University of Reading

Effects of planting density and nitrogen application on seed yield and other morphological traits of the leafy vegetable kale (Brassica oleracea).

Article

Accepted Version

Bennett, E., Gawthrop, F., Yao, C., Boniface, C., Ishihara, H., Roberts, J. and Wagstaff, C. (2013) Effects of planting density and nitrogen application on seed yield and other morphological traits of the leafy vegetable kale (Brassica oleracea). Aspects of Applied Biology, 119. pp. 201-216. ISSN 0265-1491 (special issue 'Fruits & Roots: a celebration and forward look') Available at http://centaur.reading.ac.uk/37437/

Publisher: Association of Applied Biologists

Effects of planting density and nitrogen application on seed yield and other morphological traits of the leafy vegetable kale (*Brassica oleracea*)

E J BENNETT[1], F GAWTHROP[2], C YAO[2], C BONIFACE[1], H ISHIHARA[1], J A ROBERTS[3] and C WAGSTAFF[1]

[1]*Department of Food and Nutritional Sciences and Centre for Food Security, University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP*
[2]*Tozer Seeds, Pyports, Downside Bridge Road, Cobham, Surrey KT11 3EH*
[3]*Plant and Crop Sciences Division, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD*
Corresponding Author Email: c.wagstaff@reading.ac.uk

Summary

The relationship between food security and sustainable land use is considered to be of the uttermost importance to increase yields without having to increase the agricultural land area over which crops are grown. In the present study nitrogen concentration (25 and 85 kg ha^{-1}) and planting density (6.7, 10 and 25 plants m^{-2}) were investigated for their effect on whole plant physiology and pod seed yield in kale (*Brassica oleracea*), to determine if the fruit (pod) yield could be manipulated agronomically. Nitrogen concentration did not significantly affect seed yield and it is therefore recommended that the lower concentration be used commercially. Conversely planting density did have a significant effect with increases in seed yield observed at the highest planting density of 25 plants m^{-2}, therefore this high planting density would be recommended commercially to maximise area efficiency, highlighting that simple agronomic changes are capable of increasing crop yields over a set area.

Key words: Nitrogen, planting density, kale, *Brassica oleraceae*, resource allocation, yield, seed

Introduction

Over successive years crops yields can be highly variable due to the interaction between numerous intertwined factors such as agrochemical applications, plant spacing, varietal differences, agronomic practices, integrated pest management and environmental conditions. For example, the long term Broadbalk experiment which began at Rothamstead Research in 1843 has clearly demonstrated how wheat yields have improved up to 2–3 times with changing husbandry practices, particularly NPK fertiliser application (Goulding *et al*., 2008; Rasmussen *et al*., 1998). The most successful example of improved agronomic practices having a significant and beneficial impact on crop yields comes from the “Green Revolution”, which began in the 1960s. For instance in the period 1960–2000 global grain yields doubled from 1 to 2 billion tons per year (Khush, 2001), thus highlighting the importance of this movement. Whilst it’s predicted that more developed countries (MDCs) would have been able to feed their inhabitants without the Green Revolution, on a global scale the increased yields have been attributed to raising the health status of 32–42 million pre-school children (Evenson & Gollin, 2003), thus helping to prevent starvation. As implied from the studies above, seed production and consequently final yield appears to be a highly plastic trait (Bennett *et al*., 2012), which offers both advantages and disadvantages. Whilst the ability to manipulate and enhance seed yield is essential for improving food security, the ease with which external factors can influence the process of seed production makes it more difficult to consistently achieve high yields every year.

The Brassicaceae family includes a wide range of vegetables such as broccoli, cabbage, kale and brussels sprouts, which are known to contain high levels of antioxidants, nitrates and glucosinolates. Amongst the Brassicaceae, kale leaves were found to contain some of the highest total antioxidant concentrations (2.65 mmol 100 g^{-1}) (Halvorsen *et al*., 2002) which has led to them being associated with numerous human health benefits such as reducing the incidence of cardiovascular disease and certain cancers (Stanner *et al*., 2004; Podsędek, 2007; Cartea & Velasco, 2008; Weitzberg & Lundberg, 2013). The ability to manipulate the investment of a kale plant so it favours its economically important organs, such as leaves and seeds, could help increase yields to meet the demand for the crop whilst requiring no additional land area and reducing resource inputs such as fertilisers and water. Regulating resource allocation using agronomic practice would enable kale production, and potentially that of other crops if the current work on kale can be used as a model for crop manipulation, to align with the food security goal of increasing yields without having to increasing the agricultural land area over which crops are grown. To date most research has focused on how to improve kale leaf production (Khan *et al*., 2012; Naik & Gupta, 2010), with few reports investigating ways to increase seed yield. This is because unlike other Brassicas such as oilseed rape (*Brassica napus*), kale seeds are not normally considered to be the economically important part of the plant. However given the recent rise in demand for Kale, seed breeders are now looking for ways to enhance both yields and germination efficiency from leafy vegetable crops such as kale, in order to meet the increased demand from growers. Several agronomic practices are capable of affecting final seed yield including nitrogen (N) fertilisation and planting density. N is taken up from the soil in the form of either nitrate (NO3-), ammonia/ammonium (NH3/NH4+) or urea ($CO(NH_2)_2$) and fundamental to a plants growth and survival, with deficiencies in this element resulting in growth retardation, an early senescence phenotype and reduced seed yield (Fan *et al*., 2009). In addition to being absolutely necessary it is also one of the most expensive fertilisers to apply (Masclaux-Daubresse *et al*., 2010), but despite this 85–90 million metric tonnes (MMt) is added worldwide to the soil every year (Good *et al*., 2004). Whilst global N fertiliser application is beginning to plateau, consumption is still expected to rise up to 2070 to help cultivate more crops that are needed to feed the ever growing world population (Frink *et al*., 1999). Over the last 40 years there has been an intensification of N fertiliser use which can be partly attributed to the "Green Revolution" as the superior yields achieved could only be attained due to higher fertiliser inputs. This increased reliance on N fertilisation to achieve higher yields has led to the application of excess N which can have detrimental environmental effects, potentially resulting in a substantial loss of marine life due to algae blooms (Vitousek *et al*., 2009), the advancement of global warming and ozone depletion (Wuebbles, 2009), in addition to causing problems such as lodging which actually reduce yield (Khush, 2001). Whilst the ingestion of excess nitrate/nitrite in the diet has been linked to an increased risk of developing cancer and in blue baby syndrome, methemoglobinemia (Weitzberg & Lundberg, 2013). In addition N is most effective within an optimal range and this depends upon the crop grown, as such, not all of the applied N is taken up by the plant. For instance in maize, rice and wheat on average only 38% of the applied N fertiliser is recovered in the crop (Goulding *et al*., 2008), with the remainder being lost to surface run off, leaching of nitrates, ammonia (NH_3) volatilisation or bacterial competition (Garnett *et al*., 2009), representing a substantial economic waste. In forage Brassicas, such as kale, it has been shown that the plant is capable of taking up additional N that is provided through fertilisation but when this exceeds the amount required for growth it actually has very little impact on final vegetative yield, for example when kale took up either 250 kg N ha^{-1} or 500 kg N ha^{-1} the yield remained unchanged at 25 t DM ha^{-1} (Fletcher *et al*., 2007).

In addition to the amount of fertiliser applied, planting density also plays a fundamental role in determining final yield as there will be increased competition between plants spaced closer together for resources such as water and soil minerals. It is predicted that feeding the growing world population, forecast to peak at 9–10 billion by 2050, will require doubling the area used for cropland by 2050 to maintain the current per capita food production (Waggoner, 1995). Whilst areas such as Latin American have a lot of rain fed soil most of this is rainforest and therefore unsuitable to be converted into agricultural land as this could further contribute towards climate change, therefore research into planting density is important to understand how limited land areas can be utilised to their full potential. From an economic point of view the ability to obtain higher yields without having to purchase more land would also benefit farmers. Previous work on manipulating seed yields in Arabidopsis took a destructive approach whereby pods were selectively removed from the plant to increase the source:sink (leaf:pod) ratio, this resulted in bigger pods that contained larger but fewer seeds (Bennett *et al*., 2012). To replicate this experiment in a crop on a commercial scale would not be feasible, thus one option to try and mimic the alteration of plant architecture is to plant the crops closer together as a reduction in the amount of light such plants are capable of intercepting will inevitably alter the canopy structure. Field trials involving winter oilseed rape (*Brassica napus* L.) have shown that the seed yield per plot can be significantly enhanced by increasing the planting density to 3.4 × 104 or 4.8 × 104 plants hm^{-2} (Zhang *et al*., 2012). Considering that the normal planting density for winter oilseed rape in China is between 1.8 × 104 and 2.4 × 104 plants hm^{-2}, this shows that yield could be further augmented simply by spacing the plants closer together, something which is desperately needed in China as most land suitable for agricultural purposes is already being used (Fan *et al*., 2011). Similar to N fertiliser application yield increase will plateau as the optimum plating density is reached and beyond this yield decreases will occur as the competition between plants intensifies (López-Bellido *et al*., 2005).

Few reports in the literature have considered the effects of different agronomic practices on the seed yield of the vegetable kale. Hence it has been selected for based on its leaf phenotype and not for seed yield, thus it is unlikely that the ideal conditions to maximise both leaf and seed production are identical. The objective of this study was to grow kale under different N fertiliser and planting density regimes to: (1) Determine the combined effect of N and planting density on kale seed yield as a means of ascertaining the best practice for maximising yield; (2) Identify morphological traits that can be used as early indicators of potential yield; (3) Analyse the relationship between seed yield and vegetative development.

Materials and Methods

Site description

The experiment was carried out over one growing season from July 2011 until August 2012 at Tozer Seeds, Cobham, South East England (51°19′32.8071″N, 000°24′40.6926″W). The soil type was slightly acidic sandy loam and no N fertiliser had been applied to the field the previous year. The trial field had been left fallow the previous growing season. Weather recordings (rainfall, maximum and minimum temperature) for this period were taken from the MET office, data collected at London Heathrow Airport (51°28′18.9390″N, 000°27′04.6953″W0) located 10.5 miles from the field trial and compared to averages from the previous 30 years, 1982–2012 (Table 1). This data highlights that the temperature from September 2011–January 2012 was slightly warmer than average by approximately 1.6°C. The rainfall during the study period was considerably different from the historical averages, with the winter of 2011–2012 having significantly less rainfall than normal but this was followed by a very wet spring and summer, for instance June 2012 experienced 110.8 mm of rainfall compared to the June average of 48.5 mm.

Crop management

The kale grown, Red Bull Sip seed, is a self-compatible inbred kale variety developed by Tozer Seeds Ltd, a British vegetable breeding company. Seeds were sown into trays on 10 June 2011 and germinated in a glasshouse before being transplanted into the field on 20 July 2011. Once transplanted the insecticide Spannet and a herbicide were applied at the recommended rates. Potassium sulphate and an additional herbicide application were given in March 2012 and the rest of the time the crop was hand weeded. No supplementary irrigation was given over the growing season.

Field trial design

The field trial took a block design and each block had a different N fertiliser level and kale planting density applied to it. A minimum of four plants were measured from the centre row of each block in which three staggered rows were planted. The blocks were planted 1 m apart and a 1 m^2 area of kale guard blocks, planted at a density of 10 plants m^{-2} were sown at the end of each row. N fertiliser was applied in the form of urea at three intervals, a base dressing was applied after transplanting in July 2011 and subsequent applications were given in March 2012 and May 2012 to bring the final N concentration to, 0, 105 and 210 kg ha^{-1}. However when the N concentration in the soil was tested post fertiliser application the values differed from what was expected and therefore the number of fertiliser treatments reported will be reduced to two, high N(85 kg ha^{-1}) and low N (25 kg ha^{-1}). Within the blocks three different planting densities were used, 6.7, 10 and 25 plants m^{-2}. For kale the commercial level of N fertiliser applied and planting density at Tozer seeds is 105 kg ha^{-1} N and 10 plants m^{-2} respectively.

Physiological measurements

Measurements taken from the main stem of the kale plant were as follows, pod spacing, pod abortion, plant height (August 2012), number of branches arising from the main stem and stem area. For the stem area a calliper was used to measure the diameter of the stem 10 cm above the ground in two directions at 90° to each other and the values were multiplied together to calculate stem area. Three pods representative of the pods on the main stem were sampled and measured for all the pod morphological traits, pod length, pod area and number of seeds per pod. To calculate pod area the pods were photographed and analysed using the software Image J (NIH Image). Upon harvest the plants were placed in a glasshouse and dried before being threshed and winnowed to remove the seeds from the pods. Seeds were stored at 10°C and 15% humidity to equalise the moisture content within them and kept under these conditions until they had reached a constant weight. These seeds were used to determine thousand grain weight (TGW), seed yield per plant and seed yield per hectare. After having its seed bearing pods removed at harvest the remaindered of the kale (leaves and stem) were dried to completion and weighed to calculate vegetative mass.

$$\text{Seed yield per hectare: } \frac{\text{Seed yield per plant (g)} \times \text{number of plants per block} \times 10}{\text{Block area } (m^2)} \ (\text{kg ha}^{-1})$$

$$\text{Harvest index: } \frac{\text{Seed yield per plant (g)}}{\text{Total plant biomass (g)}} \ \text{(g) (Donald \& Hamblin, 1976)}$$

$$\text{N-efficiency: } \frac{\text{Seed yield (kg ha}^{-1}\text{)}}{\text{N supplied (kg ha}^{-1}\text{)}} \ \text{(kg/kg) (Rathke } et\ al.\text{, 2006)}$$

Seed germination rate

50 seeds per plant were sown into boxes containing pleated filter paper, moistened with 50ml of water and incubated in a Fitron plant growth chamber at 20°C, 60% humidity, with a photon flux density of 200 µmol cm^{-2} s^{-1} and 16 h photoperiod. After 9 days the seeds were visually scored for normal germination according to the International Seed Testing Association (ISTA) (Don, 2006).

Statistics

Statistical analysis was performed using MINITAB version 16 (Minitab Inc., State College, PA, USA), except for the Pearson's correlation analysis which was calculated using IBM SPSS version 19 (Armonk, NY: IBM Corp). The difference in weather conditions between the year of the field trial and historical averages was calculated using a one-sample *t*-test with a significance level of P=0.05. The treatment interactions were investigated using ANOVA with general linear modelling (GLM). Analysis of individual traits was performed using a one-way ANOVA followed by Fisher's *A priori* test to determine which means were significantly different from each other. The trait means subsequently underwent a cluster analysis using ward linkage and the correlation coefficient distance measurement. The following variables were used for the principal component analysis (PCA): pod spacing, pod abortion, plant height, stem area, number of branches, vegetative mass, pod length, seeds per pod, thousand grain weight, seed yield per plant, pod area, harvest index and seed germination.

Results

The effect of nitrogen on seed yield

N-efficiency was the only trait to show a significant difference between the high and low N treatments (Fig. 1) with the low N at a planting density of 6.7 plants m^{-2} being significantly different from the high N treatment, although differences might be emphasised in this trait because the soil N concentration was used to calculate it. Whilst the kale grown at 10 and 25 plants m^{-2} doesn't show a significant difference in N-efficiency between high and low N levels the same trend exists at these higher planting densities. This demonstrates that under low N conditions the plants are able to use this resource more efficiently and are capable of achieving a high seed yield despite being N limited. The average N-efficiency is greatest when the kale were grown under the most extreme conditions, being spaced very close together (25 plants m^{-2}) and given a low amount of N (25 kg ha^{-1}) which would increase the intra-plant competition for resources. Aside from N-efficiency N levels had no significant effect on seed yield (Table 2), regardless of whether yield was expressed on a per plant or per hectare basis, as thousand grain weight, seed weight per pod, the number of seeds per pod or as harvest index. Therefore within each planting density seed yield was similar for both high and low N treatments, hence for the rest of the traits reported in this paper high and low N has been combined.

The effect of planting density on kale morphological traits

A principal component analysis (PCA) was performed to visually show the response of morphological parameters in kale to different spacing treatments (Fig. 2). Within the PCA distinct groups can be seen for the different planting densities (6.7, 10 and 25 plants/m^2). The plants grown at 10 and 25 plants m^{-2} form tighter and more distinct groupings compared to 6.7 plants m^{-2} which is more spread out across the graph. Indicating that when the plants are given more room to grow their development is less uniform.

The effect of planting density on kale architecture

Planting density affected the vegetative organs (Table 2) and altered how the kale plants grew, resulting in plant height, stem area and total vegetative mass being significantly greater when the kale is spaced further apart at 6.7 plants m^{-2} compared to 10 and 25 plants m^{-2} (Fig. 3). When the plants are provided with a larger area in which to grow the kale is less restricted which reduces the competition for resources, so the plant is able to invest more heavily in its vegetative organs. However this result is not universal as planting density affected the kale architecture on a whole plant basis but not at the level of individual organs as demonstrated by traits such as the number of branches per plant and pod length which were not significantly altered between different spacing's (Table 2).

The effect of planting density on seed yield

Planting density had a significant effect on the following seed yield parameters; seed yield per plant and seed yield per hectare (Table 2), indicating that as with the plant architecture altering the spacing effects yield on a whole plant basis but not at the level of the individual pod and seed. For instance variables such as the number of seeds per pod and seed weight per pod were not significantly altered between the planting densities. Depending on whether yield is assessed on a per plant or per hectare basis affects which planting density provides the greatest yield, as plants with more room to grow produce more seeds per plant, but densely planted kale yields more seeds per hectare (Fig. 4). On a per plant basis (Fig. 4a) the lowest and highest planting densities (6.7 and 25 plants m^{-2}) produced the greatest seed yields and although not significantly different from 25 plants m^{-2} the kale spaced furthest apart at 6.7 plants m^{-2} does produce the highest mean value. Conversely when seed yield is expressed on a kg ha^{-1} basis (Fig. 4b) the highest planting density (25 plants m^{-2}) produces a significantly greater seed yield than the kale spaced further apart at 6.7 and 20 plants m^{-2}. Demonstrating that increasing the number of plants within a set area by spacing them at 25 plants m^{-2} is not detrimental to seed yield production, plus kale grown at lower planting densities is not able to compensate for fewer plants over a given area by having each plant produce significantly more seeds or pods.

The relationship between seed yield and other traits

The relationship between all the different possible treatment combinations was analysed together in a Person's correlation matrix (Table 3). Several morphological traits are positively and significantly correlated with seed yield parameters, these are plant height, stem area, vegetative mass, pod length, number of seeds per pod, pod area and harvest index. Due to the ease with which stem area, pod length and plant height can be measured these traits could be used as early indicators of seed yield before the kale crop is harvested.

Kale appears to perform a trade-off with regards to resource allocation around the plant as it preferentially invests in the number of reproductive units as opposed to seed content. This is highlighted by the fact that seed yield (either per plant or per hectare) negatively and significantly correlates with seed germination (Table 3), indicating that as the seed yield increases less resources are available to fill each seed so there is a reduction in the number of viable seeds and therefore % seed germination. The point is further emphasized by plant height, which significantly and positively correlates with seed yield, number of branches per plant and vegetative mass. Implying that as the plant height increases this is accompanied by an increase in the number of pods per plant and hence seed yield. However at the same time plant height is significantly and negatively correlated with TGW, so as height increases the TGW decreases, implying that fewer resources are being put into seed content and instead they are preferentially getting placed into vegetative production and increasing the total number of seeds instead. An increase in seed yield is not just explained by bigger plants producing more pods because the yield is also significantly and positively correlated with the number of seeds per pod. Therefore as the number of seeds per pod increases so does seed yield.

The cluster diagram (Fig. 5) visually highlights the closest associations between different variables. This splits the variables into those mainly representing vegetative growth (pod spacing, number of branches, plant height, stem area and vegetative mass) compared to those representing pod traits (pod length, pod area, seeds per pod, seed yield per hectare, harvest index and seed germination). Showing a clear divide between vegetative and reproductive organs. The only exception to this is seed yield per plant which is more closely associated with the vegetative plant parameters such as plant height, stem area and vegetative mass. Indicating that the seed yield per plant is largely governed by these traits. However when the seed yield is assessed on a per hectare basis then it is more closely associated with the individual pod characteristics (pod length, pod area and seeds per pod). The slight anomalies are pod abortion and thousand grain weight (TGW) which are grouped together and appear to be more closely associated with the vegetative variables as opposed to the pod traits which they measure.

Discussion

Planting density has a greater effect than soil nitrogen concentration on seed yield

To date very few studies, if any, have looked at the effect of both planting density and N fertilisation on kale with regards to seed yield, instead most reports have focused on maximising leaf production for human consumption or use as a forage material for cattle (Skaland & Hillestad, 1971; Jankowiak *et al*., 1988; Chakwizira *et al*., 2011; Gent, 2002; Gowers & Armstrong, 1994; Mehwald, 1976; Fletcher & Chakwizira, 2012). Oilseed rape (*Brassica napus*) which is grown for its oil rich seeds is capable of producing higher seed yields when both N levels and planting density are increased (Zhang *et al*., 2012; Allen & Morgan, 1972), hence both of these agronomic practices were investigated in kale. The current study found that N levels had no significant effect on kale seed yield and instead planting density was the major contributor to the yield differences observed (Table 2). This result is further highlighted in the PCA analysis in which groupings can be made on the basis of planting density (Fig. 2). Based on this data it would be recommended that kale is sown close together (25 plants m^{-2}) and with minimal N input (25 kg ha^{-1}) to maximise seed yields over a given area (Fig. 4b). As individual plants are unable to produce a significantly higher yield when spaced further apart they are not capable of compensating for the higher number of plants being grown at lower planting densities (Fig. 4). Growing the plants under what might be perceived as more stressful conditions, whereby the competition for light, nutrients and water has increased is not necessarily wholly detrimental so long as the stress is not too severe. Imposing a mild water stress to wheat (*Triticum aestivum*) actually accelerated the rate of grain filling due to an increase in the concentration of the plant hormone ABA and decrease in ethylene production within the grains (Yang *et al*., 2006). A study in cotton, similar to the present experiment, also demonstrated that the combination of high planting densities and no fertiliser led to a greater reproductive allocation which determines the yield of seed crops (Sadras *et al*., 1997), plus N remobilisation is believed to be favoured under conditions where nitrite supplies are limited (Lemaitre *et al*., 2008), which might explain why the low N treatments did not appear to have a detrimental effect of seed yield. Despite the positive effects observed with a low N fertiliser regime this element is crucial for plant growth and development as it is an essential component of amino acids, nucleic acids and chlorophyll, thus reducing the fertilisation rate below 25 kg ha^{-1} would likely result in detrimental effects such as early tissue senescence and consequently lower yields. In addition N fertilisation has also been shown to positively impact upon phosphate uptake by increasing extracellular phosphatase activity, thus aiding the acquisition of other essential soil elements (Treseder & Vitousek, 2001). No significant difference in the number of seeds per pod or seed weight with regards to the treatments applied was observed (Table 2), indicating as in oilseed rape studies, that an increase in seed yield must be due to the plants producing more pods and therefore seeds over a given area as opposed to investing more resources into the seeds and increasing an individual seeds weight (Zhang *et al*., 2012; Allen & Morgan, 1972).

Soil nitrogen availability

Soil type, temperature, moisture and pH will significantly impact the concentration of N and other elements that are readily available for a plant to take up. The sandy loam soils that were present in the current trial are known for their ability to easily leach nitrate and poor N retention capacity (Defra, 2010), however this can be advantageous as nitrate is able to move throughout the whole soil matrix. The weather during the 2011–2012 growing season with regards to rainfall significantly varied from historical averages (Table 1), with a drought declared in many parts of the UK in April 2012, including the South East where the field trial was grown. This was the result of a very low amount of rainfall during the 2011–2012 winter (Oct-March). However, by April 2012 there was a remarkable hydrological transformation which resulted in April–July 2012 being the wettest in England and Wales on record (Marsh & Parry, 2012). This unusual weather pattern will have undoubtedly impacted upon the kale growth, especially as both drought and water logging are known to result in an increased rate of leaf senescence (Rosenow & Clark, 1981), the retardation of growth and development (Jensen *et al*., 2011), a decrease in photosynthesis, an alteration of the hormone balance within the plant (Zhou & Lin, 1995) and most importantly a reduction in yield (Sutton, 2009). However under wet conditions if urea fertiliser is applied, which it was in the current trial, this will undergo a rapid conversion into ammonium which can be quickly absorbed by the plant (Mendieta-Araica *et al*., 2013) thus enhancing N accumulation (Schjoerring *et al*., 1995). Rainfall also aids the incorporation of urea into the soil and can therefore reduce ammonia volatilization (Camberato, 2012). Therefore when urea is used as a fertiliser it would be most beneficial to supply supplementary irrigation following the fertiliser application to ensure that the kale can easily uptake the N and help reduce losses due to ammonia volatilisation. Although the abnormal weather conditions experienced throughout the growing season may have ultimately resulted in N leaching from the soil making it less available to the plant, hence masking differences that were expected to arise from using two N concentrations. As such the trial would need to be repeated over at least one more season to help ascertain with any certainty if N input significantly affects yield.

The effects of planting density on seed content and subsequent germination

There are two ways of partitioning resources into reproductive development, either by making more seeds with fewer reserves per seed or heavier seeds that contain additional resources beyond the minimum required for seed germination. The correlation analysis (Table 3) implies that the strategy employed by kale is to increase the number of seeds as opposed to investing more within each seed, this is highlighted as seed yield and the number of seeds per pod is positively correlated, whereas there is no correlation between seed yield and TGW. Furthermore the significantly negative correlation between seed yield and germination of the seeds generated from the trial (Table 3) implies a reduction in some component of the seed composition, a likely consequence of having to split resources between more units. Whilst maintaining reproductive organ size constant across different planting densities (Table 2) could highlight that kale utilises a mechanism to buffer the pod against stress when other vegetative organs are varying in their size and number (Harper *et al*., 1970). Whilst this goes against the Bennett *et al.* (2011) study which saw an increase in pod size and seed weight under the most extreme pod removal treatment when more resources were available, Harper *et al.* (1970) theorises that such examples are only seen when part of the plant is surgically removed. Therefore increasing planting density only has a minimal effect on seed weight.

The relationship between vegetative development and seed yield

The domestication of kale has primarily selected for vegetative leaf development and more recently increased concentrations of compounds such as antioxidants (Soengas *et al*., 2012). In the current study seed yield per plant was positively correlated with vegetative traits such as plant height, stem area and vegetative mass (Table 3), something that is also seen in other crops such as wheat (*Triticum aestivum*) (Qin *et al*., 2013). However this significance is lost when yield is expressed on a per area basis. Thus whilst bigger plants are capable of producing more seeds, although not heavier ones as the TGW was not positively associated with vegetative traits, they will also occupy more space and the increased seed yield per plant is unable to compensate for having fewer plants within a set area. Upon seed filling in some plant species, exogenous N uptake during this period is dramatically decreased as the plant becomes a closed system and as such the free N within the plant is not enough to fulfil the requirements of the seeds (Rossato *et al*., 2001). Hence the re-allocation of photoassimilates and pool of internal N from the leaves, stems and pod walls is required to complete this process (Salon *et al*., 2001; Schiltz *et al*., 2005; Masclaux-Daubresse *et al*., 2010). Consequently increasing the vegetative mass per plant and therefore photosynthetic area will naturally result in an increased seed yield, which for kale is in the form of producing more seeds as opposed to heaver seeds. Making kale, like other Brassicas, an R-strategist whose sole purpose is to produce seeds in high numbers and rapidly so that their genetic material is carried forward into the next generation (Bennett *et al*., 2012). As such determining when kale stops assimilating N could be a useful parameter to measure in future studies to indicate the last time point at which applying N fertiliser will have a beneficial effect on crop physiology. This could be achieved by applying 15N fertiliser at different time points throughout the growing season to determine when 15N stops being taken up. Due to the significantly positive correlation between stem area, plant height and pod length with seed yield per plant and for the latter trait seed yield per hectare then these phenotypic variables could be used as early indicators of potential seed yield for a particular crop of kale. Especially given the ease with which it is possible to measure such physiological parameters. In other Brassicas such as oilseed rape (*Brassic napus*) leaf area provided a good early indicator of seed yield (Xue *et al*., 1998).

Conclusion

This study provides new information about the effects of N application and planting density on the harvestable phenotype of kale and how this influences seed yield which will impact upon the agronomic recommendation adopted by seed breeders. Nitrogen is an expensive commodity to apply to kale and one which may have been over applied with regards to increasing seed yield, as such this report would recommend lower amounts of N fertiliser be used (25 kg ha^{-1}) in the future to help maximise yield whilst reducing the costs faced by the farmer and potential detrimental environmental effects resulting from run-off. Similarly kale can also be planted closer together at 25 plants m^{-2} to increase seeds yields. One must be careful however not to assume that by continually planting the kale closer together will automatically increase yield, as there is a limit to which this agronomic trait can be manipulated. At very high densities seed yield per area will decrease as the plants will not be able to undertake sufficient growth to produce reproductive structures. In addition to agronomic changes investigated in this study there is a need to develop a kale breeding program or TILLING population to find varieties that have a reduced incident of pod abortion (which was found to be between 70–80% in the current study) and show an increased resilience to environmental stress.

Acknowledgements

EB would like to thank Tozer Seeds, the University of Nottingham, and the University of Reading Endowment Trust Fund for funding her PhD.

References

Allen E J, Morgan D G. 1972. A quantitative analysis of the effects of nitrogen on the growth, development and yield of oilseed rape. *The Journal of Agricultural Science* **78**:315–324.

Bennett E, Roberts J A, Wagstaff C. 2012. Manipulating resource allocation in plants. *Journal of Experimental Botany* **63**:doi: 10.1093/jxb/err442.

Bennett E J, Roberts J A, Wagstaff C. 2011. The role of the pod in seed development: strategies for manipulating yield. *New Phytologist* **190**:838–853.

Camberato J J. 2012. Resource management and environmental impacts. In *Handbook of soil sciences*, 2nd Edn. Eds P M Huang, Y Li and M E Sumner. Boca Raton, FL, USA.

Cartea M, Velasco P. 2008. Glucosinolates in Brassica foods: bioavailability in food and significance for human health. *Phytochemistry Reviews* **7**:213–229.

Chakwizira E, Moot D J, Scott W R, Fletcher A L, Maley S. 2011. Leaf development, radiation interception and radiation-use efficiency of kale crops supplied with different rates of banded or broadcast phosphorus fertiliser. *Crop & Pasture Science* **62**:840–847.

Defra. 2010. *Fertiliser manual (RB209)*, 8th Edn. London, UK: Her Majesty's Stationery Office.

Don R. 2006. *ISTA Handbook for Seedling Evaluation*. Zurich: International Seed Testing Association.

Donald C M, Hamblin J. 1976. The Biological Yield and Harvest Index of Cereals as Agronomic and Plant Breeding Criteria. In *Advances in Agronomy*, p. 27. Ed. N C Brady. New York: Academic Press.

Evenson R E, Gollin D. 2003. Assessing the Impact of the Green Revolution, 1960 to 2000. *Science* **300**:758–762.

Fan M, Shen J, Yuan L, Jiang R, Chen X, Davies W J, Zhang F. 2011. Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. *Journal of Experimental Botany* **63**:13–24

Fan S C, Lin C S, Hsu P K, Lin S H, Tsay Y F. 2009. The Arabidopsis Nitrate Transporter NRT1.7, Expressed in Phloem, Is Responsible for Source-to-Sink Remobilization of Nitrate. *Plant Cell* **21**:2750–2761.

Fletcher A L, Brown H E, Maley S, Wilson D R. 2007. Forage production and nitrogen uptake of kale. In *Meeting the challenges for pasture based dairying*, pp. 335–342. Proceedings of the 3rd Australasian Dairy Science Symposium, Melbourne, Victoria. Ed. D F Chapman.

Fletcher A L, Chakwizira E. 2012. Developing a critical nitrogen dilution curve for forage brassicas. *Grass and Forage Science* **67**:13–23.

Frink C R, Waggoner P E, Ausubel J H. 1999. Nitrogen fertilizer:Retrospect and prospect. *Proceedings of the National Academy of Sciences of the United States of America* **96**:1175–1180.

Garnett T, Conn V, Kaiser B N. 2009. Root based approaches to improving nitrogen use efficiency in plants. *Plant, Cell & Environment* **32**:1272–1283.

Gent M P N. 2002. Growth and composition of salad greens as affected by organic compared to nitrate fertilizer and by environment in high tunnels. *Journal of Plant Nutrition* **25**:981–998.

Good A G, Shrawat A K, Muench D G. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends in Plant Science* **9**:597–605.

Goulding K, Jarvis S, Whitmore A. 2008. Optimizing nutrient management for farm systems. *Philosophical Transactions of the Royal Society B-Biological Sciences* **363**:667–680.

Gowers S, Armstrong S D. 1994. A comparison of the yield and utilization of 6 kale cultivars. *New Zealand Journal of Agricultural Research* **37**:481–485.

Halvorsen B L, Holte K, Myhrstad M C W, Barikmo I, Hvattum E, Remberg S F, Wold A B, Haffner K, Baugerød H, Andersen L F, Moskaug Ø, Jacobs D R, Blomhoff R. 2002. A Systematic Screening of Total Antioxidants in Dietary Plants. *The Journal of Nutrition* **132**:461–471.

Harper J L, Lovell P H, Moore K G 1970. The Shapes and Sizes of Seeds. *Annual Review of Ecology and Systematics* **1**:327–356.

Jankowiak J, Sikora M, Bienkowski J. 1988. Effectiveness of irrigating a fodder kale crop as related to plant density and nitrogen fertilization level. *Pamietnik Pulawski*, pp. 133–146.

Jensen E, Squance M, Hastings A, Jones S, Farrar K, Huang L, King R, Clifton-Brown J, Donnison I. 2011. Understanding the value of hydrothermal time on flowering in *Miscanthus* species. *Aspects of Applied Biology* **112**, *Biomass and Energy Crops* IV, pp. 181–189.

Khan S H, Ahmed N, Jabeen N, Afroza B, Hussain K. 2012. Variability Studies for Economic Traits in Kale (*Brassica oleracea* var. *Acephala L.*). *Advances in Plant Sciences* **25**:273–276.

Khush G S. 2001. Green revolution: the way forward. *Nature Reviews: Genetics* **2:**815–822.

Lemaitre T, Gaufichon L, Boutet-Mercey S, Christ A, Masclaux-Daubresse C. 2008. Enzymatic and metabolic diagnostic of nitrogen deficiency in *Arabidopsis thaliana* Wassileskija accession. *Plant Cell Physiology* **49**:1056–1065.

López-Bellido F J, López-Bellido L, López-Bellido R J. 2005. Competition, growth and yield of faba bean (*Vicia faba L.*). *European Journal of Agronomy* **23**:359–378.

Marsh T, Parry S. 2012. *An overview of the 2010–12 drought and its dramatic termination*. NERC/Centre for Ecology & Hydrology.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany* **105**:1141–1157.

Mehwald J. 1976. Comparison of green kale varieties. *Industrielle Obst- und Gemueseverwertung* **61**:180–185.

Mendieta-Araica B, Sporndly E, Reyes-Sanchez N, Salmeron-Miranda F, Halling M. 2013. Biomass production and chemical composition of *Moringa oleifera* under different planting densities and levels of nitrogen fertilization. *Agroforestry Systems* **87**:81–92.

Naik I A, Gupta A J. 2010. Effect of plant density and integrated nutrient management on growth, yield, quality and economics of kale (*Brassica oleracea* var. *acephala*) in temperate region. *Indian Journal of Agricultural Sciences* **80**:80–84.

Podsędek A. 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. LWT - *Food Science and Technology* **40**:1–11.

Qin X l, Weiner J, Qi L, Xiong Y C, Li F M. 2013. Allometric analysis of the effects of density on reproductive allocation and Harvest Index in 6 varieties of wheat (*Triticum*). *Field Crops Research* **144**:162–166.

Rasmussen P E, Goulding K W T, Brown J R, Grace P R, Janzen H H, Körschens M. 1998. Long-Term Agroecosystem Experiments: Assessing Agricultural Sustainability and Global Change. *Science* **282**:893–896.

Rathke G W, Behrens T, Diepenbrock W. 2006. Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): A review. *Agriculture, Ecosystems & Environment* **117**:80–108.

Rosenow D T, Clark L E. 1981. Drought tolerance in sorghum. In *Proceedings of the 36th Annual Corn and Sorghum Industry Research Conference, Chicago,* pp. 18–31. Eds H D Loden and D Wilkinson. Annual Corn and Sorghum Research Proceedings.

Rossato L, Laine P, Ourry A. 2001. Nitrogen storage and remobilization in *Brassica napus* L. during the growth cycle: nitrogen fluxes within the plant and changes in soluble protein patterns. *Journal of Experimental Botany* **52**:1655–1663.

Sadras V O, Bange M P, Milroy S P. 1997. Reproductive Allocation of Cotton in Response to Plant and Environmental Factors. *Annals of Botany* **80**:75–81.

Salon C, Munier-Jolain N G, Duc G, Voisin A.-S, Grandgirard D, Larmure A, Emery R J N, Ney B. 2001. Grain legume seed filling in relation to nitrogen acquisition: A review and prospects with particular reference to pea. *Agronomie* **21**:539–552.

Schiltz S, Munier-Jolain N, Jeudy C, Burstin J, Salon C. 2005. Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by N-15 *in vivo* labeling throughout seed filling. *Plant Physiology* **137**:1463–1473.

Schjoerring J K, Bock J G H, Gammelvind L, Jensen C R, Mogensen V O. 1995. Nitrogen incorporation and remobilization in different shoot components of field-grown winter oilseed rape (*Brassica napus* L.) as affected by rate of nitrogen application and irrigation. *Plant and Soil* **177**:255–264.

Skaland N, Hillestad R. 1971. Marrow stem kale, yield and quality. Varieties, seeding rates, harvesting techniques: 1952–1968 trials. *Forskning og Forsok i Landbruket* **22**:183–209.

Soengas P, Cartea M E, Francisco M, Sotelo T, Velasco P. (2012). New insights into antioxidant activity of Brassica crops. *Food Chemistry* **134**:725–733.

Stanner S, Hughes J, Kelly C, Buttriss J. 2004. A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutrition* **7**:407–422.

Sutton T. 2009. Functional genomics and abiotic-stress tolerance in cereals. In *Adapting agriculture to climate change,* pp. 57–64. Eds A Eaglesham and R Hardy. Ithaca, NewYork: National Agricultural Biotechnology Council.

Treseder K K, Vitousek P M. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* **82**:946–954.

Vitousek P M, Naylor R, Crews T, David M B, Drinkwater L E, Holland E, Johnes P J, Katzenberger J, Martinelli L A, Matson P A, Nziguheba G, Ojima D, Palm C A, Robertson G P, Sanchez P A, Townsend A R, Zhang F S. 2009. Nutrient Imbalances in Agricultural Development. *Science* **324**:1519–1520.

Waggoner P E. 1995. How much land can ten billion people spare for nature? Does technology make a difference? *Technology in Society* **17**:17–34.

Weitzberg E, Lundberg J O. 2013. Novel Aspects of Dietary Nitrate and Human Health. *Annual Review of Nutrition* **33**:129–159.

Wuebbles D J. 2009. Nitrous Oxide: No Laughing Matter. *Science* **326**:56–57.

Xue J, Lin M, Bell R, Graham R, Yang X, Yang Y. 1998. Differential response of oilseed rape (*Brassica napus* L.) cultivars to low boron supply. *Plant and Soil* **204**:155–163.

Yang J, Zhang J, Liu K, Wang Z, Liu L. 2006. Abscisic acid and ethylene interact in wheat grains in response to soil drying during grain filling. *New Phytologist*, **171**:293–303.

Zhang S, Liao X, Zhang C, Xu H. 2012. Influences of plant density on the seed yield and oil content of winter oilseed rape (*Brassica napus* L.). *Industrial Crops and Products* **40**:27–32.

Zhou W, Lin X. 1995. Effects of waterlogging at different growth stages on physiological characteristics and seed yield of winter rape (*Brassica napus* L.). *Field Crops Research* **44**:103–110.

Figure legends

Fig. 1. Kale N-efficiency. Bars are the mean ± SE, values that differ at the 0.05 level of significance (ANOVA followed by Fisher's *A priori* test) are labelled with different letters. For each combination of treatments n ≥ 4.

Fig. 2. Scatter plot of the first principal component against the second principal component for the kale plants at different spacing treatments (6.7, 10 and 25 plants m^{-2}). The following parameters were used for the principal component analysis; pod spacing (mm), pod abortion (%), plant height (cm), stem area (mm^2), number of branches, vegetative mass (g), pod length (mm), seeds per pod, thousand grain weight (g), seed yield per plant (g), seed yield (kg ha^{-1}), pod area (mm^2), harvest index (g), and seed germination (%). Each symbol represents 1 plant.

Fig. 3. The effect of planting density on plant architecture parameters. (a) Plant height (b) Stem area (c) Vegetative mass. Bars are the mean ± SE, values that differ at the 0.05 level of significance (ANOVA followed by Fisher's *A priori* test) are labelled with different letters. For each treatment n ≥ 9.

Fig. 4. The effect of planting density on seed yield parameters. a) Seed yield per plant (b) Seed yield (kg ha^{-1}). Bars are the mean ± SE, values that differ at the 0.05 level of significance (ANOVA followed by Fisher's *A priori* test) are labelled with different letters. For each treatment n ≥ 9.

Fig. 5. Dendogram of kale morphological traits generated by cluster analysis which visually reflects the correlations between the different variables. Variable clustering was performed using ward linkage and the correlation coefficient distance**.**

Table 1. Average monthly maximum and minimum temperatures and rainfall at London Heathrow airport (51°28′18.9390″N, 000°27′04.6953″W0) from 1982-2012. Values were compared to averages from the 30 year period, 1982-2012, and significant differences were determined by a *t*-test and indicated by an asterisk, * P<0.05, ** P<0.01, *** P<0.001. Values which are significantly higher than the 30 year average have been highlighted in light grey with black writing and those which are significantly lower have been highlighted in dark grey with white writing.

Table 2. ANOVA of variables measured in kale under different nitrogen (85kg ha^{-1} and 25kg ha^{-1}) and spacing (6.7, 10 and 25 plants m^{-2}) treatments. The variables are as follows: pod spacing (mm), pod abortion (%), plant height (cm), stem area (mm^2), number of branches, vegetative mass (g), pod length (cm), number of seeds per pod, thousand grain weight (g), seed yield per plant (g), seed yield per hectare (kg ha^{-1}) pod area (mm^2), harvest index (g), seed germination (%). F values are presented for the nitrogen effects, spacing effects and nitrogen x spacing effects. *, ** and *** indicate significance at P<0.05, P<0.01 and P<0.001 respectively.

Table 3. Pearson's correlation matrix for the traits measured in Kale under different planting densities (6.7, 10 and 25 plants/m^2). The variables are as follows: 1:pod spacing (mm), 2:pod abortion (%), 3:plant height (cm), 4:stem area (mm^2), 5:number of branches, 6:vegetative mass (g), 7:pod length (cm), 8:number of seeds per pod, 9:thousand grain weight (g), 10:seed yield per plant (g), 11:seed yield per hectare (kg/ha), 12:pod area (mm^2), 13:harvest index (g) and 14:seed germination (%).Pearson correlation coefficients are presented with * and ** indicating significance at P<0.05 and P<0.01 respectively. n = 34