⁻¹. The seed samples were counted using a Contador or Pfeuffer seed counter and since fertiliser was not being applied to the crop, the high seed rate of 220 seeds m⁻² was used to minimise the effect of plant losses due to pests such as pigeons, rabbits and slugs. The field, which had previously been left fallow, was ploughed prior to drilling. The seeds were direct-drilled to a depth of 20 mm, which was slightly less than the

usual depth of 25 to 30 mm (Vigil *et al.*, 1997), with a Oyjard drill operated by the university farm employees on 6 October 1999.

There were four replicates of each treatment and the field plots, which were 1.5*10 m in size were arranged in randomised blocks. Three metal quadrats each with an area of 0.75 m⁻² were placed at random along the length of each plot and the emergence of each seedling within each of the quadrats was recorded at regular intervals for 26 days. The newly emerged seedlings were marked by placing a coloured cocktail stick next to each seedling with different coloured sticks after each interval, which allowed new seedlings as well as those that had been lost to be identified. Humming tape was used to minimise rabbit and pigeon damage but the plants were otherwise subjected to the normal weather routines of temperature, wind and precipitation. No fertiliser was applied to the plots and they were not sprayed against weeds or pathogens.

Three growth analyses (GA) were performed during the growing season. The first growth analysis was during winter in February at growth stage 1.2, which enabled the assessment of plant loss over winter and initial plant size, the second analysis was at flowering in May at growth stage 3.0 and the third analysis was in July just before the harvest at growth stage 6.5. For the first two growth analyses all of the plants within one of the three quadrats of each plot were removed and all plants therein were analysed. For the last growth analysis the remaining quadrat was sub-sampled. The plant samples were stored in the cold store at 4°C while the growth analysis was being performed. The values for each parameter were then calculated for an area of 1 m² and 1 hectare.

The numbers of cocktail sticks that were used in the emergence counts and the numbers of plants and stems were hand-counted. The fresh weights were measured using a Sartorius Model 2574 (Calibration Precision Balance Services) balance and the dry weights were recorded on the same apparatus after the samples had been placed in paper bags in a Gannet Model L6499 (LTE Scientific) oven at 80°C for 48 hours. The leaf area was measured using a Li-3100 (Licor) area meter, which was cleaned before use and between each sample. The stem areas were calculated using the same apparatus but the values were multiplied by $\pi/2$ (McWilliam, 1998) to produce values for a cylinder; the root and stem lengths were measured using a ruler.

The chlorophyll a and b and total chlorophyll contents of the leaves and stems were determined by spectrophotometry. A 5 g sample of leaf was ground in a pestle and mortar with 10 ml acetone and 1 g acid-washed sand. The sample was then centrifuged in a Centaur 2 MSE (Shelton Technical Ltd.) centrifuge at 13,000 revolutions per minute for 10 minutes. 1 cm³ of the supernatant was removed from the centrifuge tube and placed in a plastic Fisher cuvette. The absorbances (Abs) of the supernatant at 663 nm and 645 nm were measured with a CECIL 2000 series (CE2041) spectrophotometer and were recorded. The amounts of chlorophyll a, chlorophyll b and the total chlorophyll content were calculated as shown in equations 3.1.7.1 to 3.1.7.3.

Equation 3.1.7.1	Calculation	Calculation of the chlorophyll a content		
Chlorophyll a content	Chl _(a) =	(0.0127*Abs ₆₆₃) - (0.00269*Abs ₆₄₅)		
Equation 3.1.7.2	Calculation	of the chlorophyll b content		
Chlorophyll b content	Chl _(b) =	(0.0229*Abs ₆₄₅) - (0.00468*Abs ₆₆₃)		
Equation 3.1.7.3	Calculation	of the total chlorophyll content		

Total chlorophyll content $Chl_{(a+b)} = (20.2*Abs_{645}) + (8.02*Abs_{663})$

3.2 Seed Analysis

The seed nitrogen and oil contents were determined at the ADAS laboratories in Wolverhampton in 1998 and 1999 as described below.

3.2.1 Seed nitrogen determination

The dry Micro-Dumas combustion analysis for total nitrogen in solid phases is based upon the transformation to the gaseous phase by rapid and complete flash combustion of the sample material as described below by Kirsten (1997). The plant samples were dried in a Gannet Model L6499 (LTE Scientific) oven at 80°C for 24 hours before being ground in a Cyclotec Model 1093 (Foss Tecator) sample mill to talcum powder consistency (< 250 μ m). A 2 to 4 mg sample of powder was sealed into a 10*10*10 mm tin capsule, which was placed into the combustion apparatus. Each sample was delivered separately into a quartz combustion tube containing granulated chromium III oxide catalyst held at 1200°C. A pulse of pure oxygen was admitted with each sample. All the combustible materials in the sample were burned and a constant stream of nonreactive helium swept the resulting gaseous combustion products out of the bottom of the furnace.

All the carbon in the sample was converted to carbon dioxide during the flash combustion. The nitrogen-bearing combustion products (including N_2 and various oxides of nitrogen, NO_x) were passed through a reduction column filled with chopped copper wire at 600°C. Water vapour from the sample was removed by a gas trap containing magnesium perchlorate and the carbon dioxide was removed by a second gas trap containing a carbon dioxide scrubber (sodium hydroxide on silicate carrier granules).

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Nitrogen eluted from the gradient coil (GC) column followed by the carbon dioxide and the sample gas pulses and a separate reference stream of helium was passed through a detector. The differences in the thermal conductivity between the two streams were displayed as visible peaks and were recorded as numerically integrated areas. Linear regression was then applied to the combustion of known standard materials, which yielded a regression line from which the peak areas of the unknown samples were converted into total element values for each sample.

The NA2000 Elemental Analyser (Fisons Instruments) was calibrated with solid phase reference materials in the tin capsule stage at the beginning of each run and at fixed intervals thereafter. Atropine (84% nitrogen) was used as the reference material and the total nitrogen percentage of the sample was calculated as shown in equations 3.2.1.1 and 3.2.1.2. Empty tin capsule blanks were also included periodically in each batch and any detectable carbon or nitrogen was subtracted from the sample and reference values to give a true zero baseline. Blanks allowed for the correction of traces of carbon originating from the tin capsules and for the small amount of N₂ gas that was introduced as an impurity in the oxygen pulse.

Equation 3.2.1.1	Calculation of the calibration factor, k
Calibration factor	k = (standard value/ peak area)*sample weight
Equation 3.2.1.2	Calculation of the nitrogen percentage
Nitrogen percentage	N = ((k*peak area)/ sample weight)*100

Analysis of the seed oil percentage was performed using nuclear magnetic resonance (NMR) as described below by Hornak (2003). The NMR samples were prepared by dissolving each analyte in a deuterium lock solvent in a 5 mm glass NMR tube, which was then placed within the resonant frequency (RF) coil of the probe inside the shim coils. The RF coils produced the magnetic field necessary to rotate the spins by 90° or 180° while the probe sent energy into the analyte and detected the emanating signal. The RF coils were tuned for each sample since each coil had a specific range of frequencies at which it resonated. The source RF produced a sine wave of the desired frequency, which was set by the pulse programmer. The RF amplifier increased the pulse power while the computer controlled shape and amplitude of gradient fields. The gradient amplifier then increased the power of the gradient pulses to a level sufficient to produce the necessary gradients in the magnetic field. The operator of the spectrometer gave the inputs a pulse sequence via the console terminal and the NMR spectra of the analyte were displayed on a video display; hard copies were printed out.

3.2.3 Seed yield and thousand seed weight determination

The seed yield from each mother crop was recorded and a 10 g sample was removed for determination of the thousand seed weight (TSW). The number of seeds in each sample was counted using a Contador Pfeuffer seed counter and the thousand seed weight (TSW) was calculated as shown in equation 3.2.3.1.

Equation 3.2.3.1	Calculation of the thousand seed weight (g)
Thousand seed weight	TSW (g) = $10*(1000/$ Number of seeds per 10 g sample)

3.3 Statistical Analysis

The 5% probability level was used in each analysis to class the results as significant. Since the germination (emergence and establishment) data were dependent it was not possible to use an analysis of variance (ANOVA) test alone to determine whether the differences in the germination, emergence or establishment curves were significantly affected by the seed treatment methods. To overcome this problem, Genstat[®] 5th edition (Lawes Agricultural Trust, Rothamsted Experimental Station, 2000) was used to perform a Repeated Measurements Analysis of Variance (RMAV) on the data.

RMAV can determine whether the progress of different populations with time differ significantly from each other. For example, although a high-performance seedlot may have twice the final percentage germination of a low performance seedlot, the process of germination may take the same form in both seedlots, as shown in figure 3.3.1, and this similarity would be shown by repeated measurements analysis. Alternatively, one seedlot may germinate very quickly and the other very slowly although they may both achieve the same final percentage - in which case the RMAV would show that these seedlots did not have the same pattern of germination, as shown in figure 3.3.2. RMAV can thus analyse the whole timecourse of a process but this technique was used only to determine differences in the patterns of germination and to show the error at the same timepoint of different processes, SEM (a), or the error between different timepoints along the same process, SEM (b).

Figure 3.3.1 Idealised repeated measurements of analysis of variance for seedlots A and B showing different final percentages but the same pattern of germination



Figure 3.3.2 Idealised repeated measurements of analysis of variance for seedlots C and D showing the same final percentage but different patterns of germination



Since it was also important to know how quickly the seeds or seedlings were germinating, emerging or establishing and their final percentage, the solver function in Microsoft Excel 97 was used to calculate four parameters A, B, C and M of the growth curve as defined in equation 3.3.1, for each replicate. These parameters were then used

to calculate four growth parameters for each replicate: the time to 10% (T_{10}), time to 50% (T_{50}) and the final percentage (FP) as shown in figure 3.3.3 and defined in equations 3.3.2 to 3.3.4 as well as the rate (R) of germination (G) as defined in equation 3.3.5.

Figure 3.3.3 Idealised graph showing the times to 10 and 50% and final percentage germination growth parameters



These parameters were chosen because they represented appropriate points of comparison between seed treatments. The T_{10} gave an indication of the time of the start of germination (or emergence/ establishment) and by T_{50} the seed sample would be more than half-way towards the commercial requirement of 85% germination. There is no commercial requirement for percentage emergence or establishment but since the T_{50} was used for the germination data it was reasonable to use it for these other two processes. The rate and final percentage values are self-explanatory. The T_{10} , T_{50} and FP values were not relative to the values achieved by a seedlot under a particular treatment (e.g. 95% of the maximum germination) but were real percentages, which enabled effective comparisons to be made between different treatments.

Equation 3.3.1	Equation of a general sigmoidal curve		
General sigmoidal curve equation	G		$A + C / (1 + e^{-B (X-M)})$
Equation 3.3.2	Calcul	ation of	the time to 10% germination
Time to 10% germination*	T ₁₀	=	(((LN((C/(10-A))-1)-(B*M))/-B))
Equation 3.3.3	Calcul	ation of	the time to 50% germination
Time to 50% germination [*]	T ₅₀	=	(((LN((C/(50-A))-1)-(B*M))/-B))
Equation 3.3.4	Calcul	ation of	the final percentage germination
Final percentage germination*	FPG		A + C
Equation 3.3.5	Calcul	ation of	the rate of germination
Rate of germination [*]	Rate	=	1 / T ₅₀

where: G: the percentage germination (emergence/ establishment^{*}) at time X
X: the time at which germination^{*} is measured
A, B, C and M are the sigmoidal curve parameters

Equation 3.3.6	Calculation of	f the standard e	error of the mean
Standard error of the mean	SEM =	SD/ √2	

Genstat[®] 5 version 4.2 was then used to perform an ANOVA test on the T_{10} , T_{50} , FP and rate values for each replicate, which determined whether there were significant differences between the different treatments at these specific points. Although this test also assumes that the data conforms to a normal distribution, by plotting the residuals as shown in figure 3.3.4, it was observed that the data did approximate to a normal

distribution and that this well-practised test was suitable for analysis of these data. If there were significant treatment differences or significant differences between these growth parameters, the replicate data were averaged for each treatment and the regressional analysis standard logistic curve function in Genstat[®] 5.42 was used to produce an idealised curve for each treatment as shown previously in figure 3.3.3.





The RMAV provided the standard errors of the mean, SEM (a) and SEM (b), which were plotted on the germination/ emergence/ establishment curves. The SEM values were calculated as shown earlier in equation 3.3.6. The SEM (a) is the standard error of the mean between the curves at the same time point and the SEM (b) is the standard error of the mean between consecutive time points on each curve.

Where error bars are shown directly on the data points, they are the standard error of deviation (SED). The mean percentage germination/ emergence/ establishment were not analysed since they served only as a guide to whether treatments had an effect on the data. Significant effects were thus determined from the T_{10} , T_{50} , FPG and rate parameters alone.

In contrast with the germination data the final percentage emergence (FPE) and field emergence (FPF) values were taken directly from the emergence and field raw data because the solver function overestimated the FPE and FPF of some of the treatments. Although this overestimation only affected the trend parameters of treatments in which the single treatment effect was superceded by interactive treatment effects, it was decided that for consistency, the raw data FPE and FPF values would be used in reporting the results of all of the emergence and field experiments. In some tables, where not all of the replicates reached 10 and 50% emergence/ establishment and thus the T_{10} , T_{50} and rate could not be calculated, the number of degrees of freedom is different from that for the other emergence parameters.

Unlike the field establishment data the field experiment growth analysis data were independent and Genstat[®] 5.42 was used to perform an ANOVA test on the growth analysis parameters (e.g. leaf area, chlorophyll content, root length) to determine the significance of the different seed treatment techniques.

CHAPTER IV THE EFFECTS OF SEED TREATMENT TECHNIQUES ON GERMINATION IN THE CONTROLLED ENVIRONMENT

4.1 Introduction to the Germination Experiments

The literature has reported differences in the germination responses of several varieties of oilseed rape at a range of temperatures (Kondra *et al.*, 1983; Wilson *et al.*, 1992). The base temperature of oilseed rape has been reported to vary between 0.9°C (Vigil *et al.*, 1997) and 5°C (Morrisson *et al.*, 1989) and low temperatures have been observed to cause non-linearity of the germination response (Marshall and Squire, 1996). Seed hydration prior to sowing (Cantliffe and Elballa, 1994; Noon, 1997) and seed size selection (Razzaque, 1988; Bretagnolle *et al.*, 1995) have been reported to significantly alter the speed of germination while heat treatment has been observed to reduce the final percentage seed germination (Bant and Storey, 1951).

The percentage germination of commercial oilseed rape is determined through germination tests at 20°C (International Seed Testing Association, 1993). However, the mean UK soil temperature at 20 mm depth declines from 15°C in the first week to 12.6°C in the fourth week of September and at night or if the soil is wet, the temperature can fall below 10°C (Noon, 1997). Since autumn-sown seeds are thus sown at soil temperatures of between 10 and 15°C, there is often disparity between the percentage germination as defined on the seed label and the percentage that is achieved in the field. It was thus necessary to examine the germination responses of several varieties of winter oilseed rape as well as the responses of different seedlots of the same variety at a range of temperatures. Since good establishment of winter oilseed rape is difficult to achieve, it was also important to test whether methods of seed treatment such as seed

hydration, seed size selection and heat treatment could also increase the speed and/ or final percentage germination of winter oilseed rape.

The germination responses of 9 varieties comprising 16 seedlots of winter oilseed rape that were commercially available in 1996 were examined at between 5 and 25°C. The effects of seed hydration on the germination response of seedlots of the Bristol variety of oilseed rape were examined at 5, 15 and 20°C while the effect of seed size selection was examined at 20°C. The effects of these methods on Apex seedlots grown in Sutton Bonington in 1996 and harvested in 1997 as well as heat treatment and seed nitrogen percentage were examined at 15 and 25°C.

4.2 Germination Experiments

4.2.1 The germination response of commercial varieties and seedlots to temperature

The aim of this experiment was to quantify the extent of differences in the germination response of 16 seedlots comprising nine different varieties of winter oilseed rape at different temperatures as measured by the four germination parameters (time to 10% and 50%, final percentage and rate of germination) previously described.

<u>Method</u>

For each treatment there were 5 replicates of each of the 16 commercial seedlots and 20 seeds per replicate. The seeds were germinated in the dark in the controlled growth room environment at 5, 10, 15, 20 and 25°C as previously described. This temperature range covered the temperatures that might be faced by a seed sown in northern through to temperate and semi-arid conditions, including the standard ISTA seed testing

temperature. The seedlots, which were obtained from CPB Twyford, were fungicide and insecticide treated as shown in table 4.2.1.2. The fungicide and insecticide Lindex contains lindane protects against blight (*Alternaria*) and stem rot (*Sclerotinia*). Vitavax, another fungicide and insecticide, contains carboxin to protect against blackleg (*Leptosphaeria maculans*) and flea beetles. Hydraguard, which contains deltamethrin, protects against flea beetles while Rovral, which contains iprodione, protects against stem rot (*Sclerotinia*).

<u>Results</u>

Effect of temperature on the germination response of the 9 commercial varieties

At 5°C all the 16 seedlots of the nine varieties were slow to germinate but as the temperature was increased to 25°C the rate of the germination process was increased and hence the time to the onset of germination was reduced as shown in figure 4.2.1.1.

Figure 4.2.1.1 The effect of temperature on the time to the onset of germination of the 1996 commercial seedlots (mean of nine varieties) at 5, 10, 15, 20 and 25°C



At the lowest temperature of 5°C germination started after 115 h but because some of the varieties did not achieve 10 or 50% germination, it was not possible to calculate their T_{10} , T_{50} and rate of germination. The FPG was the only calculable germination parameter for all the varieties. At 5°C some varieties (Gazelle, Falcon and Rocket) achieved a FPG of over 85% although others, such as Alpine, achieved only 9%; a mean final percentage germination of only 64% was achieved as shown in table 4.2.1.1.

Table 4.2.1.1The effect of variety on the final percentage germination of the1996 commercial varieties at 5, 10, 15, 20 and 25°C

Temperature (°C)						
	5	10	15	20	25	
Variety		Fina	l germinatio	n (%)		Mean
Gazelle	97	98	100	96	91	96
Falcon	88	96	97	99	93	95
Rocket	88	77	98	84	80	85
Apex	82	91	98	96	91	91
Synergy	60	97	91	99	92	88
Capitol	58	92	92	98	90	86
Nickel	55	74	94	90	90	81
Bristol	35	55	92	97	84	72
Alpine	9	49	89	92	93	66
Mean	64	81	94	94	89	84
Р	< 0.001	< 0.001	<0.011	< 0.001	0.071	
SED	5.600	3.541	2.986	3.350	4.590	
DF	32	32	32	32	32	-

At 10°C germination started after 65 h and five varieties (Gazelle, Falcon, Synergy, Capitol and Apex) achieved a FPG of over 85%. The average FPG increased from 64% at 5°C to 81% at 10°C. At 15°C germination started after 41 h and all of the varieties achieved more than 85% germination with an average FPG of 94%. First germination

occurred after 19 h at 20°C and the average FPG was again 94%. At 25°C first germination occurred at 15 h but the average FPG achieved at this highest temperature was 89%, which was lower than was achieved at both 15 and 20°C. With the exception of Rocket and Bristol, all the varieties achieved more than 85% germination at this temperature.

At the middle temperatures of 15 and 20°C there was significantly less variation in the FPG of the varieties than at the lower temperatures, as shown in figure 4.2.1.2. Although the highest FPG values were not obtained at 15°C by each variety, the maximal mean FPG (94%) was achieved at this temperature. The same mean FPG was also obtained at 20°C but the least variation between the varieties was observed at 15°C.

Figure 4.2.1.2 The effect of temperature on the final percentage germination of the 1996 commercial seedlots (mean of nine varieties) at 5, 10, 15, 20 and 25°C



The rate of germination $(1/T_{50})$ was plotted against temperature as shown in figure 4.2.1.3. By continuing the function back to zero, the base temperature (T_b) was shown to be 3.3°C.

Figure 4.2.1.3 The effect of temperature on the rate of germination (h⁻¹) of the 1996 commercial seedlots (mean of nine varieties) at 5, 10, 15, 20 and 25°C



The effect of temperature on the germination response of the 16 seedlots

The following data describes in more detail the effect of seedlot on varietal germination at 5 - 25°C. There were significant differences in the germination responses of the 16 seedlots to temperature as shown in table 4.2.1.2. It is important to note that the seedlots could have been grown in different locations and under different environments hence the effects obsrved could be genotypic rather than pure seedlot effects. To determine whether the effects were seedlot- or genotype-based, it would be necessary to grow each of the seedlots at the same location and then compare the germination responses.

At 5°C there were significant differences in the FPG of all of the Bristol seedlots (S1, S6 and S13) and between three of the Apex (S2, S12 and S16) and the fourth Apex (S8) seedlot. However, there were no significant differences in the FPG of either the Rocket (S4 and S15) or Synergy (S7 and S11) seedlots. At 10°C significant differences were observed only in the Bristol seedlots, which were all significantly different from each

other, while at 15°C the Bristol seedlot S13 achieved a significantly lower FPG than either S1 or S6. At 20°C the Apex seedlot S16 achieved a significantly lower FPG than the other three Apex seedlots (S2, S8 and S12) and the Rocket seedlots (S4 and S15) were also significantly different at this temperature.

Table 4.2.1.2The effect of seedlot on the final percentage germination of the1996 commercial seedlots at 5, 10, 15, 20 and 25°C

			Temperature (°C)					
			5	10	15	20	25	
Variety	Seedlot	Seed treatment		Final g	germinatio	n (%)		Mean
Alpine	S9	Vitavax RS	9	49	89	92	93	66
Apex	S2	Lindex + FS	73	88	99	98	100	92
Apex	S8	Rovral FS + H	99	95	99	100	91	97
Apex	S12	Vitavax RS	72	86	94	100	94	89
Apex	S16	Vitavax RS	83	94	99	85	77	88
Bristol	S1	Lindex + FS	55	76	94	93	72	78
Bristol	S6	Lindex + FS	41	57	94	100	88	76
Bristol	S13	Lindex + FS	8	32	87	98	92	63
Capitol	S5	Lindex + FS	58	92	92	98	90	87
Falcon	S10	Lindex + FS	88	96	97	99	93	95
Gazelle	S14	Lindex + FS	97	98	100	96	91	96
Nickel	S3	Rovral FS + H	55	74	94	90	90	81
Rocket	S4	Lindex + FS	92	74	97	91	81	87
Rocket	S15	Lindex + FS	83	79	98	77	78	83
Synergy	S7	Lindex + FS	63	96	93	98	94	89
Synergy	S11	Lindex + FS	57	97	88	99	90	86
	<u> </u>	Mean	65	80	95	95	88	84
		Р	< 0.001	< 0.001	0.001	< 0.001	0.005	
		SED	6.510	5.790	3.205	4.006	6.090	
		DF	60	60	60	60	60	

The greatest seedlot differences were observed in the Bristol seedlots S1, S6 and S13 at 5°C as shown in figure 4.2.1.4.





SEM (a) and (b) DF 23

Although the germination of S1 and S6 started at 115 h it took a further 48 h for germination to start in S13, which subsequently achieved a FPG of only 8% compared with 41 and 55% in S6 and S1, respectively. There were no further significant increases in the FPG of the seedlots after 193 h.

All three of the Bristol seedlots had been fungicide and insecticide treated in the same manner and had been grown in the same location and with the same fertiliser regimes but returning the non-germinated seeds to temperatures of 20°C did not induce their germination.

The following experiments evaluated seed treatment methods to increase germination capacity to improve seedling emergence in the field.

4.2.2 The effect of duration of seed hydration on the germination and postgerminative taproot growth of the Bristol seedlots S1 and S13 at 5 and 15°C

Seed hydration has been shown to improve low vigour *Brassica napus* seedlot germination to match that of high vigour seedlots (Zheng *et al.*, 1994). This experiment examined the potential of seed hydration to improve the germination of the low vigour Bristol seedlots S1 and S13 at 5 and 15°C.

<u>Method</u>

Each 3 g seed sample was soaked in water in the laboratory environment for 6, 12, 18, 24, 30 or 36 h at 15°C as previously described and a non-treated sample was the control treatment. Soaking in water was chosen over priming because preliminary experiments showed that priming of oilseed rape in polyethylene glycol (PEG) did not confer significant advantages over seed hydration in water (Noon, 1997). For the subsequent germination experiments, there were 5 replicates of each treatment and 20 seeds per replicate. The germination experiments were performed in darkness in the controlled growth room environment at 5 and 15°C. After eight days the lengths of the taproots of the seedlings were measured.

<u>Results</u>

The effect of seedlot and seed hydration on the final percentage germination at 5°C

At 5°C both seedlots suffered from poor germination: the final percentage germination of S13 often did not reach 10% germination and always failed to reach 50% germination thus the only calculable parameter was the FPG. Seedlot S1 had a significantly higher (P < 0.001) FPG than seedlot S13 as shown in table 4.2.2.1, which

confirmed the results of experiment 4.2.1 in which S1 consistently achieved a higher percentage germination than S13. Increasing the duration of seed hydration significantly increased the FPG to 50% (36 h treatment) compared with 28% in the control treatment (P < 0.001) although the optimal duration of seed hydration for seedlot S1 was 12 h compared with 36 h for seedlot S13.

The significant interaction between seedlot and duration of seed hydration (h) affected the FPG (P < 0.001) as shown in figure 4.2.2.1. Hydration for 12 h significantly increased the FPG of seedlot S1 to 84% compared with 48% for the control treatment but only hydration for 36 h significantly increased the FPG of seedlot S13 to 28% compared with the 7% for the control treatment.

Seed hydration thus improved the FPG of both seedlots but was unable to substantially overcome the inability of seedlot S13 to germinate at 5°C. Furthermore, although seed hydration treatments have been shown to increase biochemical and metabolic activities in seeds, it has been reported that low vigour seeds are unable to withstand the heightened level of activity and become unable to germinate. It is possible that some of the seeds from seedlot S13 were unable to survive the increased cell activity and died following soaking thus the beneficial capacity of hydration upon germination was limited by the inherent capacity of seedlot S13.

Table 4.2.2.1The effect of seedlot and duration of seed hydration (h) on the
final percentage germination of the Bristol seedlots S1 and S13 at
5°C

Duration of seed		Final percentage germination			
hydration (h)	Seedlot S1	Seedlot S13	Mean FPG		
0	48	7	28		
6	78	11	45		
12	84	6	45		
18	77	4	41		
24	71	5	38		
30	72	12	42		
36	72	28	50		
Factor means	72	11	41		
Probability	< 0	.001	< 0.001		
SED	2.3	310	4.330		
DF	5	52	52		
		T			
Adv* seedlot	P < 0.001	SED = 6.120	DF = 52		

Figure 4.2.2.1 The effect of duration of seed hydration on the final percentage germination of the Bristol seedlots S1 and S13 at 5°C (SED 4.33; DF 52)



The effect of seedlot and seed hydration on post-germinative taproot growth following germination at $5^{\circ}C$

The mean taproot length following germination at 5°C was not significantly affected by seedlot (P = 0.701) as shown in table 4.2.2.2. However, increasing the duration of seed hydration to 36 h significantly increased (P < 0.001) the length of the taproot to 4.46 cm compared with 1.62 cm in the control treatment.

Table 4.2.2.2The effect of seedlot and duration of seed hydration on the mean
taproot length (cm) of the Bristol seedlots S1 and S13 at 5°C

Duration of seed	Mean taproot length (cm)			
hydration (h)	Seedlot S1	Seedlot S13	Mean taproot length	
0	1.70	1.54	1.62	
6	2.43	1.87	2.15	
12	2.40	2.58	2.49	
18	2.51	2.54	2.52	
24	3.78	3.04	3.41	
30	3.67	3.11	3.39	
36	3.41	5.52	4.46	
Factor means	2.84	2.89	2.86	
Probability	0.	701	< 0.001	
SED	0.111		0.209	
DF	549		549	
Adv* seedlot	P < 0.001	SED = 0.295	DF = 549	

The significant interaction between seed hydration and seedlot (P < 0.001) affected the taproot length as shown in figure 4.2.2.2. The 24 to 36 h treatments significantly increased the taproot length of the S1 seedlings compared with the 6 to 18 h treatments. However, the greatest increase in taproot length of the S1 seedlings was achieved after the 24 h treatment although this was the duration that had previously produced the

smallest increase in the FPG compared with the control. Seed hydration thus affected not only the final percentage germination of the seed but also subsequent seedling growth. There was a greater effect of seed hydration on the taproot length of S13 seedlings than on S1 seedlings: increasing the duration of seed hydration to 36 h increased the taproot length to 5.52 cm compared with 1.54 cm for the control treatment. This was also the duration that had previously produced the greatest increase in the FPG of seedlot S13.





The effect of seedlot and seed hydration on the time to 10% germination at 15°C

There were no significant effects of seedlot (P = 0.993) or hydration (P = 0.204) on the time to 10% germination (P = 0.993) at 15°C as shown in table 4.2.2.3 and there were no significant interactions (P = 0.688). However, the reduction in T_{10} was greater in seedlot S1 (17 h) compared with seedlot S13 (5 h). Increasing the duration of seed hydration to 36 h reduced the mean T_{10} by approximately 11 h compared with the control as shown in figure 4.2.2.3.

Seed hydration Time to 10% germination (h) Seedlot S13 Seedlot S1 Mean T_{10} duration (h) 27.2 19.4 23.3 0 22.4 22.8 6 23.2 22.9 18.4 12 20.6 14.9 21.8 18.3 18 19.2 19.3 19.2 24 30 20.3 20.7 20.5 10.0 14.1 12.1 36 19.6 19.5 19.5 Factor means 0.993 0.204 Probability SED 2.320 4.340 43 43 DF Adv* seedlot P = 0.688SED = 6.140DF = 43

Table 4.2.2.3The effect of seedlot and seed hydration on the time to 10%
germination (h) of the Bristol seedlots S1 and S13 at 15°C

Figure 4.2.2.3 The effect of the seed hydration for 36 h on the germination response of control (C) and 36 h hydrated (36) Bristol seedlots S1 and S13 at 15°C



There were no significant effects of either seedlot (P = 0.244) or duration of seed hydration on the time to 50% germination (P = 0.149) as shown in table 4.2.2.4. However, the greatest effect was observed after seed hydration for 36 h, which reduced the T_{50} of both seedlots compared with their controls. There was no significant interaction between the seedlot and duration of seed hydration (P = 0.797).

Table 4.2.2.4The effect of seedlot and seed hydration on the time to 50%germination (h) of the Bristol seedlots S1 and S13 at 15°C

Duration of seed		Time to 50% germination (h)			
hydration (h)	Seedlot S1	Seedlot S13	Mean T ₅₀		
0	39.17	34.52	36.85		
6	42.99	37.80	40.39		
12	35.10	30.73	32.91		
18	32.13	34.68	33.40		
24	33.49	36.29	34.89		
30	35.74	32.11	33.92		
36	32.09	29.74	30.92		
Factor means	35.82	33.70	34.76		
Probability	0.	244	0.149		
SED	1	.794	3.357		
DF		43	43		
Adv* seedlot	P = 0.797	SED = 4.747	DF = 43		

The effect of seedlot and seed hydration on the final percentage germination at $15^{\circ}C$

There were no significant effects of seedlot on the final percentage germination (P = 0.117) as shown in table 4.2.2.5. There was no significant effect of duration of seed

hydration on the final percentage germination (P = 0.593). There was no significant interaction between the seedlot and duration of seed hydration (P=0.699).

Table 4.2.2.5The effect of seedlot and seed hydration on the final percentage
germination of the Bristol seedlots S1 and S13 at 15°C

Duration of seed	Final percentage germination			
hydration (h)	Seedlot S1	Seedlot S13	Mean FPG	
0	84	93	89	
6	89	87	88	
12	89	92	91	
18	92	97	95	
24	90	89	90	
30	87	91	89	
36	89	90	90	
Factor means	87	92	90	
Probability	0	.117	0.593	
SED	1	.824	3.413	
DF		43	43	
· · · · · · · · · · · · · · · · · · ·				
Adv* seedlot	P = 0.699	SED = 4.826	DF = 43	

The effect of seedlot and seed hydration on the rate of germination at $15^{\circ}C$

There were no significant effects of seedlot on the rate of germination (P = 0.364) as shown in table 4.2.2.6. There was no significant effect of duration of seed hydration on the germination rate (P = 0.264) although increased duration of seed hydration did numerically increase the rate of germination compared with the contro in both seedlots. There were no significant interactions (P = 0.925).

Table 4.2.2.6

The effect of seedlot and seed hydration on the rate of germination (h^{-1}) of the Bristol seedlots S1 and S13 at 15°C

Duration of seed	Rate of germination (h ⁻¹)			
hydration (h)	Seedlot S1	Seedlot S13	Mean rate	
0	0.02731	0.03050	0.02891	
6	0.02476	0.02753	0.02615	
12	0.02947	0.03545	0.03246	
18	0.03230	0.03065	0.03148	
24	0.03110	0.02944	0.03027	
30	0.03036	0.03206	0.03121	
36	0.03431	0.03569	0.03500	
Factor means	0.02995	0.03162	0.03078	
Probability	0	.364	0.264	
SED	0.0	01824	0.003413	
Df	43		43	
A dy* seedlot	P = 0.925	SED = 0.004827	DF = 43	
Auv seculot	1 - 0.925	510 0.004027	D1 - 43	

The effect of seedlot and seed hydration on post-germinative taproot growth following germination at 15°C

There were no significant effects of seedlot on seedling taproot length (P = 0.911) as shown in table 4.2.2.7 but seed hydration for 6 and 12 h significantly increased the taproot length (P < 0.001) of both seedlots compared with the control treatment. Although longer durations of seed hydration increased the speed of germination, shorter durations produced the maximal taproot length. The significant interaction (P = 0.022), which affected the taproot length is shown in figure 4.2.2.4.

Duration of seed	Mean taproot length (cm)				
hydration (h)	Seedlot S1	Seedlot S13	Mean taproot length		
0	22.49	22.53	22.51		
6	29.87	27.38	28.62		
12	26.41	29.06	27.74		
18	24.55	23.34	23.95		
24	24.61	21.83	23.22		
30	22.44	25.46	23.95		
36	21.88	22.20	22.04		
Factor means	24.61	24.54	24.57		
Probability	0.911		< 0.001		
SED	0.550		1.030		
DF	1220		1220		
		·····			
Adv* seedlot	P = 0.022	SED = 1.456	DF = 1220		

Table 4.2.2.7The effect of seedlot and seed hydration on the mean taprootlength (cm) of the Bristol seedlots S1 and S13 at 15°C

Figure 4.2.2.4 The effect of the seed hydration*seedlot interaction on the mean taproot length (cm) of the Bristol seedlots S1 and S13 at 15°C (SED 1.456; DF 1220)



Seed hydration for 6 and 12 h significantly increased the taproot length of the S1 seedling to 29.87 and 26.41 cm respectively, compared with 22.49 cm in the control treatment. Seed hydration for these durations also significantly increased the taproot length of the S13 seedlings to 27.38 and 29.06 cm compared with 22.53 cm in the control treatment.

The decreases in taproot length after long durations of seed hydration could be due to over-advancement of the seeds. Although seeds that had visibly germinated were discarded prior to the experiment, it is possible that the germination process may have progressed so close to the point of radicle emergence that the drying back process could either have resulted in the failure of some seeds to complete germination or resulted in relatively poor taproot growth upon germination.

4.2.3 The effect of seed hydration and seed size selection on the germination of the Bristol seedlots S1 and S13 at 20°C

The aim of this experiment was to determine whether seed hydration in combination with seed size selection could improve germination performance and the hypothesis was that seed hydration and seed size selection could improve the germination of the Bristol seedlots S1 and S13.

<u>Method</u>

Seed samples were divided into large (> 2 mm in diameter) and small (< 2 mm in diameter) samples as previously described. Each seed sample fraction was then hydrated for 18 h at 15°C as previously described and a non-hydrated sample was the control treatment. The seeds were hydrated for 18 h because this was the duration at which the highest FPG of both S1 and S13 was attained at 15°C.

For the subsequent germination experiments, there were 4 replicates of each treatment and 30 seeds per replicate. The seeds were germinated as previously described in the controlled growth room environment at 20°C.

<u>Results</u>

The effect of seedlot, seed hydration and seed size on the time to 10% germination at 20° C

There was no significant effect of seedlot on the time to 10% germination of seedlots S1 and S13 at 20°C (P = 0.527). However, both small seed size selection (P = 0.047) and seed hydration for 18 h (P < 0.001) significantly reduced the T_{10} compared with large size selection or the control treatment as shown in table 4.2.3.1.

The highest order significant interaction was between the seedlot and seed size (P = 0.027). Reducing the seed size from large to small, decreased the T_{10} although this was only significant in seedlot S1; for the control seeds of S13 the small seeds took longer to reach 10% germination than the large seeds.

Table 4.2.3.1The effect of seedlot, seed size and seed hydration on the time to10% germination (h) of the Bristol seedlots S1 and S13 at 20°C

	Time to 10% germination (h)				
Seedlot	Seed hydration	Large seed size	Small seed size	Mean T ₁₀	
S1	Control	30.33	24.35	20.13	
	Hydrated	13.95	11.89		
S13	Control	27.28	28.63	20.69	
	Hydrated	13.86	13.01		
Mean size		21.32	19.47	······	
Mean control	27.65				
Mean hydrated	13.18				
Probability	< 0.001	0.047		0.527	
SED	0.875				
DF	16				

Interactions	Probability	SED	DF
Seedlot* seed size	0.027	1.238	16
Seedlot* seed hydration	0.956	1.238	16
Seed size* seed hydration	0.629	1.238	16
Seedlot* seed size* seed hydration	0.100	1.750	16

The effect of seedlot, seed hydration and seed size on the time to 50% germination at 20° C

The effect of seedlot on the germination of seedlots S1 and S13 at 20°C was only just not significant (P = 0.056) as shown in table 4.2.3.2. Both small seed size (P = 0.002) and seed hydration for 18 h (P < 0.001) significantly reduced the T₅₀. There were no significant interactions.
Table 4.2.3.2The effect of seedlot, seed size and seed hydration on the time to
50% germination (h) of the Bristol seedlots S1 and S13 at 20°C

	Time to 50% germination (h)			
Seedlot	Seed hydration	Large seed size	Small seed size	Mean T ₅₀
S1	Control	55.2	48.4	40.9
	Hydrated	35.9	24.3	
S13	Control	51.1	47.5	36.6
	Hydrated	28.9	19.0	
Mean size		42.8	34.8	
Mean control	50.6		·	
Mean hydrated	27.0			
Probability	< 0.001	0.0	002	0.056
SED	2.090			
DF	16			

Interactions	Probability	SED	DF
Seedlot* seed size	0.567	2.96	16
Seedlot* seed hydration	0.401	2.96	16
Seed size* seed hydration	0.201	2.96	16
Seedlot* seed size* seed hydration	0.867	4.19	16

The effect of seedlot, seed hydration and seed size on the final percentage germination at 20° C

There was no significant effect of seedlot on the final percentage germination of seedlots S1 and S13 at 20°C (P = 0.962) as shown in table 4.2.3.3. Small seed size non-significantly reduced the FPG (P = 0.351) and seed hydration non-significantly increased the FPG (P = 0.381).

Table 4.2.3.3The effect of seedlot, seed size and seed hydration on the final
percentage germination of the Bristol seedlots S1 and S13 at 20°C

		Final percentage germination		
Seedlot	Seed hydration	Large seed size	Small seed size	Mean FPG
S1	Control	77	81	81
	Hydrated	87	79	
S13	Control	82	79	81
	Hydrated	82	80	
Mean size		82	80	
Mean control	80			<u></u>
Mean hydrated	82			Tel President and the second
Probability	0.381	0.	351	0.962
SED	2.230			
DF		16		

Interactions	Probability	SED	DF
Seedlot* seed size	0.856	3.15	16
Seedlot* seed hydration	0.365	3.15	16
Seed size* seed hydration	0.320	3.15	16
Seedlot* seed size* seed hydration	0.140	4.45	16

The effect of seedlot, seed hydration and seed size on the rate of germination at 20°C

Seedlot (P = 0.004), small seed size (P < 0.001) and seed hydration (P < 0.001) significantly increased the rate of germination as shown in table 4.2.3.4. Seed hydration significantly increased the rate of germination of both the large and the small seed samples. The highest order significant interaction was between seed size and seed hydration (P < 0.001) and the effect of seed size was observed in both the control and hydrated treatments. Selecting small seed size increased the rate of germination although this was only significant in the hydrated treatment. It is possible that minor differences in the seed composition or seed coat could have resulted in the interaction of

seed size with seed hydration. The small seeds germinated more quickly (lower T_{10} and T_{50}) than the large seeds. Since small seeds have a greater surface area to volume ratio compared with large seeds, they would be likely to imbibe water more quickly from their surroundings than large seeds although this was not directly tested. Faster imbibition could enable the process of germination to proceed more quickly than if water is limited and it is possible that this caused the observed reductions in the T_{10} and T_{50} and the increase the rate of germination.

Table 4.2.3.4The effect of seedlot, seed size and seed hydration on the rate of
germination of the Bristol seedlots S1 and S13 at 20°C

		Rate of germination (h ⁻¹)		
Seedlot	Seed hydration	Large seed size	Small seed size	Mean rate
S1	Control	0.01856	0.02099	0.02713
	Hydrated	0.02826	0.04069	
\$13	Control	0.01974	0.02111	0.03195
	Hydrated	0.03529	0.05164	
Mean size		0.02546	0.03361	
Mean control	0.020			• · · · ·
Mean hydrated	0.03897			
Probability	< 0.001	< 0.001 0.00		0.004
SED	0.00142			
DF	16			

Interactions	Probability	SED	DF
Seedlot* seed size	0.622	0.002009	16
Seedlot* seed hydration	0.010	0.002009	16
Seed size* seed hydration	< 0.001	0.002009	16
Seedlot* seed size* seed hydration	0.392	0.002841	16

4.2.4 The effect of seed nitrogen percentage, pod position and harvest date on seed properties of the 1997 and 1999 harvest Apex seedlots

This research moved towards more focused investigations on the higher vigour Apex variety, which was the main commercial variety in 1996, accounting for about 50% of the UK rape area. Seed analysis has shown that mother crop nitrogen management can significantly alter the seed nitrogen and oil percentage (Shamlal and Mohammad, 2000) and the aim of this experiment was to assess the effects of mother crop nitrogen management, pod position in the canopy and harvest date on the seed nitrogen and oil percentage of the Apex seedlots

The effect of mother crop nitrogen management, pod position and harvest date on the seed nitrogen content of the 1997 harvest Apex seedlots

These seedlots were collected from crops grown in Sutton Bonington in 1996 with contrasting applications of nitrogen fertiliser. Each of the mother crops received between nil and 300 kg nitrogen ha⁻¹ as shown in table 4.2.4.1 but within this range of applications the timing varied between the growth phase (G) in February and flowering (F) in March. The mother crops that received split (G/ F) applications of nitrogen received half the application during in February and the remaining half in March. By altering both the amounts and timing of the applications it was possible to determine the effect of amount and timing of mother crop nitrogen applications on the nitrogen and oil percentage of the seeds produced from each mother crop.

At harvest the crop canopy was divided into an upper section (> 1. 5 m) and a lower section (< 1.5 m). The division was made by eye as described by Mendham (1975) and pods were harvested from a 2 m² area of each section at regular intervals between 21^{st} July and 18^{th} August. The samples were dried in a polythene tunnel and the seeds were

removed by hand before being stored in airtight containers in the cold store at 4°C. By altering both the pod position in the canopy and the harvest date, the maturity of the pods and hence the maturity of the seeds contained within those pods was altered. Through selecting either late-harvested pods or pods from the lower section of the canopy, the maturity of the seeds was increased.

Table 4.2.4.1Mother crop nitrogen management of the 1997 harvest Apex
seedlots

Mother crop	Nitrogen application (kg ha ⁻¹)	Timing of application
Nil	Nil	-
80 F	80	March (flowering, F)
100 G/ F	100	50 in February (G), 50 in March (F)
160 F	160	March (flowering, F)
300 G/ F	300	150 in February (G), 150 in March (F)

Seed nitrogen percentage is a cipher for the seed protein percentage according to equation 4.2.4.1 (Stokes *et al.*, 2000).

Equation 4.2.4.1 Calculation of seed protein percentage

Protein percentage = nitrogen percentage * 6.25

Increasing the amount of nitrogen fertiliser applied to the mother crop significantly increased the seed nitrogen percentage (P < 0.001) from 2.30 to 3.61% as shown in table 4.2.4.3. By selecting pods from the lower section (< 1.5 m) of the crop canopy, the seed nitrogen percentage was also significantly increased (P < 0.001) from 2.80% (upper pods) to 3.14% (lower pods). It was also significantly affected by the interaction between the amount of nitrogen application to the mother crop and the pod position (P < 0.001). Lower pod position significantly increased the seed nitrogen percentage of all

treatments except the nil nitrogen treatment, which showed no significant effect of pod position.

Table 4.2.4.2The effect of mother crop nitrogen management and pod position
on the seed nitrogen percentage of the 1997 harvest Apex
seedlots

	Seed nitrogen percentage		
Mother crop N management	Upper pod position	Lower pod position	Mean seed
(kg ha ⁻¹) and timing	(> 1.5 m)	(< 1.5 m)	N (%)
Nil	2.289	2.311	2.30
80 F	2.445	2.627	2.54
100 G/ F	2.733	3.160	2.95
300 G/ F	3.182	3.733	3.46
160 F	3.367	3.855	3.61
Factor means	2.803	3.137	2.97
Probability	< 0.	001	< 0.001
SED	0.0	028	0.045
DF	6	2	62
N application* pod position	P < 0.001	SED = 0.064	DF = 62

The effect of mother crop nitrogen management, pod position and harvest date on the seed oil percentage of the 1997 harvest Apex seedlots

Increasing the amount of nitrogen fertiliser applied to the mother crop significantly decreased the seed oil percentage (P < 0.001) from 54.37 to 47.00% as shown in table 4.2.4.4. By selecting pods from the lower section (< 1.5 m) of the crop canopy, the seed oil percentage was also significantly decreased (P < 0.001) from 52.74 to 49.13%. The seed oil percentage was also significantly affected by the interaction between the amount of nitrogen application to the mother crop and the pod position (P < 0.001).

Lower pod position significantly decreased the seed oil percentage of all treatments except the nil nitrogen treatment, which showed no significant effect of pod position.

Table 4.2.4.3The effect of mother crop nitrogen management and pod position
on the seed oil percentage of the 1997 harvest Apex seedlots

	Seed oil per	centage (%)	
Mother crop N management	Upper position	Lower position	Mean seed
(kg ha ⁻¹) and timing	(> 1.5 m)	(< 1.5 m)	oil (%)
Nil	54.71	54.03	54.37
80 F	55.48	53.36	54.42
100 G/ F	53.06	48.53	50.79
300 G/ F	50.81	45.33	48.07
160 F	49.61	44.39	47.00
Factor means	52.74	49.13	50.93
Probability	< 0.	.001	< 0.001
SED	0.1	163	0.257
DF	6	2	62
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N application* pod position	P < 0.001	SED = 0.364	DF = 62

The timing of nitrogen application to the mother crop was also important since the 160 F nitrogen treatment produced seeds with a significantly higher nitrogen and significantly lower oil percentage than the 300 G/ F treatment as shown in figure 4.2.4.1.

It is important to note that the seeds from the mother crop that received 80 kg nitrogen ha⁻¹ at flowering had a significantly lower nitrogen percentage and a significantly higher oil percentage than those from the 100 G/ F crop that had received 50 kg nitrogen ha⁻¹ in February and a further 50 kg nitrogen ha⁻¹ in March, which was possibly due to inaccuracies in fertiliser application.

Figure 4.2.4.1

The effect of mother crop nitrogen management on the seed nitrogen and oil percentage of the 1997 harvest Apex seedlots



The seed nitrogen-oil relationship conformed to the linear expression: y = -6.5363x + 70.415 (R² = 0.895). Increasing nitrogen reduces the oil percentage, which is probably due to nitrogen delaying plant maturity (Jackson, 2000) and reducing the length of time during which oil can accumulate before harvest.

The compounding effect of pod position on the seed nitrogen and oil percentage is shown in figure 4.2.4.2: for a given nitrogen percentage the upper seeds contained more oil compared with the lower seeds.

Figure 4.2.4.2 The effect of upper and lower pod position in the crop canopy on the seed nitrogen and oil percentage of the 1997 harvest Apex seedlots



The effects of mother crop nitrogen management and pod position on the seed nitrogen and oil percentages were determined across the range of harvest dates since harvest date did not significantly affect either the seed nitrogen (P = 0.115) or oil percentage (P = 0.723) as shown in table 4.2.4.2.

Table 4.2.4.4The effect of harvest date on the seed nitrogen and oilpercentages of the 1997 harvest Apex seedlots

Harvest date	Seed nitrogen percentage	Seed oil percentage
21 st July	2.74	50.98
1 st August	2.99	51.01
14 th August	3.11	50.13
Р	0.115	0.723
SED	0.1766	1.242
DF	54	54

From this point onwards the seedlots were classed according to their seed nitrogen percentage rather than the amount of nitrogen applied to their mother crop thus in each table the values move from the lowest nitrogen/ highest oil percentage to the highest nitrogen/ lowest oil percentage.

The effect of mother crop nitrogen management on the seed nitrogen and oil percentage of the 1999 harvest Apex seedlots

These seedlots were grown in Sutton Bonington in 1998. Each of the mother crops received different amounts of nitrogen in the form of ammonium nitrate as shown in table 4.2.4.5. Following harvest in 1999 the seeds were stored in airtight containers in the cold store at 4°C to prevent deterioration.

Table 4.2.4.5Mother crop nitrogen management of the 1999 harvest Apex
seedlots

Mother crop	Nitrogen application (kg ha ⁻¹)	Timing of applications
Nil	Nil	-
100 G/ F	100	50 kg ha ⁻¹ in February (G) and March (F)
200 G/ F	200	100 kg ha ⁻¹ in February (G) and March (F)

Increasing the amount of nitrogen fertiliser applied to the mother crop from nil to 100 to 200 kg ha⁻¹ significantly increased the seed nitrogen percentage (P < 0.001) from 2.60% to 3.08% to 3.28% and significantly decreased the seed oil percentage (P < 0.001) from 51.77% to 48.87% to 47.09% as shown in table 4.2.4.6. The inverse relationship between seed nitrogen and oil percentage is shown in figure 4.2.4.3.

Table 4.2.4.6The effect of mother crop nitrogen management on the seednitrogen and oil percentages of the 1999 harvest Apex seedlots

Mother crop nitrogen management (kg ha ⁻¹)	Nitrogen (%)	Oil (%)
Nil	2.60	51.77
100 G/ F	3.08	48.87
200 G/ F	3.28	47.09
Р	< 0.001	< 0.001
SED	0.0813	0.509
DF	18	18





Comparison of the effect of mother crop nitrogen management on the seed nitrogen and oil percentage of the 1997 and 1999 harvest Apex seedlots

The highest seed nitrogen percentage was observed in the seeds from the 1997 160 F mother crop as shown in figure 4.2.4.4, which was able to transfer the majority of 160 kg nitrogen ha⁻¹ to its seeds. The seed nitrogen percentage then decreased with decreasing amounts of nitrogen applied at flowering for both the 1997 300 G/F and the 1999 200 G/F mother crops. Both the 1997 and 1999 100 G/F mother crops achieved

similar seed nitrogen and oil percentages and the nil treatments achieved the lowest seed nitrogen percentages. The 1997 80 F treatment is again anomalous in terms of its seed nitrogen and oil percentage as previously discussed but there are otherwise clear effects of fertiliser timing on the seed nitrogen and oil percentage. It is likely that only a fraction of the fertiliser applied in February is available to be transferred to the seed reserves.

Figure 4.2.4.4 The effect of mother crop nitrogen application on the mean seed nitrogen and oil percentages of the 1997 and 1999 harvest Apex seedlots



The effect of mother crop nitrogen management on the seed yield and thousand seed weight of the 1997 harvest Apex seedlots

Increasing nitrogen application to the mother crop generally increased the seed yield although the increases were not significant (P = 0.180) as shown in table 4.2.4.7. The pods harvested from the lower section of the canopy yielded significantly more weight (P = 0.013) than those harvested from the upper section of the canopy.

Table 4.2.4.7The effect of mother crop nitrogen management and pod position
on the seed yield $(g m^{-2})$ of the 1997 harvest Apex seedlots

	Seed yie	$ld (g m^{-2})$	7
Mother crop N management	Upper position	Lower position	Mean seed
(kg ha ⁻¹) and timing	(> 1.5 m)	(< 1.5 m)	yield
Nil	155.80	184.50	170.10
80 F	143.40	185.30	164.30
100 G/ F	165.80	206.40	186.10
300 G/ F	197.90	151.90	174.90
160 F	167.90	235.60	201.80
Factor means	166.10	192.70	179.40
Probability	0.013		0.180
SED	10.400		16.450
DF	62		62
			-

N application* pod position	P = 0.014	SED = 23.260	DF = 62

The seed yield was affected by a significant interaction between nitrogen application and pod position (P = 0.014). There was not a significant effect of pod position on the seed yield of the nil application mother crop while the pods harvested from the lower section of the canopy in the 300 G/ F treatment yielded significantly less than those harvested from the upper section of the canopy.

Since the yield is equal to the number of seeds*seed weight, it was possible that the upper layer may have yielded more seeds than the lower layer but that the upper seeds may have been lighter in weight. The thousand seed weight (TSW) was thus calculated as previously described and the data are shown in table 4.2.4.8.

Table 4.2.4.8The effect of mother crop nitrogen management and pod position
on the thousand seed weight (g) of the 1997 harvest Apex
seedlots

	TSW		
Mother crop N management	Upper position	Lower position	Mean TSW
(kg ha ⁻¹) and timing	(> 1.5 m)	(< 1.5 m)	
Nil	4.613	4.503	4.558
80 F	4.615	4.473	4.544
100 G/ F	4.606	4.335	4.471
300 G/ F	5.185	4.483	4.834
160 F	5.563	4.828	5.195
Factor means	4.916	4.524	4.720
Probability	< 0.001		< 0.001
SED	0.033		0.053
DF	62		62
	· · · · · · · · · · · · · · · · · · ·		
N application* pod position	P < 0.001	SED = 0.075	DF = 62

Nitrogen application to the mother crop did not significantly affect the TSW at the lower (nil, 80 and 100 kg ha⁻¹) applications. However, increasing it from 100 kg ha⁻¹ to 300 kg ha⁻¹ significantly increased the TSW (P < 0.001) as did altering the application timing from a split application (300 G/ F) to a single application at flowering (160 F). Those pods harvested from the lower section of the canopy produced significantly lighter seeds (P < 0.001) than those harvested from the upper section of the canopy.

The TSW was also affected by a significant interaction between the nitrogen application and pod position (P < 0.001). With the exception of the nil nitrogen treatment, the seeds harvested from the lower section of the crop canopy were significantly lighter than those harvested from the upper section of the canopy. Since the final yield from the lower pods was significantly greater but their individual seed weights were lighter than that from the upper pods, it is evident that more seeds were produced from the lower than from the upper pods.

The effect of mother crop nitrogen management on the seed size of the 1997 and 1999 harvest Apex seedlots

The percentage of large (> 2 mm in diameter) and small (< 2 mm in diameter) seeds of a 10g sample of seeds from the highest (3.61%) and lowest (2.30%) nitrogen 1997 harvest Apex seedlots was measured. These extremes were chosen since their comparison produced the greatest effect on the seed nitrogen and oil percentage, seed yield and TSW. Increasing the seed nitrogen percentage increased the percentage of large seeds by 19% but because of the variety between the replicates, this increase was not significant (P = 0.251) as shown in table 4.2.4.9.

Table 4.2.4.9The effect of seed nitrogen percentage on the percentage of large
(> 2mm) and small (< 2 mm) seeds of the 1997 harvest Apex
seedlots

Seed nitrogen percentage	Percentage of large seeds	Percentage of small seeds		
2.30	73	27		
3.61	92	8		
Р	0.251			
SED	0.156			
DF	(5		

Increasing the seed nitrogen percentage did not significantly increase the percentage of large seeds (P = 0.259) in the 1999 harvest Apex seedlots as shown in table 4.2.4.10.

Table 4.2.4.10The effect of seed nitrogen percentage on the percentage of large
(> 2 mm) and small (< 2 mm) seeds of the 1999 harvest Apex
seedlots

Seed nitrogen (%)	Percentage of large seeds	Percentage of small seeds		
2.60	65	35		
3.08	74	26		
3.28	72	28		
Р	0.259			
SED	0.055			
DF	60			

4.2.5 The effect of seed nitrogen percentage, pod position and harvest date on germination at 15°C of the 1997 harvest Apex seedlots

In experiment 4.2.1 the Apex variety achieved over 85% germination at all temperatures except at 5°C at which it achieved an FPG of 82%. Although significant seedlot (S2, S8, S12 and S16) differences were observed at 5 and 20°C, none were observed at the field temperatures of 10 and 15°C.

<u>Method</u>

The 1997 harvest Apex seedlots were used for this experiment. The mother crop nitrogen management regimes were 0, 80 F, 100 G/ F, 160 F and 300 G/ F kg ha⁻¹, which produced seeds with nitrogen percentages of 2.30, 2.54, 2.95, 3.61 and 3.46% respectively. Seeds were harvested from either the upper or lower half of the pod canopy, early (21st July) or late (14th August) in 1997. The effects of harvest date on the germination of the 1997 seedlots was examined since the seed nitrogen percentage had been slightly but non-significantly increased by delaying the harvest. There were 5 replicates of each treatment and 20 seeds per replicate. The seeds were germinated in the controlled growth room environment at 15°C as previously described.

<u>Results</u>

The effect of seed nitrogen percentage, pod position and harvest date on the time to 10% germination at 15°C of the 1997 harvest Apex seedlots

Increasing the seed nitrogen percentage (P < 0.001), selecting lower pod position (P = 0.004) and early harvesting (P < 0.001) significantly reduced the time to 10% germination as shown in table 4.2.5.1.

The effects of seed nitrogen could be divided into a low (2.30 to 2.95%) and high (3.46 to 3.61%) categories. Increasing the seed nitrogen percentage within either category did not significantly reduce the T_{10} but increasing it from the low to the high category significantly reduced the T_{10} by just under 3 h. Selecting seeds harvested from the lower pods reduced the T_{10} by about an hour, which was likely to be due to the nitrogen percentage since the lower pods had a significantly greater seed nitrogen percentage (P < 0.001) than the upper pods.

The relationship between the seed nitrogen percentage and the time to 10% germination is shown in figure 4.2.5.1: increasing the seed nitrogen percentage significantly reduced the T_{10} at 15°C.

Table 4.2.5.1The effect of seed nitrogen percentage, pod position and harvest
date on the time to 10% germination (h) of the 1997 harvest Apex
seedlots at 15°C

	Time to 10% germination (h)			
Seed nitrogen	Pod	Early	Late	Mean T ₁₀
percentage	position	harvest date	harvest date	
2.30	Upper	24.17	24.93	24.47
	Lower	23.38	25.39	
2.54	Upper	23.97	24.30	24.02
	Lower	23.61	24.20	
2.95	Upper	24.47	24.71	23.96
	Lower	23.09	23.58	
3.46	Upper	21.35	23.62	22.10
	Lower	21.13	22.29	
3.61	Upper	20.89	23.11	21.68
	Lower	20.65	22.08	
Mean harvest date		22.67	23.8	
Mean upper	23.55			
Mean lower	22.94			
Probability	0.004	< 0.001		< 0.001
SED	0.207	0.207		0.328
DF	108			

Interactions	Probability	SED	DF
Harvest date* nitrogen	0.071	0.463	108
Harvest date* pod position	0.942	0.293	108
Nitrogen* pod position	0.464	0.463	108
Harvest date* nitrogen * position	0.391	0.655	108

Figure 4.2.5.1 The effect of seed nitrogen percentage on the time to 10% germination (h) of the 1997 harvest Apex seedlots at 15°C



The effect of seed nitrogen percentage, pod position and harvest date on the time to 50% germination at 15° C of the 1997 harvest Apex seedlots

Increasing the seed nitrogen percentage significantly reduced the time to 50% germination (P < 0.001) as shown in table 4.2.5.2 and figure 4.2.5.2. There was no significant effect of pod position in the canopy on the time to 50% germination (P = 0.189) but early harvesting significantly reduced the T_{10} (P < 0.001) by about an hour.

There was also an interaction between the seed nitrogen percentage and the harvest date, which significantly affected the time to 50% germination (P = 0.004) as shown in figure 4.2.5.3. For both harvest dates, increasing the seed nitrogen percentage significantly reduced the time to 50% germination. The significant effect of harvest date was most visible at high seed nitrogen percentages: selecting early harvested seeds significantly reduced the time to 50% germination compared with selecting late harvested seeds. Table 4.2.5.2The effect of seed nitrogen percentage, pod position and harvest
date on the time to 50% germination (h) of the 1997 harvest Apex
seedlots at 15°C

	Time to 50% germination (h)			
Seed nitrogen	Pod	Early	Late	Mean T ₅₀
percentage	position	harvest date	harvest date	
2.30	Upper	26.94	27.70	27.29
	Lower	27.01	27.49	
2.54	Upper	27.52	27.13	27.32
	Lower	27.04	27.61	
2.95	Upper	26.11	27.63	26.80
	Lower	26.32	27.13	
3.46	Upper	24.61	27.00	25.64
	Lower	25.14	25.83	
3.61	Upper	24.55	26.76	25.33
	Lower	23.89	26.12	
Mean harvest date		25.91	27.04	
Mean upper	26.60			
Mean lower	26.34			
Probability	0.189	< 0.001		< 0.001
SED	0.180	0.180 0.180		0.284
DF	108			

Interactions	Probability	SED	DF
Harvest date* nitrogen	0.004	0.402	108
Harvest date* pod position	0.343	0.254	108
Nitrogen* pod position	0.789	0.402	108
Harvest date* nitrogen * position	0.216	0.569	108

Figure 4.2.5.2 The effect of seed nitrogen percentage on the time to 50% germination (h) of the 1997 harvest Apex seedlots at 15°C



Figure 4.2.5.3 The effect of harvest date and seed nitrogen percentage on the time to 50% germination of the 1997 harvest Apex seedlots at 15°C



The effect of seed nitrogen percentage, pod position and harvest date on the final percentage germination at 15°C of the 1997 harvest Apex seedlots

The FPG was not significantly affected by the seed nitrogen percentage (P = 0.606), pod position (P = 0.495) or harvest date (P = 0.489) as shown in table 4.2.5.3.

Table 4.2.5.3The effect of seed nitrogen percentage, pod position and harvest
date on the final percentage germination of the 1997 harvest
Apex seedlots at 15°C

	Final percentage germination				
Seed nitrogen	Pod	Early	Late	Mean FPG	
percentage	position	harvest date	harvest date		
2.30	Upper	100	98	99	
	Lower	100	99		
2.54	Upper	100	100	100	
	Lower	99	99		
2.95	Upper	96	98	98	
	Lower	99	100		
3.46	Upper	100	100	100	
	Lower	100	98		
3.61	Upper	100	100	100	
	Lower	99	100		
Mean harvest date		100	100		
Mean upper	100				
Mean lower	100				
Probability	0.495	0.489		0.606	
SED	1.614	1.614		2.552	
DF	108			· · · · · · · · · · · · · · · · · · ·	

Interactions	Probability	SED	DF
Harvest date* nitrogen	0.418	3.609	108
Harvest date* pod position	0.865	2.282	108
Nitrogen* pod position	0.382	3.609	108
Harvest date* nitrogen * position	0.627	5.103	108

The effect of seed nitrogen percentage, pod position and harvest date on the rate of germination at 15°C of the 1997 harvest Apex seedlots

Increasing the seed nitrogen percentage significantly increased the rate of germination (P < 0.001) as shown in table 4.2.5.4. Increasing the seed nitrogen percentage within the 2.30 to 2.95% (low nitrogen percentage category) or the 3.46 to 3.61% (high nitrogen percentage category) did not significantly increase the rate of germination. However, increasing the nitrogen percentage from the low nitrogen percentage to the high nitrogen percentage category significantly increased the rate of germination from 0.03695 to 0.03939 as shown in figure 4.2.5.4.

Figure 4.2.5.4 The effect of seed nitrogen percentage on the rate of germination (h^{-1}) of the 1997 harvest Apex seedlots at 15°C



Pod position in the canopy did not significantly affect the rate of germination (P = 0.205). However, the seeds that were harvested from the lower section of the canopy had a higher rate of germination than those harvested from the upper section of the canopy. Early harvesting significantly increased the rate of germination (P < 0.001), which was likely to be due to reduced ageing damage.

Table 4.2.5.4The effect of seed nitrogen percentage, pod position and harvest
date on the rate of germination of the 1997 harvest Apex seedlots
at 15°C

	Rate of germination (h ⁻¹)				
Seed nitrogen	Pod	Early	Late	Mean rate	
percentage	position	harvest date	harvest date		
2.30	Upper	0.03717	0.03619	0.03676	
	Lowcr	0.03719	0.03649		
2.54	Upper	0.03645	0.03699	0.03668	
	Lower	0.03700	0.03631		
2.95	Upper	0.03824	0.03635	0.03742	
	Lower	0.03807	0.03692		
3.46	Upper	0.04072	0.03710	0.03911	
	Lower	0.03984	0.03878		
3.61	Upper	0.04086	0.03749	0.03967	
	Lower	0.04199	0.03834		
Mean harvest date		0.03876	0.03710		
Mean upper	0.03777			······	
Mean lower	0.03809				
Probability	0.205	< 0.001		< 0.001	
SED	0.000257	0.000257		0.000406	
DF	108				

Interactions	Probability	SED	DF
Harvest date* nitrogen	< 0.001	0.000575	108
Harvest date* pod position	0.401	0.000363	108
Nitrogen* pod position	0.730	0.000575	108
Harvest date* nitrogen * position	0.205	0.000813	108

There was an interaction between the seed nitrogen percentage and the harvest date, which significantly affected the rate of germination (P < 0.001). The effects of harvest date and seed nitrogen percentage on the rate of germination are shown in figure 4.2.5.5.

Figure 4.2.5.5 The effect of harvest date and seed nitrogen percentage on the rate of germination (h^{-1}) of the 1997 harvest Apex seedlots at 15° C



For both harvest dates, the germination rate was significantly increased by increasing the seed nitrogen percentage. The significant effect of harvest date was most visible at high seed nitrogen percentages: selecting early harvested seeds significantly increased the rate of germination compared with selecting late harvested seeds.

This experiment has shown that increased seed nitrogen percentage can significantly increase the speed of germination. Since protein is needed for growth by the germinating seed, it is possible that the high seed nitrogen levels are partially responsible for the increased speed of germination as measured by the T_{10} , T_{50} and rate of germination in the high nitrogen seeds. Early harvesting also increased the speed of germination, which was probably due to reduced seed aging effects on the mother plant.

4.2.6 The effect of heat treatment, seed nitrogen percentage, pod position and harvest date on germination at 15 and 25°C of the nil and 160 F 1997 harvest Apex seedlots

The effects of heat treatment, which has been used as a method of seed sterilisation (Sorensen, 1995; Bell, 2002), was investigated to determine whether high temperature heat treatment would detrimentally affect seed germination.

<u>Method</u>

This experiment used the seeds with the lowest (2.30%) and highest (3.61%) nitrogen contents, which were produced from the nil and 160 kg ha⁻¹ nitrogen mother crop management regimes respectively. The effects of pod position in the canopy (> 1.5 m or < 1.5 m) and harvest date (21^{st} July or 14^{th} August) were also examined. Half of the seed samples were heat-treated as previously described while the other half formed the controls. There were 5 replicates of each treatment and 20 seeds per replicate. The seeds were germinated as previously described in the growth room at 15 and 25°C.

<u>Results</u>

The effect of heat treatment, seed nitrogen percentage, pod position and harvest date on the time to 10% germination at $15^{\circ}C$

Seed heat treatment significantly increased the time to 10% germination (P < 0.001) by 5 h as shown in table 4.2.6.1. Increasing the seed nitrogen percentage significantly reduced the T_{10} (P < 0.001) as did selecting seeds from upper harvested pods (P < 0.001). Harvest date did not significantly alter the T_{10} (P = 0.191).

Table 4.2.6.1The effect of heat treatment, seed nitrogen percentage, podposition and harvest date on the time to 10% germination (h) ofthe 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots at 15°C

	Time to 10% germination (h)					
Seed nitrogen	Heat	Early	Late	e F	'od	Mean T ₁₀
percentage	treatment	harvest date	harvest	date pos	sition	
2.30	Control	24.11	24.2	0 Lo	ower	25.13
	Control	20.90	23.4	6 Uj	pper	
	Heated	27.68	26.5	6 Lo	ower	
	Heated	27.27	26.8	1 Uj	pper	
3.61	Control	20.25	18.8	3 Uj	pper	22.83
	Control	20.16	22.2	7 Lo	ower	
	Heated	26.96	22.3	5 Ul	pper	
	Heated	25.67	26.1	1 Lo	ower	
Mean harvest date		24.13	23.8	2		······
Mean Control	21.77					
Mean Heated	26.18					
Mean Upper				23	3.35	
Mean Lower				24	1.60	
Probability	< 0.001	0.191 < 0.001		0.001	< 0.001	
SED	0.226					
DF	31					
Internations			1	Probability	SED	DF
				0.029	0.220	21
Heat treatment* nitrogen				0.038	0.320	31

Heat treatment* nitrogen	0.038	0.320	31
Heat treatment* harvest date	< 0.001	0.320	31
Nitrogen * harvest date	0.017	0.320	31
Heat treatment* pod position	0.015	0.320	31
Nitrogen* pod position	0.353	0.320	31
Harvest date* pod position	0.005	0.320	31
Heat treatment* nitrogen* harvest date	0.730	0.453	31
Heat treatment* nitrogen* pod position	0.118	0.453	31
Heat treatment* harvest date* pod position	0.076	0.453	31
Nitrogen* harvest date* pod position	< 0.001	0.453	31

The interaction between all four factors was not calculable because of missing values in the data. However, there was a significant interaction between the seed nitrogen percentage, harvest date and pod position in the canopy, which affected the time to 10% germination (P < 0.001). Within the seeds of high nitrogen percentage, seeds that were harvested late in the season and selected from the pods in the lower (< 1.5 m) section of the canopy took significantly longer to reach 10% germination than those selected from the upper (> 1.5 m) section of the canopy. It is possible that the high oil percentage of the upper harvested seeds protected those seeds from some of the heat damage during heat treatment.

The effect of heat treatment, seed nitrogen percentage, pod position and harvest date on the time to 50% germination at $15^{\circ}C$

The effect of heat treatment at 80°C for 48 h significantly increased the time to 50% germination (P < 0.001) by 5 h as shown in table 4.2.6.2. Increasing the nitrogen percentage from 2.30% to 3.61% significantly reduced the T_{50} (P < 0.001) by more than an hour. By selecting seeds from pods that were harvested at a height of > 1.5 m, the T_{50} was significantly reduced (P < 0.001) by an hour.

The effect of pod position in this experiment was the opposite of that observed in experiment 4.2.5 in which the pods that were harvested from lower down the pod canopy (< 1.5 m) had a faster speed of germination than those harvested from the upper section (> 1.5 m) of the canopy. Selecting late harvested seeds significantly reduced the T_{50} (P = 0.012) by just under an hour, which was also the opposite of that observed in experiment 4.2.5.

It is possible that the high oil percentage of the seeds from the upper pods could have protected these seeds from the detrimental effects of heat and thus the germination capacity of only the high nitrogen (low oil) seeds was impaired. Secondly, it is possible that heat treatment increased the breakdown of oil reserves, thus providing the germinating seedling with a ready source of energy during germination. Although these effects may have been small, by combining a reduction in the germination capacity of the high nitrogen seeds with an increase in the capacity of the low nitrogen seeds, it is possible that together they increased the germination speed of the low nitrogen seeds.

There was a significant interaction between the seed nitrogen percentage, harvest date and pod position in the canopy, which affected the T_{50} (P < 0.001). As with the T_{10} , the shortest T_{50} was achieved by the seeds of high nitrogen percentage that were harvested late in the season from the upper section of the canopy; the longest T_{50} was recorded in the low nitrogen seeds harvested early in the season from the lower section of the canopy. Table 4.2.6.2The effect of heat treatment, seed nitrogen percentage, pod
position and harvest date on the time to 50% germination (h) of
the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots at 15°C

1	[Time to 50% germination (h)				
Seed nitrogen	Heat	Early	Late	Pod	Mean T ₅₀	
percentage	treatment	harvest date	harvest date	position		
2.30	Control	26.38	26.58	Lower	27.84	
	Control	23.62	25.59	Upper		
	Heated	31.13	29.35	Lower		
	Heated	30.06	30.00	Upper		
3.61	Control	23.29	21.90	Upper	26.19	
	Control	23.02	25.03	Lower		
	Heated	31.44	27.56	Upper		
	Heated	29.08	28.21	Lower		
Mean harvest date		27.25	26.78			
Mean Control	24.43		· · · · · · · · · · · · · · · · · · ·			
Mean Heated	29.60					
Mean Upper				26.68		
Mean Lower				27.34		
Probability	< 0.001	0.0	0.012 < 0.001		< 0.001	
SED		0.178				
DF	31					

Interactions	Probability	SED	DF
Heat treatment* nitrogen	0.002	0.251	31
Heat treatment* harvest date	< 0.001	0.251	31
Nitrogen * harvest date	0.004	0.251	31
Heat treatment* pod position	< 0.001	0.251	31
Nitrogen* pod position	0.043	0.251	31
Harvest date* pod position	0.049	0.251	31
Heat treatment* nitrogen* harvest date	0.351	0.356	31
Heat treatment* nitrogen* pod position	0.392	0.356	31
Heat treatment* harvest date* pod position	0.821	0.356	31
Nitrogen* harvest date* pod position	< 0.001	0.356	31

The effect of heat treatment, seed nitrogen percentage, pod position and harvest date on the final percentage germination at 15°C

There was no significant effect of heat treatment on the final percentage germination (P = 0.646). Increasing the seed nitrogen percentage from 2.30% to 3.61% significantly increased the FPG (P < 0.001) to 100% while selecting seeds from pods that were harvested at a height of > 1.5 m, produced the same increase (P < 0.001) as shown in table 4.2.6.3. Harvest date did not significantly affect the FPG (P = 0.489).

The final percentage germination was affected by a significant interaction between heat treatment, harvest date and the pod position in the canopy (P < 0.001). Heat treating seeds that had been harvested early in the season from the lower (< 1.5 m) section of the pod canopy significantly reduced the FPG compared with the late harvest date, which was probably due to heat damage.

Table 4.2.6.3The effect of heat treatment, seed nitrogen percentage, podposition and harvest date on the final percentage germination ofthe 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots at 15°C

	Final percentage germination				
Seed nitrogen	Heat	Early	Late	Pod	Mean FPG
percentage	treatment	harvest date	harvest date	position	
2.30	Control	99	98	Lower	98
	Control	98	98	Upper	
	Heated	92	100	Lower	
	Heated	100	98	Upper	
3.61	Control	100	100	Upper	100
	Control	100	100	Lower	
	Heated	100	100	Upper	
	Heated	97	99	Lower	
Mean harvest date		100	99		
Mean Control	100				<u></u>
Mean Heated	99				
Mean Upper				100	
Mean Lower				98	
Probability	0.646	0.4	89	< 0.001	< 0.001
SED	0.483				
DF	31				

Interactions	Probability	SED	DF
Heat treatment* nitrogen	0.411	0.683	31
Heat treatment* harvest date	0.124	0.683	31
Nitrogen * harvest date	0.009	0.683	31
Heat treatment* pod position	< 0.001	0.683	31
Nitrogen* pod position	0.131	0.683	31
Harvest date* pod position	< 0.001	0.683	31
Heat treatment* nitrogen* harvest date	0.022	0.966	31
Heat treatment* nitrogen* pod position	0.112	0.966	31
Heat treatment* harvest date* pod position	< 0.001	0.966	31
Nitrogen* harvest date* pod position	0.298	0.966	31

The effect of heat treatment, seed nitrogen percentage, pod position and harvest date on the rate of germination at $15^{\circ}C$

Heat treatment significantly reduced the rate of germination (P < 0.001) from 0.04114 to 0.03352 while increasing the nitrogen percentage from 2.30% to 3.61% significantly increased the rate of germination (P < 0.001) as shown in table 4.2.6.4. By selecting seeds from pods that were harvested from the upper pods (P = 0.001) or selecting late harvested seeds (P = 0.006), the rate of germination was significantly increased.

There was a significant interaction between the seed nitrogen percentage, harvest date and pod position in the canopy, which affected the rate of germination (P < 0.001). Within the low nitrogen seeds there was no effect of harvest date on the rate of germination of the seeds that were harvested from the lower section of the canopy. However, within the upper harvested seeds, the earlier harvested seeds had a higher rate of germination than the late harvested seeds, which was probably due to reduced ageing effects. Within the high nitrogen seeds there were again varied effects of this significant interaction but in general, late harvesting produced the greatest rate of germination while early harvesting reduced the rate of germination. The highest rate of germination was recorded in the high nitrogen seeds that had been harvested late from the upper section of the pod canopy. Table 4.2.6.4The effect of heat treatment, seed nitrogen percentage, pod
position and harvest date on the rate of germination (h⁻¹) of the
2.30 and 3.61% nitrogen 1997 harvest Apex seedlots at 15°C

	Rate of germination (h ⁻¹)				
Seed nitrogen	Heat	Early	Late	Pod	Mean rate
percentage	treatment	harvest date	harvest date	position	
2.30	Control	0.03791	0.03764	Lower	0.362
	Control	0.04235	0.03911	Upper	
	Heated	0.03207	0.03410	Lower	
	Heated	0.03330	0.03336	Upper	
3.61	Control	0.04296	0.04568	Upper	0.384
	Control	0.04347	0.03999	Lower	
	Heated	0.02916	0.03629	Upper	
	Heated	0.03442	0.03547	Lower	
Mean harvest date		0.03696	0.03770		Anno de conse de la Alta
Mean Control	0.04114				
Mean Heated	0.03352				
Mean Upper		<u></u>		0.03778	
Mean Lower				0.03688	
Probability	< 0.001	0.0	006	0.001	< 0.001
SED	0.000251				
DF	31				

Interactions	Probability	SED	DF
Heat treatment* nitrogen	< 0.001	0.000356	31
Heat treatment* harvest date	< 0.001	0.000356	31
Nitrogen * harvest date	< 0.001	0.000356	31
Heat treatment* pod position	< 0.001	0.000356	31
Nitrogen* pod position	0.009	0.000356	31
Harvest date* pod position	< 0.001	0.000356	31
Heat treatment* nitrogen* harvest date	0.105	0.000502	31
Heat treatment* nitrogen* pod position	0.045	0.000502	31
Heat treatment* harvest date* pod position	0.660	0.000502	31
Nitrogen* harvest date* pod position	< 0.001	0.000502	31

The effect of heat treatment, seed nitrogen percentage, pod position and harvest date on germination at $25^{\circ}C$

At 25°C, the same effects of heat treatment, seed nitrogen percentage, pod position and harvest date were observed as have been reported at 15°C. However, although heat treatment significantly increased the T_{10} (P < 0.001) and T_{50} (P < 0.001) and significantly reduced the rate of germination (P < 0.001), it significantly increased the FPG (P = 0.011). The increase in FPG of the heat-treated seeds at 25°C was probably due to beneficial protection effects of heat shock and/ or lactate embryogenesis abundant proteins.

However, since the length of delay in germination due to heat treatment was approximately the same at both 15 and 25°C, it is interesting to note that the reorganisation of the membranes, which are likely to have been disrupted during heat treatment, was not faster at this temperature although this was not directly tested. It would be possible to test the effect of temperature on membrane reorganisation by performing a conductivity test on the cotyledons of seeds at both temperatures.

4.3 Discussion of Germination Experiments

The aims of these experiments were to examine the effect of temperature on the germination of nine commercial varieties comprised of 16 seedlots of winter oilseed rape. Following this, the effects of duration of seed hydration in water followed by drying back and large or small seed size selection on the germination of seedlots of the same variety of winter oilseed rape were examined in controlled (growth room) conditions. The effects of mother crop nitrogen management, pod position in the canopy and harvest date on the seed nitrogen and oil percentage, seed nitrogen weight, thousand

seed weight, seed yield and ratio of large to small seeds of the Apex variety of winter oilseed rape were determined. The effects of seed nitrogen percentage and heat treatment on germination were examined under controlled conditions.

Germination speed is an important component of seed vigour and low soil temperatures can reduce the percentage and the rate of germination and can increase the susceptibility of seedlings to soil-borne pathogens. In this research although the commercial varieties and their seedlots used in experiment 4.2.1 were sold according to the guidelines that they had achieved the benchmark 85% germination at 20°C (International Seed Testing Association, 1993), the germination responses of both different varieties and seedlots of the same variety varied significantly with temperature.

Between 15 and 25°C the mean FPG was greater than 85% although two of the nine varieties failed to reach 85% germination at the highest temperature. At 10°C, which is approximately the UK field temperature during late-August to early-September, the mean FPG of the majority of seedlots was only 81% while at 5°C it was reduced to 64%. Furthermore, varieties such as Rocket and Bristol did not achieve 85% germination at 20°C but did achieve it at 15°C. It is likely, therefore, that sowing these varieties in the field could result in highly variable seedling emergence and subsequent establishment.

Significant reductions in oilseed rape germination at temperatures lower than 10C have previously been reported (Nykiforuk and Johnson-Flanagan, 1994) and it was only at 15°C that all the seedlots and varieties achieved the 85% germination, which is required for commercial seedlots (International Seed Testing Association, 1993). Under increasingly colder conditions the differences between both varieties and seedlots
became increasingly visible and the genetic and physiological differences were clearest at low temperatures: at 10°C the FPG ranged from 49 to 98% and at 5°C from 9 to 97%. The base temperature, T_b , was estimated to be 3.3°C, which was the same as that reported by Marshall and Squire (1996) and lies between other estimates of 0.9 °C (Vigil *et al.*, 1997) and 5°C (Morrisson *et al.*, 1989).

These experiments have shown that variability in germination between seedlots of the same variety can be as large as the variability between different varieties. There was significant seedlot variation in the lower germination varieties such as Bristol and Apex, particularly at the lower temperatures but there were fewer effects within the higher germination varieties such as Synergy and Rocket. Contrasting effects of cultivar were also observed by Wilson et al. (1992), who reported no germination of 11 Brassica cultivars at 2°C whereas Kondra et al. (1983) had previously reported up to 91% rapeseed germination at this temperature.

It is possible that slight differences in the seed composition were responsible for the significant differences in the germination responses to temperature of seedlots of the same variety, in particular the very low germination response of the Bristol seedlot S13 at 5°C compared with S6 and S1. Since the final percentage germination of all the Bristol seedlots was reduced at 5°C, it is possible that a small difference in the fatty acid composition of S13 made it particularly vulnerable to the low temperature. Plant growth is very sensitive to temperature and it is likely that seeds undergo some metabolic changes at low temperatures. As temperatures are lowered, lipids in cellular membranes crystallise at a critical ratio of saturated to unsaturated fatty acids. Below the critical temperature they thus exist in a solid-gel state rather than a liquid-crystalline state, and

they contract (Yoshida *et al.*, 1999), which renders them susceptible to cracking upon rehydration and affects solute balances and proton transport (Dupont and Mudd, 1985).

It is also possible that S13 had a firmer seed coat than seedlots S1 and S6 and that although imbibition occurred, the embryo was not strong enough to penetrate the testa. Subtle differences within the seed such as enzyme synthesis or activation or membrane permeability changes (Salisbury and Ross, 1992) or low temperature-induced secondary dormancy could also have been responsible for the particularly poor germination response of S13 at 5°C. These differences have important implications for growers since differences in temperature responses of different varieties and individual seedlots would be likely to affect establishment, particularly in northern climates where seedbeds are likely to be colder.

Increasing the temperature brought forward the onset of germination in all the seedlots and varieties but did not always significantly increase the FPG. The highest mean FPG coupled with the least variation was observed at 15°C thus this temperature might be more appropriate to test the germination potential of seedlots while testing at 10 °C might correlate better with field performance. This experiment identified low germination varieties such as Bristol and Alpine and in particular low germination seedlots such as the Bristol seedlots S1, S6 and S13. Although only nine varieties were tested in this experiment, revision of the ISTA guidelines to test winter oilseed rape seedlots at 15 rather than 20°C might be appropriate for other varieties.

Physiological and biochemical changes occur when a seed is hydrated, which influence the rapidity, synchrony and percentage of seeds that germinate (Lang and Holmes, 1964) since seed hydration conditions can allow the repair of cell damage prior to germination. Furthermore, this research showed that hydrated seeds could be air-dried with little loss of the treatment effect provided that drying-back has occurred before radicle emergence as previously observed by Heydecker *et al.*, 1975.

As was observed in experiment 4.2.1, seedlot had a significant effect on the FPG at 5°C: S1 achieved a mean final percentage of 72% compared with 11% for S13. Seed hydration significantly improved the mean FPG of the two seedlots at this temperature increasing the mean seedlot FPG from 28 (control) to 50% (36 h seed hydration). However, the optimal duration of seed hydration was seedlot dependent with the greatest effect being observed in the lower germination (S13) seedlot. Increasing the duration of seed hydration to 36 h also significantly increased post-germinative taproot growth at 5°C.

There were no significant seedlot or hydration effects at 15°C although short durations of 6 and 12 h did significantly increase post-germinative taproot growth. The patterns of taproot growth following seed hydration for seedlots S1 and S13 were similar but the low vigour S13 needed a longer initial duration than the high vigour S1 to attain the same increase in taproot length.

Physiological studies have indicated increased rates of the metabolic processes involved in germination when primed seeds are rehydrated and after dehydration and subsequent re-imbibition, the seed germination processes resume from where they were stopped. The effects of priming are then evident as rapid radicle growth and seedling emergence: the ability of the seed to germinate and emerge under stressful environmental conditions is possibly the result of bringing the germination processes to the 'brink' of radicle protrusion (Parera and Cantliffe, 1994b). However, since hydration treatments prolong

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the phase between imbibition and germination, it is likely that physiological repair occurs during this period (Sivritepe and Dourado, 1995) as has been observed for cauliflower and Brussels sprouts (Thornton and Powell, 1992). Seed hydration treatments thus allow cells to repair membranal and other damage, which has been sustained through fluctuations in the growing or storage environments. While seeds are imbibing water it is likely that much of the cell damage is repaired and it is for this reason that germination is able to proceed promptly and more uniformly.

This research has shown that the optimal duration for the Bristol seedlots is, however, dependent on the temperature at which the seeds are germinated as well as on the seedlot quality and the measure of germination (FPG) or growth (post-germinative taproot growth). If the speed and efficiency of germination as measured by the germination parameters (T_{10} , T_{50} , FPG and rate of germination) are considered most important, then at 5°C, the optimal duration of seed hydration was 12 h for seed hydration alone since no further benefit was observed after this time. The main level interaction is misleading, however, since the significant interaction between seed hydration and seedlot altered the optimal durations to 36 h for both S1 and S13.

While longer durations may have further increased the FPG of S13, it is likely that they would have resulted in the over-advancement of S1, which would have caused the radicle to emerge from the seed testa (McWilliam, 1998). Radicle emergence prior to drying-back has been reported to make cauliflower seeds more susceptible to drying after treatment (Powell *et al.*, 2000) and to result in seedling death when the seed is subsequently germinated. This research confirmed observations by Durrant and Jaggard (1988) that the duration of sugar beet priming treatments needed to be specific for each type of seed and seedlot since seed populations are subject to inter- and intra-varietal

differences. For low germination temperatures in particular, seedlot specific durations of hydration would be necessary to obtain the highest germination performance from each seedlot.

The greatest effects on germination and post-germinative taproot growth were observed at 5°C and in the lower germination S13 seedlot, which confirmed observations by Zheng *et al.* (1994) where priming showed greatest increases in low vigour oilseed rape seedlots at low temperatures. Poor seedlots under harsh environmental conditions are more 'improved' by seed hydration treatments than good seedlots under idealised conditions because there is more deterioration in low germinating seedlots that can be repaired upon hydration in comparison with high germination seedlots, which are less damaged. It is likely that this is the reason why seed hydration did not significantly improve germination at 15°C since at this optimal temperature for germination there was the least potential for seed improvement.

Seed hydration significantly increased the speed and final percentage germination of the Bristol seedlots S1 and S13 although improvements in final percentage germination were compounded by seedlot interactions. Although there were only relatively small time savings, this may be important in dry seedbed conditions where water is limited and the speed of germination is paramount. Seed hydration also significantly increased post-germinative taproot growth and greater taproot growth could allow seeds to reach moisture deeper down the soil profile and increase the chance of seedling survival. However, both seedlot and the parameter (speed of germination or subsequent growth) significantly affected the optimal duration of seed hydration thus it would be difficult to determine a universal duration of seed hydration for all oilseed rape varieties and seedlots.

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Although seed hydration has been previously reported to be successful in improving the germination of a number of small, slow-germinating vegetable seeds including celery, parsnip and carrot (Darby and Salter, 1976), the optimal duration of seed hydration has been observed to be species and/ or seedlot dependent (Durrant and Jaggard, 1988). Furthermore, the optimal duration for maximal percentage germination of the commercial Bristol seedlots S1 and S13 was not the same as for maximal taproot length. Although a general duration of 18 h seed hydration at 15°C could be applied to a range of seedlots, this may over-advance high germination seedlots while not maximising the increase in germination potential of low germination seedlots and/ or reduce subsequent seedling taproot growth following germination at temperatures of 15°C or above.

The daily mean temperature at Sutton Bonington, UK in September ranges from 12 to 19°C while the soil temperature at 20 mm depth is about 12.6 to 15°C (Noon, 1997). UK winter oilseed rape is sown at a depth of approximately 25 mm thus the seedbed temperature would be about 11 to 14°C at sowing. The greatest increase in FPG at 15°C was observed after 18h and in the UK and northern Europe seed hydration could provide benefits in terms of the speed and final percentage germination of winter oilseed rape seedlots. However, in warmer but drier environments shorter durations of 6 to 12 h would significantly increase post-germinative growth to maximise water and nutrient resource capture. In low water environments, increased taproot length may enable seedlings to extract water from deeper in the soil profile, which could be more important for seedling survival than rapid germination.

This disparity between the optimal durations of seed hydration for maximal improvements in the germination parameters and post-germinative taproot growth is one of the problems associated with seed hydration. The effects of seed hydration and/

or priming are known to vary significantly with seedlot (Durrant and Jaggard, 1988) and this experiment has shown that this is also true for the Bristol variety of winter oilseed rape. Small seed size and seed hydration for 18 h together increased the speed of germination although there were no significant effects on the final percentage germination. The beneficial effects of both of seed size selection and hydration were compounded by seedlot effects thus although selecting small seeds would aid germination, in the commercial environment where varieties are batch primed it would be difficult to find a universal duration for winter oilseed rape.

The speed of germination was also significantly increased by selecting small seeds over large seeds of the Bristol variety of winter oilseed rape, which was probably the result of a higher rate of imbibition due to the larger surface area to volume ratio of the small seeds although this was not directly measured. Vorob'ev (1976) attributed effects of seed size to differences to the nucleic acid content of the embryo while Krishasamy and Seshu (1989) reported that oxygen uptake, dehydrogenase activity and free amino acid content correlated positively with germination rate.

Variations in the size and form of seeds have been shown to affect germination as well as emergence from deep sowing, seedling size and final yield for various grass species (Black, 1956) and alfalfa (*Medicago sativa*) (Erickson, 1946). However, for a number of species including radish (*Raphanus sativus*), cauliflower (*Brassica oleracea* var. *Botrytis*), onion (*Allium cepa*) (Black, 1958) and various herbage species (Hermann and Hermann, 1939; Kneebone and Cremer, 1955), there has been little or no correlation between seed size and percentage germination in laboratory tests (Harper and Obeid, 1967). Analysis of the seed nitrogen and oil percentages showed that both the amount and timing of nitrogen application to the mother crop were important in determining the nitrogen and oil percentages of the seeds produced. These analyses supported observations by Ayisi *et al.*, (1997) who reported that seed protein contents increased with nitrogen fertilisation. The seed nitrogen percentages of the 1997 harvest Apex seedlots varied significantly with both the amount and timing of mother crop nitrogen application and with the location from which the pods were harvested. In the 1999 harvest Apex seedlots the highest seed nitrogen percentages were again recorded in those crops that received the largest amount of nitrogen; these were also the crops that had received the highest amount of nitrogen at flowering. Although the trends in seed nitrogen and oil percentage were similar for both the 1997 and 1999 seedlots, the exact effects of mother crop nitrogen management varied with harvest year and growing location.

Analysis of the weight of nitrogen per seed showed that the increasing nitrogen application to the mother crop from nil to 160 kg ha⁻¹ significantly increased the weight of nitrogen per seed (P < 0.001) from an average of 111.60 to 199.50 μ g. There were no significant effects of pod position nor were there any interactions. The differences in seed nitrogen percentage between the upper and lower pod positions were hence due to the dilution of the same weight of nitrogen by greater oil percentages in the upper section of the canopy.

Increasing mother crop nitrogen application increased the ratio of large to small seeds although the increase was not significant due to large replicate variations. Although there was not a significant effect of mother crop nitrogen management on the percentage of large and small seeds for either the 1997 or 1999 harvest Apex seedlots, seed size has been shown to significantly affect the germination of the Bristol seedlots S1 and S13. These data also showed that the seed size can be affected by the year and growing location since the percentage of large seeds in the nil nitrogen treatment was 73% for the 1997 seedlots compared with 65% for the 1999 seedlots.

Seed size and numbers of seeds per pod in *Brassica napus* L. can be highly variable within cultivars and intra-plant variations in seed composition have been reported (Clarke, 1979). Kondra and Downey (1970) found differences in glucosinolate content of seeds from different pod positions while Bechyne and Kondra (1970) reported variations in seed oil fatty acid composition between high and low pod positions. The position on the mother plant has also been reported to affect the subsequent germination of a number of other species including *Avena fatua* (Raju and Ramaswamy, 1983), *Daucus carota* (Thomas *et al.*, 1978; Gray, 1979), *Rumex* species (Cavers and Harper, 1966) and *Trifolium subterraneum* (Halloram and Collins, 1974; Taylor and Palmer, 1979).

One explanation of these differences is that the resources are not equally allocated to all seeds thus large seeds may have different germination requirements from small seeds. Another possibility is that the seeds produced at one position, for example, the base of an inflorescence develop under different environmental conditions than those produced at another position as has been reported for *Agrostis curtisii* (Gonzalez-Rabanal *et al.*, 1994).

Selecting pods from the lower section of the pod canopy also significantly increased the seed nitrogen percentage and significantly decreased the seed oil percentage. It has been suggested that intra-plant differences in pod and seed numbers and in seed weights are

due to differences in assimilate availability within the plant (Clarke, 1979). Assimilate availability in the plant varies with time and with proximity of sink to source (Major *et al.*, 1978): leaf area is near its maximum at the start of flowering (Clarke and Simpson, 1978), which would result in a good supply of assimilates for the first pods developing on the main raceme. It is possible that the lower pods, which produced seeds with higher nitrogen percentages than the upper pods, had initially preferential nitrogen uptake, which could be due to their physical closeness to the assimilate source as has been previously reported (Bechyne and Kondra, 1970; Kondra and Downey, 1970).

Since lower pods are formed earlier and have a longer time on the plant to mature, it would also be expected that the seeds contained within these pods would have a higher oil percentage and a lower nitrogen percentage than seeds from upper pods. However, the highest seed nitrogen percentages were recorded in the seeds harvested from pods from the lower (< 1.5 m) section of the crop canopy. It is likely that although the lower pods are formed earlier, because of their higher initial nitrogen percentage, they are unable to accumulate as much oil as the upper pods in the time before harvest.

Increasing nitrogen application to the mother crop also significantly increased the thousand seed weight but did not significantly affect the seed yield. In contrast, harvesting seeds from the lower section of the crop canopy significantly decreased the thousand seed weight but significantly increased the seed yield. The lower pods thus produced significantly more, significantly lighter, significantly higher nitrogen seeds than the upper pods.

Analysis of the 1997 harvest seed nitrogen and oil percentages showed that the 160 F mother crop had the highest seed nitrogen percentage and the lowest seed oil percentage

of all the mother crops. Altering the timing of mother crop nitrogen application from the growth period in February to the flowering period in March significantly increased the seed nitrogen weight and seed protein percentage and significantly decreased the seed oil percentage.

It is likely that the mother crop, which had received 160 kg nitrogen ha⁻¹ as a single application at flowering (F), was able to transfer the majority of this nitrogen to the seed reserves during flowering since stem elongation and leaf expansion had already occurred. In contrast, the mother crop that received the split application of 150 kg nitrogen ha⁻¹ in February during the growth (G) period with a further 150 kg ha⁻¹ in March at flowering (F) would possibly only have been able to transfer the majority of the second application to the seeds. The first application of 150 kg ha⁻¹ would probably have been used for stem elongation and leaf expansion and it is likely that some of the application would also have been leached out of the soil over the winter period.

Increasing nitrogen application to the mother crop and selecting seeds from the lower section of the pod canopy significantly increased the speed of germination, which was due to seed nitrogen percentage. Significant increases in germination speed were observed between low (2.30 to 2.95%) and high (3.46 to 3.61%) nitrogen categories. Fertiliser application to parental lines has been shown to improve the percentage germination of sorghum seeds compared with the unfertilised crop (Shamlal and Mohammad, 2000). However, although high nitrogen (3.46 to 3.61%) oilseed rape seeds germinated more quickly than low (2.30 to 2.95%) nitrogen seeds, there was not a significant effect on the final percentage germination.

Pod position, which has been observed to significantly affect the seed nitrogen percentage, also affected the speed of germination. The high nitrogen seeds that were harvested from the lower section of the canopy started to germinate significantly sooner than the low nitrogen seeds from the upper section of the canopy. However, although seed germination is primarily determined by a high nitrogen percentage, harvest date also significantly influenced germination: the early harvested seeds germinated sooner than the late harvested seeds. It is possible that there were seed ageing effects on the plant as has been reported for *Protea-neriifolia* (Lemaitre, 1990) and since early harvested seeds would have had reduced seed ageing effects, this is possibly the reason for the significant reduction in the time to 10% germination.

Although oilseed rape is commercially grown for oil, an increased speed of germination would enable plants to overwinter at a more developed stage, with larger leaf and root systems. Such plants would be better able to withstand frost heave during winter and pest damage in the spring provided that their subsequent growth rate following emergence was the same as for plants from slower germinating seeds. Mother crop nitrogen management techniques that produce seeds with a high nitrogen percentage can produce faster germinating seeds for resowing than techniques that produce low nitrogen seeds. However, under field conditions or at deep sowing depths, it is possible that low nitrogen seeds might perform better than high nitrogen seeds since sustained seedling growth is likely to be maintained through the breakdown of the seed oil reserves.

Interactions between the seed nitrogen percentage, pod position and harvest date compounded the effects of the single treatments alone although in general, treatments that increased the seed nitrogen percentage increased the speed of germination. This experiment showed that differences in seed nitrogen percentage can significantly affect the germination performance of winter oilseed rape as measured by the germination parameters (T_{10} , T_{50} , FPG and rate of germination) and mother crop nitrogen management could, therefore, be used to manipulate the seed nitrogen percentage to improve germination.

Heat treatment delayed and slowed germination at 15°C, which was possibly due to the heat disruption of internal cellular membranes. Although membranes can be repaired during subsequent imbibition, extensive damage is likely to have occurred in the heat-treated seeds. Heat treatment also resulted in cracking of the seed testa and since the ease of access of water between the testa and cotyledons determines the rate of imbibition (Powell, 1989), rapid imbibition through the cracked testa would probably have damaged the cotyledon cells and increased electrolyte leakage as has been reported for soybean (*Glycine max*) (Oliveira *et al.*, 1984), peas (Powell and Harman, 1985), French bean (*Phaseolus vulgaris*) (Powell *et al.*, 1986) and long bean (*Vigna sequipedalis*) (Abdullah *et al.*, 1991).

Harrington (1918) who showed that although the germination of control lots of barley, grass and wheat was more prompt than those of dried seedlots, the differences in percentage germination were scarcely perceptible after the second day of the germination test and this research confirmed that this was true for winter oilseed rape. Harrington (1918) hypothesised that the delay in germination was due either to an increase in the time required for imbibition or for cellular reorganisation before germination could begin while Powell and Matthews (1978) attributed the loss of seed vigour in peas to reduced food reserve mobilisation due to damage to the cotyledonary cells.

Heat treatment reversed the effects of pod position and harvest date on the speed of germination but significantly increased the final percentage germination at 25°C. Ewart (1908) suggested that excessive seed drying would extensively change the protoplasm and upon remoistening, seeds would be unable to re-establish the molecular groupings essential for normal vital activity. However, although sudden temperature elevation can damage proteins and protein synthetic apparatus (Ougham, 1987), heat treatment did not reduce seed viability although since seed germination was detrimentally affected by heat treatment, it is likely that seedling emergence would similarly be delayed. However, the increased resistance of winter oilseed rape to electrolyte leakage and cotyledon damage as indicated by the maintenance of seed viability at 15°C, suggests that the final percentage seedling emergence may not be reduced at UK field temperatures.

This research indicates that *Brassica napus* seeds are inherently more resistant to imbibition damage as a result of testa cracking than other species such as soybean (*Glycine max*) (Oliveira *et al.*, 1984) and pea (*Pisum sativum*) (Powell and Matthews, 1978). Higher resistance to heat treatment is likely to be the result of a combination of factors including the production of heat shock and/ or lactate abundant embryogenesis proteins, which together are able to protect the seeds proteins from coagulation and desiccation and hence limit damage to the seed tissues. At 25°C heat treatment significantly increased the final percentage germination, which may also have been due to the effect of heat shock proteins, which could have remained in the seed after heating and could possibly have been further induced by the subsequently high germination temperature.

4.4 **Conclusions of Germination Experiments**

This chapter has shown that the germination response of winter oilseed rape varies with variety, seedlot and temperature. At low (5°C) temperatures, seed hydration for 18 h at 15°C can significantly improve the rate and final percentage germination but its effects are seedlot, temperature and parameter (germination or seedling) dependent. Small seed size selection can significantly improve the rate and percentage germination at high (15°C) temperatures in conjunction with seed hydration although in the natural environment, larger seeds may have a higher probability of germinating as has been previously reported (Winn, 1988).

By increasing nitrogen application to the mother crop and altering the timing of application from February to March, the seed protein content can be significantly increased and the seed oil percentage significantly decreased. Increased seed nitrogen percentage significantly increases the speed of germination and the beneficial effects of high seed nitrogen content were augmented by selecting seeds from pods harvested from the lower (< 1.5 m) section of the canopy and/ or by harvesting early (July) in the season. In commercial crops, however, it would be difficult to harvest only the lower pods.

Although heat treatment slightly but significantly delayed germination, its maintenance of seed viability and potential use for seed sterilisation may commercially outweigh this disadvantage. However, heat treatment significantly increased the final percentage germination at 25°C, which may increase emergence and establishment in high-temperature environments where poor establishment is a major factor limiting the yield of other tropical crops such as sorghum (Munthali, 1988).

The potential uses for these treatments include increasing fertiliser efficiency and reducing nitrogen inputs to crops, improving the synchrony and hastening the speed of germination and the control of diseases (Sorensen, 1995). This chapter has shown that it is possible to use mother crop nitrogen management to alter seed physical and chemical properties and that combined with other methods such alterations can significantly increase the speed and final percentage germination of winter oilseed rape.

The following chapter examines the effects of combinations of these techniques on seedling emergence in the controlled growth room and semi-controlled (polytunnel) environments.

CHAPTER V THE EFFECTS OF SEED TREATMENT TECHNIQUES ON EMERGENCE IN THE CONTROLLED AND SEMI-CONTROLLED ENVIRONMENT

5.1 Introduction to Emergence Experiments

In the previous chapter seed hydration, small seed size and high seed nitrogen percentage significantly increased the speed of germination while heat treatment significantly delayed germination at field temperatures. In this chapter, the effects of seed hydration and method of drying back, seed size selection, seed nitrogen percentage and heat treatment on seedling emergence are examined.

5.2 Emergence Experiments

5.2.1 The effect of seed hydration and drying method on the emergence of the Bristol seedlots S1 and S13 at 15°C

Advancement significantly increased the germination speed of the Bristol seedlots S1 and S13. This experiment examined the effect of seed hydration and method of drying on the seedling emergence of the Bristol seedlots S1 and S13 at field temperatures.

<u>Method</u>

The seeds were hydrated for 18 h at 15°C and a non-hydrated sample was the control treatment; drying back was either slow or rapid as previously described. There were 2 replicates of each treatment and 30 seeds per replicate. The seeds were sown in silver

sand at 10 mm sowing depth as previously described and placed in the dark in the controlled environment at 15°C.

<u>Results</u>

The effect of seedlot, seed hydration and drying method on the time to 10% emergence at 15° C

The time to 10% (T_{10}) emergence was not significantly affected by either the seedlot (P = 0.314), seed hydration (P = 0.247) or drying method (P = 0.903) and the mean T_{10} was 8.23 d. However, selection for seedlot S1, hydrated seed or rapid drying method numerically reduced the time to 10% emergence. There were no significant interactions between the seedlot, seed hydration technique and drying method.

The effect of seedlot, seed hydration and drying method on the time to 50% emergence at 15° C

The time to 50% (T_{50}) emergence was not significantly affected by either the seedlot (P = 0.165), seed hydration (P = 0.396) or drying method (P = 0.275) and the mean T_{50} was 10.56 d. Selection for seedlot S1, hydrated seed or rapid drying method did, however, numerically reduce the time to 50% emergence. There were no significant interactions between the seedlot, seed hydration technique and drying method.

The effect of seedlot, seed hydration and drying method on the final percentage emergence at 15°C

The final percentage emergence (FPE) was not significantly affected by either the seedlot (P = 0.124), seed hydration (P = 0.796) or drying method (P = 0.699) and the mean FPE was 82% as shown in table 5.2.1.1.

Table 5.2.1.1The effect of seedlot, seed hydration and drying method on the
final percentage emergence from 10 mm sowing depth of the
Bristol seedlots S1 and S13 at 15°C

		Final percent	age emergence	
Seedlot	Seed hydration	Slow drying	Rapid drying	Mean FPE
S1	Control	78	88	85
	Hydrated	79	93	
S13	Control	89	68	78
	Hydrated	82	73	
Mean drying		82	81	
Mean control	81			
Mean hydrated	82	<u></u>		
Probability	0.796	0.	699	0.124
SED	3.720			
DF		7		

Interactions	Probability	SED	DF
Seedlot* seed hydration	0.608	5.260	7
Seedlot* drying method	0.008	5.260	7
Seed hydration * drying method	0.318	5.260	7
Seedlot* seed hydration* drying	0.608	7.450	7

The FPE was, however, affected by a significant interaction between the seedlot and method of drying-back (P = 0.008) as shown in figure 5.2.1.1. The FPE of seedlot S1

was non-significantly increased by rapid drying-back whereas that of seedlot S13 was significantly reduced. Rapid seed drying was thus detrimental to seedlot S13. If S13 had a different seed coat fatty acid composition from S1 as has been previously postulated, it is possible that rapid heat treatment could have resulted in greater testal cracking and increased imbibition damage during germination.

Figure 5.2.1.1 The effect of the seedlot*drying method interaction on the seedling emergence of the Bristol seedlots S1 and S13 from 10 mm sowing depth at 15°C



The effect of seedlot, seed hydration and drying method on the rate of emergence at $15^{\circ}C$

The rate of emergence was not significantly affected by either the seedlot (P = 0.161), seed hydration (P = 0.508) or drying method (P = 0.235) and the mean rate of seedling emergence was 0.09595 d⁻¹ although selection for seedlot S1, hydrated seeds and slow drying method numerically increased the rate of emergence. There were no significant interactions between the seedlot, seed hydration technique and drying method.

5.2.2 The effect of seedlot, seed hydration and seed size on emergence of the Bristol seedlots S1 and S13 from 10 mm sowing depth at 20°C

The aim of this experiment was to determine the effectiveness of seed hydration (followed by drying back in the laboratory) on the emergence of large and small seeds of seedlots S1 and S13 at 20°C. The hypothesis of this experiment was that seed hydration and seed size selection could together improve seedlot emergence.

<u>Method</u>

The samples were separated into large and small size fractions as previously described before half of the samples were hydrated for 18 h at 15°C; the remaining samples were the control treatments. After seed hydration, the seeds were blotted dry before being dried back in the laboratory at 20°C. For the subsequent emergence experiments, there were 4 replicates of each treatment and 25 seeds per replicate. The growing medium was horticultural grade silver sand and the seeds were sown at 10 mm depth before the pots were placed in the dark in the controlled growth room environment at 20°C.

<u>Results</u>

The effect of seedlot, seed hydration and seed size on the time to 10% emergence from 10 mm sowing depth at $20^{\circ}C$

The time to 10% emergence was not significantly affected by seedlot (P = 0.140) although seedlot S1 was numerically faster than seedlot S13 as shown in table 5.2.2.1. Seed hydration significantly reduced the T_{10} (P < 0.001). Seed size selection just did not significantly affect the T_{10} (P = 0.062) although it was numerically reduced by small seed size selection.

The T_{10} was affected by a significant interaction between seedlot, seed hydration and seed size (P = 0.005). Since the large seeds have the most potential to be improved, it was expected that the greatest effect of seed hydration would be observed in the large seeds of seedlot S13 and the observations confirmed that this was true. The fastest treatment to reach 10% emergence was the small hydrated seed sample of seedlot S1 and the slowest treatment was the large control sample of seedlot S13.

Table 5.2.2.1The effect of seedlot, seed hydration and seed size on the time to
10% emergence (h) from 10 mm sowing depth of the Bristol
seedlots S1 and S13 at 20°C

		Time to 10%	emergence (d)			
Seedlot	Seed hydration	Large seed size	Small seed size	Mean T ₁₀		
S1	Control	3.86	4.57	3.50		
	Hydrated	3.25	2.32			
S13	Control	5.46	3.81	3.89		
	Hydrated	3.19	3.08			
Mean seed size		3.94	3.45			
Mean control	4.43			······		
Mean hydrated	2.96					
Probability	< 0.001	0.062 0.14		0.140		
SED	0.250					
DF	18					

Interactions	Probability	SED	DF
Seedlot* seed hydration	0.891	0.353	18
Seedlot* seed size	0.141	0.353	18
Seed hydration * seed size	0.922	0.353	18
Seedlot* seed hydration* seed size	0.005	0.499	18

The effect of the seedlot* seed hydration* seed size interaction on the emergence response of the Bristol seedlots at 10 mm sowing depth is shown in figure 5.2.2.1,

which shows the fastest and slowest treatments to reach 10% emergence, which bound the other data points. These treatments had a significantly different pattern of emergence (P = 0.007) from each other. In both seedlots and for both size fractions, seed hydration reduced the T_{10} although this was only significant for the small seeds of seedlot S1 and the large seeds of seedlot S13.

Figure 5.2.2.1 The effect of the seedlot*seed size*seed hydration interaction on the seedling emergence of the Bristol seedlots S1 and S13 from 10 mm sowing depth at 20°C



The effect of seedlot, seed hydration and seed size on the time to 50% emergence from 10 mm sowing depth at $20^{\circ}C$

The time to 50% emergence was not significantly affected by seedlot (P = 0.778) although seedlot S1 reached 50% emergence numerically faster than seedlot S13 as shown in table 5.2.2.2. Seed hydration significantly reduced the T_{50} (P < 0.001). Seed size selection did not significantly affect the time to 10% emergence (P = 0.540)

although small seed size did numerically reduce the T_{50} . There were no significant interactions between seedlot, seed hydration and seed size.

Table 5.2.2.2The effect of seedlot, seed hydration and seed size on the time to
50% emergence (d) from 10 mm sowing depth of the Bristol
seedlots S1 and S13 at 20°C

		Time to 50%	emergence (d)			
Seedlot	Seed hydration	Large seed size	Small seed size	Mean T ₅₀		
S1	Control	6.85	7.26	5.90		
	Hydrated	5.53	3.95			
S13	Control	7.36	7.04	6.01		
	Hydrated	4.58	5.07			
Mean seed size		6.08	5.83			
Mean control	7.13			<u> </u>		
Mean hydrated	4.78			1		
Probability	< 0.001	0.	540	0.778		
SED	0.398					
DF	18					
		D 1 1 11		DR		

Interactions	Probability	SED	DF
Seedlot* seed hydration	0.942	0.563	18
Seedlot* seed size	0.413	0.563	18
Seed hydration* seed size	0.469	0.563	18
Seedlot* seed hydration* seed size	0.096	0.796	18

The effect of seedlot, seed hydration and seed size on the final percentage emergence from 10 mm sowing depth at $20^{\circ}C$

The final percentage emergence was not significantly affected by seedlot (P = 0.809) as shown in table 5.2.2.3. Whereas seed hydration did not significantly improve germination under the harsher emergence conditions of 15° C as shown in table 5.2.1.1, it significantly increased the final percentage of seedlings at 20° C (P = 0.002) from 78% to 90%. Small seed size selection also significantly increased the final percentage emergence (P = 0.048). There were no significant interactions between seedlot, seed hydration and seed size.

Table 5.2.2.3The effect of seedlot, seed hydration and seed size on the final
percentage emergence from 10 mm sowing depth of the Bristol
seedlots S1 and S13 at 20°C

		Final percentage emergence			
Seedlot	Seed hydration	Large seed size	Small seed size	Mean FPE	
S1	Control	74	84	85	
	Hydrated	88	93		
S13	Control	69	86	84	
	Hydrated	93	88		
Mean seed size		81	88		
Mean control	78				
Mean hydrated	90				
Probability	0.002	0.0	048	0.809	
SED	3.320				
DF	21				

Interactions	Probability	SED	DF
Seedlot* seed hydration	0.721	4.690	21
Seedlot* seed size	0.887	4.690	21
Seed hydration * seed size	0.061	4.690	21
Seedlot* seed hydration* seed size	0.224	6.630	21

The effect of seedlot, seed hydration and seed size on the rate of emergence from 10 mm sowing depth at $20^{\circ}C$

The rate of emergence was not significantly affected by seedlot (P = 0.345) as shown in table 5.2.2.4. However, seed hydration significantly increased the rate of emergence (P

. date .

< 0.001). Seed size selection did not significantly affect the rate of emergence (P = 0.212) although it was numerically increased by small seed size.

Table 5.2.2.4The effect of seedlot, seed hydration and seed size on the rate of
seedling emergence (d⁻¹) from 10 mm sowing depth of the Bristol
seedlots S1 and S13 at 20°C

		Rate of seedling	g emergence (d^{-1})	
Seedlot	Seed hydration	Large seed size	Small seed size	Mean rate
S1	Control	0.1517	0.1481	0.1854
	Hydrated	0.1844	0.2575	
S13	Control	0.1344	0.1436	0.1748
	Hydrated	0.2215	0.1996	
Mean seed size		0.1730	0.1872	·····
Mean control	0.1444			·
Mean hydrated	0.2158			<u></u>
Probability	< 0.001	0.2	212	0.345
SED	0.01100			
DF	18			

Interactions	Probability	SED	DF
Seedlot* seed hydration	0.980	0.01555	18
Seedlot* seed size	0.078	0.01555	18
Seed hydration* seed size	0.314	0.01555	18
Seedlot* seed hydration* seed size	0.025	0.02199	18

The rate of emergence was affected by a significant interaction between seedlot, seed hydration and seed size (P = 0.025). The rate of emergence was significantly increased by seed hydration in all of the seed treatment combinations with the exception of the large seed of seedlot S1. In contrast with the time to 10% germination, however, the greatest rate of emergence was observed in the large hydrated seeds of S13 while the slowest rate was observed in the small control seeds of S1.

5.2.3 The effect of seed nitrogen percentage, pod position and harvest date on seedling emergence in the polythene tunnel of the 1997 harvest Apex seedlots

The aim of this experiment was to determine the effects of seed protein as altered by mother crop nitrogen management, pod position and harvest date on seedling emergence from 20 and 40 mm sowing depth in the semi-controlled environment.

<u>Method</u>

The mother crop nitrogen management regimes chosen for this experiment were 0, 80 F, 100 G/ F, 160 F and 300 G/ F kg ha⁻¹, which produced seed with nitrogen percentages of 2.30, 2.54, 2.95, 3.61 and 3.46% respectively. The seeds were harvested from either the upper (> 1.5 m) or the lower (< 1.5 m) section of the pod canopy on either 21^{st} July (early) or 14^{th} August (late). The emergence experiments were performed during August 1998 in a polythene tunnel as previously described, which exposed the seeds to field temperatures but sheltered them from the wind and rain.

The emergence experiments were carried out at 20 and 40 mm sowing depth and the sowing medium was perlite, which was less susceptible than silver sand to water logging and compaction. The perlite was initially soaked and the plants were watered each day until the pots drained freely. The sowing depths were increased from 10 mm to 20 and 40 mm since 25 mm is the usual drilling depth of winter oilseed rape (Vigil *et al.*, 1997) but in arid conditions, sowing may be as deep as 40 mm (Glen *et al.*, 1989) to maximise access to soil moisture. For each treatment there were four replicates of 25 seeds. Growth analyses (area and weight of expanded cotyledons) were performed on the 20 mm sown seedlings. The number of seeds that germinated but did not emerge

and the number of seeds that had not germinated after 17 days were recorded for the seeds sown at both 20 and 40 mm depth.

<u>Results</u>

The effect of seed nitrogen percentage, pod position and harvest date on the time to 10% emergence from 20 mm sowing depth

Increasing the seed nitrogen percentage significantly reduced the time to 10% emergence (P < 0.001) from an average of 4.25 d for the low (2.30 to 2.95%) nitrogen seeds to 3.08 d for the high (3.46 to 3.61%) nitrogen seeds as shown in table 5.2.3.1. The T₁₀ was not significantly affected by either pod position (P = 0.331) or harvest date (P = 0.083).

The time to 10% emergence was affected by a significant interaction between all the treatments (P = 0.050) as shown in figure 5.2.3.1. There was a variable effect of seed nitrogen percentage although increasing the seed nitrogen percentage generally reduced the T₁₀. In both the upper and the lower pod positions there was a clear distinction between the T₁₀ of the seedlings produced from the seeds of high (3.46 to 3.61%) and low (2.30 to 2.95%) nitrogen percentage: the high nitrogen seeds reached 10% emergence significantly sooner than the low nitrogen seeds.

The pattern of emergence of these two treatments was also significantly different (P = 0.0002): the early harvested, high (3.61%) nitrogen seeds from the lower pods emerged significantly sooner and at a higher rate than the late harvested, low (2.30%) nitrogen seeds from the upper pods.

Table 5.2.3.1The effect of seed nitrogen percentage, pod position and harvest
date on the time to 10% emergence (d) from 20 mm sowing depth
of the 1997 harvest Apex seedlots in the polytunnel

		Time to 10%	6 emergence (d)	
Seed nitrogen	Pod position	Early	Late	Mean T ₁₀
percentage		harvest date	harvest date	
2.30	Upper	4.43	4.70	4.20
	Lower	4.28	3.38	
2.54	Upper	4.21	4.42	4.46
	Lower	4.86	4.35	
2.95	Upper	3.72	4.58	4.09
	Lower	3.78	4.27	
3.46	Upper	3.38	3.00	3.26
	Lower	2.83	3.82	
3.61	Upper	2.64	3.42	2.91
	Lower	2.52	3.08	
Mean harvest date		3.66	3.90	
Mean Upper	3.85			
Mean Lower	4.09			
Probability	0.331	0.0)83	< 0.001
SED	0.136	0.136		0.215
DF		53		

Interactions	Probability	SED	DF
Nitrogen* harvest date	0.077	0.303	53
Pod position* harvest date	0.423	0.178	53
Nitrogen* pod position	0.167	0.303	53
Nitrogen* position* harvest date	0.050	0.429	53

Figure 5.2.3.1 The effect of harvest date, pod position and seed nitrogen percentage on the seedling emergence of the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots from 20 mm sowing depth in the polytunnel



The effect of seed nitrogen percentage, pod position and harvest date on the time to 50% emergence from 20 mm sowing depth

Increasing the seed nitrogen percentage significantly reduced the time to 50% emergence (P < 0.001) from an average of 5.6 d for the low nitrogen (2.30 to 2.95%) seeds to 4.5 d for the high nitrogen (3.46 to 3.61%) seeds as shown in table 5.2.3.2. Neither pod position (P = 0.714) nor harvest date (P = 0.314) significantly affected the T_{50} and there were no significant interactions.

Table 5.2.3.2The effect of seed nitrogen percentage, pod position and harvest
date on the time to 50% emergence (d) from 20 mm sowing depth
of the 1997 harvest Apex seedlots in the polytunnel

	Time to 50% emergence (d)			
Seed nitrogen	Pod position	Early	Late	Mean T ₅₀
percentage		harvest date	harvest date	
2.3	Upper	5.28	5.63	5.23
	Lower	6.08	5.13	
2.5	Upper	6.56	6.19	6.0
	Lower	5.71	5.53	
2.9	Upper	5.25	5.54	5.53
	Lower	5.48	5.86	
3.5	Upper	4.90	4.48	4.71
	Lower	4.21	5.25	
3.7	Upper	4.03	4.60	4.30
	Lower	3.72	4.84	
Mean harvest date		5.12	5.30	
Mean Upper	5.25			
Mean Lower	5.18			
Probability	0.714	0.314		< 0.001
SED	0.180	0.180		0.285
DF	53			
Interactions		Probability	SED	DF

Interactions	Probability	SED	DF
Nitrogen* harvest date	0.241	0.404	53
Pod position* harvest date	0.584	0.255	53
Nitrogen* pod position	0.411	0.404	53
Nitrogen* position* harvest date	0.207	0.571	53

The effect of seed nitrogen percentage, pod position and harvest date on the final percentage emergence from 20 mm sowing depth

The FPE was not significantly affected by the seed nitrogen percentage (P = 0.186), pod position (P = 0.110) or harvest date (P = 0.746) as shown in table 5.2.3.3.

Table 5.2.3.3The effect of seed nitrogen percentage, pod position and harvest
date on the final percentage emergence from 20 mm sowing
depth of the 1997 harvest Apex seedlots in the polytunnel

		Final percentage emergence		
Seed nitrogen	Pod position	Early	Late	Mean FPE
percentage		harvest date	harvest date	
2.30	Upper	96	100	99
	Lower	100	98	
2.54	Upper	100	99	99
	Lower	100	98	
2.95	Upper	100	100	99
	Lower	100	94	
3.46	Upper	89	98	97
	Lower	100	100	
3.61	Upper	97	96	97
	Lower	98	98	
Mean harvest date		98	98	
Mean Upper	98			
Mean Lower	99			
Probability	0.110	0.746		0.186
SED	0.719	0.719		1.137
DF	53			

Interactions	Probability	SED	DF
Nitrogen* harvest date	0.016	1.607	53
Pod position* harvest date	0.005	1.017	53
Nitrogen* pod position	0.003	1.607	53
Nitrogen* position* harvest date	0.170	2.273	53

However, the FPE was affected by a significant interaction between the seed nitrogen percentage and the pod position (P = 0.003). Seed nitrogen percentage had a variable effect on emergence within the upper harvested pods while the seeds with 3.46% nitrogen percentage had a significantly lower FPE than the other nitrogen percentages

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and/ or pod position. The seed nitrogen percentage did not significantly affect the FPE of the lower harvested pods.

The effect of seed nitrogen percentage, pod position and harvest date on the rate of emergence from 20 mm sowing depth

Increasing the seed nitrogen percentage significantly increased the rate of emergence (P < 0.001) as shown in table 5.2.3.4. Pod position did not significantly affect the rate of emergence (P = 0.479) although it was slightly numerically increased by selecting pods that were harvested from the lower (< 1.5 m) section of the canopy. Harvest date did not significantly affect the rate of emergence (P = 0.123) although it was numerically increased by early harvesting.

However, the rate of emergence was affected by a significant interaction between all the treatments (P = 0.038). There was a variable effect of seed nitrogen percentage on the rate of seedling emergence although increasing seed nitrogen percentage generally increased the rate. The seedlings produced from the high nitrogen, lower pod position, early harvested seeds had a significantly higher rate of emergence than the low nitrogen, upper pod position, late harvested seeds.

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Table 5.2.3.4The effect of seed nitrogen percentage, pod position and harvest
date on the rate of seedling emergence (d⁻¹) from 20 mm sowing
depth of the 1997 harvest Apex seedlots in the polytunnel

	Rate of seedling emergence (d ⁻¹)			
Seed nitrogen	Pod position	Early	Late	Mean rate
percentage		harvest date	harvest date	
2.30	Upper	0.000330	0.000308	0.000316
	Lower	0.000288	0.000339	
2.54	Upper	0.000266	0.000289	0.000293
	Lower	0.000303	0.000316	
2.95	Upper	0.000340	0.000314	0.000327
	Lower	0.000320	0.000334	
3.46	Upper	0.000355	0.004665	0.000373
	Lower	0.000414	0.000334	
3.61	Upper	0.000435	0.000382	0.000413
	Lower	0.000471	0.000361	
Mean harvest date		0.000352	0.000337	
Mean Upper	0.000341			
Mean Lower	0.000348			
Probability	0.479	0.123		< 0.001
SED	0.000007	0.000007		0.000011
DF	57			

Interactions	Probability	SED	DF
Nitrogen* harvest date	0.017	0.000016	57
Pod position* harvest date	0.494	0.000010	57
Nitrogen* pod position	0.788	0.000016	57
Nitrogen* position* harvest date	0.038	0.000022	57

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The effect of seed nitrogen percentage, pod position and harvest date on the time to 10% emergence from 40 mm sowing depth

The time to 10% emergence was not significantly affected by seed nitrogen percentage (P = 0.221), pod position (P = 0.169) or harvest date (P = 0.736) and the mean T₁₀ was 7.21 h. However, it was numerically reduced by selecting high (3.61%) nitrogen percentage seeds, selecting lower harvested pods or by early harvesting. There were no significant interactions between the seed nitrogen percentage, pod position and harvest date.

The effect of seed nitrogen percentage, pod position and harvest date on the time to 50% emergence from 40 mm sowing depth

Increasing the seed nitrogen percentage had a variable effect on the time to 50% emergence and the seedlings from the 2.95% nitrogen percentage seeds took significantly longer (P = 0.043) to reach 50% emergence than the other seedlings as shown in table 5.2.3.5.

Pod position did not have a significant effect on the T_{50} (P = 0.302) although it was reduced by selecting pods from the lower section of the canopy. Harvest date did not significantly affect the T_{50} (P = 0.830) although it was reduced by early harvesting. There were no significant interactions between the seed nitrogen percentage, pod position or harvest date.

Table 5.2.3.5The effect of seed nitrogen percentage, pod position and harvest
date on the time to 50% emergence (d) from 40 mm sowing depth
of the 1997 harvest Apex seedlots in the polytunnel

		Time to 50%	6 emergence (d)			
Seed nitrogen	Pod position	Early	Late	Mean T ₅₀		
percentage		harvest date	harvest date			
2.30	Upper	9.91	9.02	9.49		
	Lower	9.90	9.12			
2.54	Upper	8.17	8.48	8.65		
	Lower	8.83	9.10			
2.95	Upper	11.29	10.06	10.25		
	Lower	9.75	9.91			
3.46	Upper	8.53	9.30	8.83		
	Lower	9.36	8.15			
3.61	Upper	8.42	10.69	8.75		
	Lower	8.17	7.72			
Mean harvest date		9.23	9.15			
Mean Upper	9.39					
Mean Lower	9.00					
Probability	0.302	0.830		0.043		
SED	0.369	0.369		0.583		
DF	43					
		Duchability	CED			

Interactions	Probability	SED	DF
Nitrogen* harvest date	0.595	0.825	43
Pod position* harvest date	0.382	0.522	43
Nitrogen* pod position	0.370	0.825	43
Nitrogen* position* harvest date	0.405	1.167	43

The effect of seed nitrogen percentage, pod position and harvest date on the final percentage emergence from 40 mm sowing depth

The FPE was not significantly affected by the seed nitrogen percentage (P = 0.599), pod position (P = 0.065) or the harvest date (P = 0.522) as shown in table 5.2.3.6.
Table 5.2.3.6The effect of seed nitrogen percentage, pod position and harvest
date on the final percentage emergence from 40 mm sowing
depth of the 1997 harvest Apex seedlots at 20°C

		tage emergence		
Seed nitrogen	Pod position	Early	Late	Mean FPE
percentage		harvest date	harvest date	
2.30	Upper	97	93	91
	Lower	83	92	
2.54	Upper	95	91	92
	Lower	88	93	
2.95	Upper	83	91	84
	Lower	76	87	
3.46	Upper	99	84	85
	Lower	74	83	
3.61	Upper	100	84	89
	Lower	75	96	
Mean harvest date		87	89	
Mean Upper	92			
Mean Lower	85			, _, , _, , _, , _, , _, , _, , _, , _ ,
Probability	0.065	0.5	522	0.599
SED	3.720	3.720		5.880
DF			57	· · · · · · · · · · · · · · · · · · ·

Interactions	Probability	SED	DF
Nitrogen* harvest date	0.876	8.320	57
Pod position* harvest date	0.024	5.260	57
Nitrogen* pod position	0.930	8.320	57
Nitrogen* position* harvest date	0.626	11.770	57

There was a pod position^{*} harvest date interaction (P = 0.024), which significantly affected the FPE. The FPE of the seedlings produced from the late harvested seeds was not significantly affected by pod position but within the early harvested seeds, the seedlings produced from the upper pods achieved a significantly higher FPE than those harvested from the lower pods. While nitrogen increases the speed of emergence, oil appears necessary for emergence from deeper sowing depths.

The effect of pod position on the emergence of the early harvested seed can be seen in figure 5.2.3.2. The seeds from the upper pods started to emerge sooner and achieved a significantly higher FPE than the seeds from the lower pods although there was not a significant difference in their patterns of emergence (P = 0.1049)

Figure 5.2.3.2 The effect of upper and lower pod position on the seedling emergence of the early harvested 1997 harvest Apex seedlots from 40 mm sowing depth in the polytunnel



The effect of seed nitrogen percentage, pod position and harvest date on the rate of emergence from 40 mm sowing depth

The rate of emergence at 40 mm depth was not significantly affected by the nitrogen percentage (P = 0.105), pod position (P = 0.324) or harvest date (P = 0.826) and there

were no significant interactions. The mean rate of emergence was 0.000195 seedlings d⁻¹

The effect of increasing the sowing depth from 20 to 40 mm on emergence in the polytunnel

At 20 mm sowing depth the T_{10} was significantly reduced and the rate of germination was significantly increased by high seed nitrogen percentage. Doubling the sowing depth significantly increased and almost doubled the T_{10} (P < 0.001) and the T_{50} (P < 0.001) as shown in table 5.2.3.7. The FPE (P < 0.001) and the rate of germination (P < 0.001) were also significantly reduced at 40 mm sowing depth.

Table 5.2.3.7The effect of sowing depth on the times to 10 and 50%
emergence (d) and the final percentage and rate of emergence (d1) of the 1997 harvest Apex seedlots in the polytunnel

Sowing depth (mm)	T ₁₀ (d)	T ₅₀ (d)	FPE (%)	Rate (d^{-1})
20	3.79	5.21	99	0.008259
40	7.25	9.25	88	0.004678
Р	< 0.001	< 0.001	< 0.001	< 0.001
SED	0.230	0.198	1.9780	0.0001667
DF	99	99	117	99

The effect of seed nitrogen percentage, pod position and harvest date on the cotyledon fresh weight following emergence from 20 mm sowing depth

Increasing the seed nitrogen percentage significantly increased the cotyledon fresh weight (FWT) (P < 0.001) as shown in table 5.2.3.8 and figure 5.2.3.3. Pod position did not significantly affect the cotyledon FWT (P = 0.143). Selecting for a late harvest date significantly increased the cotyledon FWT (P < 0.001).

Table 5.2.3.8The effect of seed nitrogen percentage, pod position and harvest
date on the seedling cotyledon fresh weight (g) following
emergence from 20 mm sowing depth of the 1997 harvest Apex
seedlots in the polytunnel

	Cotyledon FWT (g)			
Seed nitrogen	Pod position	Early	Late	Mean FWT
percentage		harvest date	harvest date	
2.30	Upper	0.657	0.960	0.741
	Lower	0.592	0.752	
2.54	Upper	0.652	1.019	0.832
	Lower	0.625	1.032	
2.95	Upper	0.922	1.165	1.194
	Lower	1.595	1.094	
3.46	Upper	0.980	1.332	1.150
	Lower	0.977	1.310	
3.61	Upper	0.977	1.045	1.118
	Lower	1.112	1.339	
Mean harvest date		0.909	1.105	
Mean Upper	0.971			
Mean Lower	1.043			······
Probability	0.143	< 0	0.001	< 0.001
SED	0.0484	0.0)484	0.0765
DF		54		
Interactions		Probability	SED	DF
Nitrogen* harvest date		0.012	0.1081	54
Pod position* harvest date		0.150	0.0684	54
Nitrogen* pod position		0.037	0.1081	54
Nitrogen* position* harvest date		0.041	0.1529	54

Figure 5.2.3.3 The effect of seed nitrogen percentage on the seedling cotyledon fresh weight (g) of the 1997 harvest Apex seedlots following emergence from 20 mm sowing depth in the polytunnel



However, the cotyledon FWT was significantly affected (P = 0.041) by an interaction between all three factors. Increasing the seed nitrogen percentage combined with lower pod position and late harvest date significantly increased the cotyledon FWT compared with the combination of low nitrogen percentage, upper pod position and early harvest date, which was probably due to increased total nitrogen percentage.

The effect of seed nitrogen percentage, pod position and harvest date on the cotyledon area following emergence from 20 mm sowing depth

Increasing the seed nitrogen percentage significantly increased the cotyledon area (P < 0.001) as shown in table 5.2.3.9 and figure 5.2.3.4. Neither pod position (P = 0.646) nor harvest date (P = 0.080) significantly affected the cotyledon area. However, the cotyledon area was significantly affected (P = 0.002) by an interaction between the seed nitrogen percentage and the harvest date. Early harvesting significantly increased the cotyledon area of the high (3.46 to 3.61%) nitrogen seeds but significantly reduced the cotyledon area of the low (2.30 to 2.95%) nitrogen seeds. Although increasing the seed

nitrogen percentage non-significantly increased the ratio of large to small seeds as shown in table 4.2.4.10, it is possible that there was also some effect of seed size on the cotyledon area.

Table 5.2.3.9The effect of seed nitrogen percentage, pod position and harvest
date on the seedling cotyledon area (mm²) following emergence
from 20 mm sowing depth of the 1997 harvest Apex seedlots in
the polytunnel

	Area of cotyledons (mm ²)				
Seed nitrogen	Pod position	Early	Late	Mean area	
percentage		harvest date	harvest date		
2.30	Upper	34.17	34.22	32.90	
	Lower	30.39	32.93		
2.54	Upper	29.89	33.59	32.05	
	Lower	34.30	30.42		
2.95	Upper	37.78	41.68	37.21	
	Lower	31.23	38.14		
3.46	Upper	46.65	46.22	45.22	
	Lower	50.37	37.63		
3.61	Upper	50.40	43.36	49.89	
	Lower	62.64	43.17		
Mean harvest date		40.77	38.14		
Mean Upper	39.80			an a	
Mean Lower	39.11				
Probability	0.646	0.080		< 0.001	
SED	1.479	1.479		2.339	
DF	57				

Interactions	Probability	SED	DF
Nitrogen* harvest date	0.002	3.308	57
Pod position* harvest date	0.076	2.092	57
Nitrogen* pod position	0.173	3.308	57
Nitrogen* position* harvest date	0.262	4.679	57

Figure 5.2.3.4

The effect of seed nitrogen percentage on the seedling cotyledon area (mm^2) of the 1997 harvest Apex seedlots following emergence from 20 mm sowing depth in the polytunnel



The effect of seed nitrogen percentage, pod position and harvest date on the cotyledon fresh weight following emergence from 40 mm sowing depth

Increasing the seed nitrogen percentage significantly increased the cotyledon fresh weight (P < 0.001) as shown in table 5.2.3.10 and figure 5.2.3.5. Neither pod position (P = 0.319) nor harvest date (P = 0.792) significantly affected the cotyledon FWT.

The cotyledon FWT was significantly affected by a significant interaction between the seed nitrogen percentage and pod position (P = 0.008): increasing the seed nitrogen percentage generally increased the cotyledon FWT for both positions.

Table 5.2.3.10The effect of seed nitrogen percentage, pod position and harvest
date on the seedling cotyledon fresh weight (g) following
emergence from 40 mm sowing depth of the 1997 harvest Apex
seedlots in the polytunnel

	Cotyledon FWT (g)				
Seed nitrogen	Pod position	Early	Late	Mean FWT	
percentage		harvest date	harvest date		
2.30	Upper	0.490	0.500	0.450	
	Lower	0.327	0.485		
2.54	Upper	0.545	0.507	0.511	
	Lower	0.463	0.530		
2.95	Upper	0.468	0.567	0.588	
	Lower	0.561	0.755		
3.46	Upper	0.796	0.596	0.629	
	Lower	0.543	0.582		
3.61	Upper	0.876	0.566	0.717	
	Lower	0.756	0.672		
Mean harvest date		0.582	0.576		
Mean Upper	0.591				
Mean Lower	0.567				
Probability	0.319	0.792		< 0.001	
SED	0.024	0.024		0.037	
DF	47				

Interactions	Probability	SED	DF
Nitrogen* harvest date	< 0.001	0.0527	47
Pod position* harvest date	0.001	0.0334	47
Nitrogen* pod position	0.008	0.0527	47
Nitrogen* position* harvest date	0.804	0.0746	47

Figure 5.2.3.5 The effect of seed nitrogen percentage on the seedling cotyledon fresh weight (g) of the 1997 harvest Apex seedlots following emergence from 40 mm sowing depth in the polytunnel



The effect of seed nitrogen percentage, pod position and harvest date on the cotyledon area following emergence from 40 mm sowing depth

Increasing the seed nitrogen percentage significantly increased the cotyledon area (P < 0.001) as shown in table 5.2.3.11 and figure 5.2.3.6. Neither pod position (P = 0.086) nor harvest date significantly affected the cotyledon area (P = 0.502). There were no significant interactions between seed nitrogen percentage, pod position and harvest date.

Table 5.2.3.11The effect of seed nitrogen percentage, pod position and harvest
date on the seedling cotyledon area (mm²) following emergence
from 40 mm sowing depth of the 1997 harvest Apex seedlots in
the polytunnel

	Area of cotyledons (mm ²)			
Seed nitrogen	Pod position	Early	Late	Mean area
percentage		harvest date	harvest date	
2.30	Upper	32.72	35.57	33.47
	Lower	32.86	32.71	
2.54	Upper	33.80	30.14	32.50
	Lower	32.43	33.64	
2.95	Upper	35.89	36.98	39.84
	Lower	37.41	49.07	
3.46	Upper	45.50	45.15	46.10
	Lower	49.03	44.74	
3.61	Upper	45.90	43.11	46.80
	Lower	46.98	51.20	
Mean harvest date		39.25	40.23	
Mean Upper	38.48			
Mean Lower	41.01	· · · · · · · · · · · · · · · · · · ·		
Probability	0.086	0.502		< 0.001
SED	1.449	1.449		2.292
DF	57			

Interactions	Probability	SED	DF
Nitrogen* harvest date	0.379	3.241	57
Pod position* harvest date	0.289	2.050	57
Nitrogen* pod position	0.431	3.241	57
Nitrogen* position* harvest date	0.437	4.584	57

Figure 5.2.3.6 The effect of seed nitrogen percentage on the seedling cotyledon area (mm²) of the 1997 harvest Apex seedlots following emergence from 40 mm sowing depth in the polytunnel



The effect of increasing the sowing depth from 20 to 40 mm on seedling morphology following emergence in the polytunnel

Increasing the depth from 20 to 40 mm significantly reduced the cotyledon FWT by half (P < 0.001) as shown in table 5.2.3.12 but did not significantly alter the area (P = 0.795).

Table 5.2.3.12The effect of sowing depth on the cotyledon fresh weight (g) and
area (mm^2) of the 1997 harvest Apex seedlots in the polytunnel

Sowing depth (mm)	Cotyledon FWT (g)	Cotyledon area (mm ²)
20	1.007	39.45
40	0.579	39.74
Р	< 0.001	0.795
SED	0.0286	1.107
DF	104	117

5.2.4 The effect of heat treatment, seed nitrogen percentage and pod position on seedling emergence in the polytunnel of the nil and 160F 1997 harvest Apex seedlots

Heat treatment was observed to significantly delay germination of Apex seedlots at 15 and 25°C. The aim of this experiment was to determine the effect of heat treatment, seed nitrogen percentage and pod position on seedling emergence from 20 mm sowing depth.

<u>Method</u>

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The mother crop nitrogen management regimes were nil and 160 F, which produced seeds with 2.30 and 3.61% nitrogen percentages respectively. The pods were harvested from either the upper (> 1.5 m) or lower (< 1.5 m) section of the pod canopy and the harvest date was early (21^{st} July), which had significantly increased the speed of germination at field temperatures. Half of the seed samples were heat-treated while the other half formed the controls.

The emergence experiments were performed during August 1999 in a polythene tunnel as previously described. For each treatment there were two replicates of 25 seeds sown at 20 mm depth in perlite. The number of seeds that germinated but did not emerge and the number of seeds that had not germinated after 20 days were recorded.

The effect of heat treatment, seed nitrogen percentage and pod position on the time to 10% emergence from 20 mm sowing depth

Heat treatment significantly increased the time to 10% emergence (P < 0.001) as shown in table 5.2.4.1 while increasing the seed nitrogen percentage (P < 0.001) and selecting lower harvested pods (P < 0.001) significantly reduced the T₁₀. There was a significant interaction between the heat treatment, seed nitrogen percentage and pod position, which affected the T_{10} (P < 0.001) although there was not a significant difference in the pattern of emergence (P = 0.366). Heat treatment significantly reduced the time to 10% emergence of the high (3.61%) nitrogen seeds but significantly increased the T_{10} of the low (2.30%) nitrogen seeds. It is likely that combination of heat and subsequent imbibition damage were too great for the seeds to withstand and they consequently took longer to repair this damage than the high nitrogen seeds.

Table 5.2.4.1The effect of seed heat treatment, nitrogen percentage and pod
position on the time to 10% emergence (d) from 20 mm sowing
depth of the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots
in the polytunnel

	Time to 10% emergence (d)				
Seed nitrogen	Heat	Upper	Lower	Mean T ₁₀	
percentage	treatment	pod position	pod position		
2.30	Control	7.29	6.63	7.42	
	Heated	8.00	7.83		
3.61	Control	7.00	7.08	6.88	
	Heated	6.67	6.75		
Mean pod position		7.25	7.08		
Mean Control	7.00				
Mean Heated	7.33				
Probability	< 0.001	< 0.001 < 0.0		< 0.001	
SED	0.019				
DF	7				

Interactions	Probability	SED	DF
Heat* nitrogen	< 0.001	0.026375	7
Heat* pod position	< 0.001	0.026375	7
Nitrogen* pod position	< 0.001	0.026375	7
Heat* nitrogen* pod position	< 0.001	0.003729	7

The effect of heat treatment, seed nitrogen percentage and pod position on the time to 50% emergence from 20 mm sowing depth

Heat treatment significantly increased the time to 50% emergence (P < 0.001) by 0.33 d as shown in table 5.2.4.2 while increasing the seed nitrogen percentage significantly reduced it (P < 0.001) by 0.5 d. Selecting lower (< 1.5 m) harvested pods significantly reduced the T_{50} (P < 0.001) by 0.09 d.

Table 5.2.4.2The effect of seed heat treatment, nitrogen percentage and pod
position on the time to 50% emergence (d) from 20 mm sowing
depth of the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots
in the polytunnel

Seed nitrogen	Heat	Upper Lower		Mean T ₅₀		
percentage	treatment	pod position				
2.30	Control	8.58	8.00	8.58		
	Heated	8.88	8.92			
3.61	Control	8.04 8.13		8.08		
	Heated	8.08 8.13				
Mean pod position		8.38	8.38 8.29			
Mean Control	8.17					
Mean Heated	8.50					
Probability	< 0.001	< 0.	< 0.001			
SED	0.005167					
DF		7				

Interactions	Probability	SED	DF
Heat* nitrogen	< 0.001	0.007333	7
Heat* pod position	< 0.001	0.007333	7
Nitrogen* pod position	< 0.001	0.007333	7
Heat* nitrogen* pod position	< 0.001	0.010333	7

There was a significant interaction between the heat treatment, seed nitrogen percentage and pod position, which affected the time to 50% emergence (P < 0.001) as previously discussed.

The effect of heat treatment, seed nitrogen percentage and pod position on the final percentage emergence from 20 mm sowing depth

Heat treatment slightly but significantly reduced the final percentage emergence (P < 0.001) as shown in table 5.2.4.3. Increasing the seed nitrogen percentage did not significantly affect the FPE (P = 0.052) but selecting lower (< 1.5 m) harvested pods significantly reduced the FPE (P < 0.001) by 2%.

The FPE was affected by a significant interaction between seed nitrogen percentage and pod position (P = 0.004). The FPE of the high nitrogen seeds was not significantly affected by pod position but lower (< 1.5 m) pod position significantly reduced the FPE of the low nitrogen seeds.

Table 5.2.4.3The effect of seed heat treatment, nitrogen percentage and pod
position on the final percentage emergence from 20 mm sowing
depth of the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots
in the polytunnel

	Final percentage emergence						
Seed nitrogen	Heat	Upper	Lower pod	Mean FPE			
percentage	treatment	pod position	position				
2.30	Control 96		96	95			
	Heated	96	92				
3.61	3.61 Control 95		95	95			
	Heated	95	93				
Mean pod position		96	94				
Mean Control	96						
Mean Heated	94			· · · · · · · · · · · · · · · · · · ·			
Probability	< 0.001	< 0.	0.052				
SED	0.185						
DF			7				

Interactions	Probability	SED	DF
Heat* nitrogen	0.009	0.262	7
Heat* pod position	< 0.001	0.262	7
Nitrogen* pod position	0.004	0.262	7
Heat* nitrogen* pod position	0.226	0.370	7

The effect of heat treatment, seed nitrogen percentage and pod position on the rate of emergence from 20 mm sowing depth

Heat treatment significantly reduced the rate of emergence (P < 0.001) as shown in table 5.2.4.4. Both increasing the seed nitrogen percentage (P < 0.001) and selecting upper (< 1.5 m) harvested pods significantly increased the rate of emergence (P < 0.001). There was a significant interaction between the heat treatment, seed nitrogen percentage and pod position, which affected the rate of emergence (P < 0.001): heat treatment

significantly reduced the rate of emergence of the low nitrogen, lower harvested seeds. It is possible that the effects of heat damage combined with reduced nitrogen percentage and increased stress conditions for emergence together reduced the rate of emergence of the seedlings produced from the low nitrogen seeds.

Table 5.2.4.4The effect of seed heat treatment, nitrogen percentage and pod
position on the rate of emergence (d⁻¹) from 20 mm sowing depth
of the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots in the
polytunnel

	Rate of seedling emergence (d ⁻¹)				
Seed nitrogen	Heat	Upper Lower pod		Mean rate	
percentage	treatment	pod position	position		
2.30	Control	0.000203	0.000217	0.000243	
	Heated	0.000196	0.000195		
3.61	Control	0.000217	0.000213	0.000212	
	Heated	0.000203	0.000213		
Mean pod position		0.000205	0.000210		
Mean Control	0.000213				
Mean Heated	0.000202				
Probability	< 0.001	< 0.001 < 0.00			
SED		0.00	000001		
DF			7		

Interactions	Probability	SED	DF
Heat treatment* nitrogen	< 0.001	0.0000002	7
Heat treatment* pod position	0.002	0.0000002	7
Nitrogen* pod position	< 0.001	0.0000002	7
Heat treatment* nitrogen* pod	< 0.001	0.0000003	7
position			

Storing the 1997 harvest Apex seedlots for another (second) year before sowing in this experiment reduced the speed and final percentage emergence at 20 mm sowing depth.

Sowing in 1999 increased the T_{10} by 1.42 d and the T_{50} by 1.17 d while the FPE was reduced from 98% to 95% and the rate of emergence was reduced by 40% compared with sowing in 1998. It is possible that the variation in yearly summer temperature also affected the speed of emergence in the polytunnel and it has been widely reported that seeds lose vigour upon extended storage (Harman and Mattick, 1976; Powell and Matthews, 1981a; Powell and Matthews, 1984).

5.3 Discussion of Emergence Experiments

The aims of these experiments were to examine the effect of rapid or slow drying back and large or small seed size selection on the emergence of hydrated and control seedlots of the same variety of winter oilseed rape from 10 mm sowing depth under controlled (growth room) conditions. The effects of seed nitrogen percentage, pod position and harvest date on winter oilseed rape emergence from 20 and 40 mm sowing depth and the effects of heat treatment, seed nitrogen percentage and pod position on emergence from 20 mm sowing depth were examined under semi-controlled (polytunnel) conditions.

In the field, where plants are subject to temperature, weather, pest and disease stresses, rapid seedling emergence would allow only a short interval over which seed- and soilborne pathogens could damage or kill seedlings (Stokes, 1999, personal communication) but under controlled (growth room) and semi-controlled (polytunnel) conditions, seedling emergence of the Bristol and Apex seedlots at mean temperatures greater than 15°C varied between 69 and 100%. This research has shown that although seedlot S1 had a significantly higher final percentage and rate of germination than seedlot S13, since there were no significant effects of seedlot on seedling emergence, it is not possible to directly equate germination with emergence performance. It is possible that at deeper sowing depths, seedlot differences might be elicited but that the sowing depth of 10 mm was not sufficient for these differences to be observed.

Seedlot, seed hydration and drying method did not significantly affect the speed of emergence although the speed of emergence was numerically increased by seed hydration, rapid drying and high vigour as observed in seedlot S1. The effects of rapid drying back following seed hydration were seedlot dependent: while rapid drying after seed hydration significantly increased the FPE of the high vigour (S1) seedlot it significantly decreased the FPE of the low vigour (S13) seedlot from 10 mm depth. It is possible that this reduction could be due to the combination of heat damage and differences in the seed coat properties and/ or seed composition. Rapid air movement around the seed druring rapid drying could have exacerbated the seed coat problems with the S13 seeds, increasing damage to the testa and hence increasing imbibition damage upon germination. In a commercial environment rapid drying could result in even lower emergence of low vigour seedlots such as S13 and produce even greater variation in the final number of seedlings than was observed at germination.

When hydrated seeds are transferred to the soil almost instantaneous growth can be achieved (Heydecker *et al.*, 1975) and a second seed hydration treatment after several months of storage can further extend seed viability during normal storage conditions (Basu, 1976; Basu and Dhar, 1979; Pill *et al.*, 1991). In this research the speed and final percentage emergence of the Bristol seedlots S1 and S13 from 10 mm sowing depth were significantly increased by seed hydration for 18 h while the FPE was significantly increased by seed hydration for 18 h while the seed significantly increased by Seed hydration for 18 h while the second seed by Seed by Chippindale (1934) and Bradford, Steiner and Trawatha (1990) who reported that

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although seed priming generally accelerates the germination rate, it is not always accompanied by improvements in field emergence rates or percentages.

Although Zheng *et al.* (1994) reported that the beneficial effects of seed hydration were clearest in low germination rape seedlots, Gulliver and Heydecker (1973) observed that as the temperature increases above the base temperature, biological activity in pea and sugar beet seeds is accelerated and seeds germinate quickly. However, they also reported that low vigour seeds did not respond well to the high levels of biological activity and that the final germination percentage of low vigour seedlots was still reduced in comparison with the high vigour seeds. Furthermore, if germination has occurred at the time of drying back, there can be abnormal root development and seedling failure with the process of dehydration becoming progressively more harmful as the amount of actively metabolising tissue increases (Berrie and Drennan, 1971). It is possible, therefore, that the emergence of the low vigour seedlots was limited by their own inherent vigour potential.

Increasing the seed nitrogen percentage significantly increased the speed of Apex seedling emergence from 20 mm sowing depth although there was not a significant effect at 40 mm depth. In contrast, selection for upper pod position, which had produced seeds with a high oil percentage, significantly increased the final percentage emergence at 40 mm sowing depth although significantly larger seedlings were still produced from the high nitrogen seeds. This indicates that the high oil percentage of the upper harvested seeds might provide more energy for prolonged or sustained seedling growth or emergence from depth in comparison with the readily available but more limited source of metabolisable nitrogen in the high nitrogen seeds. A high seed nitrogen percentage appears to be important for high percentage emergence from medium (20

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mm) sowing depths although at deeper (40 mm) sowing depths the seed oil percentage is more important for sustained growth.

Increasing the sowing depth to 40 mm significantly increased the T_{10} and T_{50} and significantly reduced the rate of emergence and the FPE. The effect of increasing the sowing depth on oilseed rape has been reported to vary greatly: for the variety Tobin, depths of planting as great as 40 mm did not appear to reduce emergence whereas for Global, increased depth decreased emergence at 16°C by 5.2% for every extra cm of planting depth (Vigil *et al.*, 1997).

At 20 mm sowing depth the cotyledon FWT was significantly increased by increasing the seed nitrogen percentage or selecting late harvested seeds. Increasing the seed nitrogen percentage also increased the area of the cotyledons. At 40 mm sowing depth increasing the seed nitrogen percentage significantly increased both the cotyledon FWT and the area of the cotyledons and although the numerical differences were small, the differences were noticeable when handling the seedlings during the growth analysis. The cotyledons are the initial energy source for the germinating seed and the more energy needed by the seedling, the more cotyledon reserves are used. At shallow sowing depths the seedling is able to emerge quickly without depleting the energy reserves within the cotyledons. However, at deeper sowing depths the seedling has to use more of the reserves thus the cotyledons of the seeds sown at 40 mm sowing depth were significantly lighter than those of the seeds sown at 20 mm depth.

Once the seedling has emerged the cotyledons turn green and begin to photosynthesise, providing energy for the growing seedling. However, these data showed that although the cotyledon fresh weight was reduced after sowing at 40 mm depth compared with

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sowing at 20 mm depth, the area was not significantly affected. This suggests that either the deeper sown seedlings tried to compensate for the greater use of reserves by producing the same area of leaf for photosynthesis as the shallower sown seedlings but this was accompanied by a reduction in the cotyledon FWT, or that the initial cotyledon area is genetically pre-determined. If the former hypothesis is correct, it might be expected that selecting large seeds to sow at depth might produce larger seedlings than small seeds due to larger seed reserves.

Harper and Obeid (1967) reported that the cotyledon area of flax was positively correlated with seed size although sowing large seeds did not confer a lasting advantage. It is possible, however, that increased emergence could confer a competitive advantage over weeds, diseases and insect pests where these are a problem. Under unfavourable field conditions and particularly if combined with late sowing, the rate of emergence is important to maximise plant growth and development before the onset of winter since larger seedlings are more competitive than small seedlings and they are also more resistant to pests and diseases. However, a high final percentage emergence is important in reducing patchiness in the field and hence pigeon damage. In practice, although seeds with a high oil percentage may emerge better, the larger seedlings produced by high nitrogen seeds are possibly more likely to survive and establish in the field.

The value of heat treatment lies mainly in its potential to reduce levels of seedborne infection (Bell, 2002) but the reduction in vigour of the heat-treated seeds that was observed at germination, persisted to significantly reduce the speed of emergence as well as the final percentage emergence of Apex seedlings from 20 mm sowing depth. Since all of the seeds germinated it is likely that the repair mechanisms ameliorated some of the damage due to heat treatment but since not all of the seeds emerged, it is

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likely that the additional damage due to rapid imbibition from the perlite was too great for the seeds to repair.

5.4 Conclusions of Emergence Experiments

This chapter has shown that the emergence response of winter oilseed rape is not significantly affected by seedlot at shallow (10 mm) sowing depths. At relatively high field temperatures of 15°C, seed hydration for 18 h at 15°C and selection for small seed size can significantly improve seedling emergence.

By sowing high (3.46 to 3.61%) compared with low (2.30 to 2.95%) nitrogen seeds, it is also possible to significantly increase the speed of emergence from 20 mm sowing depths. However, at a sowing depth of 40 mm, high seed oil percentages are more important than nitrogen percentage for sustained seedling emergence.

This chapter has shown that there are fewer beneficial effects of seed hydration on seedling emergence than were observed for seed germination. However, small seed size selection still conferred some advantage over large seed size and increasing the seed nitrogen percentage significantly increased the speed of seedling emergence although the effects were smaller than those observed at germination. In contrast, the detrimental effects of heat treatment on seedling viability and vigour were greater than were observed for seed germination and at field temperatures it is possible that heat treatment would further delay field emergence and possibly reduce field establishment.

The following chapter examines the effects of seed nitrogen percentage, seed size selection and heat treatment on seedling emergence and plant establishment in the field environment.

CHAPTER VI THE EFFECTS OF SEED TREATMENT TECHNIQUES ON FIELD EMERGENCE AND ESTABLISHMENT

6.1 Introduction to Field Experiments

Increasing the seed nitrogen percentage significantly increased the speed of emergence from 20 mm sowing depth of Apex seedlots. Selection for small seed size also significantly increased seedling emergence while heat treatment significantly reduced emergence. These three methods were tested under field conditions between 1999 and 2000 to determine whether seedling emergence and/ or plant establishment could be significantly increased and whether any treatment effects persisted until harvest.

The first experiment investigated the effects of seed nitrogen percentage and heat treatment on the field emergence and establishment of the stored 1997 harvest Apex seedlots. It has been reported that seed vigour declines with ageing and storage (Powell and Matthews, 1984; Zulu, 1989) and it was likely that the 1997 harvest Apex seedlots were affected by loss of vigour and viability. The second experiment investigated the effects of seed nitrogen percentage, heat treatment and seed size on the newly harvested 1999 Apex seedlots.

6.2 Field Experiments

The Apex seed samples, which were harvested on the 21st July 1997 and the 22nd July 1999 had been subjected to different nitrogen management regimes, which had significantly altered the seed nitrogen percentage of the seeds as shown in table 6.2.1. Since the 1997 seed stocks were low, these seedlots were used to examine the effect of

seed nitrogen percentage and heat treatment in the field environment while the 1999 harvest Apex seedlots were used to examine the effect of seed nitrogen percentage, seed size selection and heat treatment.

Seed hydration was not tested under field conditions for three main reasons. Firstly, previous experiments had shown that the optimal duration of seed hydration needed to be determined for each seedlot and in the field experiments, five different seedlots were to be used. Secondly, based on the seed coat effects due to heat treatment, advancing seeds that had previously been heat-treated would probably not produce a viable seedling since there would be too much accumulated damage due to the effects of heat treatment and as a result of increased imbibition damage during seed hydration. Thirdly, the amounts of both seeds and land available for the field trial were limited and it would not have been possible to perform three field trials with the resources available. The effects of heat treatment on seedling emergence and subsequent plant establishment were assessed in the field to determine the effects on plant establishment.

6.2.1 The effect of seed nitrogen percentage and heat treatment on the field establishment of the nil and 160 F 1997 harvest Apex seedlots

The aim of this experiment was to determine the effect of seed nitrogen percentage and heat treatment on Apex seedling emergence and plant establishment under field conditions.

<u>Method</u>

The nil and 160 F kg ha⁻¹ nitrogen treatments, which produced seeds with nitrogen percentages of 2.30% and 3.61% respectively, were used in this experiment. Half of the

seed samples were heat-treated as previously described and the seeds were direct-drilled to 20 mm depth on 6 October 1999 as previously described. The emergence of each seedling within each of the quadrats was recorded at regular intervals as previously described. No fertiliser was applied to the plots and they were not sprayed against weeds or pathogens. The residual soil mineral nitrogen percentage was 165 kg ha⁻¹. Three growth analyses were performed during the course of the growing season, in February (growth stage 1.2), May (growth stage 3.0), and July (growth stage 6.5).

<u>Results</u>

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Emergence of winter oilseed rape usually takes about 9 days (Leterme, 1988), but in this experiment emergence commenced on the 7th day after sowing and the first seedling count was recorded on the 8th day. The initial plant populations were measured by counting the number of cocktail sticks of each colour that had been placed by each seedling. However, there were a number of problems with this method. Firstly, a number of cocktail sticks were broken and/ or displaced through pigeon damage. Secondly, some of the cocktail sticks were possibly duplicated because it was believed that the seedlings had not been previously marked whereas the sticks had in fact been broken or displaced by the birds. Thirdly, not all of the plants were evenly spaced, possibly because some of the drill holes had split, which made it difficult to mark the seedlings.

In the following tables the times to 10 and 50% field emergence and the rate of field emergence have been calculated relative to the total number of seedlings, which was taken as 100%, to enable a direct comparison between different seed treatment techniques, which may have produced very different final seedling numbers. In the

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following figures, however, the field emergence has been expressed as the cumulative number of seedlings.

The effect of seed nitrogen percentage and heat treatment on the time to 10% field emergence

Increasing the seed nitrogen percentage from 2.30 to 3.61% did not significantly affect the time to 10% field emergence (P = 0.067). Heat treatment did not significantly affect the T_{10} (P = 0.098) and there were no significant interactions.

The effect of seed nitrogen percentage and heat treatment on the time to 50% field emergence

Increasing the seed nitrogen percentage significantly reduced the time to 50% field emergence (P = 0.023) by more than a day as shown in table 6.2.1.1. Heat treatment did not significantly affect the T_{50} (P = 0.240) nor were there any significant interactions.

Table 6.2.1.1The effect of seed nitrogen percentage and heat treatment on the
time to 50% field emergence (d) of the 2.30 and 3.61% nitrogen
1997 harvest Apex seedlots

	Time to 50% field emergence (d)				
Seed nitrogen percentage	Control	Heat-treated	Mean T ₅₀		
2.30	11.03	11.80	11.42		
3.61	9.88	10.32	10.10		
Mean heat treatment	10.45	11.06	<u></u>		
Probability	0	.240	0.023		
SED	0.484				
DF	9				

Seed nitrogen* heat interaction	P = 0.747	SED = 0.684	DF = 9
		· ·	·····

Increasing the seed nitrogen percentage from 2.30 to 3.61% significantly increased the rate of emergence (P = 0.019) as shown in table 6.2.1.2. Heat treatment did not significantly affect the rate of emergence (P = 0.278) and there were no significant interactions.

Table 6.2.1.2The effect of seed nitrogen percentage and heat treatment on the
rate of field emergence (d⁻¹) of the 2.30 and 3.61% nitrogen 1997
harvest Apex seedlots

ſ	Rate of field emergence				
Seed nitrogen percentage	Control	Heat-treated	Mean rate		
2.30	0.091	0.086	0.088		
3.61	0.102	0.098	0.100		
Mean heat treatment	0.096	0.092			
Probability	0	.278	0.019		
SED	0.004				
DF		9			
		······			
ed nitrogen* heat interaction	P = 0.851	SED = 0.006	DF = 9		

The effect of seed nitrogen percentage on seedling emergence in the field

There was a significant effect of seed nitrogen percentage on the pattern of seedling emergence (P = 0.0187): the high nitrogen seedlings emerged sooner than the low nitrogen seedlings as shown in figure 6.2.1.1 although there was not a significant difference in the final seedling number.

Figure 6.2.1.1 The effect of seed nitrogen percentage on the mean number of seedlings (per m²) of the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots following field sowing



The effect of seed heat treatment on seedling emergence in the field

Heat treatment reduced seedling emergence in the field but because of the large differences between the individual replicates, the reduction in emergence was not significant (P = 0.870). The pattern of seedling emergence was not significantly affected by heat treatment (P = 0.2284) as shown in figure 6.2.1.2.

Figure 6.2.1.2 The effect of seed heat treatment on the mean number of seedlings (per m²) of the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots following field sowing



The effect of seed treatment on plant losses during the growing season

The percentage plant losses during the growin season for seed nitrogen percentage are shown in figure 6.2.1.3. Polynomial quadratic functions were fitted to the plant losses since their goodness of fit exceeded that of linear functions for both treatments and level of treatment. The high nitrogen seeded population suffered 32% seedling losses in the first three weeks after sowing compared with 29% of the low nitrogen seeded population and by January this loss had increased to 51% and 59% respectively. By the spring analysis in May, 61% and 66% of the original high and low nitrogen seeded populations had been lost and by harvest only 25% of the original population remained. Plant losses within the control and heat-treated populations area shown in figure 6.2.1.4. The control population suffered 27% losses compared with 37% for the heat-treated population within the first three weeks. By January the losses in the control and heat-treated populations had risen to 48% and 62% respectively and to 64 to 66% by harvest.

Figure 6.2.1.3 The effect of seed nitrogen percentage on the mean percentage plant losses between October 1999 and July 2000 of the 2.30 and 3.61% nitrogen 1997 harvest Apex seedlots following field sowing







The effect of seed nitrogen percentage on plant growth and development at growth stage 1.2 (vegetative rosette) of the 1997 harvest Apex seedlots

At the first growth analysis in February the crop was in a vegetative state at approximately growth stage 1.2. The plants were relatively small with expanding leaf

and root systems. The 3.61% nitrogen seedlings had a significantly greater leaf FWT (P = 0.049) and root FWT (P = 0.031) compared with the 2.30% nitrogen seedlings as shown in table 6.2.1.3 but there were no other differences in the seedling characteristics.

Table 6.2.1.3The effect of seed nitrogen percentage on plant growth
parameters (values per m²) of the 2.30 and 3.61% nitrogen 1997
harvest Apex seedlots at growth stage 1.2

Growth parameter	2.30%N	3.61%N	Р	SED	DF	Unit
Number of Plants	91	108	0.308	15.28	9	-
Leaf FWT	192	318	0.049	55.0	9	g
Leaf DWT	17.8	27.9	0.053	4.54	9	g
Leaf Area	1864	3028	0.119	676.4	9	cm ²
Leaf Moisture	90.99	91.11	0.854	0.678	9	%
Leaf Chlorophyll	9.19	9.95	0.703	1.930	9	μg cm ⁻²
Green Area Index	0.249	0.404	0.119	0.0902	9	-
Stem FWT	80	123	0.089	22.5	9	g
Stem DWT	5.8	9.5	0.118	2.15	9	g
Stem Area	401	641	0.147	150.9	9	cm ²
Stem Moisture	92.71	92.28	0.657	0.942	9	%
Stem Chlorophyll	6.76	5.48	0.478	1.732	9	µg cm ⁻²
Root FWT	24.2	38.9	0.031	5.78	9	g
Root DWT	2.98	4.89	0.144	1.192	9	g
Root Moisture	87.6	86.1	0.714	3.96	9	%
Mean Root Length	11.00	11.87	0.078	1.001	8	cm
Mean Root Diameter	2.14	3.90	0.130	1.039	8	cm

The effect of seed nitrogen percentage on plant growth and development at growth stage 3.0 (plant framework extension) of the 1997 harvest Apex seedlots

At the second growth analysis in May the crop framework was fully formed and was at approximately growth stage 3.0. The plants were about 1 m in height with large leaves and an extensive root system. The 3.61% nitrogen seedlings had a significantly greater leaf DWT (P = 0.001), flower DWT (P = 0.031), bud DWT (P = 0.030) and root length (P = 0.038) and a significantly lower flower moisture content (P = 0.012) than the 2.30% nitrogen seedlings as shown in table 6.2.1.4.

Table 6.2.1.4The effect of seed nitrogen percentage on plant growth
parameters (values per m²) of the 2.30 and 3.61% nitrogen 1997
harvest Apex seedlots at growth stage 3.0

Growth Parameter	2.30%N	3.61%N	Р	SED	DF	Unit
Number of Plants	75	86	0.242	8.75	7	-
Leaf FWT	952	1079	0.144	77.3	7	g
Leaf DWT	103.9	129.5	0.001	5.00	7	g
Leaf Area	54250	19564	0.227	26176.4	7	cm ²
Leaf Moisture	89.03	87.88	0.194	0.799	7	%
Green Area Index	5.40	2.00	0.227	2.62	7	-
Stem FWT	3702	4085	0.710	988.9	7	g
Stem DWT	491	507	0.730	45.7	7	g
Stem Moisture	88.12	88.43	0.352	0.306	5	%
Stem Area	14902	16586	0.158	1066.3	7	cm ²
Mean Stem length	1.166	1.157	0.900	0.0704	7	m
Root FWT	389	276	0.248	89.6	7	g
Root DWT	126.9	123.6	0.867	19.15	7	g
Root Moisture	60.7	49.9	0.206	7.69	7	%
Mean Root Length	12.7	18.0	0.038	2.08	7	cm
Flower FWT	402	177	0.232	172	7	g
Flower DWT	20.7	37.1	0.031	6.11	7	g
Flower Moisture	89.4	81.0	0.012	2.49	7	%
Bud FWT	179	212	0.147	20.6	7	g
Bud DWT	25.0	32.4	0.030	2.73	7	g
Bud Moisture	85.80	84.69	0.053	0.476	7	%

The effect of seed nitrogen percentage on plant growth and development at growth stage 6.5 (seed development) of the 1997 harvest Apex seedlots

At the time of the third growth analysis in July the crop was at approximately growth stage 6.5 and was almost ready for harvest. The plants were over a metre in height and their leaves and flowers had been replaced by filling pods. Increasing the seed nitrogen percentage did not significantly affect the growth parameters as shown in table 6.2.1.5. Increasing the seed nitrogen percentage significantly improved germination and emergence but did not significantly improve field performance.

Table 6.2.1.5The effect of seed nitrogen percentage on plant growth
parameters (values per m² unless otherwise specified) of the 2.30
and 3.61% nitrogen 1997 harvest Apex seedlots at growth stage
6.5

Growth parameter	2.30%N	3.61%N	Р	SED	DF	Unit
Number of Plants	55	55	0.923	5.02	9	-
Stem FWT	1240	1446	0.437	252.8	9	g
Stem DWT	392	433	0.583	71.7	8	g
Stem Moisture	67.3	68.3	0.848	4.95	8	%
Mean Stem Length	1.46	1.52	0.116	0.33	9	m
Pod FWT	1288	1317	0.876	183.8	9	g
Pod DWT	728	896	0.276	144.9	9	g
Pod Moisture	32.6	30.7	0.700	4.76	8	%
Number of Pods	4575	4557	0.976	561.5	9	-
Seed FWT	275	278	0.940	41.8	8	g
Seed DWT	201	207	0.829	19.4	8	g
Seed Moisture	26.3	25.5	0.783	2.97	8	%
Number of Seeds	60995	57883	0.739	9026.2	8	-
Number of Seeds/ Pod	14	13	0.409	1.267	8	-
Seed Yield	2.75	2.79	0.914	0.419	8	t ha ⁻¹

The effect of seed heat treatment on plant growth and development at growth stage 1.2 (vegetative rosette) of the 1997 harvest Apex seedlots

Heat treating seeds prior to sowing produced the effects shown in table 6.2.1.6. The seedlings produced from the heat-treated seeds had a significantly lower leaf DWT (P = 0.025), stem FWT (P = 0.037) and root FWT (P = 0.038) than those produced from the control plants. Seed heat treatment prior to sowing thus decreased the initial plant size.

Table 6.2.1.6The effect of seed heat treatment on plant growth parameters
(values per m²) of the 2.30 and 3.61% nitrogen 1997 harvest
Apex seedlots at growth stage 1.2

Growth parameter	Control	Heated	Р	SED	DF	Unit
Number of Plants	115	84	0.074	15.28	9	-
Leaf FWT	311	199	0.073	55.0	9	g
Leaf DWT	29.0	16.8	0.025	4.54	9	g
Leaf Area	3097	1794	0.086	676.4	9	cm ²
Leaf Moisture	90.43	91.67	0.101	0.678	9	%
Leaf Chlorophyll	8.93	10.20	0.527	1.930	9	µg cm ⁻²
Green Area Index	0.413	0.239	0.086	0.0902	9	-
Stem FWT	129	74	0.037	22.5	9	g
Stem DWT	9.3	5.9	0.146	2.15	9	g
Stem Area	680	362	0.064	150.9	9	cm ²
Stem Moisture	93.07	91.93	0.255	0.942	9	%
Stem Chlorophyll	6.39	5.85	0.761	1.732	9	μg cm ⁻²
Root FWT	38.6	24.5	0.038	5.78	9	g
Root DWT	4.84	3.03	0.163	1.192	9	g
Root Moisture	86.1	87.5	0.727	3.96	9	%
Mean Root Length	11.92	10.95	0.055	1.001	8	cm
Mean Root Diameter	3.78	2.26	0.183	1.039	8	cm

The effect of seed heat treatment on plant growth and development at growth stage 3.0 (plant framework extension) of the 1997 harvest Apex seedlots

Heat treating seeds prior to sowing did not significantly affect any of the growth parameters as shown in table 6.2.1.7 thus the initial disadvantages of heat-treating seeds prior to sowing were not maintained through to flowering.

Table 6.2.1.7The effect of seed heat treatment on plant growth parameters
(values per m²) of the 2.30 and 3.61% nitrogen 1997 harvest
Apex seedlots at growth stage 3.0

Growth Parameter	Control	Heated	Р	SED	DF	Unit
Number of Plants	82	79	0.725	8.75	7	-
Leaf FWT	979	1052	0.377	77.3	7	g
LeafDWT	120.0	113.4	0.229	5.00	7	g
Leaf Area	51565	22249	0.300	26175.4	7	cm ²
Leaf Moisture	87.74	89.17	0.116	0.799	7	%
Green Area Index	5.20	2.20	0.300	2.62	7	-
Stem FWT	4375	3412	0.363	988.9	7	g
Stem DWT	500	498	0.969	45.7	7	g
Stem Moisture	88.51	88.04	0.188	0.306	5	%
Stem Area	16572	14916	0.164	1066.3	7	cm ²
Mean Stem length	11.72	11.51	0.781	0.704	7	m
Root FWT	384	281	0.289	89.6	7	g
Root DWT	130.2	120.4	0.626	19.15	7	g
Root Moisture	60	50.6	0.259	7.69	7	%
Mean Root Length	13.5	17.3	0.111	2.08	7	cm
Flower FWT	393	187	0.269	172	7	g
Flower DWT	30.6	27.2	0.599	6.11	7	g
Flower Moisture	85.9	84.5	0.595	2.49	7	%
Bud FWT	196	194	0.931	20.6	7	g
Bud DWT	28.6	28.9	0.940	2.73	7	g
Bud Moisture	85.42	85.08	0.496	0.476	7	%
The effect of seed heat treatment on plant growth and development at growth stage 6.5 (seed development) of the 1997 harvest Apex seedlots

At the time of the third growth analysis in July the crop was at approximately growth stage 6.5 and was almost ready for harvest. The plants were over a metre in height and their leaves and flowers had been replaced by filling pods. Heat treating seeds prior to sowing did not significantly affect the growth parameters as shown in table 6.2.1.8. Although seed heat treatment significantly delayed germination and emergence, it did not significantly affect plant growth and development beyond G.S. 1.2.

Table 6.2.1.8	The effect of seed heat treatment on plant growth parameters
	(values per m^2 unless otherwise specified) of the 2.30 and 3.61%
	nitrogen 1997 harvest Apex seedlots at growth stage 6.5

Growth parameter	Control	Heated	Р	SED	DF	Unit
Number of Plants	58	52	0.275	5.02	9	-
Stem FWT	1489	1197	0.279	252.8	9	g
Stem DWT	433	392	0.582	71.7	8	g
Stem Moisture	69.2	66.5	0.597	4.95	8	%
Mean Stem Length	1.49	1.50	0.749	0.33	9	m
Pod FWT	1320	1285	0.851	183.8	9	g
Pod DWT	893	731	0.293	144.9	9	g
Pod Moisture	31.5	31.8	0.948	4.76	8	%
Number of Pods	4741	4391	0.548	561.5	9	-
Seed FWT	288	264	0.581	41.8	8	g
Seed DWT	213	194	0.510	19.4	8	g
Seed Moisture	25.5	26.3	0.804	2.97	8	%
Number of Seeds	61891	56986	0.602	9026.2	8	-
Number of Seeds/ Pod	14	13	0.770	1.267	8	-
Seed Yield	2.89	2.65	0.580	0.419	8	t ha ⁻¹

The effect of seed treatment interactions on crop growth parameters during the season

There were no significant interactions between seed nitrogen percentage and heat treatment that affected the growth parameters measured at either growth stage 1.2 or 6.5.

At growth stage 3.0, there was a significant interaction between the seed nitrogen percentage and seed heat treatment, which affected the leaf FWT (P = 0.020) and DWT (P < 0.001) and the root FWT (P = 0.040). Heat treatment increased the leaf FWT and DWT in the low nitrogen treatment but decreased the values of these parameters in the high nitrogen treatment. The opposite effect was observed in the root FWT.

It is possible that heat treatment affected some of the plant growth mechanisms, which resulted in the production of longer, thinner roots in the plants produced from the low nitrogen (high oil) seeds. If the seed oil percentage were protecting the internal seed tissues, heat treatment in combination with low nitrogen application to the mother crop could be used to produce plants that are better able to penetrate the soil and have a greater root surface area to volume ratio for better acquisition of water and nutrients from depth.

The effect of seed treatment on the yield components and final yield

These crops were grown under a no-fertiliser regime and the mean yield of 2.77 t ha⁻¹ compared well with the mean UK yield of 3.25 t ha⁻¹; a low yield for 2000 was 2.50 t ha⁻¹ (Nix, 2000). It is likely that the high soil mineral nitrogen percentage of 165 kg ha⁻¹ provided sufficient nitrogen for the growing crop and thus nitrogen was not a limiting factor in this experiment.

6.2.2 The effect of seed nitrogen percentage, seed size and heat treatment on field establishment of the 1999 harvest Apex seedlots

In northern areas such as Scotland, the harvest and re-sowing dates may be too close to allow re-sowing of newly harvested rapeseed and the seeds are overwintered for sowing the following year. However, in most of the UK there is sufficient time for seed cleaning and seedbed preparation between harvesting and re-sowing the winter rape crop. The aim of this experiment was to determine the effect of seed nitrogen percentage, seed size selection and heat treatment on the field seedling emergence and plant establishment of freshly harvested seeds.

<u>Method</u>

The nil, 100 and 200 kg ha⁻¹ nitrogen treatments that were harvested in 1999 and which produced seeds with nitrogen percentages of 2.60%, 3.08% and 3.28% were used in this experiment. The samples were separated into large and small fractions and half of the seeds in each seed size fraction were heat-treated as previously described. The seeds were direct-drilled to 20 mm depth on 6 October 1999 and seedling emergence was recorded at regular intervals as previously described. No fertilisers or sprays against pests and diseases were applied to the plots. Three growth analyses were performed in February (growth stage 1.2), May (growth stage 3.0), and July (growth stage 6.5) as previously described. In the following tables the T₁₀, T₅₀ and the rate of field emergence have been calculated as previously described for experiment 6.2.1

<u>Results</u>

Emergence above the soil surface commenced on the 7th day after sowing and the first seedling count was recorded on the 8th day.

The effect of seed nitrogen percentage, seed size and heat treatment on the time to 10% field emergence

Neither increasing the seed nitrogen percentage (P = 0.659) not selecting for small seed size (P = 0.512) significantly affected the time to 10% emergence However, heat treatment significantly reduced the T_{10} by just over a day (P < 0.001). There were no significant interactions.

Table 6.2.2.1The effect of seed nitrogen percentage, seed size and heat
treatment on the time to 10% field emergence (d) of the 2.60,
3.08 and 3.28% nitrogen 1999 harvest Apex seedlots

	Time to 10% field emergence (d)						
Seed nitrogen	Heat	Large	Small	Mean T ₁₀			
percentage	treatment	seed size	seed size				
2.60	Control	4.47	5.19	4.05			
	Heat-treated	3.56	2.99				
3.08	Control	4.76	4.54	4.00			
	Heat-treated	3.84	2.85				
3.28	Control	5.30	4.82	4.31			
	Heat-treated	3.39	3.74				
Mean size		4.22	4.02				
Mean control	4.85						
Mean heat treatment	3.39						
Probability	< 0.001	0.512		0.659			
SED	0.300	00 0.300					
DF	81						

Interactions	Probability	SED	DF
Heat treatment* seed size	0.501	0.425	81
Heat treatment* nitrogen	0.939	0.520	81
Seed size* nitrogen	0.623	0.52	81
Heat treatment* seed size* nitrogen	0.328	0.736	81

The effect of seed nitrogen percentage, seed size and heat treatment on the time to 50% field emergence

Neither increasing the seed nitrogen percentage (P = 0.528) nor small seed size selection(P = 0.815) significantly affected the time to 50% emergence. However, the T_{50} was significantly reduced by heat treatment (P < 0.001) as shown in table 6.2.2.2. There were no significant interactions.

Table 6.2.2.2The effect of seed nitrogen percentage, seed size and heat
treatment on the time to 50% field emergence (d) of the 2.60,
3.08 and 3.28% nitrogen 1999 harvest Apex seedlots

	Time to 50% field emergence (d)						
Seed nitrogen	Heat	Large	Small	Mean T ₅₀			
percentage	treatment	seed size	seed size				
2.60	Control	9.68	10.00	9.22			
	Heat-treated	8.71	8.50				
3.08	Control	9.76	9.86	9.26			
	Heat-treated	9.27	8.14				
3.28	Control	10.26	10.07	9.58			
	Heat-treated	8.64	9.34	-1			
Mean size		9.39	9.32				
Mean control	9.94						
Mean heat treatment	8.77						
Probability	< 0.001	0.815		0.528			
SED	0.282	0.282		0.345			
DF	81						

Interactions	Probability	SED	DF
Heat treatment* seed size	0.609	0.399	81
Heat treatment* nitrogen	0.982	0.488	81
Seed size* nitrogen	0.514	0.488	81
Heat treatment* seed size* nitrogen	0.298	0.691	81

The effect of seed nitrogen percentage, seed size and heat treatment on the rate of field emergence

The rate of field emergence was not significantly affected by increasing the seed nitrogen percentage (P = 0.525) or selecting for small sced size (P = 0.926) but it was significantly increased by heat treatment (P < 0.001) as shown in table 6.2.2.3. There were no significant interactions.

Table 6.2.2.3The effect of seed nitrogen percentage, seed size and heat
treatment on the rate of field emergence (d⁻¹) of the 2.60, 3.08
and 3.28% nitrogen 1999 harvest Apex seedlots

	Rate of field emergence (d ⁻¹)						
Seed nitrogen	Heat	Large	Small	Mean rate			
percentage	treatment	seed size	seed size				
2.60	Control	0.104	0.101	0.111			
	Heat-treated	0.120	0.120				
3.08	Control	0.105	0.102	0.112			
	Heat-treated	0.112	0.128				
3.28	Control	0.099	0.101	0.107			
	Heat-treated	0.119	0.109				
Mean size		0.110	0.110				
Mean control	0.102	· · · · ·					
Mean heat treatment	0.118						
Probability	< 0.001	0.926		0.525			
SED	0.004	0.004		0.005			
DF	81						

Interactions	Probability	SED	DF
Heat treatment* seed size	0.668	0.006	81
Heat treatment* nitrogen	0.954	0.007	81
Seed size* nitrogen	0.484	0.007	81
Heat treatment* seed size* nitrogen	0.270	0.010	81

Heat damages the seed testa and if the seeds are not physically supported the testa can split and fall off the seed during imbibition. During the germination experiments, the seeds were germinated in water, which did not offer any resistance to testa loss. During the emergence experiments the Perlite provided some support for the testa and possibly prevented some testa loss. It is hence possible that in the field the soil particles prevented many of the seed coats from falling off and that together with the reduced imbibition rate compared with the other environments and the effects of persistent heat shock responses, the rate of seedling growth in the field was increased.

The effect of seed nitrogen percentage on seedling emergence in the field

Increasing the seed nitrogen percentage significantly affected the pattern of seedling emergence (P = 0.016) as shown in figure 6.2.2.1: the seedlings produced from the 3.28% nitrogen seeds started to emerge sooner and at a higher (but non-significant) rate than the 2.60% nitrogen seeds. Increasing seed nitrogen significantly increased the FPF.

Figure 6.2.2.1 The effect of seed nitrogen percentage on the mean number of seedlings (per m⁻²) of the 1999 harvest Apex seedlots following field sowing



Although seed size did not significantly affect the pattern of seedling emergence in the field (P = 0.100) as shown in figure 6.2.2.2, the large seeds had a higher final percentage emergence than the small seeds. Although the small seeds are likely to have imbibed more rapidly than the large seeds, at deeper sowing depths, the large seeds achieved higher emergence. It is likely that in the temperate field environment, the larger seed reserves of the large seeds are more important in sustaining emergence and determining the final seedling stand than rapid imbibition.

Figure 6.2.2.2 The effect of seed size on the mean number of seedlings (per m⁻ ²) of the 1999 harvest Apex seedlots following field sowing



The effect of seed heat treatment on seedling emergence in the field

Seed heat treatment significantly increased the rate and final percentage field emergence and slightly but significantly altered the pattern of emergence (P = 0.0004) as shown in figure 6.2.2.3. Figure 6.2.2.3 The effect of seed heat treatment on the mean number of seedlings (per m⁻²) of the 1999 harvest Apex seedlots following field sowing



Effect of treatment interactions on seedling emergence

The significant interaction between size and heat treatment affected the final number of seedlings as shown in figure 6.2.2.4. The large seeds produced significantly more seedlings (P = 0.007) than the small seeds for the control and heat-treated populations.

Figure 6.2.2.4 The effect of the seed size* heat treatment interaction on the mean final number of seedlings (per m⁻²) of the 1999 harvest Apex seedlots following field sowing (SED 9.89, DF 71)



However, heat treatment significantly reduced the mean number of emerged seedlings produced from the large compared with the small seeds. There was also a significant difference in the pattern of emergence of seedlings produced from the small control and small heat-treated seeds (P = 0.0146) as shown in figure 6.2.2.5. The seedlings produced from the small, heat-treated seeds started to emerge sooner than those from the small control seeds. It was expected that there would be some delay in the emergence of the heat-treated seed, yet the small heat-treated seeds not only matched the speed of emergence of the small control seeds but exceeded it.

Figure 6.2.2.5 The effect of heat treatment on the mean number of seedlings (per m⁻²) from the small seeds of the 1999 harvest Apex seedlots following field sowing



The effect of seed treatment on plant losses during the growing season

The number of plants declined during the growing season and as with the previous experiment, polynomial quadratic functions were fitted to the plant losses. The percentage losses for seed nitrogen percentage are shown in figure 6.2.2.6. In the first three weeks after sowing the 3.25%N population suffered 45% seedling losses

compared with 54% of the 2.60%N population as shown in figure 6.2.2.6. By January this loss had increased to 46% and 64% and by the spring growth analysis in May, 60% and 71% of the original 3.28%N and 2.60%N populations had been lost and by harvest in month 9 only a third of the original population remained.

Figure 6.2.2.6 The effect of seed nitrogen percentage on the mean percentage plant losses (per m²) between October 1999 and July 2000 of the 2.60 and 3.28% nitrogen 1999 harvest Apex seedlots following field sowing



In the first three weeks after sowing the large seeded population suffered 41% seedling losses compared with 58% of the small seeded population as shown in figure 6.2.2.7 and by January this loss had increased to 42% and 59%. By the spring growth analysis in May, 59% and 66% of the original large and small seeded populations had been lost and by harvest in month 9 only a third of the original population remained. The survival of large seeded plants was thus greater than for small seeded plants.

Figure 6.2.2.7

The effect of seed size on the mean percentage plant losses (per m^2) between October 1999 and July 2000 of the 1999 harvest Apex seedlots following field sowing



In the first three weeks after sowing the control population suffered 53% seedling losses compared with 47% of the heat-treated seeded population as shown in figure 6.2.2.8 and by January this loss had increased to 54% and 48%. By the spring analysis in May, 57% and 65% of the original control and heat-treated seeded populations had been lost and by harvest in month 9 only about a third of the original population remained.

Figure 6.2.2.8 The effect of seed heat treatment on the mean percentage plant losses (per m²) between October 1999 and July 2000 of the 1999 harvest Apex seedlots following field sowing



The effect of seed nitrogen percentage on plant growth and development at growth stage 1.2 (vegetative rosette) of the 1999 harvest Apex seedlots

At the first growth analysis in February the crop was in a vegetative state at approximately growth stage 1.2. Increasing the seed nitrogen percentage from 2.60 to 3.28% significantly increased establishment as determined by the number of plants (P = 0.042) and the mean root length (P = 0.013) as shown in table 6.2.2.4.

Table 6.2.2.4The effect of seed nitrogen percentage on plant growth
parameters (values per m²) of the 2.60, 3.08 and 3.28% nitrogen
1999 harvest Apex seedlots at growth stage 1.2

Growth parameter	2.60%N	3.08%N	3.28%N	Р	SED	DF	Unit
Number of Plants	102	108	118	0.042	6.13	77	-
Leaf FWT	439	459	507	0.230	40.1	77	g
LeafDWT	38.2	38.9	45.9	0.116	4.06	76	g
Leaf Area	4071	4293	5011	0.097	447.9	77	cm ²
Leaf Moisture	91.09	91.15	90.50	0.521	0.626	76	%
Leaf Chlorophyll	7.32	8.92	10.41	0.072	1.323	77	µg cm ⁻²
Green Area Index	0.407	0.429	0.501	0.097	0.0448	77	-
Stem FWT	181.5	199.1	225.5	0.118	21.14	77	g
Stem DWT	14.84	15.80	17.59	0.255	1.674	77	g
Stem Area	832	838	967	0.243	89.4	77	cm ²
Stem Moisture	91.50	92.00	92.15	0.236	0.395	77	%
Stem Chlorophyll	6.17	6.26	5.31	0.636	1.094	77	μg cm ⁻²
Root FWT	52.3	54.6	60.1	0.227	4.59	77	g
Root DWT	6.72	6.27	6.21	0.633	0.582	53	g
Root Moisture	87.22	88.34	88.48	0.072	0.587	50	%
Mean Root Length	12.20	12.07	12.88	0.013	0.199	43	cm
Mean Root Diameter	3.005	2.864	3.342	0.085	0.2143	40	cm

The effect of seed nitrogen percentage on plant growth and development at growth stage 3.0 (plant framework extension) of the 1999 harvest Apex seedlots

By the second growth analysis the crop was at growth stage 3.0. The 3.28% nitrogen plants had a significantly greater stem DWT (P = 0.018), flower FWT (P = 0.025) and flower moisture (P = 0.034) than the 2.60% nitrogen plants as shown in table 6.2.2.5

Table 6.2.2.5The effect of seed nitrogen percentage on plant growth
parameters (values per m²) of the 2.60, 3.08 and 3.28% nitrogen
1999 harvest Apex seedlots at growth stage 3.0

Growth Parameter	2.60%N	3.08%N	3.28%N	Р	SED	DF	Unit
Number of Plants	80	80	89	0.091	4.47	63	-
Leaf FWT	1019	944	1002	0.579	75.6	63	g
Leaf DWT	116.2	99.9	112.3	0.097	7.74	65	g
Leaf Area	22782	20048	20852	0.355	1938.1	65	cm ²
Leaf Moisture	88.32	88.93	88.76	0.309	0.408	63	%
Green Area Index	2.264	2.010	2.178	0.410	0.1917	60	
Stem FWT	3899	3869	4190	0.300	226.4	61	g
Stem DWT	474.0	512.0	588.0	0.018	39.8	65	g
Stem Moisture	88.29	87.09	85.63	0.183	1.420	59	%
Stem Area	16073	14578	15751	0.173	827.2	57	cm ²
Mean Stem length	1.219	1.208	1.265	0.310	3.90	59	m
Root FWT	283	293	264	0.607	29.6	63	g
Root DWT	113.8	118.0	119.5	0.535	5.19	65	g
Root Moisture	54.5	54.7	51.0	0.451	3.23	63	%
Mean Root Length	18.9	22.2	16.6	0.570	5.28	57	cm
Flower FWT	187.2	201.9	236.4	0.025	18.04	63	g
Flower DWT	26.1	29.2	32.1	0.117	2.03	64	g
Flower Moisture	85.74	84.63	87.07	0.034	0.918	63	%
Bud FWT	199.9	184.0	200.2	0.323	12.25	64	g
Bud DWT	29.67	27.41	31.13	0.145	1.882	64	g
Bud Moisture	85.23	85.09	85.10	0.964	0.596	54	%

The effect of seed nitrogen percentage on plant growth and development at growth stage 6.5 (seed development) of the 1999 harvest Apex seedlots

At the time of the pre-harvest growth analysis in July the crop was at growth stage 6.5. Increasing the seed nitrogen percentage did not significantly affect the growth parameters as shown in table 6.2.2.6 although the increased number of 3.28% nitrogen-seeded plants at harvest was almost significant (P = 0.051).

Table 6.2.2.6The effect of seed nitrogen percentage on plant growth
parameters (values per m² unless other specified) of the 2.60, 3.08
and 3.28% nitrogen 1999 harvest Apex seedlots at growth stage 6.5

Growth parameter	2.60%N	3.08%N	3.28%N	Р	SED	DF	Unit
Number of Plants	64	67	72	0.051	3.54	78	-
Stem FWT	1483	1631	1659	0.177	100.4	77	g
Stem DWT	481.3	490.7	498.6	0.684	19.89	75	g
Stem Moisture	65.60	68.37	68.40	0.292	2.032	75	%
Mean Stem Length	1.54	1.48	1.48	0.255	3.95	79	m
Pod FWT	1440	1456	1343	0.435	93.8	72	g
Pod DWT	1031	947	951	0.495	79.5	69	g
Pod Moisture	29.4	31.4	28.9	0.666	2.90	73	%
Number of Pods	5152	4948	4709	0.382	317.2	74	-
Seed FWT	297.3	298.7	298.5	0.995	15.24	72	g
Seed DWT	220.9	222.8	230.7	0.694	12.18	72	g
Seed Moisture	25.13	25.18	21.88	0.127	1.836	75	%
Number of Seeds	61948	62229	62185	0.995	3174.2	72	-
Number of Seeds/ Pod	14	13	13	0.808	1.224	68	-
Seed Yield	2.973	2.987	2.985	0.995	0.1524	72	t ha ⁻¹

Increasing the seed nitrogen percentage between 2.60 and 3.08% did not elicit significant field responses but increasing it to 3.28% increased establishment due to increased seedling survival overwinter.

The effect of seed size on plant growth and development at growth stage 1.2 (vegetative rosette) of the 1999 harvest Apex seedlots

Selecting for seed size produced the effects shown in table 6.2.2.7. Selecting large seeds significantly increased the number of established plants (P < 0.001), leaf, stem and root FWT (P < 0.001) and DWT (P < 0.001), leaf area and stem area (P < 0.001), green area index (P < 0.001) and the mean root length (P < 0.001) compared with small seeds. Selecting for large seed size thus increased the initial size of the plants produced.

Table 6.2.2.7The effect of seed size on plant growth parameters (values per
m²) of the 2.60, 3.08 and 3.28% nitrogen 1999 harvest Apex
seedlots at growth stage 1.2

Growth parameter	Large	Small	Р	SED	DF	Unit
Number of Plants	128	91	< 0.001	5.01	77	-
Leaf FWT	552	384	< 0.001	32.8	77	g
Leaf DWT	47.8	34.2	< 0.001	3.32	76	g
Leaf Area	5392	3525	< 0.001	365.7	77	cm ²
Leaf Moisture	91.24	90.58	0.205	0.511	76	%
Leaf Chlorophyll	8.46	9.31	0.435	1.080	77	μg cm ⁻²
Green Area Index	0.539	0.353	< 0.001	0.0366	77	-
Stem FWT	247.1	156.9	< 0.001	17.26	77	g
Stem DWT	19.44	12.72	< 0.001	1.366	77	g
Stem Area	1070	688	< 0.001	73.0	77	cm ²
Stem Moisture	92.09	91.67	0.200	0.322	77	%
Stem Chlorophyll	6.09	5.73	0.687	0.893	77	μg cm ⁻²
Root FWT	66	45.3	< 0.001	3.75	77	g
Root DWT	7.58	6.22	< 0.001	0.475	53	g
Root Moisture	88.33	87.69	0.194	0.480	50	%
Mean Root Length	12.85	11.91	< 0.001	0.163	43	cm
Mean Root Diameter	3.092	3.049	0.805	0.1750	40	cm

The effect of seed size on plant growth and development at growth stage 3.0 (plant framework extension) of the 1999 harvest Apex seedlots

Selecting large seeds significantly increased the number of established plants (P < 0.001) and reproductive attributes such as the flower FWT (P < 0.001) and DWT (P = 0.001) and the bud FWT (P = 0.028) and DWT (P = 0.035) as shown in table 6.2.2.8.

Table 6.2.2.8The effect of seed size on plant growth parameters (values per
m²) of the 2.60, 3.08 and 3.28% nitrogen 1999 harvest Apex
seedlots at growth stage 3.0

Growth Parameter	Large	Small	Р	SED	DF	Unit
Number of Plants	91	75	< 0.001	3.65	63	-
Leaf FWT	965	1012	0.446	61.8	63	g
Leaf DWT	109.6	109.4	0.981	6.32	65	g
Leaf Area	21860	20595	0.427	1582.4	65	cm ²
Leaf Moisture	88.51	88.83	0.329	0.333	63	%
Green Area Index	2.182	2.119	0.689	0.1565	60	
Stem FWT	4117	3856	0.163	184.9	61	g
Stem DWT	537	513	0.467	32.5	65	g
Stem Moisture	87.20	86.80	0.730	1.160	59	%
Stem Area	15984	14951	0.132	675.4	57	cm ²
Mean Stem length	1.227	1.235	0.797	3.19	59	m
Root FWT	274	286	0.607	24.2	63	g
Root DWT	120.1	114.1	0.167	4.24	65	g
Root Moisture	51.1	55.6	0.094	2.64	63	%
Mean Root Length	17.7	20.8	0.472	4.31	57	cm
Flower FWT	237.3	179.7	< 0.001	14.73	63	g
Flower DWT	33.1	25.1	0.001	1.66	64	g
Flower Moisture	85.94	85.68	0.728	0.750	63	%
Bud FWT	206.0	183.5	0.028	10.00	64	g
Bud DWT	31.06	27.75	0.035	1.536	64	g
Bud Moisture	84.96	85.33	0.451	0.487	64	%

The effect of seed size on plant growth and development at growth stage 6.5 (seed development) of the 1999 harvest Apex seedlots

Selecting for large seeds over small significantly increased the number of plants (P < 0.001), the stem FWT (P < 0.001), stem moisture (P = 0.001) and pod moisture (P = 0.025) as shown in table 6.2.2.9.

Table 6.2.2.9The effect of seed size on plant growth parameters (values per
m²) of the 2.60, 3.08 and 3.28% nitrogen 1999 harvest Apex
seedlots at growth stage 6.5

Growth parameter	Large	Small	Р	SED	DF	Unit
Number of Plants	74	61	< 0.001	2.89	78	-
Stem FWT	1735	1447	< 0.001	81.9	77	g
Stem DWT	497.4	483.0	0.378	16.24	75	g
Stem Moisture	70.28	64.63	0.001	1.660	75	%
Mean Stem Length	1.48	1.52	0.284	3.22	79	m
Pod FWT	1444	1382	0.418	76.6	72	g
Pod DWT	970	982	0.862	64.9	69	g
Pod Moisture	32.6	27.2	0.025	2.37	73	%
Number of Pods	5032	4840	0.461	259.0	74	-
Seed FWT	304.9	291.4	0.281	12.44	72	g
Seed DWT	228.8	220.8	0.424	9.95	72	g
Seed Moisture	24.36	23.77	0.697	1.499	75	%
Number of Seeds	63528	60714	0.281	2591.7	72	-
Number of Seeds/ Pod	13	14	0.574	0.999	68	-
Seed Yield	3.049	2.914	0.281	0.1244	72	t ha ⁻¹

The effect of seed heat treatment on plant growth and development at growth stage 1.2 (vegetative rosette) of the 1999 harvest Apex seedlots

The effects of seed heat treatment prior to sowing are shown in table 6.2.2.10. Heattreating seeds significantly increased the number of established plants (P = 0.003), the leaf, stem and root FWT (P < 0.001) and DWT (P < 0.001), the leaf area (P = 0.002), stem area (P < 0.001), green area index (P < 0.001), the mean root length (P = 0.020) and the mean root diameter (P = 0.036). Heat treating seeds increased the initial plant size.

Table 6.2.2.10	The effect of seed heat treatment on plant growth parameters
	(values per m^2) of the 2.60, 3.08 and 3.28% nitrogen 1999 harvest
	Apex seedlots at growth stage 1.2

Growth parameter	Control	Heated	Р	SED	DF	Unit
Number of Plants	102	117	0.003	5.01	77	-
Leaf FWT	383	554	< 0.001	32.8	77	g
Leaf DWT	32.7	49.4	< 0.001	3.32	76	g
Leaf Area	3580	5337	< 0.002	365.7	77	cm ²
Leaf Moisture	90.78	91.04	0.613	0.511	76	%
Leaf Chlorophyll	8.50	9.27	0.474	1.080	77	µg cm ⁻²
Green Area Index	0.358	0.534	< 0.001	0.0366	77	-
Stem FWT	162.1	241.9	< 0.001	17.26	77	g
Stem DWT	12.78	19.37	< 0.001	1.366	77	g
Stem Area	722	1036	< 0.001	73.0	77	cm ²
Stem Moisture	92.05	91.71	0.302	0.322	77	%
Stem Chlorophyll	6.41	5.42	0.270	0.893	77	µg cm ⁻²
Root FWT	43.6	67.8	< 0.001	3.75	77	g
Root DWT	4.88	7.92	< 0.001	0.475	53	g
Root Moisture	88.27	87.75	0.287	0.480	50	%
Mean Root Length	12.10	12.66	0.020	0.230	43	cm
Mean Root Diameter	2.880	3.261	0.036	0.1750	40	cm

The effect of heat treatment on plant growth and development at growth stage 3.0 (plant framework extension) of the 1999 harvest Apex seedlots

Heat treatment significantly increased plant establishment and leaf FWT (P < 0.001), root FWT (P = 0.026) and the root (P = 0.013), flower (P < 0.001) and bud moisture (P

= 0.041) but significantly reduced the leaf DWT, area and GAI (P < 0.001), root length (P = 0.028), bud FWT (P = 0.002) and bud DWT (P < 0.001) as shown in table 6.2.2.11.

Table 6.2.2.11The effect of seed heat treatment on plant growth parameters
(values per m²) of the 2.60, 3.08 and 3.28% nitrogen 1999 harvest
Apex seedlots at growth stage 3.0

Growth Parameter	Control	Heated	Р	SED	DF	Unit
Number of Plants	72	94	< 0.001	3.65	63	-
Leaf FWT	1182	7995	< 0.001	61.8	63	g
Leaf DWT	130.2	88.2	< 0.001	6.32	65	g
Leaf Area	25238	17216	< 0.001	1582.4	65	cm ²
Leaf Moisture	88.82	88.52	0.382	0.333	63	%
Green Area Index	2.520	1.782	< 0.001	0.1565	60	
Stem FWT	4042	3931	0.551	184.9	61	g
Stem DWT	493.0	556.0	0.057	32.5	65	g
Stem Moisture	88.06	85.95	0.074	1.160	59	%
Stem Area	14818	16117	0.059	675.4	57	cm ²
Mean Stem length	1.262	1.199	0.053	3.18	59	m
Root FWT	253	308	0.026	24.2	63	g
Root DWT	117.0	117.2	0.957	4.24	65	g
Root Moisture	50.0	56.8	0.013	2.64	63	%
Mean Root Length	24.1	14.4	0.028	4.31	57	cm
Flower FWT	197.4	219.5	0.138	14.73	63	g
Flower DWT	30.1	28.2	0.420	1.66	64	g
Flower Moisture	84.07	87.55	< 0.001	0.750	63	%
Bud FWT	211.1	178.3	0.002	10.00	64	g
Bud DWT	33.24	25.57	< 0.001	1.536	64	g
Bud Moisture	84.63	85.65	0.041	0.487	64	%

The effect of heat treatment on plant growth and development at growth stage 6.5 (seed development) of the 1999 harvest Apex seedlots

Heat treating the seeds prior to sowing significantly increased both plant establishment at harvest (P < 0.001) and hastened seed drying and hence maturity, significantly reducing the seed moisture content (P < 0.001) compared with the control seeds as shown in table 6.2.2.12.

Table 6.2.2.12The effect of seed heat treatment on plant growth parameters
(values per m²) of the 2.60, 3.08 and 3.28% nitrogen 1999 harvest
Apex seedlots at growth stage 6.5

Growth parameter	Control	Heat	Р	SED	DF	Unit
Number of Plants	57	78	< 0.001	2.89	78	-
Stem FWT	1612	1569	0.602	81.9	77	g
Stem DWT	493.3	487.0	0.699	16.24	75	g
Stem Moisture	66.91	68.00	0.512	1.660	75	%
Mean Stem Length	1.53	1.47	0.082	3.22	79	m
Pod FWT	1445	1381	0.412	76.6	72	g
Pod DWT	984	968	0.808	64.9	69	g
Pod Moisture	31.2	28.5	0.266	2.37	73	%
Number of Pods	5062	4811	0.336	259.0	74	-
Seed FWT	306.0	290.3	0.210	12.44	72	g
Seed DWT	221.7	227.9	0.536	9.95	72	g
Seed Moisture	27.14	20.98	< 0.001	1.499	75	%
Number of Seeds	63760	60481	0.210	2591.7	72	-
Number of Seeds/ Pod	13	14	0.791	0.999	68	-
Seed Yield	3.060	2.903	0.210	0.1244	72	t ha ⁻¹

The effect of seed treatment interactions on crop growth parameters at growth stage 1.2

At growth stage 1.2 there was a significant interaction between the seed nitrogen percentage and seed size, which affected the stem chlorophyll content. Increasing the

seed nitrogen percentage significantly decreased the total stem chlorophyll content in the large seeded plants (P = 0.046) but it did not have a significant effect in the small seeded plants. The large seeded plants produced a greater stem area than the small seeded plants so possibly less chlorophyll was needed per unit area. At the lowest (2.60%) seed nitrogen percentage selecting large seeds significantly increased the total stem chlorophyll content but at the medium (3.08%) nitrogen percentage this effect was reversed. At the highest (3.28%) seed nitrogen percentage there was not a significant effect of seed size selection on this parameter.

At 3 to 4 months into the growing season, stem photosynthesis does not contribute significantly to the overall energy production of the plant. However, once the plant has flowered and the leaves have been lost, the stem makes a significant contribution to the photosynthetic capability of the plant. Higher stem chlorophyll, if maintained until leaf drop, would allow greater solar resource capture and energy conversion by the large plants compared with the small plants.

There was also a significant interaction between the seed nitrogen percentage and seed size, which affected the root length (P < 0.013). Increasing the seed nitrogen percentage significantly increased the root lengths of the heat-treated seeded plants but there was no effect on the control population. At the highest (3.28%) nitrogen level seed heat treatment significantly increased the root length but there was not an effect at the other seed nitrogen percentages. Plants with large root systems should again be more able to withstand frost heave or wind stress and longer roots would also be able to extract water and nutrients from depth thus increasing water and nutrient resource capture.

There was a significant interaction between seed size and heat treatment (P = 0.038), which affected the root DWT. Selecting large seeds over small significantly increased the root DWT of the control population but there was no effect on the heat-treated seedlings.

There was a significant interaction between the seed nitrogen percentage and heat treatment (P = 0.049), which affected the bud moisture: heat treatment significantly increased the bud moisture of the low (2.60%) and medium (3.08%) seed nitrogen populations.

There was a significant interaction between the seed nitrogen percentage and seed size, which significantly affected the number of plants (P = 0.004). Increasing the nitrogen percentage significantly increased the small-seeded population but there was no effect on the large-seeded population. Selecting large seeds at the two lower (2.60 and 3.08%) seed nitrogen percentages also significantly increased the seedling population but there was no effect at the highest (3.28%) seed nitrogen percentage.

A significant interaction between all three treatments, seed nitrogen percentage, seed size and heat treatment affected the mean stem length (P = 0.021), bud FWT (P = 0.007) and bud DWT (P = 0.005). The effects were varied, although in general selecting for large seeds significantly increased the stem length and bud FWT and DWT.

The plant number (P = 0.017), seed FWT (P = 0.028), number of pods (P = 0.006), pod FWT (P = 0.009), number of seeds (P = 0.028) and seed yield (P = 0.028) were affected by significant interaction between seed size and heat treatment. There were a significantly greater number of plants in the plots sown with heat-treated seeds than in those sown with the control seeds. Large seed size selection significantly increased the plant population of the heat-treated seeds but there was no effect of seed size on the control-seeded population. For the other parameters, the greatest weights or numbers were observed in the large control populations and the smallest were observed in the small heat-treated populations.

A significant interaction between all of the treatments affected the stem DWT (P = 0.018) and pod DWT (P = 0.009). Increasing the seed nitrogen percentage from 2.60% to 3.28% within the control treatment significantly increased the stem DWT but the treatment effects were otherwise mixed. The effects on the pod DWT were also variable but at the lowest (2.60%) seed nitrogen percentage, the large control and the small heat-treated populations gave the significantly highest DWT values; the pod DWTs of these two populations were not significantly different from each other.

6.3 Discussion of Field Experiments

The aims of these experiments were to examine the effect of seed heat treatment, seed nitrogen percentage and seed size on the emergence of Apex seedlots from 20 mm sowing depth in the field environment.

The sowing rate for this experiment was 220 seeds m^{-2} , which was almost double the usual rate of 120 seeds m^{-2} practised in UK. In the first three weeks after sowing between 27 and 37% of the seeds were lost, which may have been due to (a) seeds failing to germinate, (b) seedlings failing to emerge or (c) seedlings being lost through pigeon or rabbit damage. Winter losses were most likely due to frost damage as a result of the very late sowing date. It is also possible that some of the plant losses were due to inaccuracies in marking the new seedlings as previously discussed.

The main periods of plant loss were linked with frost conditions between growth stages 1.2 and 3.0. Winter damage is more serious than spring damage since the compensatory growth that can be made during the spring and early summer is insufficient to completely overcome the damage that is done over winter (Boag, MacFarlane Smith and Griffiths, 1990). The most crucial time for seedlings is in the early months of establishment between sowing and February, when the seedling is at risk from adverse weather and pest conditions; the hardier a seedling can be during the earlier stages of establishment, the more likely its survival until the spring.

It has been widely reported that increased planting density produces taller, thinner plants with fewer leaves compared with shorter, larger area plants at lower densities (Leach *et al.*, 1999) and that high density populations are often more susceptible to lodging and increased disease incidence (Glen *et al.*, 1989). However, following plant

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losses, the highest density of the fresh-seeded populations was 128 plants m^{-2} and the mean yield of 2.98 t ha⁻¹ compared well with the average of 3.25 t ha⁻¹ (Nix, 2000).

From the previous controlled environment research it was expected that seed protein content would have a large effect on seedling and young plant size but in practice, seed nitrogen percentage had a greater effect on improving the speed and uniformity of field emergence than on the subsequent growth of the young plants. Increasing the seed protein content significantly increased the speed of seedling field emergence of the stored (1997 harvest) Apex seedlots but did not significantly increase the speed of field emergence of the newly harvested (fresh) (1999) seedlots. However, significantly larger seedlings were produced from the fresh 3.61% nitrogen seeds than from the fresh 2.30% nitrogen seeds. Although the size increases only lasted until flowering and did not significantly affect either the yield components or the seed yield, the percentage plant loss over winter was significantly lower from the high than from the low nitrogen populations.

This research supported previous work by Shamlal and Mohammad (2000) in which field emergence was shown to be influenced by the level of fertiliser applied to the mother crop: seed harvested from fertilised crops produced seedlings with long roots and shoots and had improved field performance. Patchiness due to non-emerged plants is a problem in UK (Lunn, 2000, personal communication) and perhaps under less severe field conditions greater crop benefits would be derived from seed treatments that increased the percentage emergence rather than the speed of emergence.

Increasing the seed nitrogen percentage increased initial plant size and although seed protein content did not have any many significant effects on the growth parameters, by 3 to 4 months into the season, there was a positive trend in the leaf and stem area, fresh weights and the root diameter in response to increasing protein content although there was no significant effect on the seed yield.

The main components of the crop yield are the seed weight, the number of seeds/ pod, the number of pods/ branch, the number of branches/ plant and the number of plants/ unit area (Ward *et al.*, 1985). The yields obtained by the unfertilised 1997 harvest Apex seedlots ranged from 2.65 to 2.89 t ha⁻¹ compared with 2.90 to 3.06 t ha⁻¹ for the 1999 harvest Apex seedlots. It is thus evident that sufficient nitrogen was provided through the soil mineral nitrogen and nitrogen was not a limiting factor in these experiments since without fertilisation these crops almost obtained the average UK yield of 3.25 t ha⁻¹; a low yield in 2000 was 2.50 t ha⁻¹ (Nix, 2000). Furthermore, an adjacent experiment with up to 300 kg nitrogen fertiliser ha⁻¹ did not have an increased yield (Lunn, 2001, personal communication). The level of maize harvest obtained at the end of the season partially depends on the quality of the seeds planted by farmers (Ajayi and Fakorede, 2000) and the lower than average 1997 harvest Apex yields were probably a result of reduced seed vigour following storage for two years before sowing.

Although high seed nitrogen percentage did not have significant effects beyond the emergence phase of growth, since the time between sowing and emergence is hazardous, the greater the speed of germination and emergence, the fewer water and pest problems the seed will suffer. An increased rate of germination and emergence also maximises the establishment period of the seedlings and the more established a plant is going into the winter period, the more likely it is to survive over winter and emerge intact and undamaged in the spring.

It was expected that although the small seeds would imbibe water faster than large seeds and would germinate sooner as had been previously observed, the large seeds might be better able to emerge from depth and would thus achieve a higher percentage emergence and increased plant establishment. This was shown to be true for the 1999 harvest Apex seedlots under field conditions: large seed size selection significantly increased seedling emergence in the field and the large-seeded population suffered fewer overwinter losses than the small-seeded population. Selecting large seeds significantly increased plant establishment and plant size although the effects on plant size were reduced during the season.

It has been reported that large seeds often produce relatively large seedlings (Gross, 1984; Stanton, 1984; Hendriz *et al.*, 1991) and that large seedlings can enhance survival probability and provide increased fitness of adult plants, particularly in competitive conditions (Stanton, 1984; Wulff, 1986). Ahmed and Zuberi (1973) also reported that the seed size and plant weight after 30 days were positively correlated, which indicated that larger seeds produced plants with higher vigour. Furthermore, the plants from small seeds flowered later than those from large seeds and the size of seeds planted and size of seeds produced were significantly correlated (P = 0.05).

In this research selecting large seeds increased establishment and increased leaf, stem and root areas at the time of the early growth analysis, which should have maximised solar, nutrient and water resource capture. More of the plants produced from the large seeds survived through the winter and they produced a greater weight of flowers and buds than the plants produced from the small seeds thus there were more potential pod sites. Mendham, Shipway and Scott (1981) reported that large seeds increased the leaf area at flowering and this was similarly observed in this experiment although there was only a significant increase in the weight of potential pod sites at this stage. The increase in stem area will have made the large plants more resistant to frost heave and this is possibly why the large-seeded populations suffered fewer losses overwinter than the small-seeded populations. There did not appear to be a noticeable effect of seed size on the date of flowering although in this research the flowering date was not monitored.

It has been also reported that rape seeds graded according to size produced plants that differed in seed yield in spaced plantings under field conditions in Bangladesh (Ahmed and Zuberi, 1973). Fontes and Ohlrogge (1972) found that although the size of soybean seed had no effect on emergence as determined by seedling count, the large seeds showed the largest mean grain yield per plant, while the small-seeded treatment had the lowest yield. In this research however, although the large seeds produced significantly larger plants than the small seeds the extent of these differences declined during the season and there was not a significant effect of seed size on the ultimate seed yield. This was possibly due to the compensatory nature of oilseed rape, which occurred in the plants grown from the small seeds. It has similarly been observed that although growth parameters may be increased and thus a yield or quality improvement is expected, in practice the lower population plants compensate in their growth and produce a greater number of pods, more seeds per pod, higher seed weights or nitrogen/ oil percentages (Davies *et al.*, 1994).

Although the small seeds appeared to be more resistant to heat treatment, which may have been due to differences in the seed coat or seed composition, smaller plants have large spaces between them in which pigeons can sit, so delayed sowing can increase pigeon damage (Anon 1, 2000) and there can also be a decrease in the number of leaves, the weight of the roots and the thickness of the root neck (Jasiñska *et al.*, 1987) as was observed at growth stage 3.0. The root data are consistent with Temu (1994) who postulated that large Bambara groundnut (*Vigna subterranea* L.) seeds produced heavier roots than small seeds because more reserves were necessary to support the higher growth rate of the large-seeded plants.

Seed heat treatment of the 1997 harvest stored seedlots prior to sowing did not significantly delay field emergence but did significantly reduce seedling size at G.S. 1.2, which was possibly due to greater initial deterioration following storage coupled with heat damage. However, beyond this point these differences were not maintained and although these populations suffered greater plant losses than the control populations, there were no significant effects of heat treatment on either the yield components or the final yield.

It is important to note that the 1997 harvest Apex seedlots used in this experiment had been stored for two years in less than optimal conditions and it was likely that their initial vigour had decreased during storage as has previously been reported (Barton, 1961; Abdalla and Roberts, 1968). Although it might be expected that seed treatment techniques would have a greater effect on stored seeds than on fresh seeds, seed thermotolerance declines with age (Howarth, 1990), which could have made the stored

seeds more susceptible to heat damage.

In contrast, heat treatment significantly increased the speed and final percentage field emergence of the newly harvested (1999) seedlots and significantly reduced plant losses and significantly increased plant establishment of the 1999 seedlots. It was unexpected that heat treatment, which significantly delayed seed germination in controlled conditions and seedling emergence in the polytunnel did not delay field emergence of the fresh seedlots. However, germination measurements made in constant temperature environments are not always relevant to the field where soil temperatures vary diurnally: Soman and Peacock (1985) observed that some sorghum lines, which fail to germinate in incubators at 40°C, will germinate and emerge in soils of the same mean temperature.

In the fresh-seeded populations, the heat treated plants had a significantly greater weight and area of leaves and stems, significantly more roots and heavier, longer and thicker roots than the control seeds. The heat-treated plants were noticeably larger in the field as well as when handling the plants during the growth analysis. The thicker stems of the plants produced from the heat-treated seeds would have better resisted frost heave than those grown from the control plants and would have helped to reduce population losses overwinter but the physiological effects on the leaf weights and area, and the root fresh weight and length were only maintained until flowering.

Mendham, Shipway and Scott (1981) reported that techniques for improving the yield of late-sown crops should be directed mainly at increasing the leaf area and this could be achieved through increasing the speed of establishment, the number of plants per m² and the size of individual leaves. Heat treatment significantly increased the leaf area thus maximising solar resource capture while the larger stems made the plants stronger. The significantly greater root number and root length of the plants produced from the heat-treated seeds would have aided their water and nutrient resource capture and made them more able to withstand frost heave and wind stress during the winter. However, although at harvest the number of plants in the heat-treated plots was significantly greater than in the control plots, there were no significant effects of heat treatment on the final seed yield.

6.4 Conclusions of Field Experiments

This chapter has shown that high seed nitrogen percentage, large seed size and heat treatment can increase the speed of fresh seedlot field emergence and can significantly increase field establishment. Plant losses can be significantly reduced by high seed nitrogen percentage, large seed size and heat treatment and these treatments generally increase, although not always significantly so, the initial plant size. The increased sizes of plant components produced from high nitrogen seeds were not maintained until harvest and there was no significant effect of seed nitrogen percentage on the final yield. However, this does not mean that such growth differences would not be maintained under poorer growing conditions. Larger seeds produced significantly larger seedlings and plants but although the increases in the sizes of some components were maintained until harvest, the final seed yield was not significantly greater from large seeds. Heat treatment, which reduced the plant size from older seeds but increase that of fresh seeds, did not significantly affect the yield but did significantly increase plant establishment.

The variability of the oilseed rape crop and its ability to compensate for crop damage can make the interpretation of experiments difficult. Leach *et al.*, (1994) reported that there were no consistent effects of spring nitrogen rates or timing on the yield or yield components of winter oilseed rape and in these experiments there were no significant effects of increased establishment, size or reproductive capacity on the final yield. Increasing the seed nitrogen percentage, selecting large seeds or heat treating seeds significantly increased the taproot length at growth stage 1.2 for the 1999 harvest Apex seedlots, which would have increased nitrogen uptake from the soil before nitrate leaching occurred from the shallow root zone in the late autumn. The major yield restriction on autumn sown crops, which produce small plants overwinter, is the amount of vegetative growth, especially leaf area, that can be made before flowering in May. At flowering, the UK temperature is too low for rapid growth and leaf expansion and leaves are shaded out by flowers and developing pods, which are only able to support the growth of their own seeds (Mendham, 1975). Selecting large seeds significantly increased the leaf area of the 1999 harvest Apex seedlots at growth stage 3.0 while heat treatment significantly increased the leaf area overwinter at growth stage 1.2 but significantly reduced it at growth stage 3.0.

Variations in the rate of germination have important implications for crop production in areas such as the semi-arid tropics where there can be prolonged exposure to high temperatures and increased risk of dehydration and clearly a rapid growth rate is advantageous in such environments (Mohamed *et al.*, 1988). Under good seedbed conditions, small, high nitrogen seeds would germinate quickly but if seedbed conditions are poor and seeds need to be sown at depth to ensure sufficient moisture, large, high oil (low nitrogen) seeds might produce a better crop stand.

Vigorous seedling emergence is important for crop production (Sepaskhah and Ardekani, 1978). This research has shown that it is possible to use seed nitrogen percentage, seed size and heat treatment to significantly increase seedling and plant establishment in the field and to significantly alter plant morphology to increase resource capture. However, under the environmental conditions of this experiment, the true potential of these treatments was not tested and further field experiments in a variety of stressed (temperature, water, nutrient, seedbed) environments are necessary.

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CHAPTER VII FINAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Final Discussion and Conclusions

The difficulty in achieving early establishment is one of the major problems for oilseed rape and the production of vigorous seedlings has been reported to be a worthwhile agronomic target to minimise yield losses in late-sown crops (Matthews *et al.*, 1988). Establishment is the point at which a seed produces a plant that is capable of producing new seeds and between sowing in the autumn and establishment during the spring, plant populations in oilseed rape are reduced (McWilliam, 1998). Successful establishment is governed by a combination of inherent seed and environmental factors (Rao *et al.*, 1987) and although several factors have been studied (McWilliam *et al.*, 1995; Bullard *et al.*, 1996; McWilliam *et al.*, 1998), up to 30% of seeds fail to establish, which can cost the UK farming industry about £50 million in bad years (Nix, 1997). However, if the success of each stage can be improved through targeted "seed management" it should be possible to approach maximal establishment of the winter-sown rape crop.

All commercial seedlots in Europe must meet minimum germination standards (Powell *et al.*, 1997) and the current guide to the germination potential of the seed is that more than 85% of the seed will germinate at 20°C (International Seed Testing Association, 1993). However, the research described in this thesis has shown significant differences in the germination parameters (T_{10} , T_{50} , FPG and rate of germination) of nine varieties comprised of sixteen seedlots of winter oilseed rape at this temperature as well as differences in the optimal temperature for germination.

Although increasing the temperature generally increased the final percentage germination of the majority of seedlots and the highest mean final percentage of the seedlots was obtained at 15 and 20°C, it was at 15°C that there was the least variation between the sixteen seedlots of the nine varieties. Since the aim of the ISTA germination test is to give the highest germination for a seedlot under optimal conditions, this could be achieved by revision of the ISTA guidelines to test oilseed rape germination at 15 rather than at 20°C although this would be unlikely to predict field emergence, which differs markedly between high and low vigour seedlots. At lower temperatures, the final percentage germination ranged from 49 to 98% (10°C) and 9 to 97% (5°C) and it is these conditions that are most likely to reveal field emergence potential thus testing germination at these temperatures could constitute a stress-based physiological vigour test.

Seed hydration in water at 15°C significantly increased the speed of germination and emergence. This research confirmed reports that the optimal duration of hydration for a seedlot varies with the temperature at which the seedlot is germinated following hydration (Zheng *et al.*, 1994) since the effects of seed hydration were more profound at 5 than at 15°C. The data also confirmed that seed hydration was more beneficial in increasing the speed of germination and emergence of low than high germination seedlots as has also previously been reported (Durrant and Jaggard, 1988; Zheng *et al.*, 1994). The most likely reason for this is that the poor germination seedlots have the most potential for improvement through seed hydration since they are relatively more deteriorated or damaged than high germination seedlots.

Seed hydration results in more uniform germination, especially at lower germination temperatures (Heydecker et al., 1975). The onset of germination is associated with

ribonucleic acid (RNA) production and structural protein synthesis and during hydration treatments it has been observed that deoxyribonucleic acid (DNA) replication and hence cell division and expansion are delayed for several hours, which allows repair processes to be completed (Bray *et al.*, 1989; Clarke and James, 1991). It is likely, therefore, that hydrated seeds are better able to germinate because following hydration treatments they are less deteriorated than the non-hydrated (control) seeds. In this research seed hydration also produced significant morphological effects by altering post-germinative taproot growth although the optimal duration of seed hydration for maximal taproot length was not the same as the optimal duration for maximal final percentage germination; this may also be the result of differential repair mechanisms.

Although seed hydration techniques have the potential to improve oilseed rape germination and subsequent seedling emergence under low soil temperature conditions (Zheng *et al.*, 1994) the commercial use of seed hydration requires the calibration of individual seed lots and necessitates the uniformity of moisture amongst individual seeds during treatment (Durrant and Jaggard, 1988). This research has confirmed that although seed hydration can induce the rapid germination and emergence of winter oilseed rape, the optimal duration of hydration varies with seedlot, temperature and the desired physiological outcome.

In general, only a small number of seeds dispersed from a plant germinate into seedlings and an even smaller proportion survives to maturity. Many studies have shown that seed size affects a number of characteristics that affect the probability of survival to reproduction such as percentage germination (Alexander and Wulff, 1985), the time to emergence (Winn, 1985; Wulff, 1986), plant size (Parrish and Bazzaz, 1985; Wulff, 1986) and growth rate (Marshall, 1986). Furthermore, seeds produced on the same plant

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may vary in size, shape or colour and these variations may be associated with different germination responses (Baskin and Baskin, 1998).

Large seeds of *Aegilops spp.* (Maranon, 1989), *Beta vulgaris* (Wood *et al.*, 1980) and *Prunella vulgaris* (Winn, 1988) have been shown to germinate to higher percentages than small seeds since their high food reserves have enabled seedlings to grow vigorously with great root and shoot lengths (Shamlal and Mohammad, 2000). However, this research did not confirm that this was true for winter oilseed rape and neither was it true that germination was independent of seed size as has been reported for *Dactylis glomerata* (Bretagnolle *et al.*, 1995) and *Zea mays* (Eagles and Hardacre, 1979).

For the winter oilseed rape varieties and seedlots tested, seed germination was significantly increased by selection for small seed size, which is likely to be due to increased water uptake upon imbibition as a result of a high surface area to volume ratio. Beneficial effects of small seed size selection have also been reported for *Festuca pratensis* (Akpan and Bean, 1977) and *Rumex crispus* (Maun and Cavers, 1971). However, in contrast with these other reports, small rape seeds were observed to have a higher speed but not necessarily final percentage germination than large seeds, which confirmed observations by Stickler and Wassom (1963) and Harper and Obeid (1967).

Seed size has also been shown to be important in determining the soil depth from which seedlings can emerge and within triticale (*Triticosecale rimpaui* Wittmach), barley (*hordeum vulgare* L.), maize (*Zea Mays* L.) and cluster bean (*Cyamopsis psoralioides* DC): smaller seeds show faster emergence than larger seeds (Dhillon *et al.*, 1976). The research confirmed that the speed and final percentage emergence from 10 mm sowing depth were significantly increased by selection for small seeds but that in the field, the large seeds produced higher plant populations and significantly larger seedlings than the small seeds. In many species, large seeds give rise to larger seedlings than are produced by small seeds because they have a greater store of reserve energy that can be used by the seedling in the early stages of growth: amino acids from protein are the initial energy source for the polysome formation and protein synthesis that is essential for radicle growth and protrusion (Bewley and Black, 1983).

Larger seeds thus tend to produce seedlings that are more likely to survive to maturity than seedlings from smaller seeds (Carleton and Cooper, 1972; Marshall, 1986; Wulff, 1986) although this is not always the case. Wulff (1986a) hypothesised that the smaller seedlings derived from smaller seeds may be less subject to predation although it has been observed that the survivorship of seedlings from large *Garcinia mangostana* seeds can be higher than that of seedlings from small seeds (Hume and Cobin, 1946). There is also evidence that large rape seeds are less susceptible than small seeds to flea beetles (Lunn, 2002, personal communication) although these field sowings were not affected by this pest. This research confirmed reports by Major (1977) that seed size did not affect the seed yield of field-grown plants.

Selecting small seeds with a high surface area to volume ratio thus increased the speed of germination and emergence although the plants produced were significantly smaller than those produced from the large seeds, which was probably due to lower initial seed reserves. At shallow sowing depths the effects of faster germination due to small seed size were significant but at field sowing depths and under field conditions large seeds obtained a higher final percentage emergence and significant increases in plant establishment during the growing season.

The nutrition of parental sorghum lines with nitrogen has been reported to have a significant influence on seed protein content and to be successful in improving seedling establishment in the field (Shamlal and Mohammad, 2000). The seed nitrogen, size and thousand seed weight analyses of the Apex variety of winter oilseed rape has shown that increasing the seed nitrogen percentage significantly increased the thousand seed weight and non-significantly increased the ratio of large to small seeds. The higher seed yield from the lower section of the canopy was the result of greater numbers but lighter seeds than were obtained from the upper section of the canopy.

Selecting seeds on the basis of the nitrogen percentage was successful in significantly increasing the speed of germination although the final percentage germination was not significantly affected. Significant effects of seed nitrogen percentage on emergence were also obtained when the five initial seed nitrogen categories were divided into two broader, high nitrogen and low nitrogen, categories. At 20 mm sowing depth increasing the seed nitrogen percentage of the Apex seedlots significantly increased the speed of emergence but at 40 mm sowing depth the effects of high percentage seed nitrogen were less visible. Increasing the seed nitrogen percentage but at 40 mm sowing the seed nitrogen percentage significantly increased plant establishment and initial plant size but did not significantly affect the final seed yield of the field-grown crop.

Increasing the seed nitrogen percentage and ready supply of metabolisable proteins thus increased the speed of germination and emergence from shallow sowing depths although at deeper sowing depths the high oil percentage of the low nitrogen seeds, which allowed sustained growth, aided their successful emergence. Plant losses over winter were also significantly reduced by sowing high nitrogen seeds and the main effects of high seed nitrogen percentage on plant growth were to significantly increase

the leaf and root area available for solar, water and nutrient resource capture from seedling emergence until flowering. Sowing high nitrogen seeds could hence be used to increase the yield of non-seed parts, which for other *Brassica* crops are more important than the seed yield.

Heat treating seeds significantly delayed germination and emergence, probably because of the extra time needed for membrane repair following heat and imbibition damage. All organisms respond to heat by inducing the synthesis of a group of proteins defined as heat-shock proteins (HSP), which in plants are nuclear-coded and translated on cytosolic ribosomes (Adamska and Kloppstech, 1991; Vierling, 1991; Kruse and Kloppstech, 1992). Although the functions of HSPs are not well understood, it is known that they help to protect organisms against heat damage (Debel *et al.*, 1997). The increased expression of small (molecular mass 15 to 30 kDa) heat-shock proteins (sHSP) under heat shock conditions of 20°C and their protective effect on cell viability at elevated temperatures, suggest that they may have a function in the formation or maintenance of cytosolic proteins through recognising and binding proteins that unfold during thermal stress thus protecting them from irreversible aggregation (Jakob *et al.*, 1993)

Dry heat at temperatures between 60 and 100°C can cause seeds of species such as *Glycine clandestina* to become permeable (Baskin and Baskin, 1998) and tropical soil temperatures, which can reach 64°C (Peacock *et al.*, 1993), can limit the production of crops such as pearl millet (*Pennisetum glaucum* (L.) R. Br.) (Soman and Peacock, 1985). However, there is evidence that heat shock proteins are involved in the development of thermotolerance (Howarth, 1990) and combinations of partial hydration in water followed by heat shock (36 to 38°C) for 1 hour have been observed to produce

the optimum increase in germination and thermotolerance in tomato and pepper seedlings (Sánchez *et al.*, 2001). Baker (1969) also reported that for seeds in general, the thermal tolerance to heat treatment declines with age resulting in a reduction in the average seedling emergence.

The physiological effects of heat treatment were greater in the high nitrogen seeds, which suffered from increased testal cracking and hence greater imbibition damage than the low nitrogen seeds, which may have been due to differences in the seed composition. Heat treatment delayed seedling emergence but there was probably a compounding influence of the initial seed vigour. In this research, heat treatment for 48 hours at 80°C significantly reduced germination vigour although at 25°C it significantly increased the final percentage germination and hence viability compared with the control seeds. Although there were some deleterious effects of heat treatment on emergence vigour, heat treatment significantly increased the speed of emergence and plant establishment in the field. In the fresh seedlots heat treatment significantly reduced plant losses over winter and significantly increased plant establishment throughout the growing season of the crop although it increased the amount of damage to stored seedlots. The physiological effects of heat treatment on the plant leaves of the 1999 harvest seedlots were maintained until May when significant increases in the reproductive potential of the plants were observed although these increases were not translated into significant yield improvements. It is important to note that although the production of heat shock proteins is a common phenomenon, Ougham and Stoddart (1986) reported that while both heat-tolerant and heat-susceptible sorghum lines are capable of synthesising HSPs, the time at which they were first synthesised varied.

7.2 Concluding Remarks and Recommendations

The aim of this research was to improve the establishment of winter oilseed rape. This was achieved through determining the extent of variation in germination capacity between different varieties and different seedlots of winter oilseed rape and examining the effects of seed nitrogen percentage, seed hydration, seed size selection and seed heat treatment on germination, emergence and establishment.

Planting poor quality seeds can be costly (Byrum and Copeland, 1995) and producers rely on the results of the standard germination tests, which are printed on the seed tag, to give them reliable information when making planting decisions (Happ *et al.*, 1993). This research showed that although seed germination tends to increase with increasing temperature, the highest mean final percentage and smallest variation in percentage germination were obtained at 15°C. A revision of the ISTA seed testing procedures to test winter oilseed rape seeds at 15 rather than 20°C might thus enable a better comparison between different varietal and seedlots germination and provide a higher correlation between germination and emergence performance to provide growers with a more realistic expectation of likely field emergence and establishment.

The combination of factors involved in seed hydration makes it difficult to recommend a specific duration time for the optimal improvement of oilseed rape germination and emergence. Furthermore, there were clear detrimental effects of storage on the germination and emergence of the Apex seedlots used in this research, which might outweigh any beneficial effects of subsequent seed hydration if the seeds were saved and treated before sowing the following year because the seeds would have been too damaged to allow sufficient repair during the soaking treatment. However, poor emergence and establishment are a major constraint on crops in semiarid areas (Harris, 1996) due to irregular planting depth, soil crusting, poor soil quality (Duthoit, 1999) and limited water availability and seed hydration might thus improve the speed and final percentage of germination and emergence of winter oilseed rape in these areas. Since the effects of seed hydration are more noticeable in poor conditions (Lush and Groves, 1981), seed hydration would most benefit crops in marginal conditions (Hampson, 1999) and the effects of seed hydration on improving oilseed rape germination, emergence and establishment should be tested in semi-arid conditions.

In northern Europe, autumn-sown oilseed rape often receives 30 kg nitrogen ha⁻¹ by the end of September as seedbed nitrogen and the remainder in the spring at the beginning of growth and at stem elongation (HGCA, 1998). However, in contrast with crops such as sorghum for which fertiliser regulation is one of the most well known approaches to regulate the physical and biochemical proportions of the resulting seed for progeny field performance (Shamlal and Mohammad, 2000), there are no specific guidelines for growing oilseed rape seeds for resowing.

Faster germination reduces the risk of pathogen attack and temperature stresses and results in quick establishment and early canopy formation (Mabika, 1992). This research has shown that by altering the seed composition of winter oilseed rape it is possible to enhance germination and emergence under controlled and, more importantly, under semi-controlled and field conditions, which could enable growers to manipulate the seed nitrogen percentage for their prevailing environmental conditions. Rapid emergence would be most desirable under arid conditions where water is limiting whereas high final percentage emergence would be most beneficial under temperate conditions with combinations of high levels of pests and disease or northern climates

with very low field temperatures. Since increases in nitrogen fertiliser reduce the oil percentage of winter oilseed rape, this author recommends that the residual soil mineral nitrogen is measured prior to sowing and the amount and timing of nitrogen input to the crop is balanced accordingly.

It is important to determine the reasons for high nitrogen enhancement of germination and emergence through performing more detailed seed analyses. The three growth analyses in this research were performed at important stages in the crop lifecycle but they were not sufficient to adequately monitor the effects of seed treatment on plant growth and development. It would thus be helpful to assess the effects of mother crop nitrogen management through sub-sampling the crop at each stage in its lifecycle to enable more precise recommendations for timing mother crop nitrogen management to be made. Although the seeds harvested from the lower pods were observed to have higher seed nitrogen percentages than those harvested from the upper pods, the yield of seeds was greater from the lower pods. Early harvesting when the lower pods have filled may thus produce greater commercial oil yields due to greater seed numbers as well as maximising seed stocks for resowing. Crop lodging and fertiliser costs could also be reduced while earlier harvesting would increase the time available to prepare the land for the next crop.

Grading rapeseed before planting could be of great value to the farmer, if the sowing depth is (a) deeper than 20 mm, to ensure sufficient water availability to the germinating seed, (b) the soil profile is dry at the surface but moist at depth, for example, in semiarid conditions or (c) if a constant sowing depth is difficult to achieve because of the drilling technique or seedbed conditions.

Although heat treatment increased the initial plant size, which may have helped to counteract the effects of frost damage, under warm, damp conditions, the loosening of the seed coat as a result of heat treatment may render the seeds more vulnerable to bacterial and fungal infections at an early stage. A study of the effects of heat treatment on the physical seed coat and internal tissues should be performed to determine the cause of vigour loss at field temperatures of stored seeds but an increase in the vigour of fresh seeds at high temperatures. It has been reported that the phospholipid content of pea seed membranes declines with age (Powell and Matthews, 1981a) and a determination of phospholipid content would be useful to determine whether high temperature heat treatment produces some of the same effects as ageing. It is also important to determine the production and role of heat shock proteins in *Brassica napus* seeds since their role in thermotolerance may be important for crop establishment in tropical environments as has previously been reported for tomato and pepper (Sánchez et al., 2001). In addition, it is important to separate the two components of heat treatment and determine whether it is the high temperature or the extreme drying that affects germination, emergence and establishment of winter oilseed rape.

The field conditions in Sutton Bonington between 1999 and 2000 were not representative of many of the environments in which oilseed rape is grown - there was sufficient soil water and enough residual soil mineral nitrogen to ensure that neither water nor nitrogen supply restricted seed germination and subsequent plant growth. Furthermore, the temperatures over which the plants grew and developed were temperate and pests and diseases did not affect the crop although early seedling losses due to pigeons and rabbits were high. Before recommendations can be made for growing winter oilseed rape in non-temperate environments, it would be desirable to determine the effects of these treatments in a range of environments from northern to

tropical conditions. Although significant yield effects were not achieved using these techniques, under harsher conditions greater benefits of these techniques might be expected.

This thesis has shown that there is the potential for improving winter rapeseed quality through a variety or combination of mother crop nitrogen management, seed hydration, seed size selection and seed heat treatment techniques. The speed and final percentage of the germination, emergence and establishment processes can be significantly improved through these techniques either alone or in combination but the method of seed treatment should be tailored to the desired outcome.

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