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ESTABLISHMENT OF OILSEED RAPE: SEED CROP MANAGEMENT EFFECTS ON SEED QUALITY AND SEEDLING PERFORMANCE

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by

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Contents

List of Figures and Tables

Abstract

Establishment of oilseed rape is difficult and can account for up to 50% of the total cost of growing the crop. This difficulty is due to small seeds and poor seedbed conditions on the heavy clays where 60% on the national crop is grown. The aim of this project was to assess the potential for improving establishment by improved seed quality and seedling vigour, by examining the influence that management of the mother crop might have on seed germination and seedling performance. The main hypotheses tested in the work were that:

- Maturity of seed affects their subsequent germination and emergence performance
- Seed protein/oil content can be manipulated by husbandry/harvesting strategy
- Seed protein/oil content influences germination and emergence
- Seed selection and treatment processes can improve germination and emergence performance

Seed maturity (measured by seed harvest date) had very little effect on germination performance. Whilst hand-separated immature (orange) seeds showed significantly lower germination than mature (black seed), the immature seed content in the samples was not large enough to affect performance. Thousand seed weight, oil and protein content also did not vary with seed date indicating that the ‘sink’ for protein and oil was full by the time sampling began. In contrast, nitrogen management of the mother crop had significant effects on seed properties. Increasing nitrogen fertiliser increased thousand seed weight (mainly in the upper part of the canopy) and seed nitrogen content. There was generally an inverse linear relationship between the proportion of oil and nitrogen in the seed, with the highest proportion of oil in the sparsely fertilised crop. The highest seed nitrogen contents were in those crops which received medium amounts of nitrogen just before flowering, rather than those which received larger doses earlier in development. The nitrogen and oil balance of the seed was found to significantly affect germination and emergence performance, with less time elapsed to 50% germination or emergence in high nitrogen content seed. Seed with higher nitrogen contents also produced larger cotyledons. Assessment of the potential for improving establishment by seed selection and treatment procedures showed that advancement (soaking in water before drying back) and selection of seeds above 2 mm in diameter both improved germination and emergence over untreated and unselected seed. In conclusion, the work has shown the potential for manipulation of seed physiochemical properties (such as thousand seed weight and oil/protein ratio) by husbandry of the mother crop, producing the best quality seed by carefully defined nitrogen management. In addition, there is potential to further improve seed quality by selecting for large seeds and by processing them with treatments such as advancement. Assessment of these techniques for improving seed quality is continuing in HGCA Project 1349, ‘Improving establishment in oilseed rape by seed crop husbandry, seed selection and seed treatment’.

Summary

Sustainable rape growing (maximising light harvesting efficiency for optimal final seed yield but minimising the influence of weeds, pests and diseases) depends on the achievement of uniform plant populations at optimum density. This is equally important for rape grown for industrial as well as for food use. About half the total cost of growing rape is associated with the establishment period, a time when there is great uncertainty about the outcome, especially for the 60% of the national crop sown into heavy clay soils where cereal residues have been incorporated.

Poor establishment or patchy areas directly reduce the sustainability of rape growing by increasing the reliance on autumn herbicides. This leads to uneven plant development, less uniform crop growth and less even ripening, necessitating greater use of pre-harvest desiccants. In some circumstances larger applications of nitrogen fertiliser may be applied in the spring to try and hasten canopy expansion but this has its own associated environmental and economic costs. Rapid and synchronous establishment generally reduces the need for broadcast molluscicides (e.g. methiocarb) which are proven to have a detrimental effect on wildlife (e.g. field mice), and negative effects on food chain dynamics. Re-drilling incurs costs in terms of additional seeds, additional labour, tractor hours, fossil fuels and agro-chemicals expended in establishing a second crop, and may also disrupt beneficial soil-living invertebrates. In a re-drilled or patched crop it is likely that reliance on agro-chemicals will be higher still because of the greater pressure from pollen beetle and other insects migrating from earlier flowering crops to later, less developed sowings. At the other end of the spectrum from thin, patchy crops, excessive plant establishment resulting from use of high ‘insurance’ seed rates also creates its own problems. Excessive interplant competition increases crop height and lodging leading to pod abortion and uneven ripening in the lower portions of pod canopies, which will increase reliance on desiccants.

Establishment of rape in autumn provides a substantial challenge. A significant area of rape is grown on unstable and heavy clay soils (Bullard *et al.* 1996). Usually the previous cereal crop has dried out the soil profile. Rainfall can be so low and evaporative losses from the soil so high that the young seedling is extremely vulnerable to desiccation. The seed is small (~2mm diameter) and thus carbohydrate reserves are low. Failure to sow rape soon after the cereal has been harvested results in a loss of yield (Scott *et al.*, 1973), so appropriate cultivations must be sandwiched into a relatively short period. The aim must be to improve the speed and uniformity of the establishment process. HGCA has funded work to examine the effects of cultivation on the complex interactions at the seed / soil interface and progress in this area is presented in Bullard *et al*. (1996). However, developments in cultivation and machinery cannot provide all the answers, and there is the need to look for more strategic areas of improvement. Previous HGCA-funded studies have established that seed selection, in order to retain the larger seed, and seed advancement by pre-

germinating, can both lead to increases in emergence 'vigour'. Selecting larger seed produced seed with greater energy reserves that can emerge from greater depth, thus overcoming problems associated with the seed dropping deep in the soil profile when the soil is dry and cloddy. Advancement increases the speed of germination in the field, thus getting the seed through the stage where it is vulnerable to desiccation more rapidly.

Although selecting and pre-treating seed can significantly improve performance, there is little time between harvest of the seed crop and drilling of the following commercial crop to implement these procedures. In those instances where seed is not 'carried over' from one season to the next, we must seek methods of manipulating the mother crop to improve seed performance or selectively harvest portions of the crop which contain the fittest seed, if indeed we can demonstrate that there are differences within the crop canopy. It is surprising, but currently, there is little indication that seed crops are managed similarly to those grown for food and industrial use. In order to examine the opportunities for seed crop manipulation, the objectives of this project were to

- Determine uniformity of seed maturity from contrasting crop canopies at different times.

- Test for effects of stage of maturation or provenance on:
    a) seed coat colour
    b) seed dry weight
    c) protein and oil deposition
    d) germination and early seedling growth

- Begin an investigation of the potential for improving seed maturity and fitness for resowing in immature or variable seedlots by seed selection or treatment procedures.

In the experiments reported here, an examination was made of the patterns of germination and emergence of rape seedlings to examine the influence that management of the mother crop might have on variability of seedling performance and to see whether this might be a possible explanation of the considerable variation in performance found between seedlots of a variety (Stokes *et al*. 1998). It is clear from our work on canopy management that nitrogen fertiliser has a dominant effect on the number of pods initiated, size of the rape canopy and, as a consequence, the amount of sunlight penetrating to the lower pods in the canopy. Thus, in probably the majority of seed crops, there is large disparity in the receipt of sunlight, growth of seeds, and the uniformity of maturation within the canopy.

Very little effect of maturity was found, with the only impairment being the reduced germination capacity of orange seeds, which form a very small fraction of the total. However, from the work reported here, it is clear that there is scope to manipulate the chemical composition of the seeds, especially the content of nitrogen and the balance between oil and protein, through strategic application of N. It appears that the quality of seed resulting from large applications of N at the more conventional timings (mid February and mid March) can be achieved with smaller doses applied just before flowering. This has important implications for growers of seed crops. First, it shows that high protein seed can be produced without the risk from large, early applications creating excessively large canopies and the associated penalties of wide variations in maturation. Second, it increases the probability that the fewer pods will contain seed of larger size and that this will lead to an automatic improvement in well filled seeds.

Increases in seed nitrogen (thus protein) content, almost doubling in some cases, were achieved with relatively small reductions in the weight of oil per seed. Thus, the energy reserve for early seedling growth and emergence would be little affected. However, the gain from increased protein appears to offer a range of benefits. First, imbibition is more rapid and there appeared to be a linear relationship between N% in the seed and the time to both 50% germination and 50% emergence. This improvement is particularly important because rape is usually sown into seedbeds where the risk from desiccation is high and any saving in time during the imbibition stage reduced the period of risk from evaporation before the seedling had roots sufficiently deep to access moisture reserves. Similarly to the results from the earlier work (Stokes *et al*. 1998) the advantages of advancing the seed to the point of germination was substantiated.

There were considerable benefits from removing the smaller fraction of the seed from some samples, especially those from crops were nitrogen supply was smaller than normal. In some cases emergence was improved two fold by selection of the fraction of larger seed.

Overall, this preliminary work on manipulation of seed quality through management of the mother crop has confirmed that there is worthwhile scope to continue the examination of control of seed provenance to improve seed quality. The aim of the next phase of these studies is to extend the work to examine the scope to improve seed maturation without the confounding influence of nitrogen supply. This next phase is being continued under funding from HGCA, MAFF CPB-Twyford and Germain's (HGCA project 1349: 'Improving the establishment of oilseed rape through seed crop management, seed selection and seed treatment').

Introduction

Sustainable rape growing (maximising light harvesting efficiency for optimal final seed yield but minimising the influence of weeds, pests and diseases) depends on the achievement of uniform plant populations at optimum density. This is equally important for rape grown for industrial as well as for food use. Over 90% of the 500,000 ha of UK rape grown in 1999 was autumn sown and cost the agricultural industry in excess of £50 million to establish (Nix, 1997). There is great uncertainty associated with the establishment period, especially for the 60% of the national crop sown into heavy clay soils where cereal residues have been incorporated. If too few plants establish, canopy expansion during autumn will be restricted, reducing growth and the ability of the crop to compete with weeds and pigeons. Patchiness in thin stands exacerbates the problem by reducing the uniformity of both crop growth and development, leading to less uniform ripening, often necessitating the greater use of pre-harvest desiccants. In extreme cases, total crop failure requires re-sowing or change in the cropping sequence. Crops of winter rape are at risk of poor establishment throughout the whole of the six month period from September through to February and in the worst years establishment can be so poor that up to 30% of rape crops have to be abandoned.

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

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

The risk of failure during establishment is high because the seed is small (and thus has few energy reserves) and production of suitable seedbeds difficult, especially where straw has to be incorporated into relatively dry heavy clay soils typical of the conditions in which the majority of rape is grown. In field and laboratory conditions, Bullard *et al*., (1996) and McWilliam *et al*. (1998) have examined the physical and biotic forces which operate either singularly or in combination to impair establishment. Losses can occur in any one or all of three phases - sowing to germination, germination to first emergence and from emergence through to the establishment of a plant that will survive to contribute to yield. Pressures exerted on the seed and seedling often result from a combination of forces because most rape is grown on unstable, heavy clay soils where the previous cereal crop has dried out the soil profile and cereals residues have to be incorporated. Rainfall can be so low and evaporative losses from the soil so high that the young seedling is extremely vulnerable to desiccation.

These previous studies by the University of Nottingham/ADAS Centre for Research in Agronomy have shown that mechanical solutions alone will not reliably overcome establishment deficiencies because the factors that effect germination and seedling emergence processes seldom act in isolation (Bullard *et al.*, 1996). These factors have been characterised in detail by McWilliam *et al*. (1998). From these studies which defined the impact of seed size, seed depth, soil temperature, aggregate size and moisture availability on the seed/seedling it was concluded that the best way to achieve more reliable seed establishment was to improve the quality of the seed itself. To this end we have developed a number of hypotheses related to seed performance which should allow a conceptual 'ideotype' seed to be developed. The scope for the improvement of establishment through seed selection and pre-treatment was first demonstrated by Stokes *et al* (1998). The performance of a range of seedlots used by growers in 1996 varied markedly in the speed and uniformity of germination, particularly at temperatures similar to those experienced in the field. Variation between seedlots of the same variety was as large as the difference between varieties, and in some cases, treatment with fungicide and insecticide reduced germination. Seedlots showing more rapid germination emerged better from deeper sowing. The reasons for failure are uncertain; primary dormancy seems not to be involved but it is possible that certain seedlots may be particularly prone to enter secondary dormancy (Lutman *et al.* 1994). An understanding of the causes of this variation in seedlot response to temperature was considered crucial for determining the oilseed rape 'ideotype'.

From within these commercial seed grades, selecting the half with larger seeds resulted in individual seed weight being increased by 30%. Associated with this increase in size, the seed was able to emerge better from deeper drilling, as would be the case in dry conditions when growers tend to place the seed at depth where moisture is more available.

The speed of germination, emergence and early seedling development were all improved by seed advancement, achieved by soaking in water for 12-24 hours before drying back to the original moisture content. There was no adverse effect of advancement until the radicle emerged when drying back substantially reduced subsequent root growth. There was strong evidence that the potential benefit from seed advancement was greatest when seeds were sown 3cm deep or deeper, a zone in which many seeds have been found in growers' fields. Advancement also increased the number of seeds which germinated where osmotic stress was caused by dry soil, fertilizer N or leachates from decomposing straw.

There were indications that the improvements possible from each individual step, i.e. seedlot selection, seed grading and seed advancement, might combine to give a large overall benefit in specific situations. Thus, these preliminary observations suggest that oilseed rape could be graded or selected following harvest to improve establishment success. In a continuation of these studies this current five month research project aimed to further define the relationship between seed characteristics and seed performance by relating the maturity condition and protein content of the seed at harvest to subsequent performance. Does maturity have an effect on subsequent performance, and if so is that related to the absolute oil/protein content of the seed or the ratio of these products? If so, does the optimum differ from normal ware crops? Also, although selecting and pre-treating seed can significantly improve performance (Stokes *et al*. 1998), there is little time between harvest of the seed crop and drilling of the following commercial crop to implement these procedures. In Scotland, the time is so short that seed has to be carried over winter. At present, there is little indication that seed crops are managed any differently to those grown for food and industrial use. Analysis of commercial seedlots, sown by growers in 1996, has shown that there is much variation from seed to seed in stage of maturity (as indicted by the colour of the seed coat), and that black seed shows improved germination and early seedling growth. In some seedlots, the black (most mature) seed accounts for about 70-80% of the seedlots and therefore almost immediate benefits could accrue from growing crops to improve the uniformity of maturity and increase the proportion of black seed. The aim of the work reported here was to begin an investigation of the potential for the management of the mother seed crop to significantly manipulate the quality of the resultant seed and thereby contribute to the variability observed in the performance of some seedlots of the same variety. If seed provenance can be shown to significantly affect quality for resowing and establishment, there can then be the formulation of specific advice to growers to enable them to produce the best quality seed.

Objectives

The objectives of this project were to:

- Assess progress of maturation in the upper and lower portions of the rape pod canopy.
- Examine for improvement in uniformity of maturity from producing seed in more open canopies.
- Test for effects of stage of maturation on:

  e) seed coat colour
  f) seed dry weight
  g) protein and oil deposition

h) germination and early seedling growth

- Begin an investigation of the potential for improving seed maturity and fitness for resowing in immature or variable seedlots by seed selection or treatment procedures.

Materials and Methods

General methodology

Before initiation of this project, samples of rape seed were collected from crops under the HGCA project 'Canopy Management on Oilseed Rape' (1579) with contrasting applications of fertiliser nitrogen (N) chosen to produce a wide range of canopy structures. Samples were taken from July through to mid August from the upper and lower portions of 2 m^2 areas of the crops grown at the University of Nottingham which had received either no fertiliser nitrogen, 100, or 300 kg/ha N applied in equal splits in February and in March, or 160 kg/ha applied in a single dressing at the start of flowering (160F). The sample dates were 18, 21 and 24 July and 1, 4, 11, 14 and 18 August. Samples from the two layers of the canopy were harvested *in situ*, firstly from the top half and then from the lower half. The samples of pods were dried in a well-ventilated horticultural polythene tunnel and the seeds were threshed by hand as soon as the samples were sufficiently dry. To prevent deterioration of the seedlots, the dried seed was stored in airtight containers in a cold store at 4 ^{o}C. Samples remaining from 1996 included seed that were taken from the whole crops receiving either nil, 100, or 200 kg/ha N (in equal splits) or from an unfertilised crop which had been defoliated in early spring to produce a smaller than optimum canopy.

Seed physiochemical characteristics and maturity

For the seed collected from the experiments at the University of Nottingham in 1997 the yield of seed per m^2, moisture content after drying and thousand seed weight (dry matter) were calculated for all samples of seed. Samples were sent to ADAS Laboratories (Wolverhampton) for determination of percentage oil (NMR method) and percentage nitrogen (Dumas) content which allowed the calculation of the weight of oil and
nitrogen per seed. Protein percentage of the seed is equivalent to N% x 6.25.

As the seed samples had to be collected within the work programme of the Canopy Management project and dried to equivalent moisture content for storage before the start of this project, the anticipated change in maturity over time was not found. Therefore, samples of seeds of different coat colour (orange, orange/brown, brown/black or black) were selected from the parent samples and the effect of 'maturity' (*i.e.* coat colour) was investigated by germination testing of these seedlots as described below.

Germination tests

These were undertaken in controlled environment conditions. Initially, germination of the seed from different harvest dates and different parts of the canopy from the mother crop treatments with different N applications were compared. Later, non-selected seeds, non-advanced seeds were compared in tests with selected/advanced seeds both singularly and in combination. These tests were conducted at either 15 or 20°C. Twenty-five or 30 seeds were placed on 2 sheets of Whatman No. 1 filter paper, 6 ml of water was added and the lid was replaced. In a preliminary experiment (Stokes *et al.*, 1998), the volume of water applied was found to have negligible influence on the progress to germination. Between 4 and 8 ml, there was no observed effect. There were between 3 and 5 replicates per individual treatment but, as most experiments were factorial combinations, there were as many as 50 replicates per main factor effects in some experiments. These methods followed the protocols developed in the previous project (Stokes *et al.*, 1998).

Emergence tests

These were conducted in horticultural silver sand in 12 cm diameter pots. The required depth of sand was added to the seed so that the surface of the sand was at the same height in each pot, regardless of the depth that the seed was sown. In the emergence tests, water was applied as necessary, to ensure the seedlings were not droughted. The number of emerged seedlings (cotyledons opening) was monitored on a regular basis, with the percentage emergence calculated from the records of initial numbers of seeds sown.

Seed selection and seedling development in compact and dry soils

To begin investigating the potential for improving seed maturity and fitness for resowing by seed selection and treatment, seeds saved from 1996 were studied. The parent crops received nil N, 100 kg/ha, 200 kg/ha or were from a nil N crop that was defoliated to reduce canopy size further. The samples of seed were either ungraded (mixed) or were graded into fractions over 2mm diameter (large) or less than 2mm diameter (small) by sieving with a 2 mm square mesh sieve. All seeds (25 per treatment) were sown on a firm seedbed and each row was fixed with a light covering of finely sieved soil before adding the 6 cm topsoil layer. Soil compaction was achieved using a heavy wooden tamping block. Water was supplied to field capacity immediately after sowing, but then no water was applied for the next six days, after which water was applied regularly to be non limiting.

The pots were placed into a controlled environment at 15°C. There were 8 replicates of the 12 treatments arranged in a randomised block design. Measurements of emergence (cotyledons above surface of peat and starting to unfolded) were made every 12 hours for the first 6 days and then daily thereafter. To help identify the initial stages of drought stress, 3 identical soil blocks (unsown) were set up within the experiment design. Soil cores were taken from the 3 unsown soil blocks and the gravimetric soil moisture content was thus recorded at stratified layers through the soil profile on each of the first 6 days of the experiment. Growth stage assessments started at the first sign of emergence (6 days after sowing). A 10 point scoring system was used to record early seedling development (Table 1). The scores from the 10 point system were used to calculate the Seedling Rate of Development Index (SRDI). These measurements started on the first day of emergence and ended 16 days later. At final harvest, the fresh weight of one bulked sample per treatment per block was recorded. Each sample was desiccated in an oven set at 100ºC for 48 hours and the dry weight subsequently recorded. Due to waterlogging, four of the blocks were discarded prior to the final analysis.

Table 1: Scores for Steiner's Seedling Rate of Development Index (SRDI).

Score	Description of growth stage
1	Hypocotyledonary arch emerged.
2	Cotyledons fully expanded from soil but still in contact with each other.
3	Cotyledons unfolded.
4	Cotyledons fully expanded and unifoliate leaf apparent.
5	1st true leaf exposed.
6	1st true leaf exposed; 2nd true leaf apparent.
7	1st and 2nd true leaves exposed.
8	1st and 2nd true leaves exposed and 3rd true leaf apparent.
9	1st, 2nd and 3rd true leaves exposed.
10	1st, 2nd and 3rd true leaves exposed and 4th true leaf apparent.

Seed selection and advancement techniques

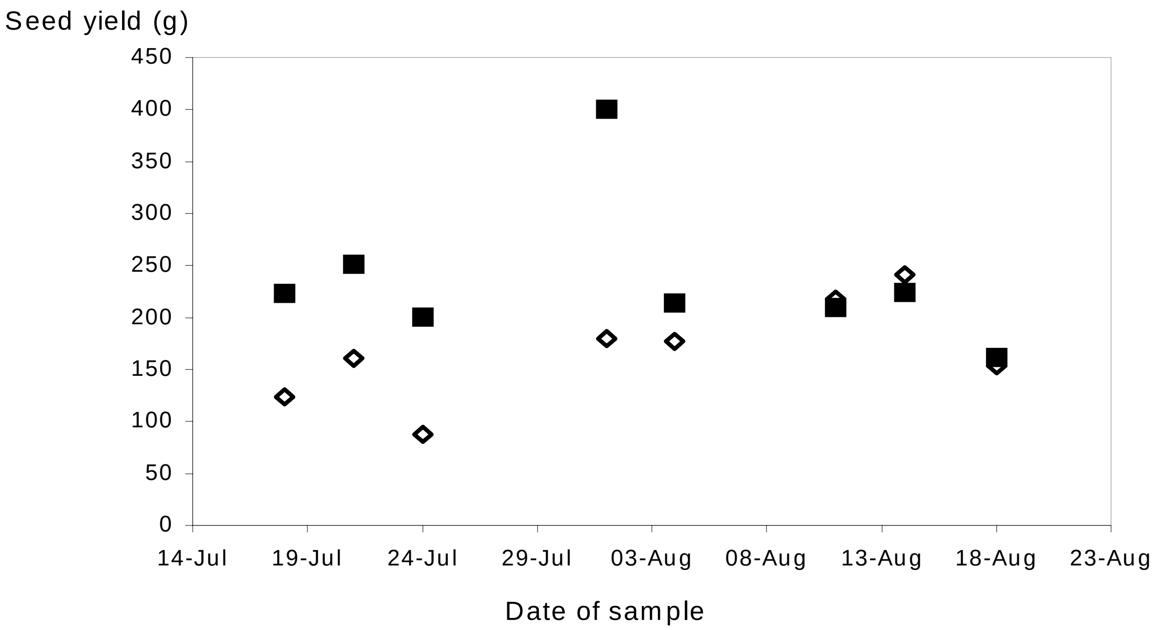
Seeds were selected as described above and were assessed in emergence tests in drying and compacted soils. All advancement techniques used in this study followed the same procedure to that reported by Stokes *et al*. 1998). Seed was placed in water at 15°C for the required duration of advancement (generally 18 h), after which the seeds were removed, blotted dry and dried to a constant weight between 20 and 25°C. This drying usually took approximately 2 hours in a dry room environment but slightly less in forced draft conditions. In a preliminary experiment, the rate of drying of advanced seed was doubled without any detriment to subsequent germination. When compared to water, advancement in 5% and 10% solutions of polythene glycol gave no improvement and so was therefore discontinued.

Results

Progress of maturation in the canopy and effects on seed quality

The sampling procedure used in this study (hand harvesting the upper and lower pod halves of a 2 m^2 area of crop), resulted in too much variation in harvested seed weight through the sampling period to allow accurate analysis of any changes in yield as a consequence of late seed filling or seed loss through shatter. There were no progressive effects on yield with time, as shown for the 300 N crop (Figure 1). There were no significant effects on any of the other crops analysed (data not presented).

Figure 1: Effects of maturity of the mother crop on seed yield in the top (◊) and bottom (■) halves of the pod canopy (300 N applied)



The effect of sample date on thousand seed (dry matter) weight (Figure 2) and on seed nitrogen percentage (Figure 3) and oil percentage (data not shown) was very similar. Again, the points formed a broad scatter and there were no consistent significant progressive effects of sample date ('maturity') on thousand seed weight or nitrogen percentage. The data in the Figures is for the 300 N crop; the other crops analysed (data not shown) showed a similar pattern.

Figure 2: Effects maturity of the mother crop on thousand grain (dry matter) weight in the top (◊) and bottom (■) halves of the pod canopy (300 N applied)

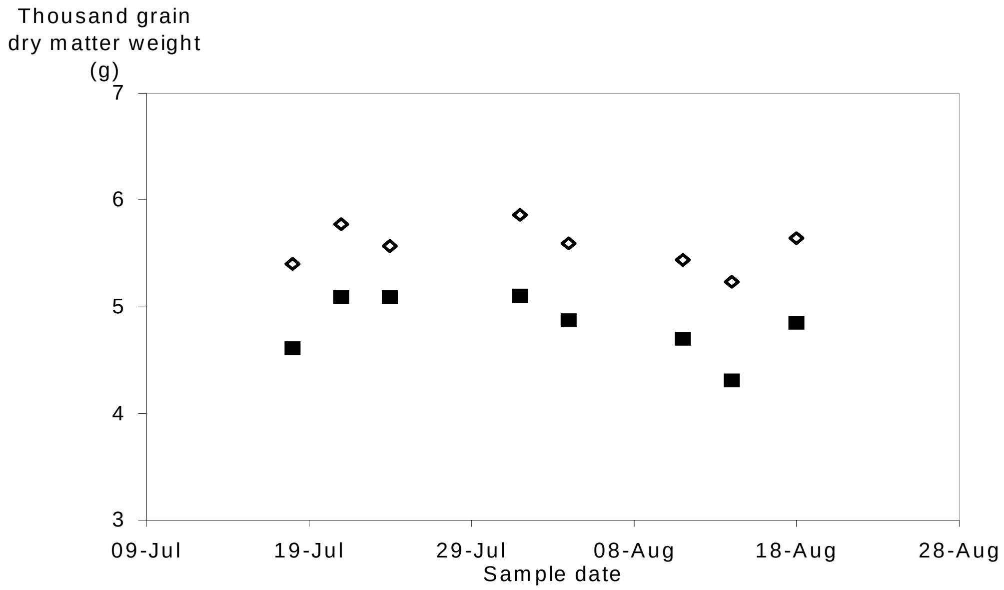
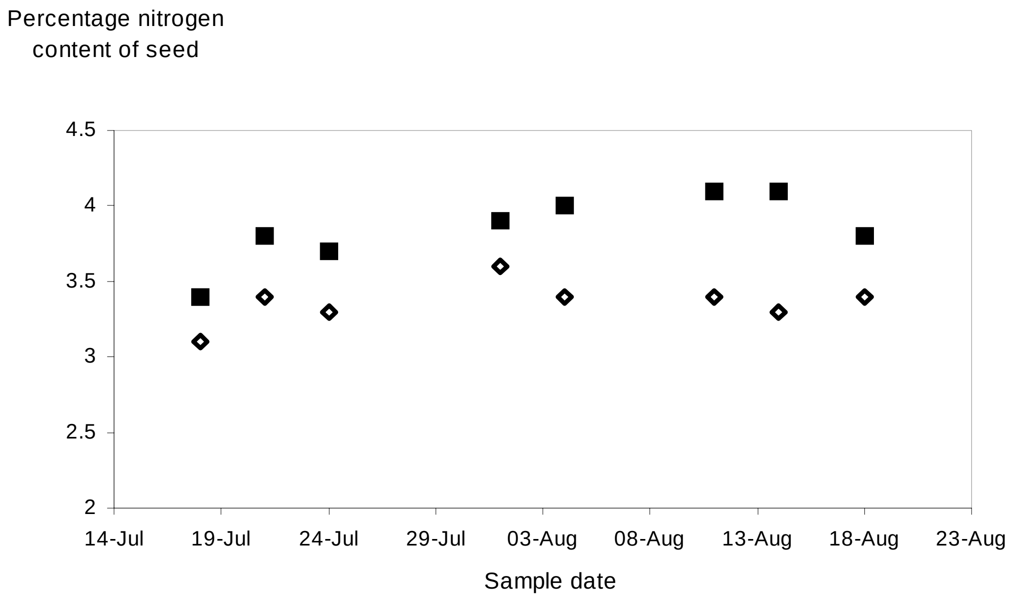
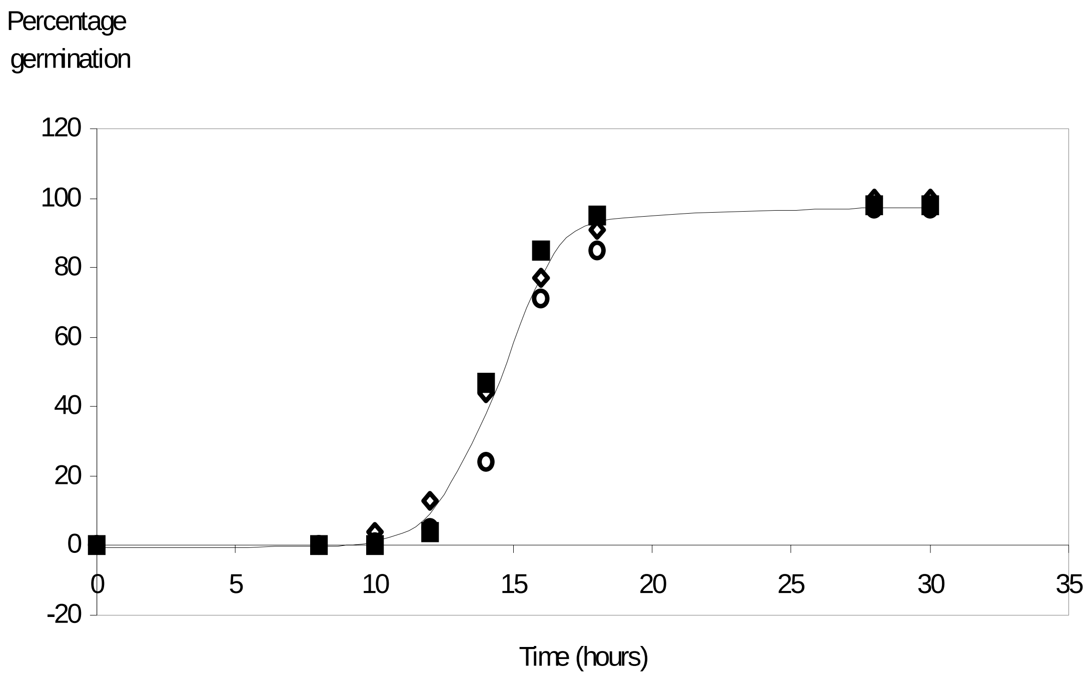


Figure 3: Effects maturity of the mother crop on percentage nitrogen content in the top (◊) and bottom (■) halves of the pod canopy (300 N applied)



From these initial analyses it was evident that the anticipated differences in seed maturity due to serial sampling through time were not present, either because when sampling began in early July most seed was already 'mature' or because differences disappeared in the drying and storage process. This was further confirmed by comparison of the germination profiles of the seed lots from different dates. Fitting logistic curves to the germination data with time, using Genstat statistical software, showed that most of the variance (>95%) could be explained by fitting a single logistic curve to all the germination data, regardless of harvest date. Plotting separate parallel curves or individual curves did not significantly improve ($P>0.05$) the amount of variance accounted for, indicating no significant difference in germination profile with harvest date. In the example shown below in Figure 4, 98.7% of the variance was accounted for by the single curve shown: individual curves for each harvest date increased the amount of variance accounted for by only 1%. The Figure shows the germination test results at 25°C for the upper part of the canopy with 300N applied. The results were similar for the other crops, the lower canopy fraction and for germination tests completed at 15°C.

Figure 4: Percentage germination at 25°C with time for samples from upper part of canopy of crop with 300 N applied, harvested on 21 July (◊), 1 August (■) and 14 August (O) 1997. The bold line shows the highly significant ($P<0.001$) logistic curve accounting for 98.7% of the variance in the data.



Due to the lack of significant differences in maturity, it was therefore not possible to directly assess the progress of maturation in the canopy or to assess improvement of uniformity in more open canopies as originally hoped. However, germination profiles of seed batches selected on the basis of their different coat colours (Table 2) showed a significant effect of maturity. Fitting of logistic curves in Genstat was not possible, so an analysis of variance of the final percentage germination after a 68 hour germination test was done. The data were transformed (standard angular transformation) to ensure normality.

Table 2: Transformed percentage germination after 68 h germination (15°C) of black, black/brown, brown and orange coloured seeds chosen from two seedlots (S1 and S2). Untransformed data shown in brackets. S.E.D (21 d.f.) = 2.14, L.S.D (5%) = 4.29.

Colour	Black	Black/brown	Brown	Orange
Seedlot 1	73.8 (92.0)	67.3 (85.0)	70.9 (88.0)	65.4 (82.0)
Seedlot 2	70.5 (88.0)	66.6 (83.0)	72.3 (90.0)	57.8 (71.0)

Thus, although the similarity in maturity of the stored samples available to the project precluded a full analysis of the effects of maturity on seed quality, this experiment indicated the potential for seed maturity to affect quality for re-sowing. It is intended that further investigations into the effects of maturity on quality of rapeseed for establishment will continue in the following SAPPIO project.

Effects of stage of maturation, canopy position and mother crop nitrogen applications on seed characteristics

As indicated in the preceding section, there were no significant effects of harvest date ('maturity') on the seed characters. Therefore, the sample dates were used as replicate determinations of the properties studied to allow analysis of variance to identify effects of position in the canopy and mother crop nitrogen application on seed quality. In each case the F ratio of the replicate stratum in the ANOVA table was studied, and no significant effects were found ($P>0.05$).

The effects of these canopy position and mother crop nitrogen applications on thousand seed (dry matter) weight are shown in Table 3. The effects of N on thousand seed dry matter weight were highly significant, with greater seed weights in the samples receiving nitrogen than in the nil nitrogen treatment and the greatest seed weights in the treatment which received 160 kg/ha N just prior to flowering. As previous work (Stokes *et al.*, 1998) has shown benefits for emergence (particularly from depth) with larger seed, this result indicates some potential for improving seed quality through mother crop management, by later application of nitrogen.

Table 3: Analysis of variance of effects of N applied to the mother crop on thousand seed weight in the top and bottom halves of the pod canopy (S.E.D. d.f. = 34)

N application	Position in canopy			
	Top	Bottom	Mean	N effect *P*<0.001, S.E.D 0.056
Nil	4.61	4.50	4.56	
300	5.18	4.48	4.83	Position *P*<0.001, S.E.D. 0.093
160F	5.56	4.83	5.20	
Mean	5.12	4.60		Interaction *P*<0.001, S.E.D. 0.16
Grand Mean			4.86	

The position in the canopy also had a significant effect, with greater thousand seed weights in the top half of the canopy compared to the bottom, except in the nil nitrogen treatment where the difference was not significant.

Analysis of the seed for nitrogen and oil content at the ADAS Wolverhampton laboratories revealed similar effects of canopy position and mother crop nitrogen application on the chemical composition of the seed (Tables 4 and 5).

Table 4: Effects of nitrogen applied to the mother crop on seed percentage nitrogen content in the top and bottom halves of the pod canopy (S.E.D. d.f. = 34)

N application	Position in canopy			
	Top	Bottom	Mean	N effect *P*<0.001, S.E.D. 0.054
Nil	2.30	2.33	2.31	
300	3.19	3.74	3.46	Position *P*<0.001, S.E.D. 0.044
160F	3.36	3.85	3.61	
Mean	2.95	3.30		Interaction *P*<0.001, S.E.D. 0.076
Grand mean			3.13	

Nitrogen fertilisation (compared to nil nitrogen) significantly increased seed percentage nitrogen content, with an additional slight increase in nitrogen content in the late 160F application compared to the 300 N application. Except in the nil nitrogen treatment, seed from the lower portion of the canopy had a higher percentage nitrogen content than seed from the upper portion of the canopy (and thus a higher protein content, given as %N x 6.25).

Table 5: Effects of nitrogen applied to the mother crop on seed oil percentage in the top and bottom halves of the pod canopy (S.E.D. d.f. = 34)

N application	Position in canopy			
	Top	Bottom	Mean	N effect $P<0.001$, S.E.D. 0.271 Position $P<0.001$, S.E.D. 0.221 Interaction $P<0.001$, S.E.D. 0.383
Nil	54.71	54.02	54.37	
300	50.80	45.32	48.06	
160F	49.61	44.39	47.00	
Mean Grand mean	51.71	47.91	49.81	

The trends on oil content were the inverse of the effects observed on nitrogen content. Nitrogen fertilisation reduced the percentage oil content per seed, with the latest application resulting in the lowest oil content. Oil content was lowest in the bottom portion of the canopy (i.e. in the seed that had the highest proportion of nitrogen).

Table 6: Effects of nitrogen applied to the mother crop on weight of N (μg) per seed in the top and bottom halves of the pod canopy (S.E.D. d.f. = 34)

N application	Position in canopy			
	Top	Bottom	Mean	N effect $P<0.001$, S.E.D. 3.86 Position $P>0.1$, S.E.D. 3.15 Interaction $P>0.1$, S.E.D. 5.46
Nil	112.2	111.0	111.6	
300	176.0	179.3	177.6	
160F	199.9	199.1	199.5	
Mean Grand mean	162.7	163.1	162.9	

There was a highly significant effect of mother crop nitrogen application on the weight of nitrogen per seed, with a near two-fold increase when comparing the late application to the nil nitrogen treatment, and a 10% increase caused by the late application of 160 kg/ha compared to early split application of 300 kg/ha. There was no effect of canopy position (and no nitrogen x position interaction) on the weight of nitrogen per seed. Thus the difference in nitrogen percentage between the canopies observed earlier would appear to be due to dilution of the same weight of nitrogen by larger oil reserves in the upper portion of the canopy. This would be expected due to the strong linear relationship found between oil and nitrogen percentage (Figure 5) when larger numbers of samples were submitted.

Table 7: Effects of nitrogen applied to the mother crop on weight of oil (µg) per seed in the top and bottom halves of the pod canopy (S.E.D. d.f. =34)

N application	Position in canopy			
	Top	Bottom	Mean	
Nil	2682	2590	2636	N effect *P*<0.001, S.E.D. 30.3
300	2798	2176	2487	Position *P*<0.001, S.E.D. 24.7
160F	2940	2296	2618	Interaction *P*<0.001, S.E.D. 42.8
Mean	2807	2354		
Grand mean			2580	

The effect of nitrogen treatment on weight of oil per seed was seen as a lower weight of oil in the 300 N canopy compared to the nil and 160F treatments. Although the 160 F and 300 N application had greater weights of oil in its upper canopy half compared to the nil N treatment, the weight of oil in the seeds of the bottom half was far smaller. In the 160F treatment the much greater oil content of the upper canopy offset this effect on mean oil content. As would be expected from the relationship between oil and nitrogen (Figure 5), the oil content in the upper half of the canopy, which had greater protein, was higher than that in the lower half of the canopy.

Figure 5: Relationship between nitrogen and oil concentrations (as percentage dry matter) in seed harvested from the top (open squares) and bottom (filled squares) halves of pod canopies of crops receiving a wide range of nitrogen applications. Fitted linear regression lines for the top and bottom halves of the canopy are shown.

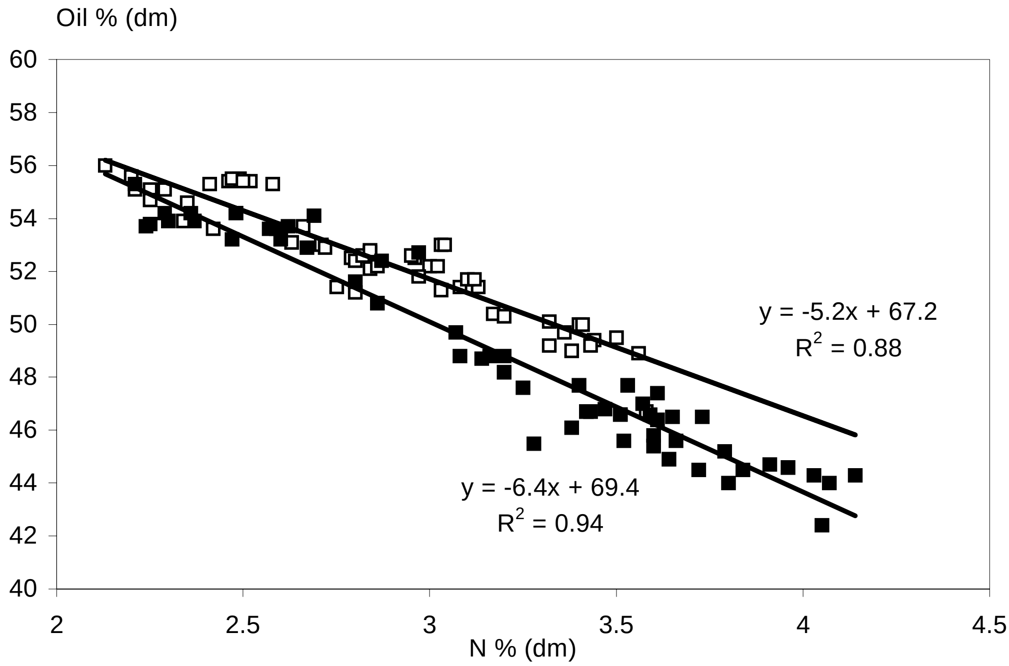


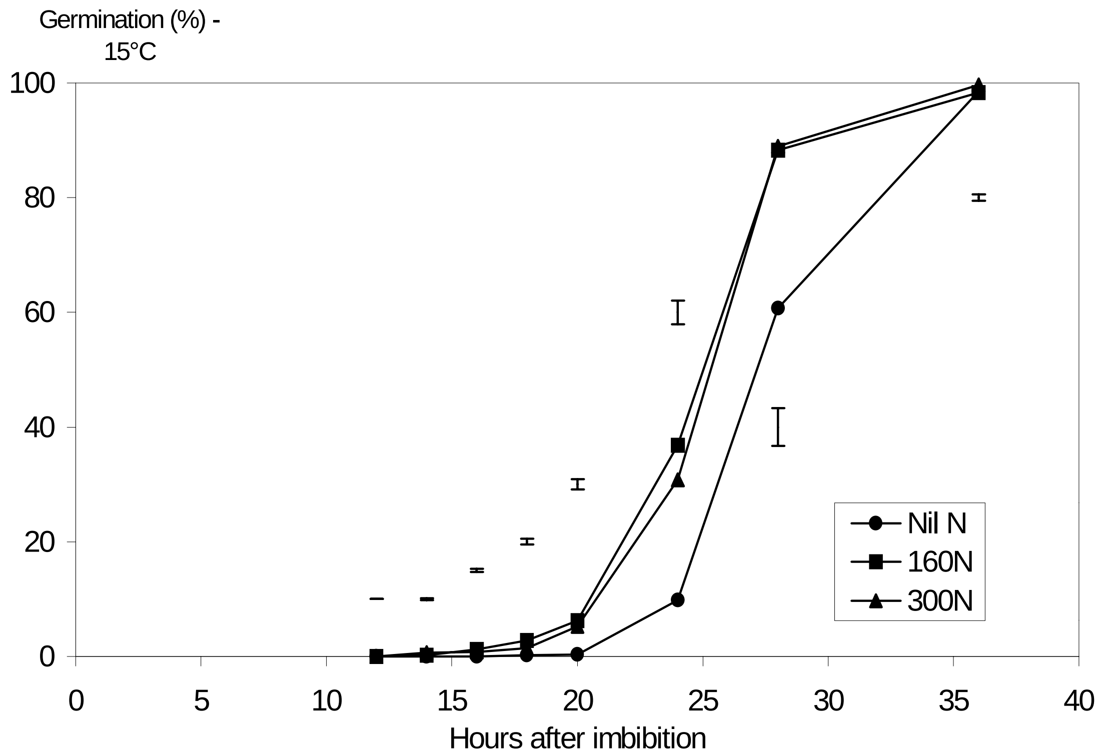
Figure 5 shows the strong, significant linear relationship between the percentage oil and nitrogen contents of

a large range of crops with different mother crop nitrogen management regimes. As percentage nitrogen increased, percentage oil decreased. There was a significant difference +between the lines fitted to samples from the upper and lower portions of the canopy, with higher percentage oil contents for a given nitrogen content (and *vice versa*) for the upper compared to the lower part of the canopy. This reflects the results for effect of canopy position discussed earlier.

Effects of seed characters (manipulated by mother crop nitrogen application) on seed germination

The effect of the chemical composition of seed on germination at 15°C is shown in Figure 6. Germination was slower between 20-30 hours in the nil N seed (2.3%N/54.4% oil) compared to the 300 and 160F N (3.5% N/48.1 % oil, 3.6% N/47% oil respectively) which were not significantly different. However, the same final percentage germination levels were reached. Similar results were seen in the 25°C germination tests (data not presented).

Figure 6: Effect of nitrogen application to the seed crop on the pattern of germination of the harvested seed at 15 ^{o}C (error bars show S.E.D.)



Effects of seed characters (manipulated by N applied to the mother crop) on seed emergence

Figure 7 shows the effect of seed chemical characteristics on emergence from a depth of 2 cm. As would be expected from the germination data reported above, emergence from the low nitrogen/high oil seed of the nil N treatment was significantly slower than from the seed from the nitrogen-treated crops. However, no difference in percentage emergence could be discerned between the two nitrogen treatments and all seed lots eventually reached the same final value.

Figure 7: Effect of nitrogen application to the mother crop on the pattern of emergence of the seedlings from
2cm in light sand (error bars show S.E.D.).

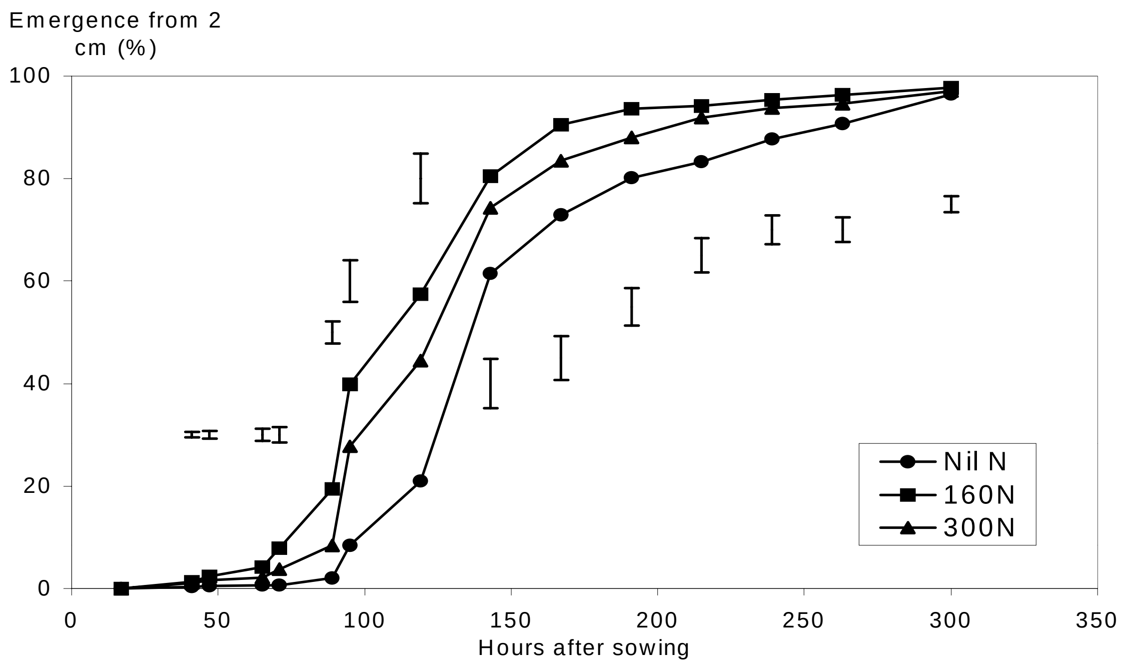
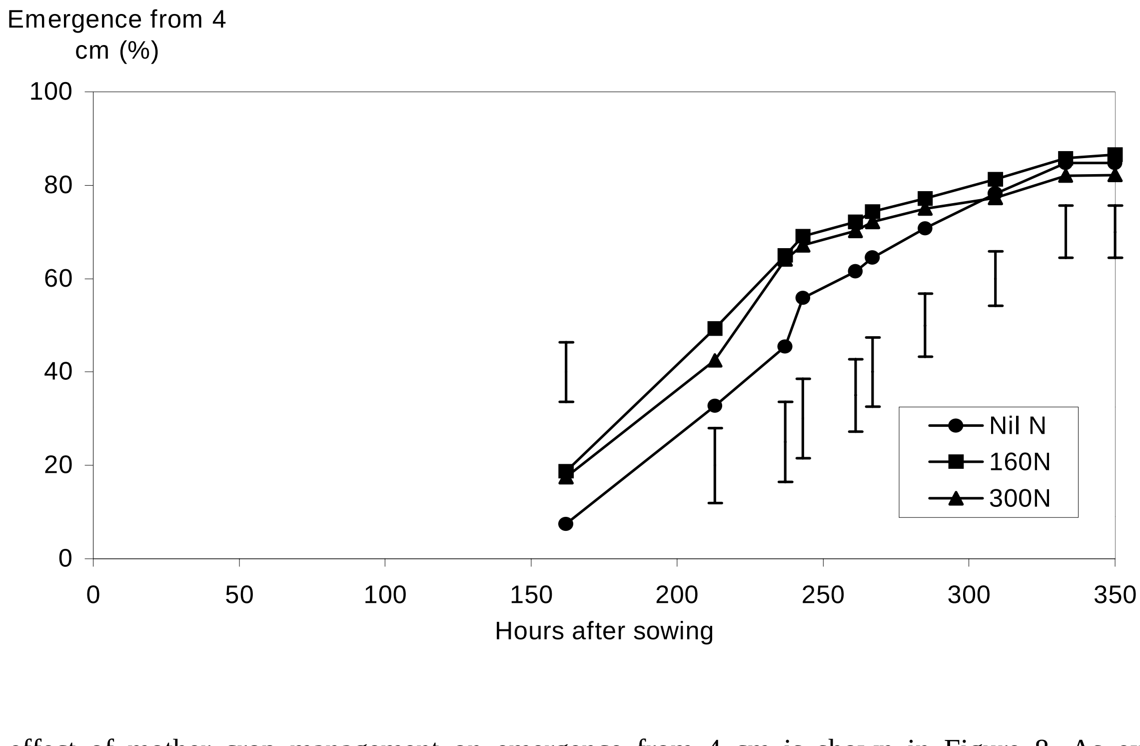


Figure 8: Effect of nitrogen application to the mother crop on the pattern of emergence of the seedlings from

4cm in light sand (error bars show S.E.D.)



The effect of mother crop management on emergence from 4 cm is shown in Figure 8. As expected, emergence from 4 cm took significantly longer for all seedlots than emergence from 2 cm. Subjectively, the seed from the nitrogen-treated crops emerged before that from the nil N treatment. However, the standard errors were too large to show significant differences and the final percentage emergence was uniformly high at > 80%.

Calculation of the time to 50% germination or 50% emergence did reveal significant effects of seed chemical composition on germination and emergence. Figure 9 shows the time to 50% emergence in relation to the seed nitrogen content. A significant regression line (*P*<0.05) could be fitted to the data with an R^2 of 0.88, showing that the time to 50% emergence decreased with increasing nitrogen percentage.

Figure 9: Relationship between percentage nitrogen in seed and the time to 50% germination at 15°C

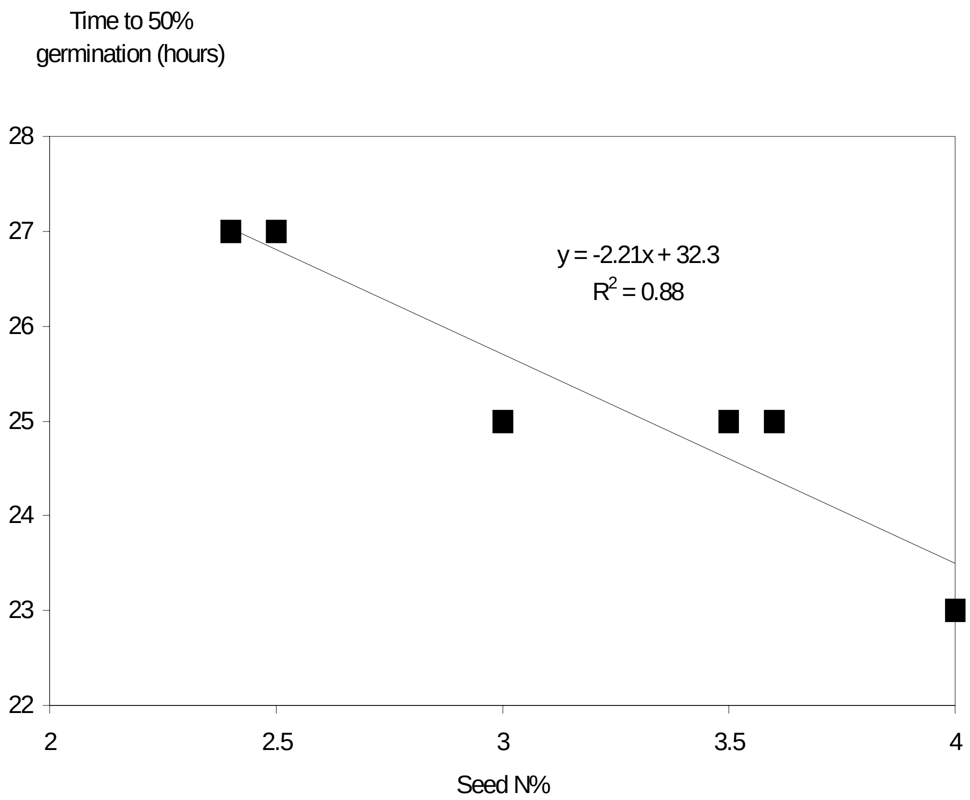
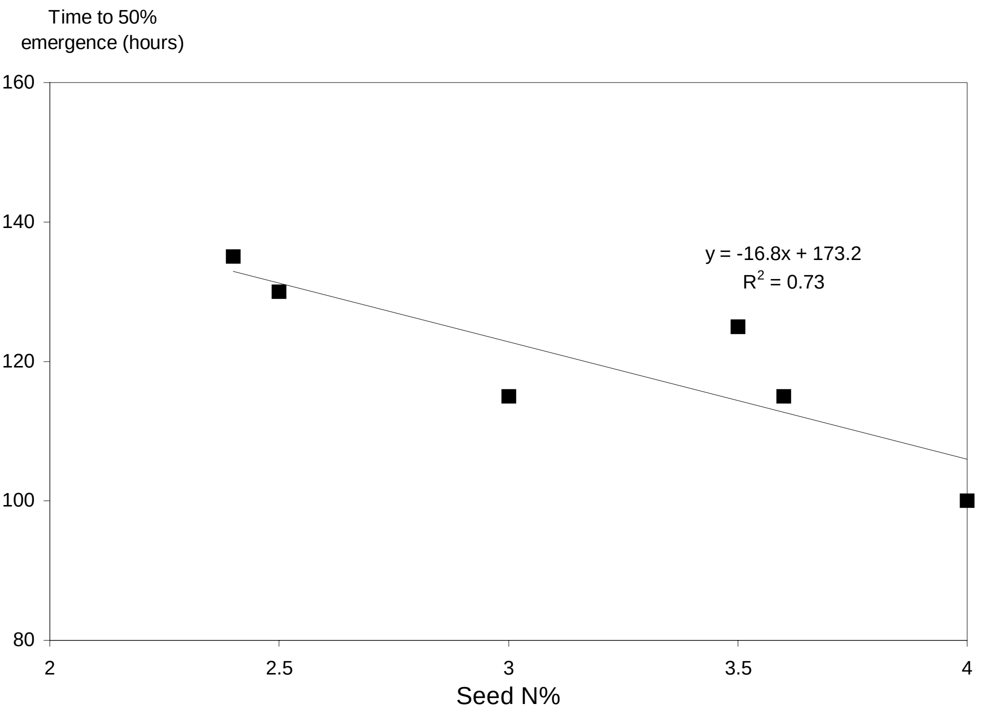
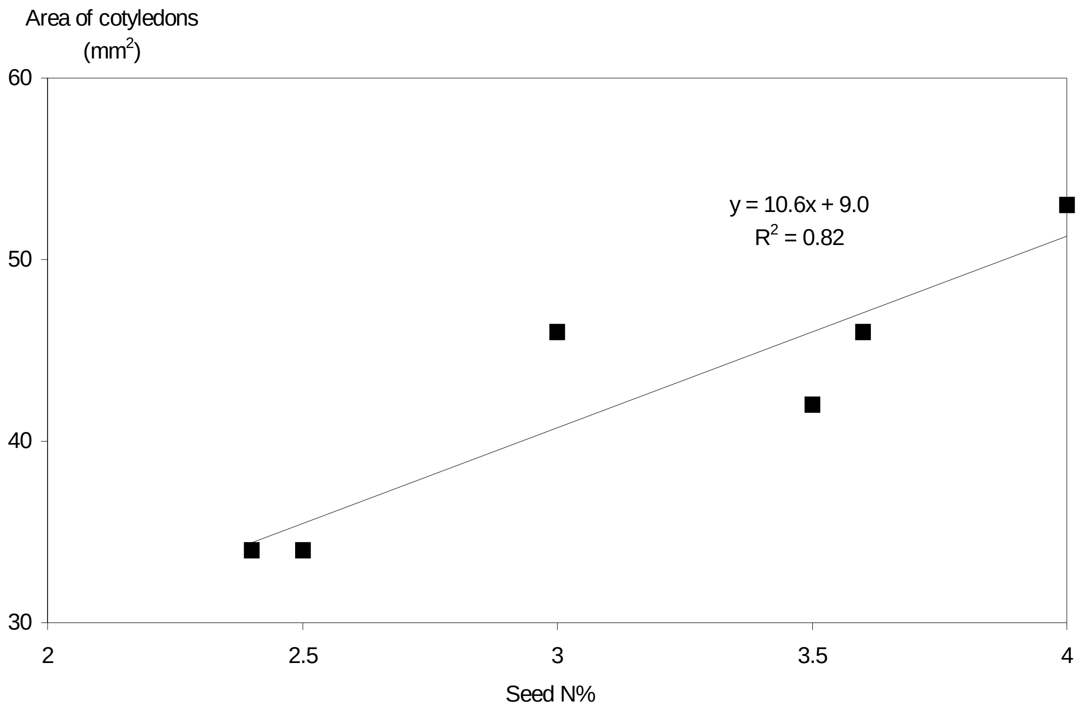


Figure 10: Relationship between percentage nitrogen in seed and the time to 50% emergence.



Similarly, the time to 50% emergence (Figure 10) was also significantly (P<0.05, R^2 0.73) correlated with seed nitrogen content: a reduction in about 20 h of the time to 50% emergence could be achieved with an increase in seed nitrogen content from 2.5 to 4%. A growth analysis after the emergence test also indicated an significant increase (*P*<0.05, R^2 0.82) in the size of cotyledons emerged from seed with high nitrogen content compared to those with lower nitrogen content (Figure 11). The earlier emergence and production of larger cotyledons more quickly could give high nitrogen content seed an advantage in the establishment process.

Figure 11: Area of cotyledons emerged from seed of different percentage nitrogen content.



Investigation of the potential for improving seed maturity and fitness for resowing.

(a) Size selection

Analysis of data collected from all eight replicated blocks of this experiment revealed non-significant effects for size and treatment. However, seed germination did not appear to be even across the eight replicated blocks. Problems associated with the initial saturation process caused pools of water lie in the centre of four of the replicate blocks. This may explain the poor seed germination recorded around the central areas of these blocks which skewed the results. As a result, these four of the original blocks were discarded, and the remaining data was reanalysed. The SRDI results are shown in Table 8.

Table 8: Steiner's seedling rate of development index (SRDI), calculated at the end of the assessment period

	Large seed	Mixed seed	Small seed	Mean 5% LSD = 2.5 (3 d.f)
Nil N	4.09	3.53	1.68	3.10 b
100 kg N	9.96	8.74	3.59	7.43 a
200 kg N	7.34	4.81	2.99	5.05 a,b
defoliated	3.63	5.80	3.20	4.21 b
Mean 5% LSD = 2.17 (2 d.f)	6.25 a	5.72 a	2.87 b	

Provenance (P=0.010) and seed size (P=0.007) showed highly significant differences.

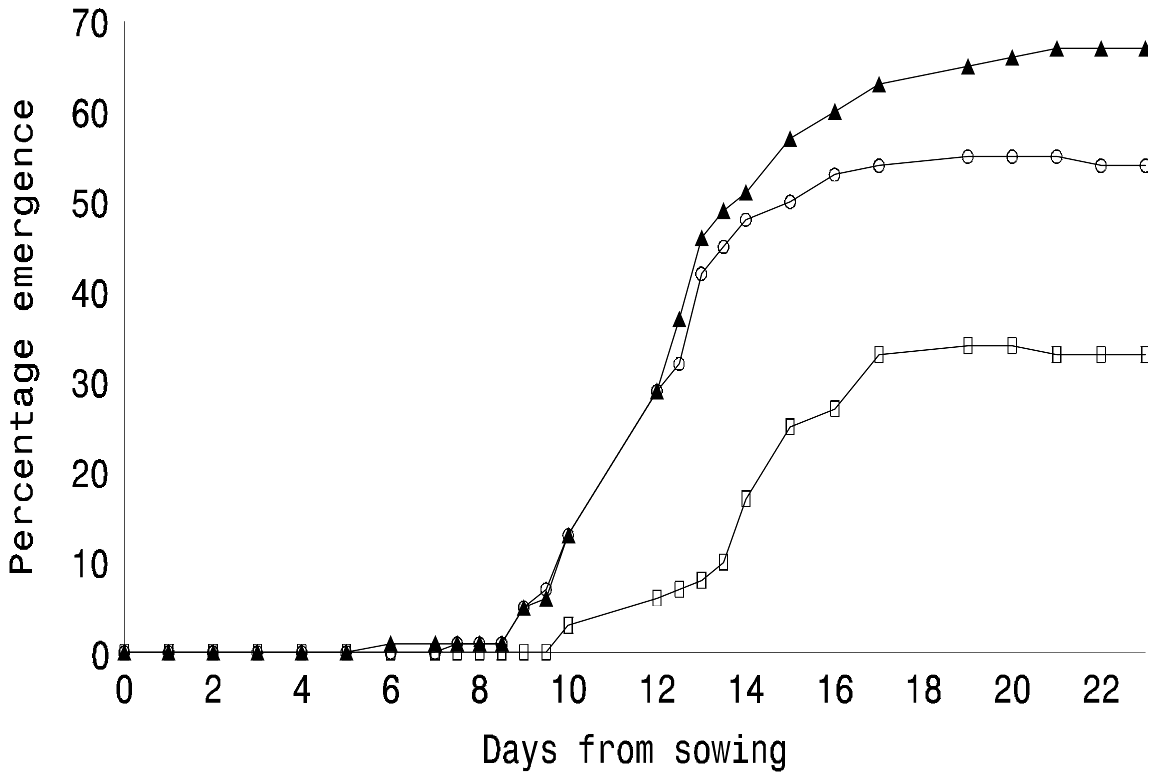
There were benefits (higher SRDI values) associated with large or mixed seed compared to small seed, and with nitrogen application compared with no nitrogen application. There also appeared to be a benefit with 100 kg N compared to 200 kg N treatments, although at borderline significance. The results indicate that the benefit from size selection could be more to do with removal of small seeds than with selection of large seeds. Table 9 overleaf shows that there were also benefits for dry matter accumulation due to size selection and nitrogen application. Seed size (p= 0.001) and provenance (P=0.024) showed significant differences. This was consistent with SRDI results. Seedlings emerged from small seeds had significantly lower dry weights at the end of the assessment period compared to seedlings from the mixed or large seeds and again, the 100 kg N treatment gave the largest dry matter accumulation.

Table 9: Seedling shoot dry weight measured at the end of the assessment period.

	Large seed	Mixed seed	Small seed	Mean 5% LSD = 0.696 (3 d.f)
Nil N	1.43	1.22	0.43	1.02
100 kg N	2.65	2.67	0.78	2.03
200 kg N	2.28	1.45	0.95	1.56
defoliated	0.98	1.60	0.90	1.16
Mean 5 % LSD = 0.596 (2 d.f)	1.83	1.74	0.76	

The effect of seed size on the emergence profile can also be seen in Figure 12 below, with a faster rate of emergence from large and mixed seed compared to small seed, and a greater final percentage emergence in the order large>mixed>small. Broadly similar results were seed for the same comparison for the other nitrogen treatments (data not shown).

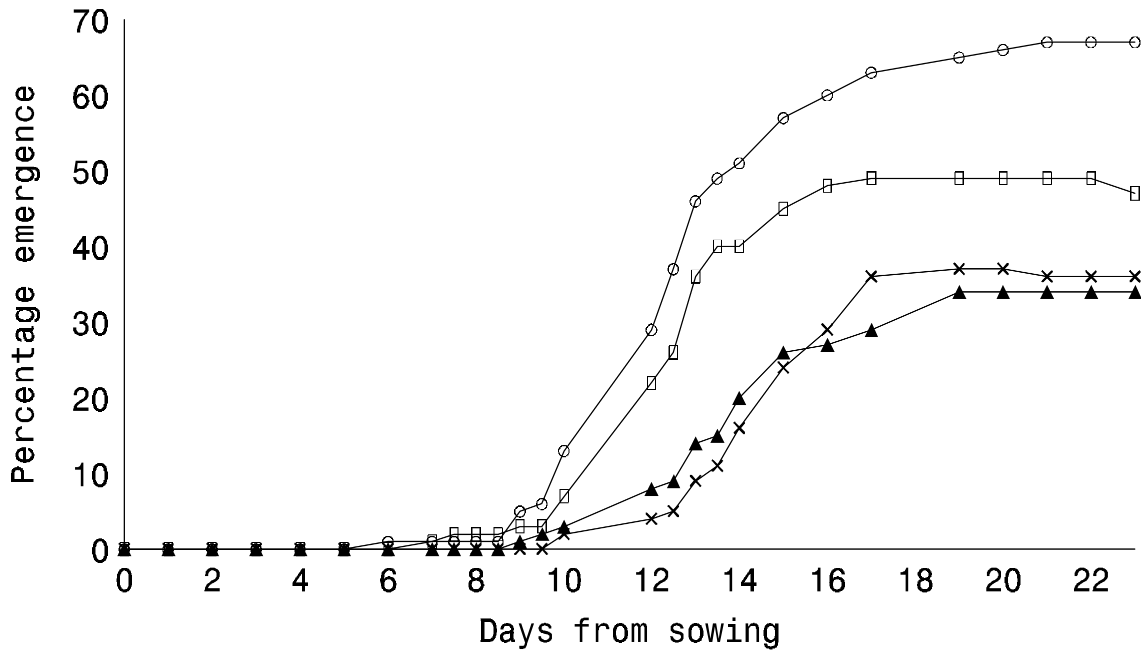
Figure 12: Seedling emergence progress for 100 kg N canopy management; large seed (▲), mixed seed (O) and small seed ( ).



For the nil N treatment, there were no differences between the emergence of the large and mixed seeds, although there was less emergence from the small seeds and for the defoliated nil N treatment, emergence was better from the mixed than from the large seeds. The emergence from the 200 kg N treatment was very similar to that shown in Figure 12, but with markedly better emergence from large than from mixed seeds.

A comparison of the emergence from large seed of the four nitrogen treatments is shown in Figure 13. Emergence was greatest from the 100 kg N treatment, followed by the 200 kg N treatment and the nil N and defoliated nil N treatments, which were not significantly different.

Figure 13: Seedling emergence progress for large seed from the 100 kg N (O) 200 kg N( ), nil N (▲) and nil N defoliated (x)



There was considerable difference in the final percentage emergence of the 100 kg/ha and 200 kg/ha N crops, with greater final emergence in the former. This indicates that an optimum level of nitrogen fertilisation is required to maximise seed quality, rather than blanket high applications where deleterious effects on canopy structure may play a role. Stubbings (1998) showed that the seed weight in the 200 kg/ha treatment was lightly lower than in the 100 kg/ha N treatment (4.47 mg dry matter per seed compared to 4.65 mg/dm). This was probably due to mutual shading by higher numbers of pods in the 200 kg/ha N treatment with a resultant unfavourable change in the size distribution of seeds compared to the 100 kg/ha N treatment causing poorer emergence. These tests did however indicate advantages of both seed size and mother crop nitrogen management for improvement of emergence from soils in drying and compacted conditions.

(b) Seed advancement

When large seed (Figure 14) from the 200 kg N treatment were advanced and then compared to non-advanced seeds in an emergence test in horticultural silver sand, more rapid emergence and a greater final percentage emergence was found in the advanced compared to the non-advanced seeds. A similar result was found for the mixed seed (data not shown). However, for the small seed (Figure 15), no significant difference was found between advanced and non-advanced seed. This result shows that incremental advantages for emergence could be gained by seed size selection and advancement.

Figure 14: Emergence profiles for large seed from 200 kg N treatment, advanced ( ) and non-advanced seed (▲)

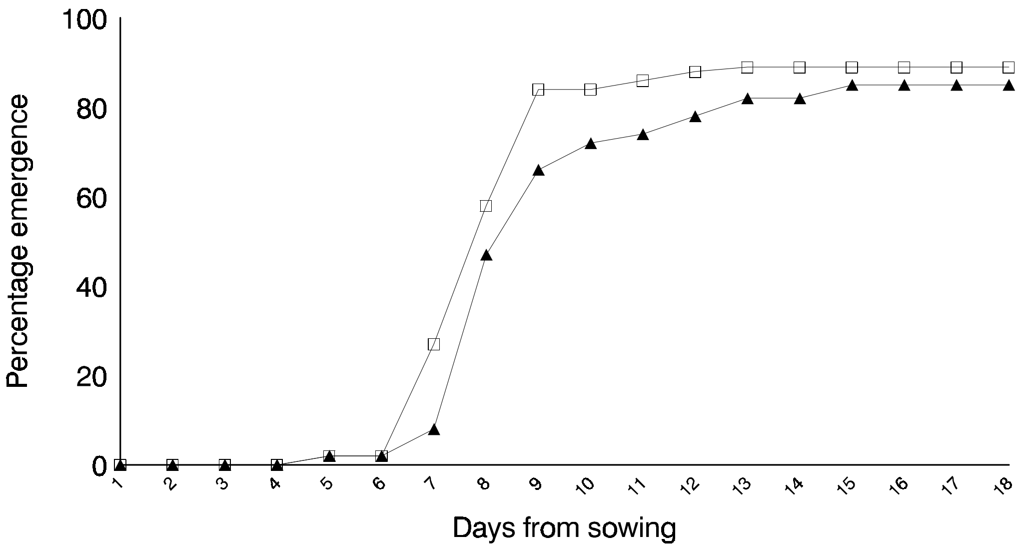
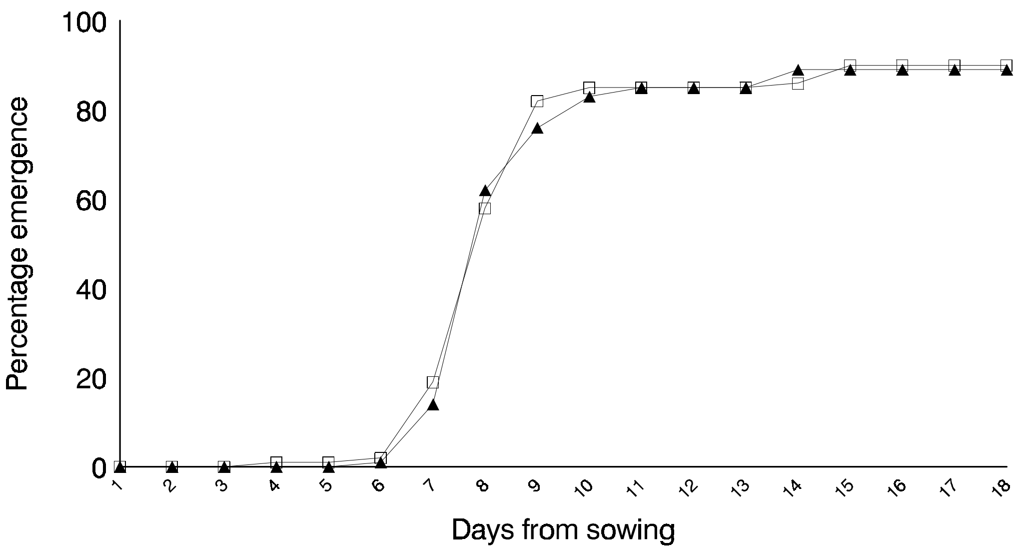


Figure 15: Emergence profiles for small seed from 200 kg N treatment, advanced ( ) and non-advanced seed (▲)



Discussion

The hypotheses tested in this work were that:

1. Maturity of seed affects their subsequent germination and emergence performance
2. Seed protein/oil content can be manipulated by husbandry and harvesting strategy.
3. Seed protein/oil content influences germination and emergence

If we were able to support any of these hypotheses, the impact would be three-fold. Firstly it would provide additional information on how seed batches should be screened following harvest in order to further increase fitness. Secondly, it might indicate that the mother crop could be manipulated either before or during harvest so that no intermediary post-harvest selection was needed, but an increase in fitness was still demonstrated. The importance of the latter was that it would enable establishment improvements even where insufficient time for seed pre-treatment was possible. Thirdly, and as a direct result of the second point, it would indicate that a departure from conventional practices which do not differentiate the husbandry of ware crops (for oil) and seed crops (for re-sowing) was required. Regardless of whether the hypotheses were accepted or rejected, the information gained would be of direct relevance to the ongoing LINK (SAPPIO) project (Project 1349: Improving the establishment of oilseed rape through seed crop management, seed selection and seed treatment) which is aiming to demonstrate field scale improvement in oilseed rape establishment through selection and pre-treatment.

Maturity, as measured by seed harvest date, was only shown to exert a small effect on subsequent seedling performance, where the least mature (orange) seed suffer delayed germination. Orange seed tend also to be the smallest, and there is a strong correlation between the effects reported here and those previously observed for small seed (Stokes *et al*, 1998). The general lack of impact of seed collection date on subsequent performance is presumably because the seed 'sink' for oil and protein was already full by the time of the first seed harvest date. Thus, the only difference was the moisture content of the seed at harvest; earlier harvested seed were effectively dried down more rapidly than would be encountered under field conditions. This was not seen to have an adverse effect on subsequent germination performance. Given the very small proportion of orange seed in all seedlots examined, the opportunities to exploit this information appear limited, and most orange seed would be captured in any grading (by size or weight) process.

An alternative, untested, method for evaluating the maturity of the seeds may be to record the fluorescence of the seeds, which is directly related to the chlorophyll content of the seed; greener seeds being those that are less mature. This would have the advantage of assessing maturity of fresh material rather than dried. However, the results presented here do indicate that maturity effects would still be small.

As a consequence, modification of harvest time does not appear to be a fruitful avenue to be pursued further in the quest for improved seed vigour through provenance manipulation.

The impact of fertiliser nitrogen on seed composition could be summarised thus:

- Increasing nitrogen fertiliser content was seen to increase seed mass. This was predominantly due to increased seed size in the top half of the crop canopy, with very little effect being shown in the lower half of the canopy.
- Increasing nitrogen fertiliser also produced seed with greater quantities of nitrogen in them, although the weight differential seen between the top and bottom halves was due to increased oil in the seeds from the top of the canopy.
- Cotyledon size was related to nitrogen content of seed.
- Generally, the proportion of nitrogen in the seed was inversely related to the amount of oil.
- The highest proportion of oil was contained in crops that had been sparsely fertilised.

The nitrogen in the seed was a direct measure of the quantity of structural proteins and enzymes in the seed. The quantity of oil was related to the energy reserves in the seed. The ideotype seed should have high quantities of nitrogen, and thus large cotyledons in order to exploit resources in the autotrophic phase as well as possible, but also have sufficient carbohydrate reserves in order to tolerate extreme conditions. Increases in seed protein, almost doubling in some cases, were achieved with relatively small reductions in the weight of oil per seed. Thus, the energy reserve for early seedling growth and emergence would be little affected. However, the gain from increased protein appears to offer a range of benefits. First, imbibition is more rapid and there appeared to be a linear relationship between N% in the seed and the time to both 50% germination and 50% emergence. This improvement is particularly important because rape is usually sown into seedbeds where the risk from desiccation is high and any saving in time during the imbibition stage reduced the period of evaporation before the seedling had roots sufficiently deep to be at little risk. In line with the results from the earlier work (Stokes *et al*. 1998) the advantages of advancing the seed to the point of germination was substantiated. As a consequence of this work, we propose that the resowing fitness of an oilseed rape mother crop can be optimised by harvesting the top half of the canopy from a crop that has received large quantities of nitrogen fertiliser, at least some of which is applied late, at flowering. This would also provide the best seed for subsequent selection or pre-treatment. Harvesting only half the crop may present technical difficulties, and will almost certainly result in the sacrifice of the bottom half of the canopy. However, given that the entire seed production for a variety may come from a single field, the benefits of producing more fit seed may outweigh the loss of a few tonnes of ware crop.

It appears that the quality of seed resulting from large applications of N at the more conventional timings (mid February and mid March) can be achieved with smaller doses applied just before flowering. This has important implications for growers of seed crops. It shows that high protein seed can be produced without the risk from large, early applications creating excessively large canopies and the associated penalties of wide variations in maturation.

There were considerable benefits from removing the smaller fraction of the seed from some samples, especially those from crops where nitrogen supply was smaller than normal. In some cases emergence was improved two fold by selection of the larger seed.

Conclusion

This short-term work has demonstrated significant benefits for germination and emergence of oilseed rape from mother crop management (N application), seed size selection and seed advancement which show there is significant potential for improving the quality of seed available to producers. The potential improvements in germination and emergence from seed selection / advancement which have been demonstrated in the current project and the previous HGCA funded projects (Report no. OS29) are being further developed and tested with LINK (SAPPIO) and HGCA funding (Project No. 1349). These laboratory and field based studies will provide first indications of whether the strategic combination of specific treatments will produce more than additive advantages, particularly where stresses are severe. Overall, this preliminary work on manipulation of seed quality through management of the mother crop has confirmed that there is worthwhile scope to continue the examination of control of seed provenance to improve seed quality.

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