Growth Studies with Hemp (*Cannabis sativa* L.)

A thesis presented in partial fulfillment of the requirements for the degree of

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Arthur Randall Gibson

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Growth studies with hemp (*Cannabis sativa* L).

Abstract

The effect of latitude, sowing date and density on yield, height and growing time were studied by sowing two varieties of hemp for seed at three sites in New Zealand. Predation and poor germination hampered results. Latitude effects on height could not be confirmed but Anka, unlike Finola, suffered a reduction in height with later sowing. Anka was the larger plant with higher seed yield, but the dwarfed Finola gave a higher harvest index. Thus seed yield for later sowing is reduced for cv Anka, unchanged for cv Finola. Plant density data allowed estimated yields at 30, 60 and 90 plants.m^{-2} to be determined. At 30 plants.m^{-2}, seed yield in both varieties was reduced with later sowing, and confirmed Anka with the greater gross yield against Finola having the higher harvest index. At 60 and 90 plt.m^{-2}, the results confirmed only that Anka is the bigger plant. Earlier sowing than the 1st sowing in our trial (October) could be an advantage. Higher sowing rates than 40-50 plt.m^{-2} would be of little advantage for Anka, but rates higher than 90 plt.m^{-2} should be explored for Finola. The asymptotic yield maximum for Anka falls dramatically with later sowing, whereas Finola does not. This suggests double cropping might be explored, with an early sowing of Anka followed by Finola. Growing time data indicated the lower latitude site had longer growing times overall. Day length sensitivity (shorter growing time with later sowing) was confirmed for Anka, but Finola was unaffected. This suggests Finola is a day-length neutral plant. Leaf production in both varieties was higher at higher latitudes, and was reduced by later sowing date.

A second experiment examined the nitrogen and phosphorus uptake of a fibre hemp cultivar (EIL1) with respect to three sowing dates and two latitudes. Harvests were taken monthly to establish growth trends. From biomass figures, equations were established for leaf/stem relationships. Uptake values (on a dry matter basis) tended to stabilize as the plant matured at: N(stem) 3.04%, N(leaf) 4.5%, P(stem) 0.26% and P(leaf) 0.45%. High initial concentration of both N and P fell rapidly. For maximum uptake seed should be sown early (October or earlier) and plants grown as long as possible (5 months or more).

Acknowledgements

I would like to thank my supervisor, Dr. M. A. Nichols, for his unstinting advice and encouragement. His knowledge and experience were invaluable, when apparently incomprehensible data drove me to the point of despair. Financial support from the Foundation for Research in Science and Technology was invaluable, simply because without that support, and that of my mentor, Ashley Berrysmith of NZ Freshcuts (land and personnel for trials), the whole project would have been impossible. The help and advice of Massey University staff, from both the Soils Laboratory (chemistry analysis), and Plant Growth Unit (laboratory services) were vital for preparation of data. Last, but of course not least, I must thank my fiancée Susan Lawrence for her support. As my most ardent critic, she provided advice when I needed it, stress when I didn't (or didn't think I did) and above all belief in myself when I had none. Without all these extraordinary people the project would not have begun, let alone been completed. I thank them all.

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1. Introduction

Industrial hemp (*Cannabis sativa* L) is a potentially valuable crop, which represents an emerging industry in New Zealand (McIntosh 1998, McPartland et al. 2004). The history of hemp is very old however, and is even mentioned by Herodotus, the "father of history" as a source of cloth in burial ceremonies (Herotodus 73-75). Rollins (1731), as part of a diatribe on the need for a productive working life, refers to the usefulness of hemp in clothing – in context apparently for some 4000 years. This is confirmed by Lu and Clarke (1995) who present a detailed account of the history and use of hemp in China from circa 4000BC. They report the cultivation and use of hemp in clothing, paper, food and medicine. They give details of sowing dates, fertiliser requirements, plant densities and cultivation dating from some 200 years BC. Clearly hemp cultivation was widespread and well understood in pre-biblical time, at least in China. Captain James Cook (1779) refers to New Zealand flax in a comparison to hemp "There is one plant that serves the natives instead of hemp and flax, and which excels all that are applied to the same purposes in other countries." Thus hemp has seen many centuries of use, in Western society and ancient Greece, as well as in China, in clothing, rope, oil and medicine (although medicinal properties may refer primarily to high THC varieties).

In more recent times, research effort has been somewhat sparse, if increasing. Work is reported in various aspects of fibre reinforcement in automotive construction (Gulati and Sain 2005), and ballistic reinforcement (Wambua *et. al.* 2007).

Detailed work is also reported on the biochemical or nutritional characteristics of hemp oil and its constituents (Tang *et. al.*, 2006, Calloway 2004, Calloway, 2006, Leizer *et. al.* 2000 and others), and this effort also appears to be increasing.

On the subject of the growth of hemp plants, less work seems to have been reported, although again the effort is apparently increasing. Some comment is made of the genetic variability of hemp, emphasized by statements that the plant is dioecious, with enough variability to select monoecious varieties (Moliterni *et. al.,* 2004). Moliterni noted that monoecious varieties will revert in time to dioecious, a situation which must be monitored. Plant density and self thinning effects in relation to fibre yield and quality has been reported (Werf *et. al.* 1995, Drew *et. al.* 1977). Werf found that self thinning in hemp is more pronounced than in other plants. One trial (Lehoczky, 2006) reports the effect of hemp intrusion into maize as a weed, and Bennett *et. al.* (2006) reported the effect of variety and harvest time on fibre quality.

Little appears to have been reported on the nitrogen and phosphorus uptake potential of hemp, although Amaducci *et. al.* 2002 reported a hemp trial on plant density (30-250 $plt.m^{-2}$) and N fertilization (100-200 $kg.Ha^{-1}$), finding no significant differences in yield between (fibre) varieties.

This project was intended to examine the growth parameters of industrial hemp, in an attempt to derive the most appropriate growing conditions (planting date, latitude and density) for the production of hemp seed oil in New Zealand. Given the paucity of publicly available information on hemp growth for seed in this country, a preliminary trial was devised to identify areas for further, more detailed study.

Concurrently with our trial, however, results from an undergraduate experiment indicated that hemp may also be of benefit as a bioremediation crop (Gibson and Nichols in prep). A second experiment was undertaken to explore this possibility, by sowing the same cultivar on two sites. This investigation therefore involves two distinct experiments.

Experiment 1: three (arable) sites, two replicates, two varieties, four sowing dates and three sowing densities.

Experiment 2: two (effluent disposal) sites, one cultivar, three sowing dates, three sowing densities and monthly harvests.

2. Experiment 1: Growth Parameters of Industrial Hemp (Cannabis sativa L.) for Seed in New Zealand.

2.1. Introduction

As a commercially useful plant, hemp has been used for centuries for cloth and rope, and as food and medicine (Lu and Clarke, 1995). More recently, the beneficial effects of hemp seed oil have attracted more detailed attention (Callaway, 2004, Callaway *et. al.* 2005) and hemp is currently being cultivated for this purpose (Tang *et. al.* 2006, McIntosh 1998, McPartland *et. al.* 2004.). Little has been published on the growth parameters of the plant in New Zealand, and in particular there seems little known of the effects on seed harvest index of latitude, plant height, maturation time and yield, although Struik *et. al.* (2000) did report latitude effects in relation to fibre. A detailed model development project under controlled laboratory conditions is also reported (Lisson *et. al.* 2000), based on the APSIM-hemp protocol, which derives a full model encompassing all major growth factors, except latitude. Further, the cultivars used in their trial were different than available to us, and any relationship to the current trial could not readily be assessed.

A growth analysis experiment (Gibson and Nichols in prep) has been conducted under hydroponic greenhouse (NFT) conditions, but these results may have little bearing on field parameters, and clearly contribute nothing to the effects of latitude or sowing densities. The current experiment was intended to address some of these issues, and define future areas by sowing at different latitudes, at different dates and sowing densities, with particular emphasis on oil, (i.e. seed) rather than fibre (stem) production. Latitudes covered in this New Zealand study ranged from 35° 14'S (Kerikeri) to 40° 22'S (Massey), being the broadest range logistically feasible, and available.

2.2. Materials and Methods

2.2.1. Sites and Cultivars

The cultivars used were cv Anka and cv Finola at Massey University Tiritea campus (Fruit Crops Unit, longitude 175° 37'; latitude 40° 22'S); Rangiriri, South Waikato, longitude 175° 07'; latitude 37° 26'S and Kerikeri, Bay of Islands, longitude 173° 56'; latitude 35° 14'S. Anka is a Canadian bred, monoecious cultivar, certified for cultivation in Canada from 1999. It is medium height, and can be harvested for either oil or fibre, under appropriate conditions for each. Finola is a cultivar developed and bred in Finland specifically as an early flowering, short stemmed, oil producing dioecious variety. It has been certified for cultivation in Canada since 1999.

Seeds in our trial were direct drilled into prepared plots, as follows. Two replicates of 1.25m wide plots were sown, each containing three 2.0m sub-plots end-to-end, at three sowing densities for each cultivar, with a 1.0m guard plot at each end. Consecutive sowings, one month apart, were sown alongside the previous sowing. Harvest samples of 1.0m^2 each were taken from the centre of each plot, such that each sample had guard rows surrounding it. Bird netting was used to cover the freshly sown area.

Seed beds were not routinely irrigated. This was to ensure natural growing conditions, and to avoid unwanted soil/water/plant interactions due to the close proximity of earlier sowings. Water was applied under excessively dry conditions.

Regular (monthly) site visits were scheduled for maintenance, sowing and harvesting.

2.2.2. Problems and Limitations

Several problems were encountered during the growing phase of the trial, which impacted on the extent and quality of the data collected.

2.2.2.1. Seed quality

Due to delays in licensing, import and availability, sowing at the planned starting date (September) was not possible. Sowing therefore commenced in October 2005, using residual Anka and Finola seed from an earlier trial. In fact some seed was 4 years old at purchase (thus 5 years at sowing) which resulted in poor germination rates. We increased sowing rates throughout the experiment as this problem became apparent.

2.2.2.2. Predation

Bird netting was not totally effective, and other predators, including (but not limited to) slugs, springtail, possums, mice and pukeko also contributed to low plant densities.

2.2.2.3. Watering in

Seeds were not routinely watered in, to minimize crossover interaction on the adjacent, previously sown plots. In some cases current soil moisture may not have been sufficient to ensure good soil/moisture contact with the germinating seed.

2.2.2.4. Fertilisation

The sites were either fertilized, or believed to be of adequate fertility. This may have been insufficient in some cases. Where poor germination was encountered, however, weeds grew well. Thus fertiliser application should not have been the dominant problem at these sites.

2.2.2.5. Wind damage

A severe wind event at the Rangiriri site, some weeks after first sowing, affected some plants. The smaller (Finola) plants were damaged to some degree by this, but Anka appeared unaffected. In some aspects of data reduction, this produced anomalous results, in which case Finola data was removed. In most cases, however, the damage did not appear to seriously affect results, and data was therefore retained.

The above factors, or interactions between them, resulted in losses – the first sowing at the Kerikeri site, and the first three sowings at Massey. Newer seed, available toward the end of the trial, was used for a late sowing at Massey in late January. Anka produced harvestable seed, but replicates were notably different. Finola did not produce a crop, possibly due to severe weed infestation. Data from this sowing was inspected in all aspects of data reduction, but was excluded due to unacceptably high errors, and lack of equivalent data from other sites.

Harvest was conducted as closely as possible to the "80% ripe" stage. The geographic spread of sites and problems of determining ripeness remotely made this difficult. This, and the loss of ripe seed due to shattering and bird predation, resulted in some errors in the seed harvest data.

From each plot at each site, the following data were recorded:

- Average height at harvest (m),
- Weight of above ground biomass (stem, foliage and seeds).

- Sowing and harvest dates.
- Plant density ($plant.m^{-2}$).

2.2.3. Analytical Methods

Data were analysed based on the premise that plants grow exponentially. Transforming data to natural logarithms will thus produce a straight line, from which a linear regression yields intercept and slope for interpretation. The regression also yields an estimate of error for statistical analysis. Similarly, an asymptotic limitation to growth such as competition for light, nutrient or other resource can be modeled by a reciprocal transform. Again, the resulting linear regression allows interpretation of the intercept and slope of the line.

The regression data were analysed by analysis of variance (SAS Institute Inc., Cary, NC, USA version 9.1). Initially, for each analysis, a full factorial model including all main effects and interactions was prepared. The least significant F ratio from this analysis was then removed, and the process iterated until only factors at the 5% significance level remained. This model was then examined.

2.2.3.1. Height analysis

Height (m) was recorded and analysed.

2.2.3.2. Time

Time to harvest was recorded in days. Sowing time was recorded as months from first sowing (October).

2.2.3.3. Seed yield

Seed yield and above-ground biomass were converted to per plant, and transformed to natural logs. These data were plotted, displaying the seed to biomass ratio and highlighting anomalous data due to seed loss from bird strike and shattering.

The following protocol was devised to accommodate this loss of seed. Linear regressions of (ln) seed weight v (ln) biomass were calculated for each cultivar with an assumed slope of 1.0. Where the (exponentiated) seed weight was less than 25% of the estimated seed weight (based on the regression equation) that data was discarded. Removal of this data altered the regression relationship, and the process was iterated until no further data warranted removal. The residual dataset thus obtained was analysed, and the significance model examined.

2.2.3.4. Plant density effects

It was assumed that at high plant densities, internal shading would result in an asymptotic yield maximum. This could be modeled by a reciprocal transform according to the 'density equation' $1/w = A\rho + B$ (Drew and Flewelling, 1977), where ρ is plant density in plants.m^{-2}, and w is the plant yield. Plant density was therefore regressed against reciprocal above ground biomass yield (per plant) from each site, sowing date and variety.

Replicates were combined. Suspect data was scrutinized by (a) plotting data from each variety (i.e. combining data from all sowings and sites) and (b) plotting data for each sowing and variety (i.e. combining site data). These plots highlighted anomalous data due to errors in labeling, late harvesting and other handling errors. Massey data was found to be unusable due to paucity and high variability, and was excluded from further analysis of this parameter.

Intercepts (yield with no competition) of plant density regression coefficients so derived were analysed by analysis of variance. Analysis of slope data (yield with maximum competition) was not possible due to the selection procedure as discussed. The regression equations were however used to calculate expected productivity values at selected plant densities of 30, 60 and 90 plants.m^{-2}, based on the range of densities observed. These values (for reciprocal expected yield) were analysed, regressed and the resulting equations used to calculate a trend line at interpolated values (0 – 90) for each cultivar. The reciprocal slope from these equations is presented as the asymptotic maximum yield for each cultivar.

2.2.3.5. Growing time

Days to harvest was analysed with respect to month of planting, from October through January. Some unavoidable error was introduced due to slight variation in sowing date at several disparate sites. Due to logistic difficulties, harvesting could be two weeks from ideal ripeness.

2.2.3.6. Leaf weight

Leaf weight was recorded as per plant, and compared to the total above ground biomass. Data was converted to natural logs, analysed by analysis of variance and examined.

2.3. Results and Discussion

2.3.1. Height analysis

The significance model (appendix A) was derived. Only sowing data from Rangiriri and Kerikeri were included. The (5%) significant effects found were for Site, Variety and the sowing * variety interaction.

2.3.1.1. Site effect

The means (Table 1) show a significant site effect, with the more southerly site taller by 24.6%. In the absence of Massey data (the most southerly site) this effect cannot be reliably linked to latitude (a trend requires at least 1 degree of freedom, or at least 3 points), and thus requires further study. The differences observed could be due to uncontrolled local variables such as soil type, drainage, moisture regime and climate, rather than latitude.

Table 1 *Site Means of Overall Plant Height*

Site Means	
Site	**Mean (m)**
Kerikeri	0.65
Rangiriri	0.81

Note: Combined mean heights of anka and finola cultivars.

2.3.1.2. Variety effect:

Variety means (Table 2) clearly reflect the expected overall height difference in these two varieties.

Table 2 *Varietal Means of Overall Height*

Varietal Means	
Variety	**Mean (m)**
Anka	1.01
Finola	0.45

Note: Combined mean of Rangiriri and Kerikeri sites, and all sowings.

The height of Anka, reported here at 1.01m, was observed on-site as often considerably taller than this. Reasons become clear in Figure 1 below, and are discussed in that context

2.3.1.3. Sow-variety interaction

The means are presented in Figure 1.

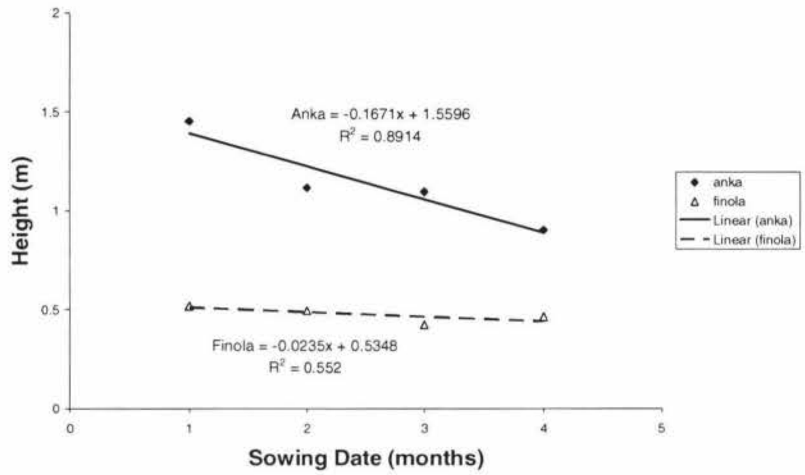

Figure 1 The effect of sowing date on varietal height.

This figure shows that while Finola height is virtually unaffected by later sowing, Anka is markedly so. It could be speculated that Finola, unlike Anka, is a day length neutral flowering variety. This is unusual for hemp, possibly related to the extremely high latitudes (Finland) where Finola was developed. Further trials, with extended sowing dates (and possibly laboratory trials) would help to clarify this, but the possibility could exist for double cropping if Finola (or a similar) variety is involved in late sowing.

The height data presented above elicits some information, some possibilities and some indications for further work. Since stem growth normally ceases at floration, it follows from Figure 1 that Anka is a day-length sensitive plant, but Finola may not be. Finola was developed in Finland (Callaway, 1996) specifically as an early flowering variety, and it is perhaps unsurprising that this is so. If true, the result highlights the natural genetic variability of hemp (Moliterni *et.al.* 2004), and offers encouragement to developers breeding varieties for specific growing conditions.

Of interest is the relatively low average height of Anka (Table 2) compared to Finola. The reason becomes clear with reference to Figure 1; Anka can be grown to some 1.5m with early sowing, but will attain less than 1.0m if sown late. For oil seed production this could be significant. Anecdotal accounts of harvest difficulties with taller plants highlight the need for a management tool to minimize these problems, and later sowing can clearly achieve this. However the impact of low height on seed yield limits this advantage, as discussed in section 2.3.2 below.

The height difference shown (Table 1) between sites is also of interest. Studies have shown some biochemical responses to latitude, notably THC/BCD ratios (Leizer *et. al.* 2000), and it could be that height is also affected. It is unfortunate that loss of data at our most southerly site precluded statistical confirmation of this (at least three sites are required to indicate a trend), but the possibility certainly warrants further study. If confirmed, a further management tool would be available indicating high latitude sites for fibre production, and low latitudes for oil seed production. Note that this result relates to height only, not specifically seed yield, which is discussed below.

2.3.2. Seed yield analysis

The graphed final dataset, selected as described above (section 2.2.2.3), is shown in Figure 2.

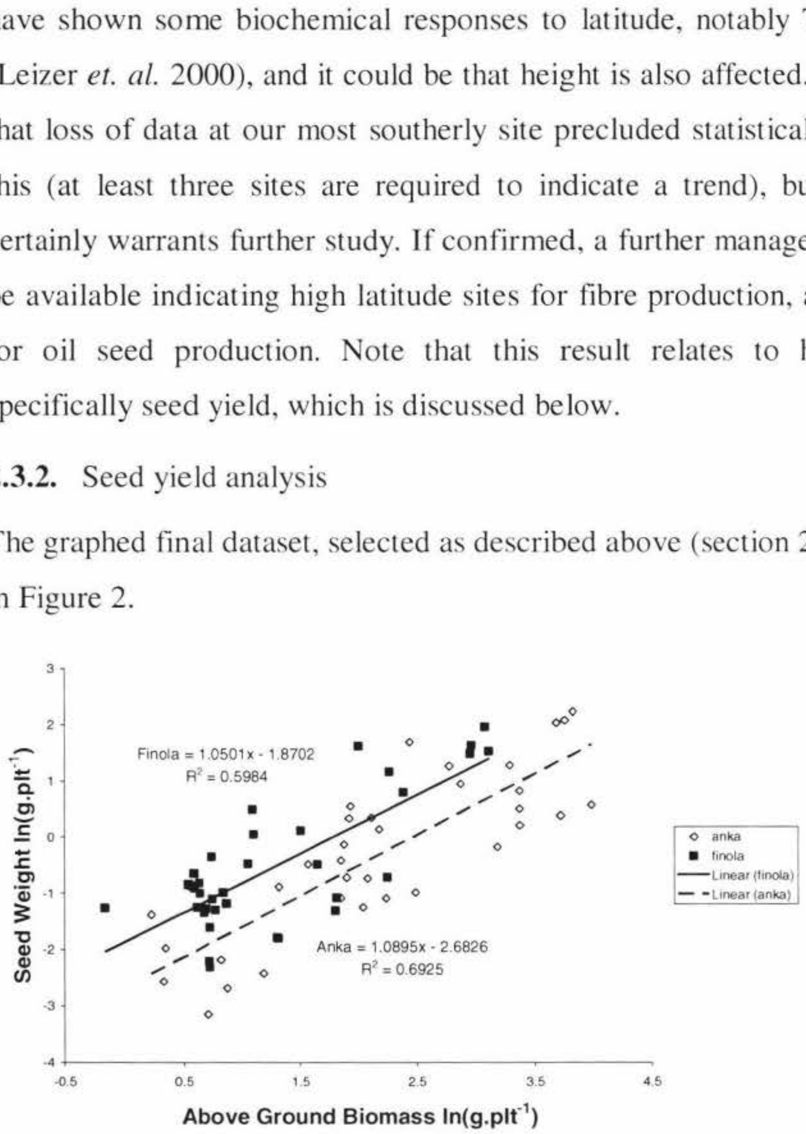

Figure 2 Final dataset of ln(seed) v ln(total biomass), with damaged data removed

This shows the clear difference between varieties, and that the selected data (for both varieties) has a slope overall very close to 1.0, as defined by the transform and selection process. With the average varietal slope set to 1, the intercept for individual points, as (ln)seed yield/biomass (g) can be calculated as follows;

(ln)intercept = ln(seed) – (ln)biomass.

The intercept differences displayed between cultivars are as follows;

Finola intercept = (exp) -1.8702 = 0.154 g(seed).g(biomass^{-1})

Anka intercept = (exp) – 2.6826 = 0.054 g(seed).g(biomass^{-1})

Thus finola has a higher harvest index, by a factor of almost 3. Anka is a larger plant however, and further analysis is required to confirm which cultivar is the higher producer on an area basis. This point is discussed below.

2.3.3. Plant density

From the basic density equation, $1/w = A\rho + B$, where ρ is plant density in plants.m^{-2}, regressions were obtained for each site, sowing and variety (replicates combined), yielding an intercept (reciprocal yield with no competition) for each variable. Slope data (reciprocal yield at asymptotic density limit) could not be directly analysed, as the slope had been arbitrarily set to 1.0 during the selection process (section 2.2.3.3). Analysis of variance (intercept) produced the following results, including sowing date and variety effects, as shown in appendix B.

2.3.3.1. Sowing

Massey data was again unusable, and Rangiriri sowing one (October) data was excluded due to wind damage. Kerikeri sowing one data was also lost. The results (Figure 3) therefore contain three points, which do however illustrate a clearly significant trend.

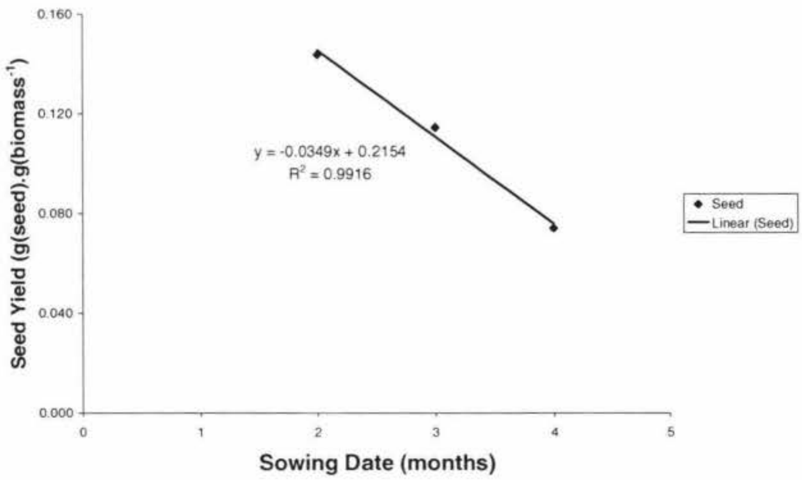

Figure 3 Effect of sowing date on expected seed yield at intercept (no competition)

This figure demonstrates the overall effect of later planting times on expected harvest index for the two cultivars (combined) under study, assuming no competition between plants.

This confirms the importance of early sowing, as reported above in relation to height, and provides a guide to how effective such a strategy could be.

2.3.3.2. Variety

Overall means for Anka and Finola harvest index, are presented in Table 3, including data converted from reciprocal means.

Table 3 *Overall intercept means*

Variety Effect On Seed Yield (Intercept)		
Variety	**Mean (ln)**	**Mean (exp)**
Anka	-2.6771	0.069
Finola	-1.7786	0.169

Note: Mean (ln) extracted from data in Appendix B.

This table shows the higher harvest index of Finola compared to Anka, with no competition. Finola harvest index is some 2.4 times that of Anka under these conditions.

2.3.3.3. Slope (productivity with maximum competition)

The estimation of maximum productivity from this data was not possible, as discussed above (section 2.3.3). Data variability and seed losses required an arbitrary assumption of an overall slope of 1.0 to extract statistically significant effects. Although technically possible to examine internal differences due to site, sowing date and variety, relating any such conclusions to real world conditions is not practical, and thus not reported. In any future trial, with more controlled and consistent data, such an analysis may well be appropriate.

2.3.4. Plant density effects at selected calculated densities

Biomass productivity data estimates were prepared for densities 30, 60 and 90 plants.m^{-2}, and analysed separately to preserve data independence.

2.3.4.1. Density 30 plants.m^{-2}

Analysis gave significant effects for sow, variety and the sow * variety interaction (appendix C), as follows.

2.3.4.1.1. Sowing date

Results of the effect of sowing date on total above ground biomass are shown in Figure 4.

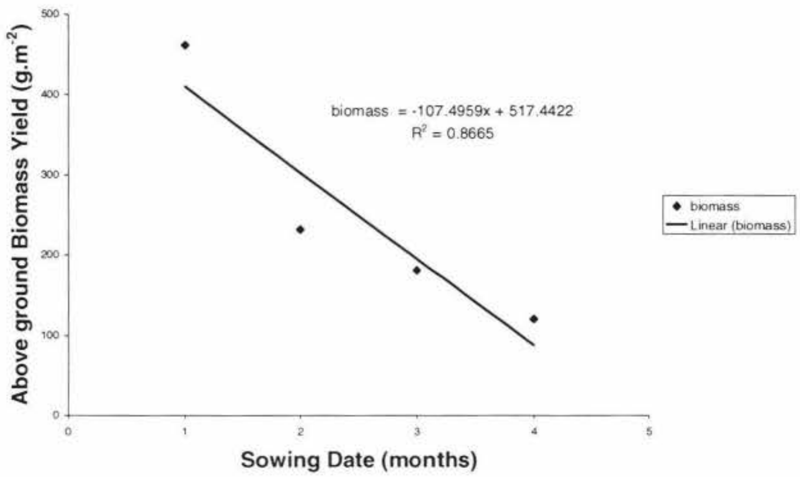

Figure 4 The effect of sowing date on total above ground biomass at 30 plants.m^{-2}.

This figure confirms a reduction in total above ground biomass yield with later sowings. It will be noted that the sowing * variety interaction is significant at this density level which, since both varieties perform differently, implies that this finding should be considered in conjunction with the interaction analysis below (section 2.3.4.1.3).

2.3.4.1.2. Variety effects.

Data from the two varieties are presented in Table 4.

Table 4 *Effect of Variety on Above Ground Biomass*

Effect of Variety on Yield (30 Plants.m^{-2})	
Variety	**Mean**
Anka	321.2
Finola	115.3

Note Higher productivity of Anka at 30 plants.m^{-2}

This table shows that Anka has an expected biomass yield nearly three times that of Finola (ratio 2.8). This again confirms Anka as the larger plant, and suggests that Finola could probably be sown at higher densities than Anka.

2.3.4.1.3. Sow * Variety interaction

Results of this interaction are shown in Figure 5.

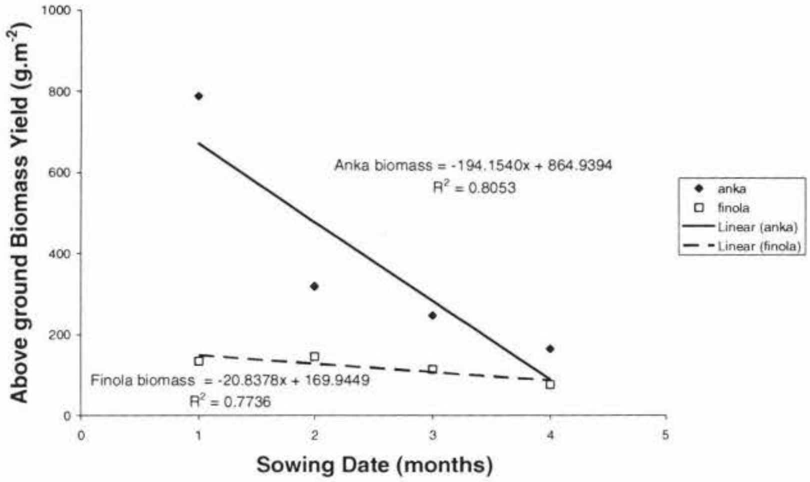

Figure 5 Interaction effects of both varieties against sowing date at 30 plants.m^{-2}.

This figure dramatically demonstrates the difference in effect of sowing date for the two varieties (at this density level). Anka productivity is markedly reduced by later sowing dates, whereas Finola reduces very little. Figure 5 also effectively resolves the issues raised in Figures 3 and 4, highlighting the different response to sowing date by the two varieties. This could be significant if double cropping is contemplated, as late sowing of Anka is clearly unproductive compared to Finola.

2.3.4.2. Density 60 plants.m^{-2}

Results are presented in appendix D, indicating the significant effect of variety alone.

2.3.4.2.1. Variety:

Means for this effect are shown in Table 5

Table 5 *Variety Effects of Density at 60 Plants.m^{-2}.*

Effect of Variety on Yield (60 Plants.m^{-2})	
Variety	**Mean**
Anka	376.7
Finola	156.5

This table shows that the overall above ground biomass at 60 plants.m^{-2} is in similar ratio between varieties to that at 30 plants.m^{-2} (ratio 2.4), but at increased level for both varieties. Thus at 60 plants.m^{-2}, the asymptotic yield limit for biomass productivity has not been approached by either variety.

2.3.4.3. Density 90 plants.m^{-2}

Results at this sowing density are presented in appendix E.

2.3.4.3.1. Variety

Means for the only significant effect (variety) are presented in Table 6.

Table 6 *Variety Effects at Density of 90 Plants.m-2*

Effect of Variety on Yield (90 Plants.m^{-2})	
Variety	**Mean**
Anka	403.9
Finola	180.5

Again, the difference between varieties is in similar ratio to that found at 30 and 60 plants.m^{-2} (ratio 2.2), and is again numerically higher in both varieties than for 30 or 60 plants.m^{-2}. Since progressively higher yields are recorded for 30 60 and 90 plants.m^{-2}, it follows that the asymptotic yield limit is not reached at these levels. Due to lower than expected plant densities in this trial, calculations at densities above 90 were not warranted, but should be explored in further trials. The derived equations, however, were combined with seed yield data to calculate trend lines for seed yield as shown in Figures 6 (Anka) and 7 (Finola).

2.3.4.4. Summary of Plant Density Effects

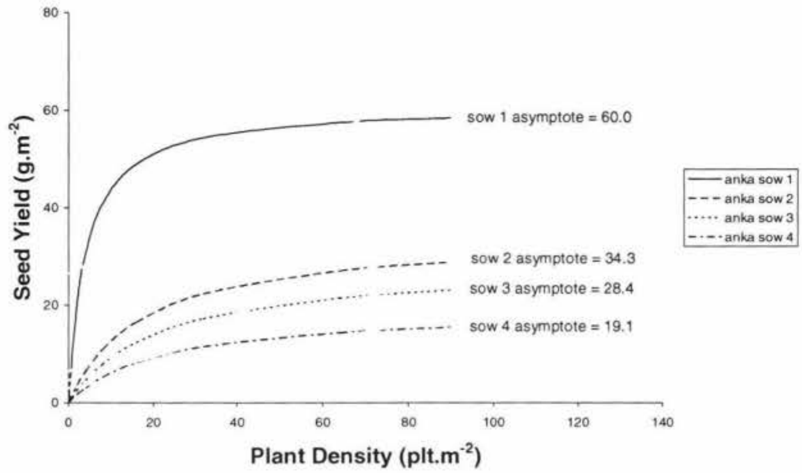

Figure 6 Seed yield trend line for Anka, showing asymptotic maxima

Figure 6 clearly shows an asymptotic trend for Anka, illustrating that earlier sowing dates provide considerably higher yields. The possibility that earlier sowing could be even more productive is clearly shown. It is also clear that sowing densities for Anka greater than 40 plants.m^{-2} give progressively less response with later sowing. Thus earlier sowing will attain higher seed yield with lower densities.

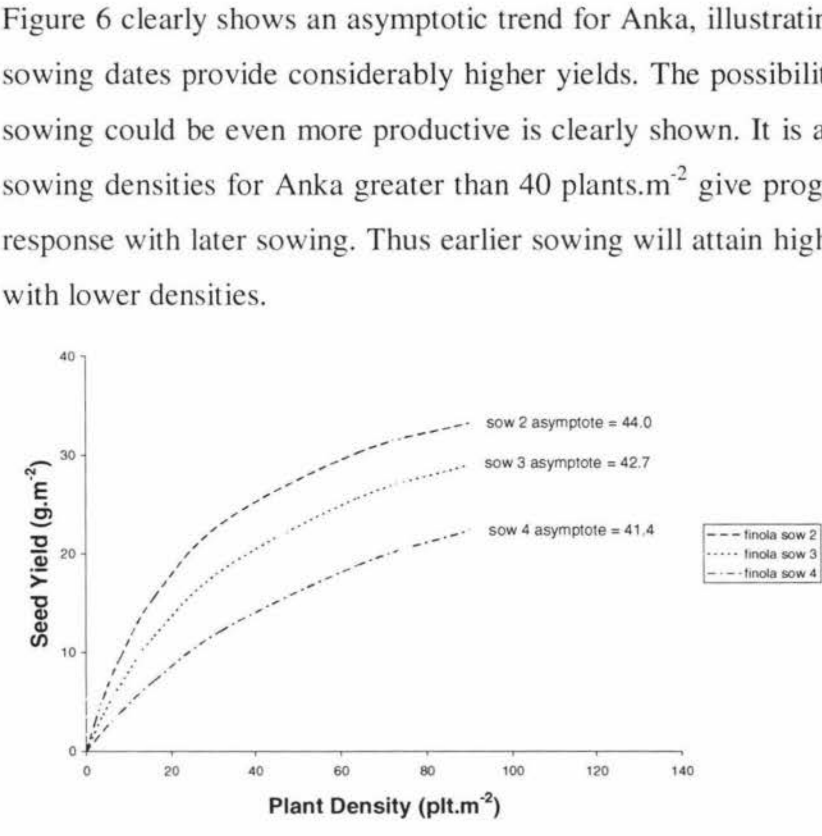

Figure 7 Seed yield trend line for Finola, showing asymptotic maxima

Figure 7 illustrates that Finola also exhibits higher yields for earlier planting, with the possibility of higher yields for even earlier sowing dates, as does Anka. Only three sowings are presented for Finola, due to missing and damaged data. The trend lines indicate that Finola could benefit from higher plant densities than derivable from our data, possibly in excess of 100 plants per square metre. Again, higher densities will be required with later sowing to maintain high yields.

The asymptotic seed yield limits (exponentiated slope from the derived equations) for both Anka and Finola are compared in Figure 8, showing trends for the two cultivars.

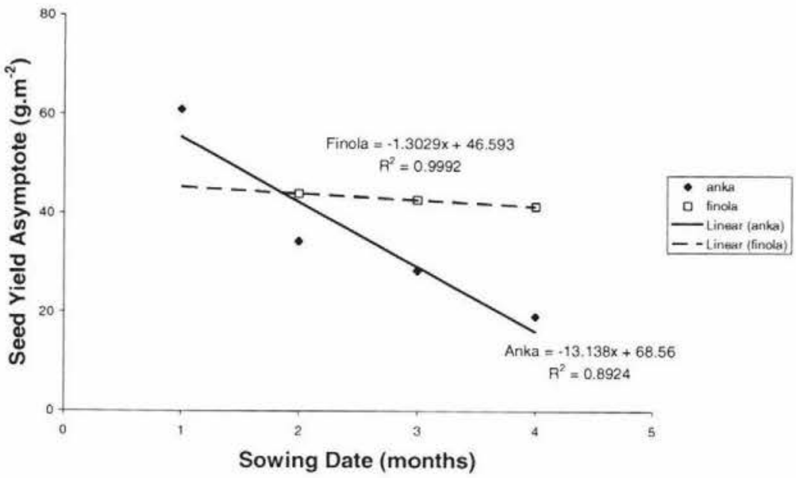

Figure 8 Asymptotic seed yields for varieties v sowing date

This figure clearly illustrates the effect of sowing date on seed yield performance of Anka and Finola. Anka productivity clearly reduces with later sowing, whereas Finola does not. Thus Anka requires an early sowing date to perform well, possibly earlier than achieved in our trial (October), but Finola performance is effectively unaffected. It will be recalled that early sowing of Anka results in taller plants, and that this could impact on harvesting problems; this would not be true of Finola.

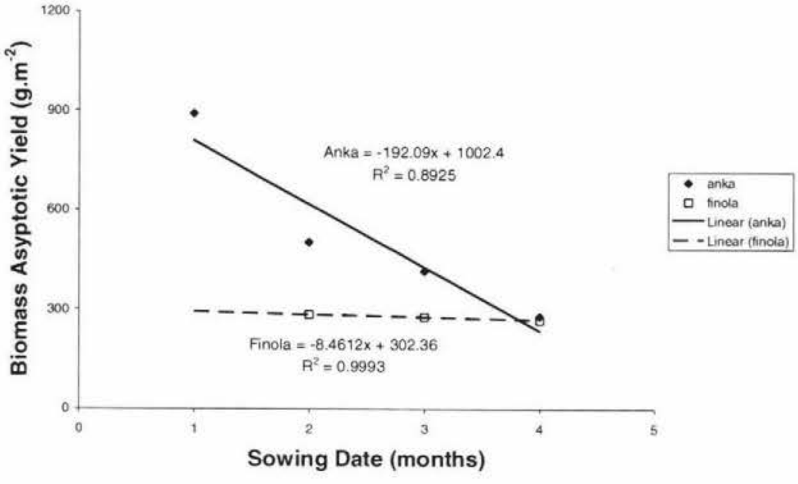

Figure 9 Effect of sowing date on biomass for Anka and Finola

The figure shows the biomass yield reduction for Anka with delays in sowing date, where Finola is again essentially unaffected. When compared to Figure 8, it also shows that Finola harvest index is higher, when planted late. This confirms the possibility for double cropping with Anka sown early, followed by Finola.

This possibility should be explored with further trials, extending the overall growing period (possibly required to allow adequate growing time for both varieties), and identifying any harvest problems due to height which may occur.

2.3.5. Growing time analysis

The model resulting from these data included site, sow, and the site * sow interaction, as presented in appendix F. These effects, significant at the 5% level, were based on omitting all Massey data, and the first sowing at Rangiriri.

2.3.5.1. Site effect

The means for site data are presented in Table 7

Table 7 *Site Effect for Growing Time to Harvest.*

Site Effect for 'Days To Harvest'	
Site	**Mean**
Kerikeri	76
Rangiriri	59

The growing time would appear to be significantly shorter at Rangiriri than at Kerikeri, which is considerably to the North. This result could indicate a latitude effect but, in the absence of the Massey data (the most southerly site) to establish a trend, this could not be verified. If such a latitude effect could be established, it would impinge on the double cropping concept mentioned above. It may, for example, be possible to double crop at higher latitudes, but not at lower. Conversely, the earlier sowing dates feasible in warmer climates (lower latitudes) could negate this. Further trials are required to clarify the extent and importance of these effects.

2.3.5.2. Sowing date effect

The results are presented in Figure 9. A clear reduction in growing time with later planting is apparent, notwithstanding high variability.

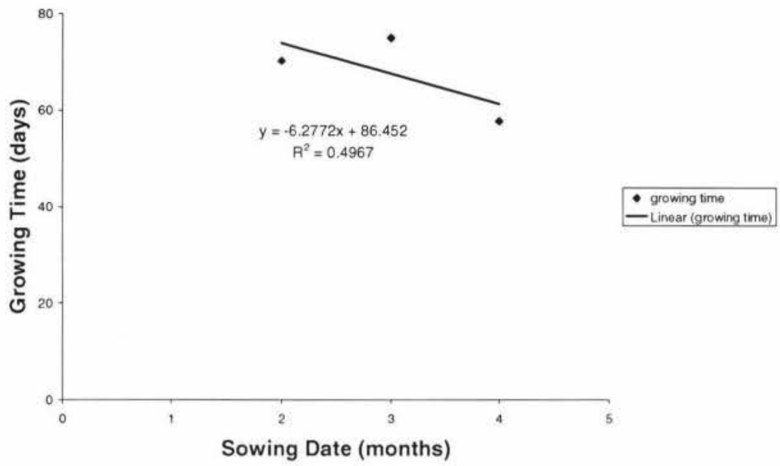

Figure 10 Effect of sowing date on growing time to harvest

Again, this result requires clarification. In the absence of clear varietal differences (discussed in section 2.3.5.3), either variety could be day-length sensitive. In which case early sowing of the first day length sensitive crop would not necessarily result in earlier harvest. Other indications, as outlined above, suggest that Anka, but not Finola, could be day-length sensitive. If so, early sowing of Anka would provide higher yield, but not necessarily earlier harvest. This requires a determination of the latest feasible sowing date, which is not clear from the data.

2.3.5.3. Site * sowing interaction

Means for this effect are presented in Figure 11.

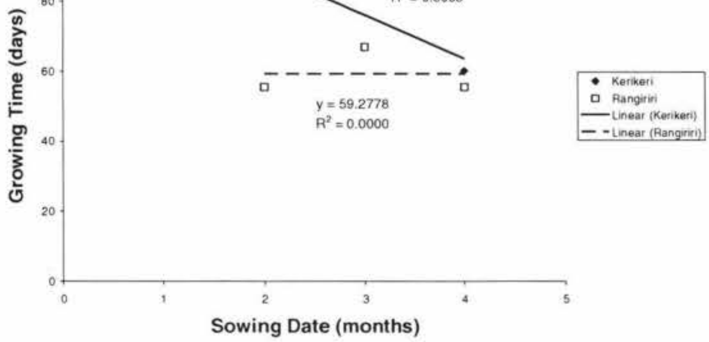

Figure 11 Sowing date versus growing time interaction effect between sites

Despite inherent errors, and paucity of data, this figure clearly shows the difference in growing time between the two varieties. It would appear that Finola can be sown as late as January and provide an adequate crop.

Growing time therefore, depends on variety and sowing date, with the possibility of a latitude effect. Certainly, it has been shown that latitude affects some biochemical processes (Leizer et. al. 2000), and these results could indicate more fundamental effects. Clearly a more detailed investigation into day-length sensitivity and latitude effects is warranted, at least for the varieties studied here.

2.3.6. Leaf weight analysis

Leaf weight (natural log) was plotted against (natural log) total above ground biomass, as shown in Figure 12.

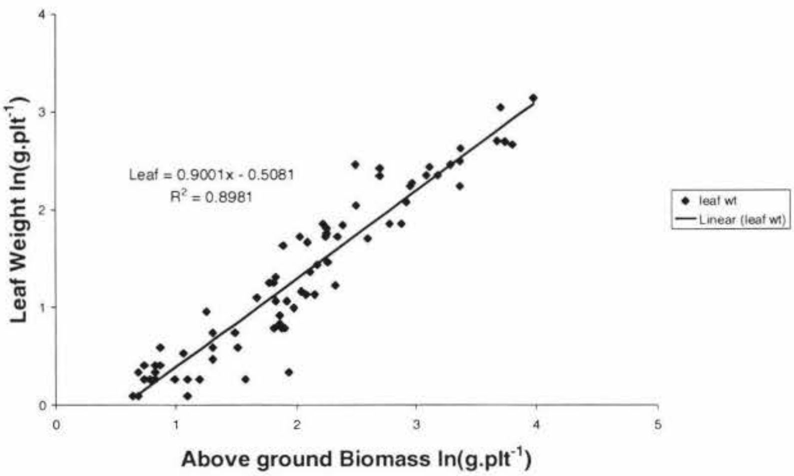

Figure 12 Leaf weight per plant plotted against total above ground biomass

Figure 12 shows a clear correlation between total leaf weight and above ground biomass, irrespective of variety, with a slope of 0.9001. Thus as the (natural log) total weight rises, the natural log leaf weight rises less, by a factor of 0.9. As weight is a function of height, it follows that as the plant matures, it allometrically changes from leaf to stem production. This could be important as a factor in quality fibre production or, assuming a difference in chemical uptake between leaf and stem (discussed in section 3.3.2), in bioremediation.

Analysis of the ratio of (ln) leaf wt and (ln) above ground biomass, with Massey data excluded, yielded results as shown in appendix G. Significant effects included site and sowing date, and the interactions site * sow, and site * variety.

With variety not significant as a main effect, the interaction of variety and site was not considered further.

2.3.6.1. Site effect

Means for this effect are shown in Table 8.

Table 8 *Leaf / Biomass Ratio Means for Kerikeri and Rangiriri.*

Leaf/Biomass Site Effect		
Site	**Mean**	**Mean(exp)**
Kerikeri	0.49	1.63
Rangiriri	0.67	1.96

Mean differences for this effect, with Rangiriri being higher by 37% as natural logs, 20% as the exponent, could be due to latitude effects. As before, loss of data from the Massey site precludes a clear conclusion but, together with other results in this thesis, indicate the need for further detailed studies into latitude effects.

2.3.6.2. Sowing date effect

This effect is demonstrated in Figure 13.

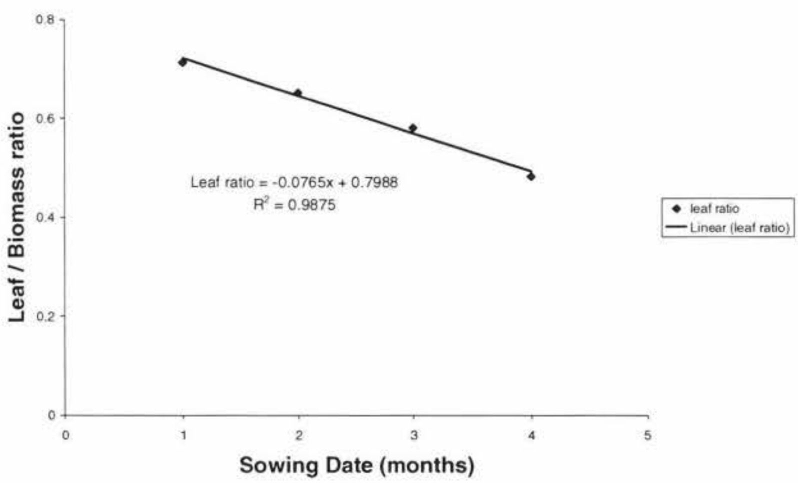

Figure 13 Effect of sowing date on leaf/biomass ratio

Figure 13 clearly shows an effect of planting date on the (ln) leaf wt, (ln) above ground biomass ratio, which decreases with later sowing date. This could be a significant result, given that allometric changes, and therefore growth distribution could affect stem and fibre quality. It will be noted, however, that later sowing dates result in lower height maxima, lower seed yields and lower above ground biomass for Anka.

This result, therefore, could reflect a plant response to changing day-length, vernalization, and the need to harvest more daylight during shorter day-length regimes. It is clear, however, that random events such as rainfall and sunshine hours cannot explain the clear trend shown. Further, unmonitored variables such as soil type, drainage and fertility levels are also not responsible, as they did not change during the experiment. Thus latitude remains the likely candidate, by mechanisms not clear from the data. Future, more detailed investigations are required to explain these results, and other allometric inferences.

2.3.6.3. Site * sowing date interaction

Means for this effect are shown below in Table 9

Table 9 *Site v Sowing Date Interaction for Leaf/Biomass Ratio.*

Leaf/Biomass Site * Sowing Date		
Site	**Sow**	**Mean**
Kerikeri	2	0.53
Kerikeri	3	0.41
Kerikeri	4	0.51
Rangiriri	1	0.71
Rangiriri	2	0.76
Rangiriri	3	0.70
Rangiriri	4	0.46

This table illustrates the wide variation of (ln) leaf wt v (ln) above ground biomass compared over the two sites reported. No simple trend is apparent in this case, and the results may be due to confounding and uncontrolled factors.

2.4. Conclusions

Plants sown at the higher latitude (Rangiriri) grew 24.6% taller than at the lower latitude (Kerikeri). Absence of precise data from the southernmost site (Massey), and consequent loss of the third point required to establish a trend, precluded verification of a direct link to latitude however, and a more comprehensive trial is required to exclude environmental effects. Shorter plants could be advantageous for harvesting of seed however, and confirmation of this finding could affect appropriate sites for cultivation, either for seed (high latitude) or stem (low latitude). Lower seed yield under these conditions could result, negating this advantage.

The taller variety, Anka, was shown to grow nearly twice as tall as Finola, but more significantly, the height of Anka is considerably reduced by later sowing. Finola was not greatly affected. If final height is a consideration when planning cultivation, whether for stem (fibre) or seed (oil) care should be taken in selection of site, variety and sowing date.

Anka is the larger plant, but biomass and seed production was considerably reduced by later sowing. In contrast, Finola biomass and seed production was unaffected by delayed sowing. The possibility therefore exists for increasing overall productivity by double cropping, with Anka followed by Finola. Extended sowing dates could improve this possibility, and should be explored in further trials.

Plant density analysis showed that, without competition, seed yields conformed to the directly measured findings, indicating that under the conditions of our trial, relatively little competition occurred. Analysis of above ground biomass at 30 plants.m^{-2}, showed yield reduced with later sowings by some 75%. Thus a considerable penalty in biomass (and therefore seed yield) is attracted by delays in sowing date. As the smaller (albeit more productive) plant, Finola biomass productivity was virtually unaffected by later sowing, thus highlighting the dramatic effect of later sowing of Anka. If later sowing is envisaged, whether due to weather constraints or proposed double cropping, Finola should be encouraged compared to Anka. At 60 plants.m^{-2}, and at 90 plants.m^{-2}, Anka produces considerably more biomass than Finola, and each variety produces more than at lower densities. In neither case is the asymptotic yield (yield with maximum competition) approached, although Anka seed yield shows relatively minor response to densities above 50 plants.m^{-2}. Densities above 90 plants.m^{-2} for Finola should be explored in further trials.

Analysis of growing time to harvest showed that the lower latitude site (Kerikeri) required longer growing time than did the higher latitude (Rangiriri) site. Clear correlation with latitude could not be established, due to loss of data from the highest latitude (Massey) site. The difference was substantial however (76 days v 59 days) so the possibility should be explored in further trials. Growing time was also significantly affected by sowing date, to a similar degree. Again, early sowing data was unavailable, but sowing in November required 76 days to mature on average, sowing in January only 60. It was found that Anka was again greatly affected by later sowing, but Finola was not. Thus it would appear that Finola is not a day-length sensitive plant, but Anka conforms to the norm for hemp.

Comparing leaf weight (ln) with above ground biomass (ln) showed a correlation, with a slope of 0.9. Thus as the plant increases in size, relative leaf production falls, by a factor of 2.5. The ratio of ln(leaf weight) to ln(above ground biomass) was shown to be significantly affected by site and variety. The lower latitude site gave a higher ratio, and the ratio was lower with later sowing date. Any possible latitude effect could not be confirmed in the absence of precise data from the highest latitude site under study.

3. Experiment 2: Growth and nutrient uptake of industrial hemp (Cannabis sativa L.) in nutrient rich effluent.

3.1. Introduction

The potential of bioremediation as an effluent purification method has long been known. McColl and Gibson (1979) found that down-slope runoff through grass root mat assisted removal of nitrogen and phosphorus from drainage water. This is an on going subject of research, as shown by Sawabe *et. al.* (2006), who identified matches between target pollutants and appropriate plants. In recent times, studies have been conducted on sewage effluent (nitrogen and phosphorus) purification (Yang Lei; *et. al.* 2001), comparing different wetland substrates using napier grass as a test plant.

Little has been reported in the literature regarding the growing of industrial hemp for the removal of nitrogen and phosphorus from nutrient rich solutions, although hemp is well documented as a medium for removal of heavy metals (Tlustos *et al.* 2006) and hydrocarbons (Liste, 2006). For several years, hemp has been used in a project to purify soils, including the radioactive contaminated soils near Chernobyl (Charkowski 1999; Khan 2005).

In a recent study (Gibson & Nichols in prep), the authors have defined the growth and nutrient uptake characteristics of industrial hemp under NFT hydroponic conditions. The inference was that hemp may have value as a commercially useful bioremediation crop, particularly in the removal of N and P. Little has been reported on N and P uptake in hemp, but a study has been reported on uptake by forage grasses in North Eastern U.S. Ketterings, *et. al.* (2006) reported rates for N, on average over several fertiliser application rates, of 0.0200 $g(N).g(DM)^{-1}$, and for P, 0.0029 $g(P).g(DM)^{-1}$, and Fosu (2004) investigated nitrogen accumulation and release by sunn hemp, calopo mucuna and devil bean.

This section of the thesis examines the growth and nutrient uptake characteristics of hemp under New Zealand field conditions.

3.2. Materials and Methods

3.2.1. Sites and Cultivar

Two New Zealand sites were used, one at Feilding (Lat 40° 14'S), utilizing tertiary treated sewage effluent as feedstock, and one at Ashburton (Lat 43° 54'S), using effluent from a major meat processing plant.

The same cultivar (EIL1) was used at both sites. EIL1 is a dioecious fibre type cultivar developed in Queensland by EcoFibre Industries Ltd

Three sowings were made at each site. Each sowing at each site comprised three 10m by 2m plots, at three plant densities. The 3 sowings were direct drilled into unprepared soil (Feilding) and into cultivated soil (Ashburton) at monthly intervals. Random $1.0m^2$ destructive harvests were taken monthly from each 10 x 2m plot, starting one month after sowing. These samples were weighed, leaf and stem weights recorded, sub samples dried at 80C for 48 hours and weighed. The sub samples were fine ground, and analysed for total nitrogen (Keldahl) and total phosphorus (Phosphomolybdate). Plant numbers for each plot and harvest were also recorded, thus all data could be analysed on per plant, and on per m^2 basis.

3.2.2. Problems and Limitations

3.2.2.1. Herbicide damage

Several plots were inadvertently damaged by herbicidal spray during the Feilding trial. This limited the usefulness of data from this site for density analysis, and exacerbated the data weighting limitations inherent to the experimental design.

3.2.2.2. Weighting limitations

Successive harvests on successive sowings imply fewer harvest samples available from later sowings. Thus 5 samples were available from sowing 1, 4 from sowing 2 and 3 from sowing 3. In fact losses due to herbicidal spray resulted in only 1 sample being available for harvest 5. This affected the statistical validity of averaged results.

3.2.3. Analytical Methods

Statistical analyses were performed to the same protocol and with the same software as for experiment 1. As both exponential growth and asymptotic limits to growth were observed, and the effects are independent, both transforms (natural logarithm and reciprocal) were applied where applicable (section 3.2.4).

In order to test for differences between sites, sowing dates and harvests, sample data were converted to per plant and natural log (ln) transforms performed. However as interest in this project is in nutrient uptake on an area basis, all results were only finally considered on an area basis.

Given that sowings were performed at different plant densities, results on an area basis would be valueless without compensation for this variation. For this reason, and because some data was lost, results were 'standardized' to 30 plants.m^{-2}. As dry matter yield is fundamentally asymptotic due to shading and self thinning, reciprocals were taken of total (ln) dry matter per square metre, and plotted against plant density. The resulting linear regression formulae were used to estimate total dry matter yield at 30 plants.m^{-2}. By this means, values for each harvest, sowing and site could be regressed against harvest date. Due to variations and anomalies in sowing dates between sites, Ashburton sowing date data was adjusted by 1 month to compensate, allowing all available data to be used in a single calculation.

3.2.4. Allometric Relationships

Allometric analysis between leaf and stem was based on the relationship of (ln) leaf and (ln) stem against total weight. The resulting regression equations were used to calculate leaf and stem weights for each harvest, sowing and site.

3.2.5. Nutrient uptake

Total (ln) nitrogen and (ln) phosphorus data, for both leaf and stem, were regressed against (ln) leaf and (ln) stem biomass data respectively to derive an expected uptake nutrient value. From these regressions, and the biomass data outlined above, uptake values for both nitrogen and phosphorus were derived, on a per harvest basis, irrespective of sowing date or site.

3.3. Results and discussion

The following data were recorded for each site, plot and harvest:

- Leaf dry weight (g)
- Stem dry weight (g)
- Plant numbers (count)
- Total Nitrogen for leaf and stem (%)
- Total Phosphorus for leaf and stem (%)

3.3.1. Allometric Relationships

Leaf weights (natural log) were plotted against stem (natural log) weights, as shown in Figure 14, on an area (square metre) basis.

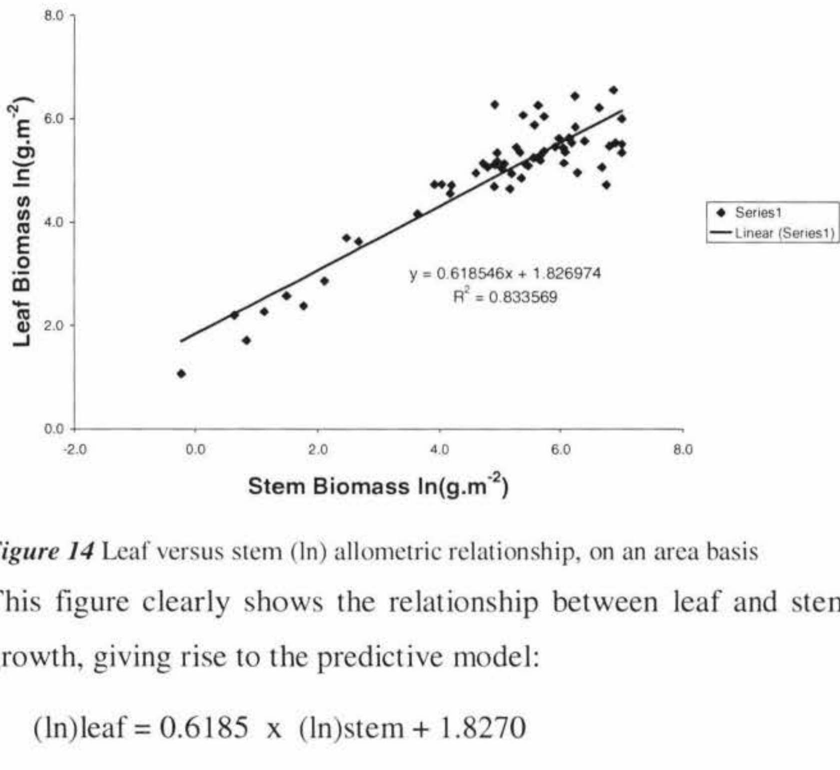

Figure 14 Leaf versus stem (ln) allometric relationship, on an area basis

This figure clearly shows the relationship between leaf and stem during plant growth, giving rise to the predictive model:

$$(\ln)\text{leaf} = 0.6185 \times (\ln)\text{stem} + 1.8270$$

Further, the slope (less than 1.0) illustrates the trend, throughout growth, of an allometric change from dominant leaf production to dominant stem production.

This is more clearly demonstrated when stem and leaf (ln) weights are plotted against total (ln) weight, as shown in Figure 15 (per plant basis), and Figure 16 (area basis).

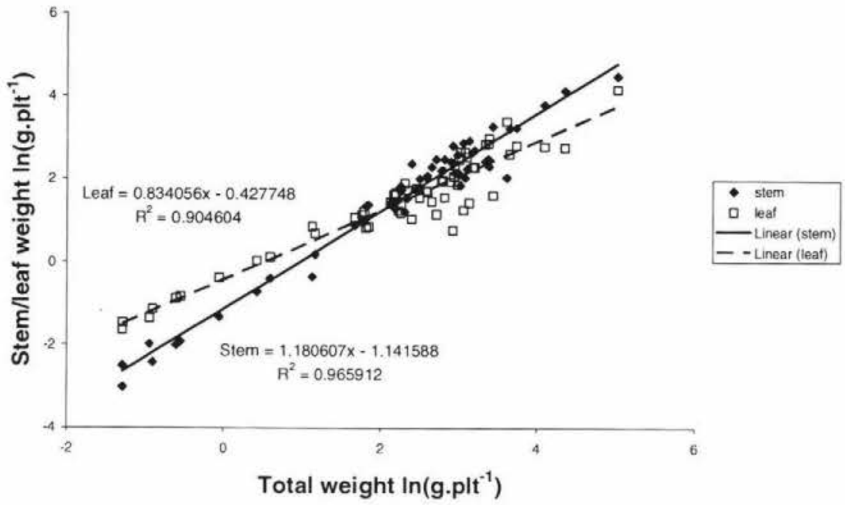

Figure 15 Stem and leaf (ln) weights v total (ln) plant weight

The equivalent chart, Figure 16 below, illustrates a similar trend on an area basis.

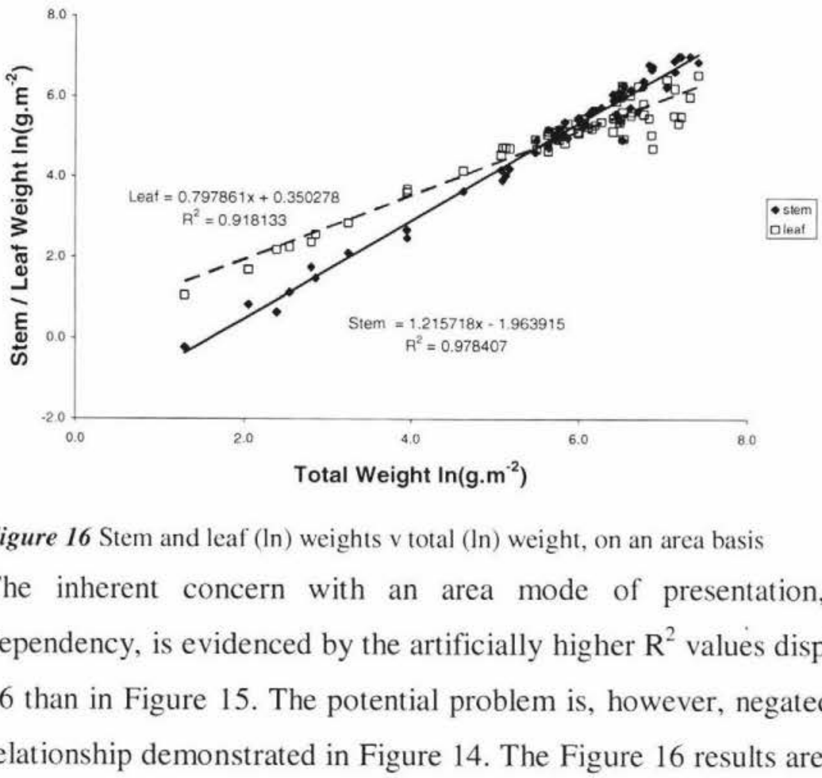

Figure 16 Stem and leaf (ln) weights v total (ln) weight, on an area basis

The inherent concern with an area mode of presentation, internal data dependency, is evidenced by the artificially higher R^2 values displayed in Figure 16 than in Figure 15. The potential problem is, however, negated by the similar relationship demonstrated in Figure 14. The Figure 16 results are more useful as predictive models.

It will be noted that these relationships are true across both sites, and all sowings and harvests. Thus, the pattern of growth of hemp is independent of these parameters, and can be estimated from the following equations:

(log)leaf = 0.834056 x (log total weight) - 0.427748

(log)stem = 1.180607 x (log total weight) - 1.141588

3.3.2. Nitrogen and phosphorus uptake.

Nitrogen and phosphorus uptake was calculated as discussed.

3.3.2.1. Stem and leaf nitrogen uptake

Both stem and leaf N uptake were compared with total N uptake (natural log), on an area basis $\ln(g.m^{-2})$ as shown in Figure 17.

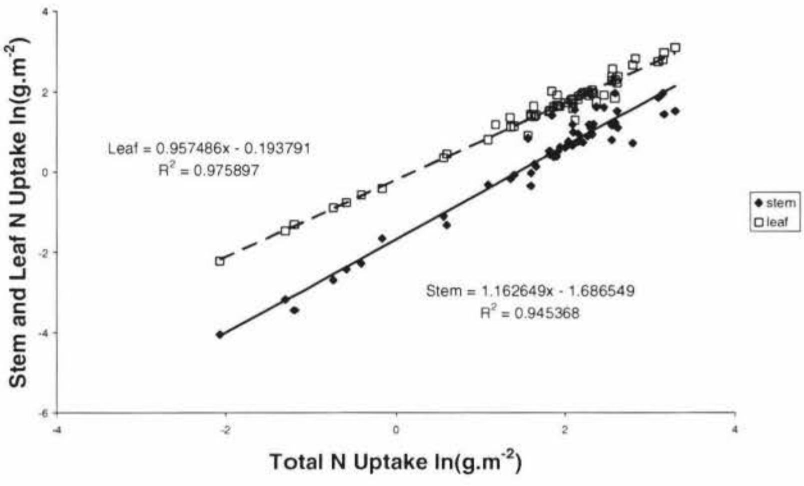

Figure 17 Relationship between stem and leaf N, and total N (natural log)

This relationship shows the overall higher level of N in leaves than in stem, and the differing slopes apparent as the plant grows (ages). While the regression lines do not cross in the range under study, as they do for the biomass data shown above, a similar trend is apparent in both.

3.3.2.2. Stem and leaf phosphorus uptake

Correspondingly a similar pattern emerges when the phosphorus data is plotted (Figure 18), but at a significantly lower level.

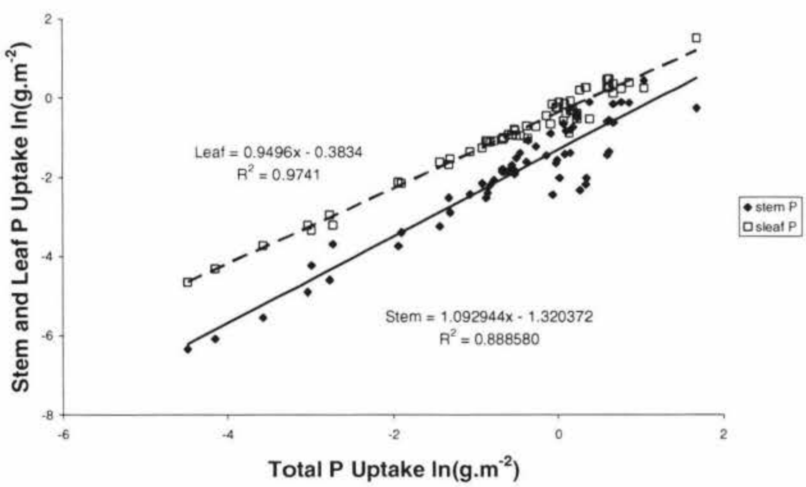

Figure 18 Relationship between stem and leaf P, and total P (natural log)

It can be seen that P follows a similar relationship to that of N, and once again the lines converge, but do not cross in the range of our data. Comparison of Figures 17 (N) and 18 (P) show the differences in uptake levels for the two nutrients.

Stem and leaf uptake rates for both nitrogen and phosphorus were calculated (on an area basis) in Figures 19 and 20.

3.3.2.3.N and P uptake for stem

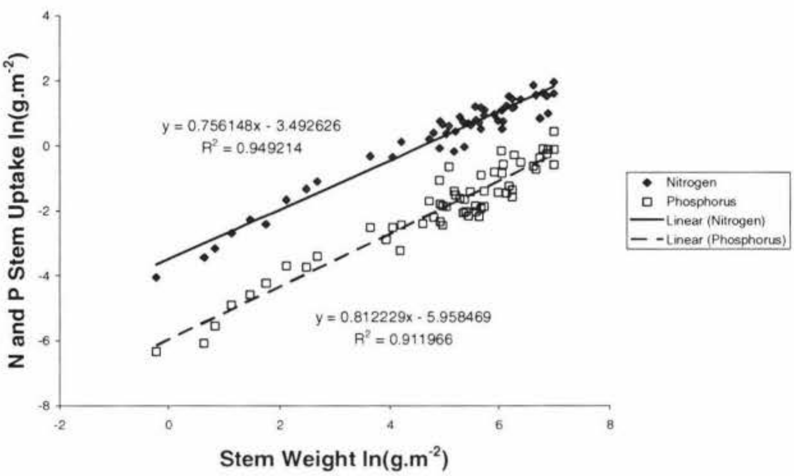

Figure 19 Stem N and P uptake rates v stem weight, on an area basis

For stem uptake, it is clear that N and P are taken up at similar rates for a given size of plant, as evidenced by the closely parallel regression trend lines. It will also be noted that the intercept for each element corresponds to the (natural log) uptake of that element for 1 g of stem dry matter (natural log 0 = 1.0, so the intercept represents this value). Thus

Stem nitrogen uptake = 0.0304g. dry matter stem

Stem phosphorus uptake = 0.0026g. dry matter stem

3.3.2.4. N and P uptake for leaf

Uptake values for N and P are presented in Figure 20.

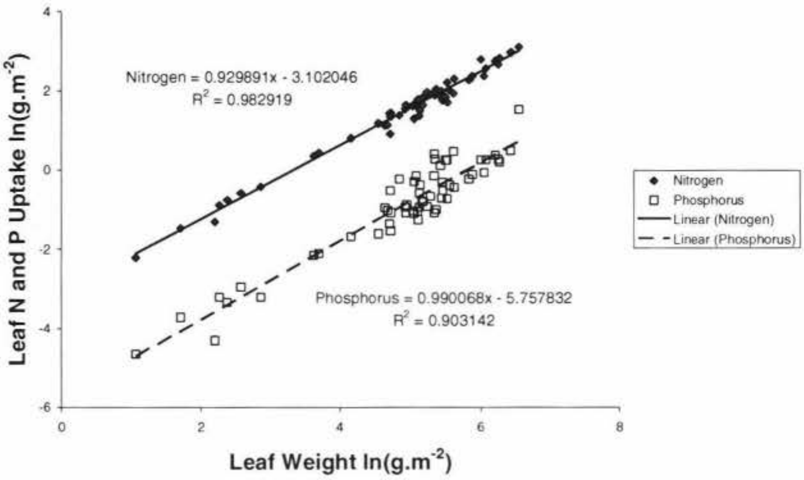

Figure 20 Leaf N and P uptake v Leaf wt, on an area basis

Once again, the lines are closely parallel, indicating that hemp takes up N and P in leaves at virtually the same rate irrespective of plant size. Again, the intercepts indicate a model for N and P uptake, as discussed above.

Leaf nitrogen uptake	= 0.0450g. dry matter leaf
Leaf phosphorus uptake	= 0.0032g. dry matter leaf

3.3.3. Harvest date effects

The effect of harvest date (in months) on total N and P uptake is presented in Figure 21.

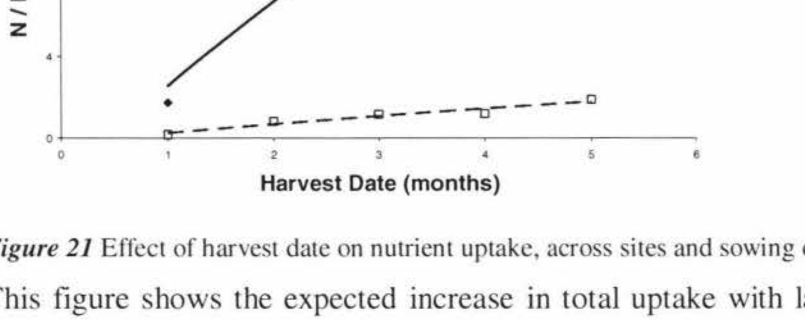

Figure 21 Effect of harvest date on nutrient uptake, across sites and sowing dates

This figure shows the expected increase in total uptake with later harvests and larger plants. A quadratic line has been added for clarity. Since both nitrogen (%) and phosphorus (%) reduce for both stem and leaf over the growth cycle of the plant, Figure 21 indicates that the rapidly increasing total biomass more than compensates. It is thus clear that allowing the plants to reach maturity will optimize nutrient removal from the growth medium, to the asymptotic maximum illustrated and discussed above.

Total nitrogen and phosphorus uptake estimates for each of five successive harvests were calculated as outlined above, with results shown in Table 10. It should be noted that these estimates were based on combined results of three successive sowings. Thus the harvest data contained progressively fewer data points, and the last (5th) only 1. The later harvests should therefore be considered indicative only, due to the inherent weighting constraints outlined in section 3.2.2.2.

Table 10 *Nutrient uptake ($g.m^{-2}$) for 30 plant.m^{-2}*

	Nutrient Uptake ($g.m^{-2}$) for 30 Plant.m^{-2}			
	Nitrogen		Phosphorus	
Harvest	Leaf	Stem	Leaf	Stem
1	1.24	0.28	0.11	0.03
2	5.05	1.60	0.48	0.18
3	6.19	2.06	0.60	0.24
4	9.27	3.39	0.92	0.41
5	13.33	5.32	1.35	0.66

This table, subject to the constraints indicated, shows the steadily increasing uptake values for both N and P. Thus the rapidly increasing biomass in both leaf and stem more than compensate for the observed reduction in both N and P concentrations over the life of the plant.

3.4. Conclusions

3.4.1. Leaf/stem dry weight

The dry weight allometric relationship of stem and leaves (on a per plant basis) in industrial hemp can be described by the formulae:

Leaf weight = 0.834056(total weight) - 0.427748

Stem weight = 1.180607(total weight) - 1.141588

This is true, with high probability, irrespective of latitude (41°S to 43°S), sowing date (October to December) or harvest date (monthly from one month after sowing). The (natural log) leaf dry weights decrease with respect to (natural log) stem dry weight as total (natural log) above ground biomass increases. Although this thesis is concerned with hemp seed oil (experiment 1) and nutrient uptake (experiment 2), this allometric relationship, in relation to density effects found in experiment 1, could be significant for hemp fibre production.

When leaf and stem weights are considered on an area basis, the equivalent formulae are:

(ln)leaf = 0.797861(ln total weight) + 0.350278

(ln)stem = 1.215718(ln total weight) - 1.963915

These results explain a high level of total variability, irrespective of latitude, sowing date or harvest date. When considered in conjunction with density effects, as discussed in section 2, this could be relevant to fibre production.

3.4.2. Nitrogen and phosphorus uptake.

Leaf and stem uptake values for nitrogen and phosphorus were compared against total plant N and P respectively. These showed a similar trend to the weight figures reported above, but with less pronounced tendency to converge at higher plant weight.

Stem nitrogen and phosphorus uptake values were compared to total stem weight, and equivalent results calculated for leaves. The results showed the following relationships:

Stem N uptake= (exp) -3.492626 = 0.0304 $g.m^{-2}$ D.M. stem

Stem P uptake = (exp) -5.958469 = 0.0026 $g.m^{-2}$ D.M. stem

Leaf N uptake = (exp) -3.102046 = 0.0450 $g.m^{-2}$ D.M. leaf

Leaf P = (exp) -5.757832 = 0.0032 $g.m^{-2}$ D.M leaf

Plotting values for these equations as leaf/stem ratios against total weight gives a consistent pattern for the two elements, with nitrogen being consistently some 3-5 times the uptake rate of phosphorus. The relationships show initial high uptake rates, settling to a stable, lower value as the plant matures. The values for leaf nitrogen and phosphorus compare favorably with the work of Ketterings *et. al.*

(2006) for grass of 0.0200 g(N).g(DM)-1 and 0.0029 g(P).g(DM)-1 respectively. Thus nitrogen uptake by hemp leaf would appear to be more than twice that for grass. Even for stem (not directly comparable to grass) uptake is some 50% higher. However the equivalent rates for phosphorus are similar to those for grass. Hemp would thus appear to be a favorable alternative to grass in terms of N and P uptake, while still producing a potentially valuable product as an output.

Sites differences could well be due to differences between nutrient feedstock and local soil/climate effects, but it can be stated that optimum removal is achieved by sowing as early as possible (October or earlier), and harvesting as late as possible (5 months or longer). The high biomass accumulation in hemp more than compensates for the high concentration of both Nitrogen and Phosphorus in young plants.

4. General Conclusions

The two experiments in this project, while apparently similar, were in fact designed for complementary purposes. Experiment 1 focused on seed production, and the growth and density parameters involved; experiment 2 primarily explored nitrogen and phosphorus uptake from effluent irrigation. Experiment 1, with two varieties, provided insight into factors of height, growing time, harvest index, and plant density effects, and gave indications of latitude effects on these factors. More importantly, the two varieties (cv Anka and cv Finola) gave information on the interactions between these effects, particularly varietal differences (sections 2.3, 3.3). This was possible, notwithstanding, problems due to poor seed quality, predation, weed infestation and wind damage, all of which contributed to low yield and density, and missing plots.

Results were achieved despite these problems by statistical modeling, based on the premise that plants grow exponentially. Transforming data to natural logarithms will thus produce a straight line, from which a linear regression yields intercept and slope for interpretation, with an estimate of error for statistical analysis. Similarly, an asymptotic limitation to growth such as competition for light, nutrient or other resource can be modeled by a reciprocal transform. Again, the resulting linear regression allows interpretation of intercept and slope of the line. As these two effects are independent, both can be used as applicable, as was done to delineate allometric relationships (section 3.3.1), where both exponential growth and internal shading occurred.

By these means it is concluded that the harvest index of Anka falls dramatically with later sowing, where the Finola harvest index does not. This different result between cultivars is reflected in biomass, growing time, seed yield and height, and indicates the strong possibility that Finola is a day-length neutral plant. Experiment 2, with only one cultivar (cv EIL1), could not provide this level of insight, but chemical analysis showed that greater quantities of nitrogen and equivalent quantities of phosphorus, can be extracted compared to grass. It was shown that the exponentially increasing biomass yield more than compensates for the reduction in %N and %P throughout the growth cycle of the plant. Successive harvests throughout the plant growth cycle, provided insight into allometric changes in chemical composition, complementary to the single harvest data available from experiment 1. Conversely, plant density data from experiment 1 was used to delineate density effects, where data from experiment 2, with several plots damaged by herbicidal spray, could not.

5. Future Work

Both these experiments were intended as preliminary trials, to define parameters for more exhaustive future investigations, and to identify useful areas of further study.

The main points identified include:

- Latitude effects. Some evidence was obtained indicating latitude effects in plant height, growing time and harvest index. This was inconclusive, primarily due to loss of data at our most southerly site (experiment 1) and the more northerly site (experiment 2). Further trials are required, at more sites, to confirm any such effect.
- Sowing date. Strong evidence was found indicating potential improvements in productivity and yield from an extended growing period, possibly leading to double cropping potential using different varieties. Effects of frost damage in spring, vernalization and floration inhibiting growth were not investigated however, and further trials are required to establish effective sowing date limits.
- Plant density. Varietal differences and asymptotic limits were established, but it is clear that these limits were not approached at the densities achieved in our trial. Further trials at higher densities are required to refine yield limits.
- Varieties. Very clear varietal differences in yield have been established with two varieties in experiment 1, particularly with respect to sowing date, and allometric

relationships with one variety in experiment 2. A trial with a wider range of varieties is required, to identify and quantify the extent of these points of difference.

- Nutrient uptake. Our results confirm that considerable quantities of nitrogen and phosphorus can be removed from nutrient rich effluent. No information on varietal differences was gained however, and further trials with different varieties are clearly warranted.

A trial or series of trials can be envisaged involving a wide range of cultivars, latitudes, plant densities, sowing dates and effluent levels. Our data confirms that such trial(s) are warranted. This was one of our main objectives, with the expectation throughout our trial period of a realistic resolution of New Zealand licensing regulations (at that time under consideration) for growing hemp. In fact the regulations, after a four year delay, were finally promulgated in July 2006 (Misuse of Drugs (Industrial Hemp) Regulations 2006), and came into effect August that year. The main thrust of the new regulations is to severely inhibit any further trials in this area. The new licensing requirements involve $550 per site (plus GST), an additional $100 *if the crop is to be grown for research or breeding purposes* (my italics). A further clause involves additional costs for testing of each plot for THC levels, and a requirement that the crop be not sown within 5km of a residential zone. Thus to find suitable sites, and conduct trials as indicated by our results, will require considerable additional funding, making any such research prohibitively expensive without private funding.

References

Amaducci, S., Errani, M., Venturi, G. (2002). Response of hemp to plant population and nitrogen fertilisation. Italian Journal of Agronomy 6 (2) : 103-111

Bennett, S.J., Snell, R., Wright, D. (2006). Effect of variety, seed rate and time of cutting on fibre yield of dew-retted hemp. *Industrial Crops and Products, 24 (1): 79-86.*

Charkowski, E., (1999). Hemp "Eats" Chernobyl Waste, Offers Hope for Hanford retrieved on 15/01/2006 from http://hemp.net/news/9901/06/hemp_eats_chernobyl_waste.html.

Callaway, J.C., Laakkonen, T.T. (1996). Cultivation of cannabis oil seed varieties in Finland. *J Int Hemp Assoc 1996:3(1):32-34*

Callaway, J.C., (2004). Hempseed as a nutritional resource: An overview. *Euphytica 140 (1-2): 65-72.*

Callaway, J., Schwab, U., Harvima, I., Halonen, P., Mykkanen, O., Hyvonen., P., Jarvinen, T. (2005). Efficacy of Dietary Hempseed Oil in Patients with Atopic Dermatitis. *Journal of Dermatological Treatment. 16:87-94.*

Cook, J., (1779) Narrative of the Voyages Round the World; Chapter II: Narrative of Captain Cook's First Voyage Round the World.

Drew, T. J., Flewelling, J. W., (1977). Some Recent Japanese Theories of Yield-Density Relationships and their Application to Monterey Pine Plantations. *Forest Science, Volume 23, Number 4, 1 pp. 517-534(18).*

Fosu, M., (2003) Nitrogen accumulation and release by sunn hemp, calopo, mucuna and devil bean in semi-arid Ghana. *Agricultural and Food Science Journal of Ghana 2 : 141-153*

Gulati, D., Sain, M. (2006). Fungal-modification of natural fibers: A novel method of treating natural fibers for composite reinforcement. *Journal of Polymers and the Environment 14 (4): 347-352.*

Herodotus: *The History* (Chap. 73-75); Univ. Chicago Press; David Greene, transl.; ISBN 0-226-32770-I.

Ketterings, Q. M., Godwin, G., Kilcer, T. F., Barney, P., Hunter, M., Cherney, J. H., Beer, S. (2006). Nitrogen, phosphorus, potassium, magnesium and calcium removal by brown midrib sorghum sudangrass in the Northeastern USA. *Journal of Agronomy and Crop Science 192 (6) : 408-416.*

Khan, A.G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology 18 355–364*

Lehoczky, E., Nemeth, T., Reisinger, P., Radimszky, L., Komives, T. (2006). Effect of weediness on the water content of the soil: A field study. *Communications in Soil Science and Plant Analysis 37 (15-20): 2673-2678.*

Leizer, C., Ribnicky, D., Poulev, A., Dushenkov, S., Raskin, I. (2000). The Composition of Hemp Seed Oil and Its Potential as an Important Source of Nutrition. *Journal of Nutraceuticals, Functional & Medical Foods Vol. 2(4)*

Lisson, S.N., Mendham, N.J., Carberry, P.S. (2000). Development of a hemp (Cannabis sativa L.) simulation model 1. General introduction and the effect of temperature on the pre-emergent development of hemp. *Australian Journal of Experimental Agriculture 40 (3): 405-411*

Lisson, S.N., Mendham, N.J., Carberry, P.S. (2000). Development of a hemp (Cannabis sativa L.) simulation model 2. The flowering response of two hemp cultivars to photoperiod. *Australian Journal of Experimental Agriculture 40 (3): 413-417*

Lisson, S.N., Mendham, N.J., Carberry, P.S. (2000). Development of a hemp (Cannabis sativa L.) simulation model 3. The effect of plant density on leaf appearance, expansion and senescence. *Australian Journal of Experimental Agriculture 40 (3): 419-423*

Lisson, S.N., Mendham, N.J., Carberry, P.S. (2000). Development of a hemp (Cannabis sativa L.) simulation model 4. Model description and validation. *Australian Journal of Experimental Agriculture 40 (3): 425-432*

Liste, H.H., Prutz, I. (2006). Plant performance, dioxygenase-expressing rhizosphere bacteria, and biodegradation of weathered hydrocarbons in contaminated soil. *Chemosphere 62 (9): 1411-1420*

Lu, X., Clarke, R.C. (1995). The cultivation and use of hemp (*Cannabis sativa* L.) in ancient China. *Journal of the International Hemp Association* Vol. 2 No. 1.

McIntosh, D. J., (1998). The 5 minute guide to industrial hemp in New Zealand. *New Zealand Hemp Hemp association: pp62.*

McColl, R.H.S., Gibson, A.R., (1979). Downslope movement of nutrients in hill pasture, Taita, New Zealand. 2. Effects of season, sheep grazing, and fertiliser. *New Zealand Journal of Agricultural Research 22 (1) : 151-161.*

McPartland, J. M., Cutler, S., McIntosh, D. J. (2004). Hemp production in Aotearoa. *Journal of Industrial Hemp 9 (1) : 105-115*

Misuse of Drugs (Industrial Hemp) Regulations 2006 and the Misuse of Drugs Amendment Regulations 2006.

Moliterni, V.M.C., Cattivelli, L., Ranalli, P., Mandolino, G., (2004). The sexual differentiation of Cannabis sativa L.: A morphological and molecular study *Euphytica. 140 (1-2): 95-106*

Rollin, C., (1731). Rollin's Ancient History: Egypt. Of The Egyptian Soldiers and War.

Sawabe, A., Takeda, R., Komemushi, S., (2006). Phytoremediation: searching for plants with high environmental purification capacity. *Memoirs of the Faculty of Agriculture of Kinki University 39 : 1-8*

Struik, P.C., Amaducci, S., Bullard, M.J., Stutterheim, N.C., Venturi, G., Cromack, H.T.H. (2000). Agronomy of fibre hemp (Cannabis sativa L.) in Europe. *Industrial Crops and Products 11 (2-3): 107-118*

Tang, C.H., Ten, Z., Wang, X.S., Yang, X.Q. (2006). Physicochemical and functional properties of hemp (Cannabis sativa L.) protein isolate. *Journal Of Agricultural And Food Chemistry 54 (23): 8945-8950.*

Tlustos, P., Szakova, J., Hruby, J., Hartman, I., Najmanova, J., Nedelnik, J., Pavlikova, D., Batysta, M. (2006). Removal of As, Cd, Pb, and Zn from contaminated soil by high biomass producing plants. *Plant, Soil and Environment 52 (9) : 413-423*

Wambua, P., Vangrimde, B., Lomov, S., Verpoest, I. (2007). The response of natural fibre composites to ballistic impact by fragment simulating projectiles. *Composite Structures 77 (2): 232-240.*

Werf, H.M.G. van der, Wijlhuizen, M., Schutter, J.A.A. de. (1995) Plant density and self-thinning affect yield and quality of fibre hemp (Cannabis sativa L.). *Field Crops Research 40 (3) : 153-164.*

Yang, L., Chang, H.Ting., Huang, M.N.L. (2001). Nutrient removal in gravel- and soil-based wetland microcosms with and without vegetation. *Ecological Engineering 18 (1) : 91-105*

Appendix A.

Final 5% significance model for height at harvest, from Rangiriri and Kerikeri (sowings 2, 3 and 4).

Rangi/Keri height

Dependent Variable: height height

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	6.41447698	1.06907950	34.85	<.0001
Error	64	1.96348077	0.03067939		
Corrected Total	70	8.37795775			

R-Square	Coeff Var	Root MSE	height Mean
0.765637	23.84665	0.175155	0.734507

Source	DF	Type I SS	Mean Square	F Value	Pr > F
site	1	0.46018791	0.46018791	15.00	0.0003
variety	1	5.46667081	5.46667081	178.19	<.0001
sowing*variety	4	0.48761826	0.12190457	3.97	0.0061

Rangi/Keri height

Level of site	N	------------height---------- Mean	Std Dev
Kerikeri	35	0.65285714	0.32402407
Rangiriri	36	0.81388889	0.35246299

Level of variety	N	------------height---------- Mean	Std Dev\
anka	36	1.00694444	0.25971214
finola	35	0.45428571	0.13249386

Level of sowing	Level of variety	N	------------height---------- Mean	Std Dev
DEC2005	anka	12	1.07500000	0.19598237
DEC2005	finola	12	0.40416667	0.08106769
JAN2006	anka	12	0.85000000	0.18090681
JAN2006	finola	11	0.48636364	0.15666989
NOV2005	anka	12	1.09583333	0.32083579
NOV2005	finola	12	0.47500000	0.14538351

Appendix B.

Model derived for reciprocal seed yield (intercept) across sites, sowings and varieties;

Rangi/Keri seed (ln) yield

Dependent Variable: intercpt intercpt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	13.44751580	4.48250527	8.98	<.0001
Error	44	21.95604562	0.49900104		
Corrected Total	47	35.40356142			

R-Square	Coeff Var	Root MSE	intercpt Mean
0.379835	-31.97603	0.706400	-2.209155

Source	DF	Type I SS	Mean Square	F Value	Pr > F
sow	2	3.81596311	1.90798155	3.82	0.0294

variety	1	9.63155270	9.63155270	19.30	<.0001

Rangi/Keri seed (ln) yield

Level of sow	N	intercpt Mean	intercpt Std Dev
2	20	-1.93967701	0.79772658
3	13	-2.16786360	0.76243213
4	15	-2.60424407	0.94570378

Level of variety	N	intercpt Mean	intercpt Std Dev
anka	23	-2.67713262	0.80182449
finola	25	-1.77861510	0.69486452

Appendix C.

Analysis of variance and means for plant density effects at 30 plants.m^2.

Rangi/Keri density 30

Dependent Variable: yield30 yield30

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	431391.3654	61627.3379	11.55	0.0042
Error	6	32027.3824	5337.8971		
Corrected Total	13	463418.7478			

R-Square	Coeff Var	Root MSE	yield30 Mean
0.930889	33.46921	73.06091	218.2929

Source	DF	Type I SS	Mean Square	F Value	Pr > F
sow	3	163129.1726	54376.3909	10.19	0.0091
variety	1	148400.1373	148400.1373	27.80	0.0019
sow*variety	3	119862.0555	39954.0185	7.48	0.0188

Rangi/Keri density 30

Level of sow	N	yield30 Mean	yield30 Std Dev
1	2	461.569267	461.205799
2	4	232.033669	131.334485
3	4	180.919042	91.937951
4	4	120.287881	59.089717

Level of variety	N	yield30 Mean	yield30 Std Dev
anka	7	321.249271	218.223995
finola	7	115.336572	69.866944

Level of sow	Level of variety	N	yield30 Mean	yield30 Std Dev
1	anka	1	787.691015	.
1	finola	1	135.447519	.
2	anka	2	319.073215	65.149952
2	finola	2	144.994124	131.141889
3	anka	2	246.877333	38.261891
3	finola	2	114.960750	80.571728
4	anka	2	164.576391	47.846197
4	finola	2	75.999371	18.429239

Appendix D.

Analysis of variance and means for plant density effects at 60 plants.m^2.

Rangi/Keri density 60

Dependent Variable: yield60 yield60

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	47.1548318	47.1548318	5.64	0.0351
Error	12	100.3381214	8.3615101		
Corrected Total	13	147.4929533			

R-Square	Coeff Var	Root MSE	yield60 Mean
0.319709	65.07221	2.891628	4.443721

Source	DF	Type I SS	Mean Square	F Value	Pr > F
variety	1	47.15483185	47.15483185	5.64	0.0351

Rangi/Keri density 60

Level of variety	N	-----------yield60----------- Mean	Std Dev
anka	7	6.27898740	3.84806644
finola	7	2.60845486	1.38398154

Appendix E.

Analysis of variance and means for plant density effects at 90 plants.m^2.

Rangi/Keri/Massey density 90

Dependent Variable: yield90 yield90

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	174733.3619	174733.3619	5.39	0.0386
Error	12	388740.2491	32395.0208		
Corrected Total	13	563473.6110			

R-Square	Coeff Var	Root MSE	yield90 Mean
0.310100	61.59577	179.9862	292.2054

Source	DF	Type I SS	Mean Square	F Value	Pr > F
variety	1	174733.3619	174733.3619	5.39	0.0386

Rangi/Keri/Massey density 90

Level of variety	N	-----------yield90----------- Mean	Std Dev
anka	7	403.923595	238.484289
finola	7	180.487212	88.967890

Appendix F.

Analysis of variance and means for growth time (in days) to harvest

Rangi/Keri Days to harvest no sowing 1

Dependent Variable: growingdays growingdays

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	5	10618.35382	2123.67076	60.90	<.0001
Error	65	2266.57576	34.87040		
Corrected Total	70	12884.92958			

R-Square	Coeff Var	Root MSE	growingdays Mean
0.824091	8.714680	5.905116	67.76056

Source	DF	Type I SS	Mean Square	F Value	Pr > F
site	1	5254.964498	5254.964498	150.70	<.0001
sow	2	3538.063119	1769.031559	50.73	<.0001
site*sow	2	1825.326203	912.663102	26.17	<.0001

Rangi/Keri Days to harvest no sowing 1

Level of site	N	---------growingdays--------- Mean	Std Dev
Kerikeri	35	76.4857143	11.9148238
Rangiriri	36	59.2777778	8.9494169

Level of sow	N	---------growingdays--------- Mean	Std Dev
2	24	70.2500000	15.1147783
3	24	74.9166667	11.9379434
4	23	57.6956522	5.4140791

Level of site	Level of sow	N	---------growingdays--------- Mean	Std Dev
Kerikeri	2	12	85.0000000	0.0000000
Kerikeri	3	12	83.0000000	0.0000000
Kerikeri	4	11	60.0909091	7.0064905
Rangiriri	2	12	55.5000000	1.7320508
Rangiriri	3	12	66.8333333	12.4669259
Rangiriri	4	12	55.5000000	1.7320508

Appendix G.

Leaf weight and total above ground biomass data analysis of variance (5%) model.

Rangi/Keri/Massey leaf ratio

Dependent Variable: ratio ratio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1.29817176	0.16227147	7.00	<.0001
Error	57	1.32079277	0.02317180		
Corrected Total	65	2.61896453			

R-Square	Coeff Var	Root MSE	ratio Mean
0.495681	25.29028	0.152223	0.601903

Source	DF	Type I SS	Mean Square	F Value	Pr > F
site	1	0.53548550	0.53548550	23.11	<.0001
sow	3	0.26873919	0.08957973	3.87	0.0138
site*sow	2	0.27965420	0.13982710	6.03	0.0042
site*variety	2	0.21429286	0.10714643	4.62	0.0138

Rangi/Keri/Massey leaf ratio

Level of site	N	ratio Mean	ratio Std Dev
Kerikeri	25	0.48655105	0.18257610
Rangiriri	41	0.67223908	0.17912721

Level of sow	N	ratio Mean	ratio Std Dev
1	12	0.71362398	0.13255284
2	19	0.65281308	0.19331291
3	19	0.58107766	0.21510936
4	16	0.48238542	0.17968898

Level of site	Level of sow	N	ratio Mean	ratio Std Dev
Kerikeri	2	9	0.53452132	0.22137676
Kerikeri	3	8	0.41127830	0.12700497
Kerikeri	4	8	0.50785726	0.17943313
Rangiriri	1	12	0.71362398	0.13255284
Rangiriri	2	10	0.75927566	0.06775549
Rangiriri	3	11	0.70456810	0.17932692
Rangiriri	4	8	0.45691359	0.18843990

Level of site	Level of variety	N	ratio Mean	ratio Std Dev
Kerikeri	anka	15	0.55138252	0.16591230
Kerikeri	finola	10	0.38930386	0.16899158
Rangiriri	anka	20	0.63489008	0.18708716
Rangiriri	finola	21	0.70780955	0.16792295