# Parametrising and customising the SimPlE model for *Miscanthus* germination and emergence

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## Abstract

*Miscanthus* an Asian perennial and promising biomass crop has reduced uptake due to the cost of establishment. Planting the crop is currently done by rhizome, while a seed established crop could greatly reduce initial costs involved in establishment (Jørgensen & Schwarz, 2000; Scurlock, 1999). In order to best use seed based hybrids small-scale seed testing must be extrapolated to allow prediction of success in a variety of climates. Computational modelling has been used in *Miscanthus* yield predictions (Clifton-Brown, Lewandowski, & Jones, 2000; Hastings, Clifton-Brown, Wattenbach, Mitchell, & Smith, 2009), to predict suitable sowing times, treatments, and locations and could be used in *Miscanthus* germination for seed based establishment.

A version of the SimPlE model (SIMulation of PLant Emergence) (Dürr et al., 2001, 2003) was modified for *Miscanthus* and parametrised. The parametrisation used data from several experiments that identified the impacts of the main effects on germination, primarily water and temperature. This model was used to estimate the germination and emergence of *Miscanthus* seed, which should give a good indication of the subsequent, established of *Miscanthus*.

The thermal requirements for germination of thirteen *Miscanthus* genotypes were parametrised from a thermal gradient plate experiment. A laboratory test provided the base soil-water content required to germinate *Miscanthus*. The thermal gradient elongation experiment on two genotypes parameterised early growth. The controlled environment cluster sowing into soil experiment provided later stem elongations. The basic functions of clods and soil surface crusting were added to the model but were not yet parameterised for *Miscanthus,* as seed was not drilled into the soil. Further parametrisation can be added to account for more effects of field sowing on the seed or the effect of sowing the seed below the soil surface.

The models estimates of germination were compered to seed germinated in a variety of environments form lab to field. Overall, the model predicted germination across all environments to an R2 of 0.73; however, the model still requires more parametrisation to allow full predictions of *Miscanthus* germination in real sowings.

## Introduction

*Miscanthus* is a C4 Asian perennial and promising high yielding biomass crop (Clifton-brown et al., 2001; Hastings et al., 2009; Heaton et al., 2012; Heaton, 2004; Nixon & Bullard, 2001). Planting the crop by seed instead of rhizome would greatly reduce initial costs promoting uptake (Jørgensen & Schwarz, 2000; Scurlock, 1999). Seed based hybrids are being developed however sowing in the correct hybrids in the correct climate and conditions is vital for success. The seed must be capable of germinating and establishing at a suitable time scale to take advantage of the first growing season.

Mulch film is a commercial crop product that could prove useful in *Miscanthus* establishment, by providing seeds with additional thermal time. During the establishment phase, film can act in a similar way to a degradable glasshouse; and consequently provide an increased yield (Easson & Fearnehough, 2000; Farrell & Gilliland, 2011). It can be applied to fields along rows, appearing similar to a layer of perforated cling film (for details see 2.1f above). It has been claimed that using mulch film increases early soil temperatures by approximately 10˚C at the surface, dropping to 5˚C at 10 cm deep, thereby halving the time to crop emergence (Farmers Guardian, 2008). From this report, Maize under film can achieve 20% higher yields as well as producing a more reliable crop, and thus has been economically sound for farmers (Farmers Guardian, 2008). Mulch film was tested with Maize in China and was found to increase the soil water and the crop harvest mass (Zhou et al., 2009). Also the farming of Maize in Ireland is expanding west and north with the use of film; this could be replicated on marginal sites across the UK, particularly within Scotland (Dr Trevor Gilliland - Head of AFBI plant establishment, via (Farmers Guardian, 2008)). The increase in dry mass of Maize under film in Ireland was primarily due to the thermal boost mulch film gave the soil (Farrell & Gilliland, 2011). This exemplifies the ability to grow crops outside of normal geographical range, and has particular implications for *Miscanthus*, the aim with which is to target the largest possible geographical area.

It has been observed anecdotally that *Miscanthus* seeds may germinate better on firm soil than freshly tilled soil; there are observations of seeds germinating on tyre tracks yet not in plots. This may be due to the size of the seed leading it to be washed down into the soil substructure. It may be relevant that informal observations of *Miscanthus* suggest that germination in the lab, on wet paper or in water, is high but that germination in pots or in the field is much lower. This may be due to a lack of seedling emergence force (Brunel-Muguet et al. 2011) failing to let the emerging hypocotyl reach the surface. As studied in sugar beet by Dürr & Aubertot, (2000) average emergence forces within species determine which samples survive in which soils. Testing whether there is a negative impact on germination and establishment based on sowing by sowing method will be important to solve this problem. Previously *Miscanthus* seed was shown to establish better when drilled into the soil than when broadcast over the soil, possibly because the seeds benefit from improved hydraulic contact with the soil (Christian, Yates, & Riche, 2005).

There is a wide variation in *Miscanthus* genotypes between and within species in the wild (Deuter, 2000; Songstad et al., 2010, Chapter 7). This variation has been used through breeding mainly to produce bigger *Miscanthus* plants, and to a lesser extent to extend the range of *Miscanthus*. The Aberystwyth University breeding program has many seed accessions that could be characterised by response to germination conditions. Thus far, breeding has not focused on the potential germination range of hybrid seed; however, direct sowing will make this a more important factor, though not as important as biomass and overwintering.

The main physical environmental triggers for seed germination are water potential (hydrating the seed) and temperature (allowing metabolic processes that also signal germination). The water content of the soil is partly responsible for the water potential, though soil type is also influential. The soil type also influences temperature through density, water content, and albedo. Understanding how *Miscanthus* seed germination changes based on real world soil water and temperature will be vital to direct sowing.

As a C4 plant, *Miscanthus* is more suited to tropical than temperate climates; however, it can naturally grow in temperate environments unlike many C4 plants (Clifton-Brown et al., 2001; Hastings, Clifton-Brown, Wattenbach, Mitchell, Stampfl, et al., 2009; Naidu et al., 2003). Because the physical limits on germination are temperature and water, which are genotype dependent, new geographic ranges should be calculable for interspecific hybrids. For example Maize seed has been bred to grow successfully in northern Europe despite also being a C4 grass (Clifton-Brown et al., 2011).

For *Miscanthus* to be direct sown in a range of environments and soils, producing a model to assess its germination and emergence from small-scale trials would improve understanding of crop sowing. Existing crop yield models have been applied to crops of *Miscanthus* (Stričević et al., 2015); custom models of climate and soil have more often been applied to demonstrate the potential yield over time (Hastings, Clifton-Brown, Wattenbach, Mitchell, & Smith, 2009). Modelling could also help optimise the effects of treatments and growing conditions on plug plants, the current (Clifton-Brown et al., 2016) technology for future seed based hybrids. This could be important when optimising on a larger scale with hormones such as in section 4 above, or adjusting the temperature required in the greenhouse for uniform emergence. As predicted with priming, a short warm time in the greenhouse could serve to synchronise germination in all the seedlings.

MiscanFor (Hastings, Clifton-Brown, Wattenbach, Mitchell, & Smith, 2009) and before that MiscanMod (Clifton-Brown et al., 2000) predicted the yield of *Miscanthus* crops. A model of the emergence may make planting by seed drilling predictable.

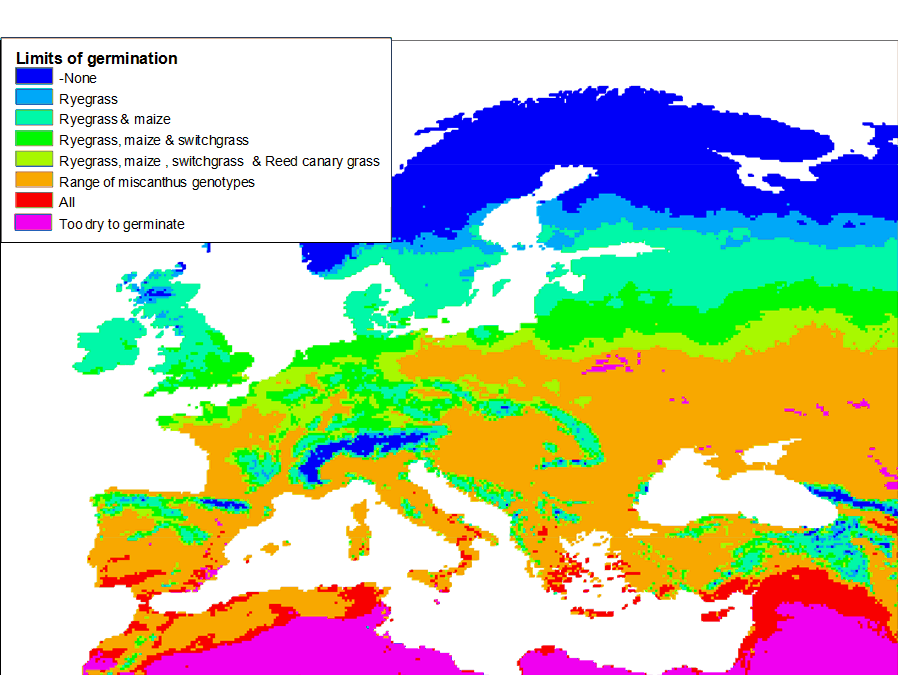


Figure 6‑1: Map of seed propagation limits in Europe for Miscanthus and other selected grasses (Clifton-Brown et al., 2011).

Modelling factors which influence seed germination such as seedling emergence force (Dürr & Aubertot, 2000) or temperature, result in data which, when applied to a known system, has the capacity to give broad predictions. Figure 2 shows the result of a thermal gradient experiment modelled onto European temperature ranges (Clifton-Brown et al., 2011). As with temperature modelling, other variables rely upon knowledge of the crop agronomy and physiology to create a model that is capable of simulating outcomes in the real world. To do this modelling, basic data about the crop responses to the environment must be obtained (Clifton-Brown et al., 2000). However, through small scale testing information can be added to the model that will show how a procedure can affect the crop in a wide variety of real world conditions. A good example of a small scale test that can be applied is taking a measure of seedling emergence force to show what an emerging hypocotyl can penetrate; this builds into a wider model of field aggregate size (Dürr & Aubertot, 2000). However, there may be no simple way of expressing how germination changes with time (Garcia-Huidobro, Monteith, & Squire, 1982) (see section 2.1a above).

## Parameterisation

### Methods & Materials

Germination of seeds was determined by eye when the radical has visibly emerged (Bewley, 1997; Ellis, Hong, & Roberts, 1985).

#### Soil Water Content

For comparisons of germination between the laboratory model and field to be better assessed, 100 MX300 seeds were placed Petri dishes containing around 85 g of 3 mm sieved, autoclaved and dried Aberystwyth soil. This was repeated 24 times; the dishes were then numbered randomly and the mass of each was recorded and the mass of soil in each calculated. Then each of the 24 dishes had SDW (sterile distilled water) added to 1 of 6 soil water percentages (4, 8, 12, 16, 20, 24), these were chosen to represent the soil moisture range as recorded by reflectometers during sowings in Aberystwyth in 2013. The dishes were then placed in a random grid into a germination cabinet at 25°C and 60 RH [max] for 15 days.

Germination was then counted at 5, 7, 12, and 15 days. Each time the germination was counted, the dishes were weighed and the amount of water lost was calculated; SDW was added to replace it, this did not account for the mass of the growing seedlings, which should have been low compared to the dish and the soil. By monitoring the water loss over differing periods, it could be observed whether opening the dishes to count the germination lost most of the water, or if it was lost from venting while in the cabinet. At the end of the experiment, the soil with seedlings was dried and a mass was recorded to determine the errors accumulated over the course of the experiment.

Any effect soil water had on germination was checked with a Friedman test using time of count as the blocking factor. To calculate the minimum water for germination, Ψ would be required (Bradford, 1990); however, because this is difficult to measure in field sites seed germination to hydrotime was not used in the analysis. Instead an approximate linear model was made between proportions of seeds germinated and soil W/V, in order to calculate a minimum. This will only truly reflect a minimum for this soil, so may misinform the model. However, because the main model evaluations are done in the same soil this should not be a problem in this study. External to this study the model may need more refinement or a parameter for soil type, as Ψ is difficult to collect.

#### Thermal Gradient for Seed Germination

To provide detailed information on *Miscanthus* seed germination and allow for a calculation of base temperature, the same primed and control *Miscanthus* seed as sown in the UK field trials (section 6.2a) and the genotypes from the ‘Multi Genotype Direct Sowing Trial’ along with eight promising hybrids; were assessed on a thermal gradient plate (Grant Instruments Ltd. Cambridge, UK). At each temperature, 60 seeds were placed on wet blue roll for the primed and control seed batches. Seeds were germinated under constant fluorescent light at a PAR of ~70 μmol m-2 s-1 as measured by Clifton-Brown et al., (2011); this was combined with a constant temperature to avoid diurnal effects.

The bar was monitored by a ‘Campbell CR10PW’ dater logger, which was used to confirm the consistency of the temperatures throughout the experiment. A glass thermometer and a multi-meter (CA5233 Chauvin Arnoux) thermocouple were used to calibrate and assess the temperatures at the beginning and the end of the test; the temperatures varied by 1°C or less within each set of cells. This was particularly necessary to check because there was a 1-2°C variation in the thermal gradient plate thermocouples at the same temperature; this may have been due to corrosion from extended use in a wet environment. During the experiment the thermal gradient plate was kept as wet as possible while avoiding floating the seeds (done using the wick system adding tap water every 48 hours).

##### Seeds

The thermal gradient plate could test up to fourteen seed lots; the genotypes chosen for characterisation were picked based on diversity and using genotypes that could information other experiments. Therefore, the main SYN55 and MX300 seed lots were used on the thermal gradient. Then seed lots SYN56, SYN58, SYN16, and SYN17 were used to mirror the seed in the multi genotype experiment (section 6.2b above). Newer seed were also used to inform future development of *Miscanthus* breeding: GNT1, GNT2, GNT3, GNT4, GNT5, GNT22, and GNT36. Primed seed (section 2.1g) were included to determine the effect of priming in a controlled environment. The seeds were not surface sterilised to provide the model with more accurate parameterisation of germination.

##### Temperature Range

The thermal gradient plate was set to create a temperature gradient to approximate the range of field temperatures seen in the field trials in 2013 from the two UK field sites. Diurnal fluctuations were not used because the field sowings had been conducted throughout the year so there was no consistent day length. The field temperatures fluctuated a lot from -5°C to 42°C; however, these extremes were not maintained for long so the thermal gradient temperatures were derived from running 6-hour averages on the data. From this the temperatures 4.6°C and 33.3°C were reached. These were simplified to give regular spaces of 2 degrees per cell on the thermal gradient (5 to 31°C).

##### Counting

Counts were done manually every day at the same time and visibly dead (very mouldy/squashy) seed was removed at approximately 150-hour intervals to stop mould spreading to other seeds and highly squashy seeds disintegrating beyond recognition. Germination of seeds was determined by eye when the radical had visibly emerged (Bewley, 1997; Ellis et al., 1985). The experiment was ended once no seed had germinated for 3 days. The end procedure was similar to Clifton-Brown et al., (2011); all-remaining seeds after the test was ended were given a temperature boost to 30°C to determine viability. Unlike Clifton-Brown et al., (2011) this was done only for 3 days after the germination had stopped. However, as soon as the higher temperature germination started dropping the tweezer method (Borza, Westerman, & Liebman, 2007) was used to determine firmness. The temperature boost and the tweezer method were done to help establish which remaining seed were firm and/or viable; any firm seeds that did not germinate may have been in a deep state of dormancy. At the end of the test all seeds were placed into three categories according to their condition at the end of the test: Germinated, Mouldy or Viable.

##### Reliability & Analysis

The thermal gradient plate has been shown to be a reliable non water-limited way of assessing the effect of temperature on germination (Clifton-Brown et al., 2011). A snapshot of germination at seven days was used, chosen as a standard germination comparison time, this tested which seed lot, and which temperature performed best on the thermal gradient. This was done with both poisson and negative binomial generalized linear models (Venables & Ripley, 2002).

Using the range of temperatures and rates of germination on the thermal gradient plate the base temperature for the seeds were interpolated with information from Dr Ruth Sanderson at Aberystwyth University based on the work in Clifton-Brown et al., (2011). This was done by fitting a logistic curve to the proportion of seeds germinated along an axis of thermal time with a default base temperature (0°C); from this curve, the point of inflation (T50) could be extracted. 1/T50 gives the rate of germination for each cell on the thermal gradient plate; a linear regression could then be done to find the intercept from all the rates (1/T50) for each seed lot. Outliers outside 4/*n* of Cooks distance were removed (Bollen & Jackman, 1985, p. 268) and a robust regression from the ‘MASS’ R package was used (Venables & Ripley, 2002). However, this method removed too many points, and did not remove some high values that seemed to be lifting the robust models; therefore, the data was trimmed to remove growth rates above 0.05. The intercept of this was then used as an approximation for the seed lots’ base temperature because it is the temperature where the germination rate reaches zero (Gummerson, 1986). Confidence intervals were bootstrapped to this intercept using estimated standard error (this used the ‘boot’ package (Davison & Hinkley, 1997)).

#### Thermal Gradient for Seedling Stem Elongation

An experiment was conducted to parameterise the effect of temperature on seedling stem elongation. Both SYN55 and MX300 seed were used because those are most compliant with the other experiments. Seed were not sterilised, in line with the first thermal gradient plate experiment. 400 SYN55 and 230 MX300 seed were germinated in the dark at 25°C on wet blue roll, to produce 196 germinated seeds. After 72 hours, the germinated seeds were moved to the thermal gradient plate (Grant Instruments Ltd. Cambridge, UK) with lighting added by Clifton-Brown et al., (2011). There they were placed in a pre-determined random arrangement of rows across the temperature gradient, seven for each genotype. Each temperature interval had two germinated seed placed in it (for redundancy); these seed were measured as they were placed for stem/leaf elongation. The same temperature range was used as ‘Thermal Gradient for Seed Germination’ experiment (section 6.2e above), 5 to 31°C in fourteen 2°C increments, but to reduce risk of stem brakeage the seed were measured at decreasing intervals rather than daily (0, 1, 2, 4, 7, 11, 16, 22, 29, 37, 46 Days). The same lighting arrangement was used as in the ‘Thermal Gradient for Seed Germination’. The water on the thermal gradient plate’s wick system was regularly topped up. The seeds’ health was scored at the end using the same system as the germination tests used (Appendix K, without fluorescence imaging).

The difference between the seed lots’ elongation rates was tested with a three-way ANOVA. For a selection of the central temperatures, t-tests or Kruskal-Wallis rank sums were done to check if there was a difference between the seed lots for the difference between the final elongations of the stems.

The results were analysed to find the base elongation temperature. This was done by modelling the elongation rate against temperature. The rate could be calculated by dividing the final elongation change by the final time elongation; however, it was decided to be more reliable to calculate the elongation rate per hour between each time point and the next then average these results. This method requires less reliance on accuracy in the final (most difficult) elongation measurement and may account better for the rate of elongation varying with time. The base elongation temperatures were calculated for each seed lot from the germination rate using a linear model; the outliers, where Cooks distance was less than 4/*n*, were removed (Bollen & Jackman, 1985, p. 268).

A three way ANOVA was also done on the calculated elongation rate (logged for normality). This was used to test the relative significance and interactions of genotype time and temperature.

### Results

#### Soil Water Content

It was important to determine the effect of soil moisture on germination, and from this to determine a minimum level of soil moisture required. Figure 6‑30 shows the pattern of germination changing from a shallow line to a more typical s-shaped germination curve as soil moisture increases. The seed only approaches 50% germination for the 0.24 water (W/V); 100% was achieved at the same temperature on the thermal gradient plate (section 6.3e). Over all the germination times, there was a significant effect of water (W/V) (Friedman’s rank sum, P < 0.01).



Figure 6‑30: The percentage of germination measured at 25°C, over a range of soil moisture levels. Second order polynomials have been added as lines with standard error bars on each point to depict the variation in the four replicates.

The minimum soil water content required for germination was 0.062 W/V. This was calculated from the intercept of a linear model of the fifteen-day germination count against water concentration (Figure 6‑31). The model was highly significant (P < 0.0001), and the R2 for the fit was 0.77.



Figure 6‑31: A graph of the germination (at 15 days) by soil moisture, with a linear model added to calculate base germination and with a 95% confidence interval. The blue zone shows the range of field water W/V recorded in Aberystwyth sowings 1-7 with and without film (section 6.3a), and the red line is the intercept of the model.

About 0.5% of water was lost per day from the dish (Figure 6‑32 & Table 6–3). Therefore, because the water top-ups were every 2-3 days there was some variability. During this time, the water percentage could have been lower than the target level.



Figure 6‑32: The mean soil water measured for each set of dishes at each time, dishes were topped up with the calculated amount of water at each time. The discrepancies between the four replicates are represented with standard error bars.

As most dishes contained around 100 g of soil, the average amount of water added at the start was close to the W/V percentage (see Table 6–3).

Table 6–3: Mean water loss per day in μL and the percentage water, with standard errors for the four replicates over the fifteen days. Information is given for each water percentage 4 to 24%.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Proportion of Water per Dish | Water Quantity (W/V) | | | | | | | | | | |
| 04% |  | 08% |  | 012% |  | 016% |  | 020% |  | 024% | |
| Loss per Day W/V (%) | 0.34±0.15 |  | 0.43±0.15 |  | 0.51±0.22 |  | 0.59±0.26 |  | 0.50±0.19 |  | 0.54±0.21 | |
| Loss per Day (mL) | 0.29±0.13 |  | 0.36±0.13 |  | 0.53±0.23 |  | 0.54±0.23 |  | 0.53±0.20 |  | 0.53±0.17 | |
| Mean Starting Water (mL) | 3.4 |  | 6.7 |  | 12.5 |  | 14.9 |  | 21.8 |  | 24.8 | |

#### Thermal Gradient for Seed Germination

A thermal gradient plate was used to parameterise the germination of fourteen seed-based hybrids at a wide range of temperatures. The temperature probes placed at approximately 5, 18, and 31°C recorded stable temperatures (Figure 6‑33) and checks at the start and end of the test confirmed the temperature range [for details see Appendix R].



Figure 6‑33: Temperature variation across the thermal gradient plate for three sensors at 5, 18, 31°C temperature and across all 40 days of the experiment. Mean temperatures were logged every thirty minutes. The standard deviations are 5°C ± 0.15, 18°C ± 0.46, and 31°C ± 0.36. [For the calibration of the thermal gradient plate, see Appendix Q]

Figure 6‑34, visually confirms that ~25°C appears to be the best temperature for germination in most seed lots. The generalized linear models both agreed on a best seed lot of GNT2 and an optimal temperature of 25°C. At seven days, there were also noticeable differences between the minimum germination temperature and the maximum germination percentage (Figure 6‑34).



Figure 6‑34: The germination of the seed lots at 7 days on the thermal gradient plate. The points have a third order polynomial added.

Figure 6‑35 shows there was a lot of variation in total viability of the seed, dependent on genotype; mostly as total viability increases so does germination. The ratio between total viable seeds and germinated seeds indicates the temperature range for germination in that genotype.



Figure 6‑35: The status of each seed that had been on the thermal gradient plate at the end of the test, ordered by total germinated plus viable. Viable seed was seed that germinated when the temperature was increased, while firm seed was un-germinated at the end of the test.

GNT5 germinated better when kept cold and wet on the thermal gradient plate before having the temperature increased, than it did at a constant 25°C (Figure 6‑35). The primed seed has less total germinated seeds than unprimed SYN55, and more seeds in the firm (unchanged) category by the end of the thermal gradient plate experiment (Figure 6‑35).



Figure 6‑36: The end state for each seed lot at each temperature.

In Figure 6‑36, primed seed also has the most firm seed at the conclusion of the experiment. There appears to be little negative effect from priming seed on germination in Figure 6‑36; however, primed has a small uptick at 7°C that un-primed SYN55 dues not. When only primed and un-primed SYN55 were tested against each other using a generalized linear model with a negative binomial distribution across all times and temperatures, priming did significantly affect the model (P < 0.0001). However, the benefit was to the unprimed seed as seen in Figure 6‑36.

Figure 6‑36 clearly shows where genotypes thrive and what the rates of mould are; notable is that some seed lots experience the higher rates of mould at hotter temperatures, such as GNT5 and to a lesser extent GNT1, while most seed lots have more mould at the colder temperatures.

Several GNT’s such as 3, 5, 2 and 36, show excellent recovery and germination after being kept cold and wet for 40 days while older seed lots, such as SYN55 and SYN17 particularly, go mouldy at these cold temperatures (Figure 6‑36).



Figure 6‑37: Three exemplar seed lots show the calculation of germination of viable seed by thermal time using a default base of 0°C [for all plots see Appendix Q]. Lines have been coloured to represent the thermal gradient plate temperatures.

Figure 6‑37 shows the thermal time (base 0°C) for viable seed germination rates for three very different seed lots; the logistic model was fitted to these curves. The logistic models’ starting parameters were found by fitting a self-starting model then taking the values and refining them through iteration.



Figure 6‑38: The robust linear models plotted on the germination rates of each seed lot over the thermal gradient plate. The x-intercept represents the estimation of base temperature.

The lowest calculated base temperature was SYN17, which was 1.39°C (Table 6–4). SYN17’s estimate in Figure 6‑38 was disrupted by higher than expected germination rates at 5 and 7°C; however, because germination was observed at these temperatures, and the rates were not unrealistically high, the points were left in.

Table 6–4: Base temperatures calculated for each seed lot on the thermal gradient plate, with confidence intervals.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Seed Lots |  | Estimated Base Temperature (°C) |  | Bootstrapped 95% Confidence interval | | |
|  |  | Lower |  | Higher |
| Primed |  | 8.88 |  | 6.61 |  | 10.22 |
| SYN55 |  | 7.39 |  | 5.52 |  | 08.60 |
| SYN56 |  | 8.51 |  | 6.09 |  | 09.84 |
| SYN58 |  | 9.33 |  | 6.25 |  | 09.68 |
| SYN16 |  | 8.50 |  | 6.19 |  | 12.50 |
| SYN17 |  | 1.39 |  | -6.13 |  | 24.27 |
| GNT1 |  | 6.86 |  | 7.62 |  | 11.65 |
| GNT2 |  | 9.92 |  | 4.73 |  | 10.74 |
| GNT3 |  | 7.66 |  | 5.61 |  | 08.51 |
| GNT4 |  | 7.59 |  | 5.48 |  | 09.03 |
| GNT5 |  | 6.57 |  | 4.72 |  | 11.34 |
| GNT22 |  | 7.85 |  | 5.91 |  | 10.41 |
| GNT36 |  | 7.97 |  | 3.00 |  | 09.32 |
| MX300 |  | 8.67 |  | 4.78 |  | 11.62 |

The estimates of base temperature for seed germination averaged at 7.65°C across all the seed lots (Table 6–4).

#### Thermal Gradient for Seedling Stem Elongation

The thermal gradient plate was again utilised, this time to parameterise stem elongation in two genotypes. A stable temperature was again maintained (for graph see Appendix S). The elongation of the seeds increased with time and temperature (Figure 6‑39), the fastest elongating temperature over all times was 25°C, where seedlings grew at 0.095 mm per hour. 25°C also had the peak elongation change at 11 days (Figure 6‑40); at the end of the experiment this peak was 29°C with 25°C second. Peak growing time over all temperatures was from 24 to 48 hours, elongating at 0.052 mm per hour. Overall elongation peaks at 25°C and between 24 to 48 hours with seedlings elongating an average of 0.127 mm per hour.



Figure 6‑39: Seedling elongation over time between the two seed lots, at six temperatures chosen to show the differences between the seed lots over a range of temperatures. The boxes represent the difference in the fourteen seed of each genotype at each temperature. SYN55 (blue) and MX300 (red) with time plotted on a square rooted scale, with fitted loess curves.

SYN55 seed elongated more than MX300 at most temperatures (Figure 6‑39). However, the difference between the seed lots was not significant when this range of six temperatures was tested at the final measurement time with a t-test (except 15°C which was tested with a Kruskal-Wallis rank sum) (P = 0.67, 0.23, 0.19, 0.65, 0.21, 0.86 for 11, 15, 19, 23, 27, & 31°C). When a generalized linear model with a negative binomial distribution tested all time points, there was not a significance in the difference between the likelihood functions of the model (P = 0.087).



Figure 6‑40: Total change in seedling elongation on the thermal gradient plate by the eleventh day (264 hours). MX300 and SYN55 elongations have been combined into twenty-eight seeds per temperature.

Germination rate as shown in Figure 6‑41 shows no visually apparent trend, with MX300 or SYN55 growing faster overall. The elongation rate for all seeds has twin peaks at both 25 and 29°C, with similar low points at 23, 27, and 31°C. The curve shown in Figure 6‑41 could be smoothed to level out after 21°C and before 9°C, with the major influence of temperature on elongation rate between these points.



Figure 6‑41: A boxplot of the mean hourly elongation rate for seedlings by seed type and temperature. The replicates were averaged; errors represent the elongation rate variation over the nine time points in the experiment. Black x’s show the average elongation rate of each temperature.

When elongation rate (see Figure 6‑41) was compared with a three-way ANOVA for factors of time, temperature, and seed lot (all logged for normality) there was a significant effect of seed lot (P < 0.05), with the SYN55 elongating 2.5 μm h-1 faster than MX300. Genotype did not produce any significant interactions with time (P = 0.65) or temperature (P = 0.45). Temperature had a highly significant effect on elongation rate (P < 0.0001); this effect can be expressed as a difference of 4.6 μm h-1 °C-1. Time also had a significant effect on the rate of germination (P < 0.05). However, time was more significant as an interaction with temperature (P < 0.0001).



Figure 6‑42: Models of the thermal gradient plate elongation per hour for SYN55 and MX300, averaged over the number of hours between readings, then averaged over all.

The mean rates of germination (seen in Figure 6‑41) were averaged, and then the result was modelled using a linear model (Figure 6‑42) to calculate a minimum temperature for elongation. The minimum temperature for elongation was 5.62°C for SYN55 and 6.73°C for MX300 using this calculation. Removing outliers using Cooks distance only affected the base estimate for MX300 (from 5.96°C).



Figure 6‑43: The total elongation plotted against thermal time measured from the base temperature for each genotype, plotted with a second order polynomial curve with a confidence interval in grey.

When the base temperature was used to calculate thermal time in degree-days (Figure 6‑43), elongation was linear with thermal time levelling out after 500°Days. SYN55 still showed higher elongation but the slopes were the same during the linear part of the curve.

It was observed at the end of the experiment that when the heat was turned up to 25°C across the thermal gradient bar most of the seeds in the low (5 and 7°C) temperatures, that had not become mouldy, revived and started growing.

## Model Variables

A full description of the modal parameters can be found in Durr et al., (2001), hear is a brief description of the model parameters and there methods of acquirement. The litrichere (Reff) (Reff) (Reff) shows a variety of techniques for determining the variables for the modal.

### Computational Modelling of Germination

A *Miscanthus* model was created based on the SimPlE model (SIMulated Plant Emergence) (Dürr et al., 2001, 2003), this model by Dürr et al., was originally written in C and produced a mathematical seedbed in soil with stones positioned in the bed to simulate obstacles. The model was run on a daily loop of soil temperatures to determine the thermal time accumulated by each seed, and then its growth up around clods and through the soil surface (Dürr et al., 2001, 2003). The model has been used for many crop and weed plants from mustard to weed beet (Brunel-Muguet et al., 2011; Brunel et al., 2009; Constantin et al., 2015; Dorsainvil et al., 2005; Sester et al., 2007).

A version of this model was created with Python to allow an object-oriented approach. To this, a graphical user interface was added with R using Shiny, this sent inputs to the Python program. In this system, R saves user-selected data from Shiny into CSV files and passes these to a Python program, which runs the model. The Python program can run independently; however, when doing so it must be configured manually through input CSV files.

The model operates by producing a seedbed of clods and seed (Figure 6‑3); the clods are added in size categories as in Dürr et al., (2001), normally starting with the largest clods and working towards the smallest. This makes them easier to place, because the smallest clods would limit the range of larger clods. Size of clod was assigned by placing clods in sets which represent sieve sizes, in line with Dürr & Aubertot, (2000). For example, a clod in the 5 mm set would not pass through a 5 mm sieve, and would be the right length to pass through the sieve size above. Each clod is given as L, h and l dimensions, though different to Dürr & Aubertot, (2000), L, the longest axis, represents the sieve size above.

Dürr et al., (2001) makes it clear that using ellipsoids is better: "This [ellipsoids] is more realistic than a spherical shape, and influences the hypocotyl length and the probability for seedlings to encounter a clod". Although ellipsoids are used for this model, it was excessively complicated to determine the intersections of ellipsoids in Python, especially because *Miscanthus* is not sown below the soil. Therefore, to calculate when clods overlapped, they were simplified into spheres. The radii of the spheres as calculated in equation (7) could then be used to determine easily if clods would overlap.

|  |  |  |
| --- | --- | --- |
|  |  | (7) |

Clods can still be rotated through the primary (L) axis, so that seedlings encountering clods experience varying levels of difficulty in circumventing them. This also allows for full implementation of ellipsoids if needed in the future. The clods were then placed and rotated randomly to create variation in the seedbed, and their positions relative to other clods checked to prevent overlaps.



Figure 6‑3: A basic diagram of each section of the model and what it does [for more information see Appendix P]. Within the section that is run each day, each seed is checked.

To allow for different soil types, the placement of a clod on the z-axis can be given a probability of being fully or partially above the soil surface. For example, a recently ploughed field may have more surface clods. Seeds are then added randomly to the seedbed at the sowing depth, and the seeds check their position so no two seeds occupy the same square millimetre and that the seeds were not placed in a clod. The seeds placement can be controlled on the y-axis, to provide one or two rows of seed to represent a field sowing better.

After the seed is placed, its thermal time requirement is selected proportionately from a user input distribution of thermal times by proportion of seeds germinated. The same thing is done with soil water volume required to germinate. This is different to how the SimPlE model used water, as an on/off requirement using water potential (Brunel-Muguet et al., 2011). All this is done on day zero (Figure 6‑3), then the seedbed advances by one day at a time. Then for each day, rainfall data allows a sub model to calculate if the soil surface has a crust and if that crust is wet or dry. This sub model works as outlined by C. Dürr et al., (2001) as based on earlier work (Dürr & Boiffin, 1995; Tamet et al., 1996).

Then each seed is advanced one day: First, the model has a seed death/loss factor that has been added to account for the seed being lost into the sub soil, so each un-germinated seed has a random chance of dying. This is to account for situations where the seed sit for a long time in the soil before conditions become ideal, and when they do, the seed does not seem to germinate and emerge. Then if the seed is alive, the thermal time it has gathered since sowing is calculated; if this is more than the number selected for that individual seed the water level is tested and must be equal or above the selected seed’s requirement, for it to be marked as germinated [Appendix P]. If not it will wait until the next day. If the seed does not become lost in the soil it will pass the thermal time and retry the water the next day, this will continue until lost or germinated. Once the seed is germinated, the lost in the soil factor will no longer apply to it; however, a separate death factor is used for if the soil becomes dry enough to damage the seedling. *Miscanthus* seedlings seem to go through a secondary thinning process, which is only seen in soil based sowings, probably due to lack of water. Therefore, this model included a chance of young seedlings dying if the soil was dry, if the soil was drier than a seedling’s base soil moisture for germination it had a 1 in 5 chance of dying, which was re-calculated each day. However, each day it lived the probability of dying (*p*) is reduced (8), where *Nd* is number of days alive and *py* is the probability of dying yesterday.

|  |  |  |
| --- | --- | --- |
|  |  | (8) |

In Dürr et al., (2001), after germination the early growth of the seedling is calculated by a weibull function; however, *Miscanthus* early growth better fitted a second order polynomial, this was determined from elongation data on the thermal gradient plate, where a second order polynomial fitted with an R2 of 0.92 (Figure 6‑4). Each time the seedling grows the position of the new tip is calculated, if this tip is inside a clod, the seedling may be stopped. Firstly, if the clod is under a set clod minimum size the seed can proceed unimpeded, if not the seed may be stopped from advancing. If the seed hits near the centre of the clod and the clod’s first angle (incline along the longest axis) (L) is near to flat, the clod is determined to be impassable and the seed will wait 5 days and then die. However, if the clod is passable the seed will attempt to circumvent the clod each day for up to five days, during this time it is not growing taller. After five days, the seed will die if it has not passed the clod. In the original model the seed’s ability to pass the clod was determined by random, either following the line of the ellipsoid or dying (Dürr et al., 2001).



Figure 6‑4: Parameterisation of early growth model, using data from the ‘Thermal Gradient for Seedling Stem Elongation’ experiment (section 6.3f).

When the seed reaches the soil surface, a sub model, the same as Dürr et al., (2001), determines if it can emerge. This is based on rainfall, and previous state of the soil crust. As with the clods, if the seedling is stuck under the soil crust for five days it is counted as dead. The model for growth after emergence of true leaves in the SimPlE model was not used, because there is no clear distinction in growth patterns for *Miscanthus* (which is a monocot). This was therefore based on the extra elongation seeds achieved on the ‘Thermal Gradient for Seedling Stem Elongation’, which at each time point was linear per degree-day (Figure 6‑5). A linear model of growth was estimated from this (grey line in Figure 6‑5); this was used for seedlings after they achieved the maximum growth (~5 cm) using the early growth model.



Figure 6‑5: An estimation of the linear equation needed for late stage growth, based on thermal time (data from the ‘Thermal Gradient for Seedling Stem Elongation’ experiment (section 6.3f)).

The model was tested without clods because the only testing with soil above the seed was with sieved soil in a controlled environment, so clods nor surface crusting have been fully tested. However, they are still implemented to make the model more expandable.

The model was trained and compared each time on sets of data from different experiments. It can be compared to three types of growing environment: Firstly, the Petri dish with blue germination paper, which is closest to the thermal gradient plate from which the model’s germination values are based; here seed loss can be turned off; water, clods, and crusty surfaces are also not an issue. The modelling was repeated six times to give the same replication as the comparison experiment. Germination was compared with a Friedman Rank Sum using time as the blocking factor, to the results of six of the controls from results in Physical & Chemical Germination Factors (section 4.4), using both a specific (25°C), and all temperature range model. Wilcox tests were also used to compare the model to the real experiments for final germination and elongation.

Secondly, there is the controlled environment where water is not an issue but other aspects work similar to the field. This was then compared to the section ‘Sowing Methods Experiment’ (section 6.3c), because this tested sowing beneath the soil. ‘Seed Competition Soil Experiment within a Controlled Environment’ (section 5.2b above) was not used because the effect of seed competition is not included in the model, and its post emergence growth was used as a comparison during model development. To compare emergence and elongation across all times, likelihood ratios were compared between generalized linear models (Zeileis & Hothorn, 2002) produced with and without modelling (real or modelled data) as a factor. Final 31-day emergence and elongation were compared using Wilcox tests.

Thirdly, there is the field environment. This has the most hurdles to overcome, although, the model still does not have to account for stones or a crusty surface because the seeds were sown on the top of the soil in a groove. This was compared to both the ‘Direct Sowing Agronomy Trial’ (section 6.3a) and ‘Multi Genotype Direct Sowing Trial’ (section 6.3b), which allowed for comparison of a selection of seed lots. The modelling of both trials was tested as with the ‘Seed Competition Soil Experiment within a Controlled Environment’, by comparing generalized linear models with and without if the data was real as a factor. The emergence of seedlings were also compared at the closest time to 45-days, this seemed the best end time for a comparison because after 45-days tillering affected the accuracy of field counts.

Across all levels, an R2 was tested between mean predicted final results for each type; 40 or 45 days in the field, 31 days in the controlled environment, and 11 days in the lab.

### Variables

The Input components for the SIMPLE modal can be divided into 4 main groups of variables Seeds, Climate, Soil, and Sowing.

#### Seed Characteristics

The seed characteristics are the distribution of thermal time for seed germination and elongation, the seed mass at sowing (Dorsainvil et al., 2005) and the parameters of the seed elongation function. {add ref and explanation of the wable? Elongation function}

#### Climate Variables

The mean daily soil temperature and the rainfall, although it the original modal rainfall was only used to determine the soil crust (Reff) later uses of the modal have included a water potential component(Reff).

#### Sowing

To determine the chances of clod collision and distance for hypocotyl to travel to reach the surface the distribution of seeds in the seed bed is needed. This requires both in depth and in frequency.

#### Soil Texture

The soil texture is used to determine the physical barrier to germination. These are the soil texture, to predict soil crust, and the aggregate size and distribution to modal the chances of the emerging seed meeting a clod in a 3D environment. Surface crust was also predicted and a chance of failure to emerge through it was applied if it was in place.

### methods of acquirement

#### Seed Characteristics

The seed was assessed using a thermal gradient bar (model) this provides extensive information on the model’s most important variable thermal time. Laboratory measurements of seeds at set ages and photographs throughout early growth were also used to determine the hypocotyl elongation parameters.

#### Climate Variables

Data from miscanthus sowing field sites germinating in 2013 was used for the temperature and rainfall/water potential requirements.

#### Sowing

The sowing, soil texture, and distribution of clods components are not used as the miscanthus seed is sown on the soil surface. However these components where included in the modal so the relative effect of soil structure and sowing depth could be assessed if sowing practises where to change.

## Model Testing Experiments

### Methods & Materials

Samco Grey mulch film was used to cover seed and seedlings, this film is a 7 μm starch film, which breaks down under UV exposure (Samco Agricutural Manufacturing Ltd, 2014). The film has a ‘pinhole 20’ aeration that facilitates both ventilation and for the plant to push through the film.

#### Multi genotype agronomic test with and without film

This experiment was similar to the ‘Direct Sowing Agronomy Trial’ above, but focused on the differences between *Miscanthus* genotypes for direct sowing in the UK. This experiment was a smaller, concurrent direct sowing field trial using five seed crosses and primed seed was carried out in Aberystwyth (West UK), with all plots replicated four times, both under mulch film and without film. This trial consisted of six seed types: primed seed (see section 2.1g above), four crosses from Texas crossing blocks (SYN55, SYN56, SYN58, & SYN16), and SYN17 which was crossed under glass in Aberystwyth. The SYN55 is widely used in this study, as is the primed SYN55; the other crosses were chosen to have parents from a range of climates. Comparing direct sown seed in Aberystwyth with and without film should give an idea of how seeds/seedlings with different temperature requirements respond. Also sowing the different crosses under film will investigate the effect of the thermal boost film should give. Seed were sown in compressed open grooves made with a v-shaped length of wood. This trial also used 300 seed per row to ensure a measurable difference in germination/ emergence. Sowing took place on the 30th of May 2013.

Field plots were scored for seedling emergence every week for 4 weeks, then biweekly. The emergence total was recorded for film-covered seeds; however, this was less accurate for the reasons explained in section 6.2a. These plots were also photographed regularly to give a better understanding of the ground, weeds, and plant growth patterns.

Overall emergence was tested with a generalized linear model using a negative binomial distribution. Unlike section 6.2a above, emergence was otherwise scored using the 40-day count, the counts were synchronised from sowing for all replicates, and counts were not made until day 33. Therefore, day 40 allowed plenty of time to pass without the tillering complication indicated to occur at approximately 45 days (section 6.2a). The emergence data was then logged for normality and analysed with a two-way ANOVA. This was done to allow for a Tukey’s HSD post hoc test, the results of which could be compared to Tukey’s HSD results for final year wet weight, to give a general idea of to what extent the groupings were maintained once the plants were grown.

Plots were harvested using a forage harvester at the start of the third year. Larger plots had fresh weights taken, and then a subsample (~600 g) was used to calculate moisture content, and work out DW. These samples were lost so fresh weight data was used to analyse plot success. This data was analysed without failed plots (zeros) using a two-way ANOVA and a Tukey’s HSD; the effect of film was further tested with zeros included using a Kruskal-Wallis rank sum.

#### Sowing Methods Experiment

An experiment was used to test the sowing method used in the field. Drilling *M. sinensis* seeds has previously been shown to be more successful than broadcast sowings as the seeds have better soil contact (Christian et al., 2005); but observations indicated seed sown beneath the soil had very low emergence. Seed sown in the two trials above (sections 6.2a & 6.3b), were sown in open grooves and had relatively low germination rates.

This experiment used 330 mm lines of 100 seed, to approximate the same sowing density as 6.2a and 6.2b above (300 seeds per meter), each tray having four lines of seed sown using four different sowing styles randomly ordered. This experiment used the same cabinet and monitoring setup as the ‘Seed Competition Soil Experiment within a Controlled Environment’ experiment (section 5.2b above) but to 31 days rather than 32. The film was removed at 22 days. This was done using SYN55, which should grow faster than the MX300 seed in section 5.3b, resulting in an early harvest. For this test, soil from the field (experiments 6.2a & 6.2b) was used after being sieved and autoclaved. For one random line of seed in each tray, the soil was compacted to make a groove as done in the field experiments; for the three other comparison sowing styles, a soil-covered groove was used, and a surface sowing and finally a soil covered surface sowing. The soil was wet by adding water to the troughs the seed trays stood in, the grooves were made, and the seed was applied by shaking from an envelope as performed in the field. The trays were then covered with the same mulch film used in the field. This was removed and reapplied when taking measurements. At decreasing intervals (2, 4, 7, 10, 14, 22, 31 Days) the seeds’ emergence/tillering and the elongation of the seedling were measured. When necessary and when the seed were measured the water in the reservoir troughs was topped up, to keep this experiment none water limited. At 31 days, the plants were dug up and the soil was washed off. Above ground elongation, plant number, tiller number, and the elongation of each tiller was recorded because the Seed Competition Soil Experiment within a Controlled Environment experiment (section 5.2b above) showed that elongation was valid for small seedlings and was more variable than last ligule. The above and below ground plant material for each row was washed and separated, then dried (70°C for 48 – 72 h) and weighed, to obtain above and below ground dry masses.

The emergence and elongation data used one treatment with four levels, and was analysed with a Friedman’s test over time (this used the mean value at each time point for each treatment). This was broken down using one time point with two Kruskal-Wallis rank sums for groove and soil covering or a two-way ANOVA depending on normality. The final measurements of above and below ground dry weight and root length treated the sowing method as two pairs of treatments: groove sowing vs. atop ground and under soil vs. on the surface. A two-way ANOVA was used for the analysis; if the results did not fit a normal distribution a transformation was used.

### Results

#### Sowing Methods Experiment

This experiment tested the effect of several different in-soil sowing methods. All sowing methods had less than 40% of seeds emerge (see Figure 6‑27), less than the laboratory tested germination rate of ~58% for SYN55 in section 6.3e below, but not as low as germination in the real field sowings such as those in 6.3a and 6.3b above. Using the mean over time emergence data, a Friedman rank sum found a significance of sowing method with time as a random factor (P < 0.001) (Figure 6‑27).



Figure 6‑27: Percentage of emerged seed identified over the experiment, standard error bars shown in dark grey depict the variation in the four replicates. The SYN55seeds were germinating in a constant light at environment 25°C.

Figure 6‑27 shows emergence counts remained stable after 96 hours, therefore, emergence at 96 hours was tested using two Kruskal-Wallis rank sums; these found the effect of the soil covering was significant (P < 0.001) yet not the effect of the groove (P = 0.46). As seen in Figure 6‑27 the soil covered seed performed worse than the uncovered seed.



Figure 6‑28: Elongation of seedlings averaged for each rep, standard error bars, of differences between replicates, shown in dark grey illustrate the variation in the four replicates. Seedlings were grown in constant light at 25°C.

Using time as a random effect, the Friedman’s test found a significant effect of treatment on stem elongation (P < 0.05). The final elongation time of 744 hours (the right most bar in Figure 6‑28) was chosen to analyse further using a two-way ANOVA (data logged for normality), this found no significance of groove or soil covering (P = 0.08 & P = 0.42).

Root elongation at the end of the test gave a similar result when logged and tested with a two-way ANOVA; it was not significant for either groove or soil covering (P = 0.06 & P = 0.66).



Figure 6‑29: Above (green) and below ground (brown) dry biomass at the end of the experiment (31 days). The standard error between replicates is shown in the grey error bars illustrative of the variation in the four replicates. Bellow ground biomass has been inverted to give a view of overall biomass.

The above ground biomass, below ground biomass and total biomass were all logged to produce a normal distribution and tested with two-way ANOVA’s against grooves and soil covering. All three showed a significant effect of groove (all P < 0.05) (see Figure 6‑29). However, none of the dry masses had a significant effect from soil covering (above ground P = 0.73, below ground P = 0.26 & total P = 0.72).

#### Multi Genotype Direct Sowing Trial

The results for the Aberystwyth ‘Multi Genotype Direct Sowing Trial’ are detailed below; these were collected with Mr Ashman of Aberystwyth University.

This experiment tested five *Miscanthus* genotypes and primed *Miscanthus* SYN55 seed in a direct sowing site in Aberystwyth. This aimed to test the relative success of the seed lots and the effect of film under which all plots were replicated. This trial was sown next to the ‘Direct Sowing Agronomy Trial’ at the same time as the second sowing (late-May). This trial was not individually monitored for environmental data but temperature and rainfall data from the metrological station nearby was collected [shown in Appendix O]. There was only ~40 mm of precipitation in June but there was adequate soil moisture as seen in Figure 6‑6 above, due to the ~70 mm of precipitation in May.



Figure 6‑23: Seedling emergence as counted in spring/summer 2013; film (blue) and control (black), with all six seed sets (labelled). Counting was carried out weekly for five weeks after thirty-three days, then on an occasional basis. Standard error bars have been added to represent the variation in the eight plots four replicates with film and four without.

Mean emergence in all seed lots at 40 days was less than 10%; differences between mulch film and no film were significant over time (P < 0.0001) with film notably higher in most seed lots (Figure 6‑23). Extra emergence occurred after 60 days; this is not shown extensively in Figure 6‑23, which cuts off at 80 days, because this is mostly a counting error due to tillering. The generalized linear model likelihood ratios also found a significant difference between the genotypes. These effects where investigated further at 40 days from sowing to discount the massive effect of time.



Figure 6‑24: Boxplot of emergence of seed at 40 days after sowing. Boxes represent the variation between the four replicates. Separated into film and control treatments, primed seed is on the far left. The seed categories from a Tukey’s HSD are shown along the bottom for each seed lot / genotype.

At 40 days after sowing the seed should have mostly emerged and produced a visible sign, so this time point was analysed with a two way ANOVA. This showed a significant effect of seed lot (P < 0.001) but no overall significance of film (P = 0.85) and no significant interaction with film (P = 0.4). The complex effect of film can be seen in Figure 6‑24, where film’s effect varies dependent on genotype. Using a Tukey HSD post hoc analysis, primed and unprimed SYN55 significantly differed (P < 0.05) with more emergence for primed seed (Figure 6‑24). The Tukey’s HSD with just the genotypes put SYN56 and SYN16 in the same group, and SYN16 with SYN17; all except SYN17 had overlapping groupings (Figure 6‑24).



Figure 6‑25: Boxplot of total wet biomass generated by each seed lot, film, and control. Mass taken at the end second year in a standard spring (March 2015) harvest time. Boxes represent the variation in the four replicates.

When harvest wet mass was analysed with a two-way ANOVA with seed lot and film as factors, both had a significant effect on wet mass, being P < 0.05 for seed lot and P < 0.01 for film (Figure 6‑25). This occurred even when failed plots were removed from the analysis. The genotypes followed a similar distribution under film, with SYN56 being the only one to change order. SYN56 and SYN58 performed disproportionately well under film. SYN16 and SYN17 did not thrive in either circumstance, despite being cold suited *M. sinensis* synthetic crosses. A Tukey’s HSD produced two groupings, one with all the seed lots and a lower set of SYN16, SYN17 and primed seed.



Figure 6‑26: Change in end of wet weight between film covered and control plots as harvested at the start of the third year. Standard error shown for the variation in the four replicates.

The film always improved the mean wet harvest weight as seen in Figure 6‑26 above and detailed in Table 6–2 below. For untransformed fresh weights with failed plots included, there was still a significant result for the overall difference in film vs. control when analysed with a Kruskal-Wallis rank sum (P < 0.01).

Table 6–2: The improvement of the six seed lots’ wet weight with the application of film at sowing, measured at the end of the second year at harvest time (March 2015). The standard error to the right is between the four replicates. A percentage increase of film on control is also shown on the right.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Seed lot** |  | **Mean film Effect (Kg)** | **±SE** |  | **Mean film Improvement (%)** |
| Primed SYN55 |  | 01.69 | 0.58 |  | 0,441 |
| SYN55 |  | 05.53 | 2.86 |  | 0,077 |
| SYN56 |  | 14.51 | 4.38 |  | 0,332 |
| SYN16 |  | 03.61 | 0.45 |  | 1,053 |
| SYN58 |  | 11.12 | 2.17 |  | 0,830 |
| SYN17 |  | 00.73 | 0.37 |  |  |
|  |  |  |  |  |  |
| **Mean** |  | **6.2** | **3.60** |  | **547** |

## Comparison of Model

A computational model was produced based on SimPlE and parameterised for *Miscanthus* seed using the experiments in this study. The model was tested at three scales by graphing the result and comparing with Wilcox tests between the real germination and emergence, and the model results; the comparisons are always done with data from experiments that were not used to parameterise the model. The three scales of testing were the laboratory level (Figure 6‑44), controlled environment (Figure 6‑45), and field (Figure 6‑48 & Figure 6‑47).



Figure 6‑44: Testing the model against the mean value of laboratory germination testing (from the controls in six of the Physical & Chemical Germination Factors results 4.4) (black). The model was run with 64 seed on a none water limited surface at 25°C twelve times, six at using the ‘Thermal Gradient for Seed Germination’ 25°C data (red) and six times using all the input thermal data (as would be used in the field) (orange). A 95% loess line has been added to highlight the trends while boxes characterise the variability between six replicates.

Two models were used to simulate the laboratory germination results in Chapter 4, these were compared to the real laboratory germination results at the last time point (the eleventh day). The first model was a restricted temperature model, only parameterised with the 25˚C data from the ‘Thermal Gradient for Seed Germination’ experiment; the second model used all the thermal gradient data (Figure 6‑44). A t-test was used for this comparison, because the data for the last day was centred on a mean. Both for the main model and the 25°C model there was no significant difference to the real laboratory germination (P = 0.23 & P = 0.16). In order to compare across all the times a Friedman test was used for both models, this showed no significant difference for the all temperature model (P = 0.76), but there was significance for the 25°C configured model (P < 0.05).

Overall, as apparent in Figure 6‑44, the 25°C model better matches the germination rate over the first four days, but both models capture the seed germination percentage by day eleven. The elongation at day eleven when it was measured in the real test was 10.95 ± 1.6 mm (SE); this was lower than, but close to, the day eleven elongation for all temperature modelling (14.27 ± 0.3 mm SE). The model using 25°C data produced taller day eleven seedlings (20.09 ± 1.6 mm SE); however, the real seed was growing in the confines of a Petri dish. When tested with two Wilcox tests the overall model was not significantly different from the real elongation (P = 0.065), while the 25°C model was (P < 0.01).

The next scale tested was germinating in soil in a controlled environment. This was modelled and tested against the results of the ‘Sowing Methods Experiment’. Two models were run using the soil water and temperature data from the ‘Sowing Methods Experiment’, one for seed under a soil covering and one for uncovered seed.



Figure 6‑45: Emergence of seed sown in the control environment (from section 6.3c above), compared against the models. Modelling was done with 200 seed and was repeated four times (the number of replicates in the 6.3c experiment), at both surface sown and 5 mm covered with soil. The groove effect was not modelled, because it was not significant. Standard Deviation bars have been added.

As is clear from Figure 6‑45 there was a significant difference between the modelled results and the real emergence by the final time point for both soil covered and surface sown seed (P < 0.01). The model overestimates germination in the soil by ~20%; this is with the added model factor of a 1% chance of mortality per day. When emergence was tested with the generalized linear model, the effect of whether the data was real or from the model produced a significant difference (P < 0.0001). The model emergence lagged ~4 days behind the real data (Figure 6‑45). The rate of emergence was about the same between the model and the real data.



Figure 6‑46: Model elongation of seedlings compared to the seedlings in the 6.3c experiment, with standard error bars demonstrating the variability of the four replicates. Two distinct sowings, soil covered (orange) and surface (black), were used.

Emergence produced similar results, with the model lagging behind reality but producing a similar slope (Figure 6‑46); however, the slope diverged around 5 cm elongation where the real sowings started a second growth phase and the model did not keep up. The elongation in the real test compared to the model seedling height was significantly different at the end (P < 0.01) and overall (P < 0.0001). It can also be seen in Figure 6‑46 that the model has no difference in the heights of the under soil seed compared to the surface sown seed, because this is not currently a parameter in the model.



Figure 6‑47: Modelling (green) compared by climate to the Direct Sowing Agronomy Trial experiment (section 6.3a) (red). Fourth order polynomial lines with added confidence intervals (dotted) over the six plots. Mean temperature between all film and no film sensors was used. The real data (green) was combined between priming treatments.

The third scale tested was field sowings. The model was run on the ‘Direct Sowing Agronomy Trial’ environmental data and modelled emergence using the same numbers of seed and replicates. This model predicted the germination with an excessive lag in early-May but was within the confidence interval at 100 days for both film and control (Figure 6‑47). In the late-May sowing the model did predict the under film failure but did not predict the success in the control. Lastly, when modelling the mid-June sowing the model predictions were too low for both film and control but were worse under film (Figure 6‑47).

When tested with a generalized linear model there was a strong significance to the result for if the data was modelled or not (P < 0.0001). However, testing the 45-day counts (the closest field measurement to 45 days was chosen) for each sowing using Kruskal-Wallis rank sum tests gave a better picture; here only the test for the mid-June sowing under film was significantly different between the model and reality (P < 0.05). This may be due to the high field variability and low germination (Figure 6‑47).

The comparison to the ‘Multi Genotype Direct Sowing Trial’, shown in Figure 6‑48, also has the model under-predicting germination. However, after 45 days in the field the seedling tillering may have artificially inflated real germination counts. It is also clear the soil moisture may have a large effect on the model, particularly because the film sowings always do worse in the model, but in reality, the film improved all the sowings. This will be affected by the data the model used, because it used the environment data for film and control from the ‘Direct Sowing Agronomy Trial’ (section 6.3a), where the film was laid 3 weeks earlier.



Figure 6‑48: Modelling against the results from the ‘Multi Genotype Direct Sowing Trial’ (section 6.3b) with and without the film treatment, using five genotypes (and primed seed) that were calibrated for the model in the ‘Thermal Gradient for Seed Germination’ (section 6.3e above). The model (green) was run four times for each film seed combination (the same as the number of field replicates); standard error bars have been added.

The model does predict the relative success of the different seed lots, with SYN55, SYN56, and SYN58 performing best, while primed seed, SYN16 and SYN17 performed worse (Figure 6‑48). When tested, the generalized linear model showed a significant difference the model and the real data (P < 0.0001). At 40-days, there were only significant differences in the model compared to reality for film sowings of SYN55 and SYN16, and control sowings of SYN56 and SYN58.



Figure 6‑49: A comparison between the final measurements of the model and all of the real experiments, which have been labelled.

Across all the results of each experiment for the single time point used in the analysis, the R2 between the model and the real tests was 0.73. This was heavily influenced by if the experiment was from the lab, controlled environment, or field (Figure 6‑49).

## Discussion

#### Elongation Performance

The elongation of the seedlings, not the height, is what the model predicts, this was because the early growth model is always calculating elongation of small seedlings (less than 5 cm), and may be dealing with pre-emergent seedlings. The secondary growth model is currently quite crude and predicts elongation up to ~20 cm; this was not designed for predicting a whole year’s growth. For these reasons, the model was not compared to the field’s end of year height measurements.

In the lab testing, the main model non-significantly over estimated the elongation of the seedlings (3.32 mm); this could have been an effect of the seedlings being in a contained Petri dish. With the controlled environment, the model closely mirrored elongation of seedlings under ~2 cm. However, longer seedlings, rapidly diverged from the model ending up at 31 days only ~30% the size of the real seedlings, this shows a failing in the secondary growth model. Although, the elongation reached by the modelled seeds of ~5 cm when real seeds had elongated to ~16 cm, may show a better estimation of height to the last ligule, because a height of 5 cm would correlate to an elongation of ~20 cm in the ‘Seed Competition Soil Experiment within a Controlled Environment’ (section 5.3b above).

Overall, the model well represents early growth, but is less accurate in later growth. However, the early model has not been fully tested with *Miscanthus* seedlings growing up though soil. While it may be difficult, on a thermal gradient, a time lapse of seedlings growing up through soil could be used to better calculate pre-emergence seedling growth. This may not be necessary, unless it becomes routine agronomic practice to sow *Miscanthus* under soil.

#### Germination Performance

The model is required to predict germination over a wide variety of seed conditions. Early iterations of the model predicted lab germination very accurately, particularly when running the model using only the 25°C thermal gradient data; this was due to the similarities between the thermal gradient plate data from which was used to train the model, and the lab conditions. However, the success of the lab prediction did not account for the field sowing results, where emergence was over estimated by more than 10x. After introducing parameters for the probability of seed loss in the soil and the probability of seedling death, as well as a properly parameterised soil water model, the results presented were more accurate. This model is still inaccurate, particularly in predicting the controlled environment experiments, which sits between the lab and the field (where the germination was over-estimated by ~20%). However, while the slope of germination lagged behind reality, it was still a similar overall germination rate. With further parameterisation of why the seed does not germinate in soil conditions, it may be possible to lower total germination in the controlled environment soil while reducing the lag. The field is now somewhat under-represented for emergence, but the model predicted germination best in the first sowing, which had the most accurate data. Again, further parameterisation could be of benefit in the field, but would need to account for the controlled environment as well.

The model will likely remain unsatisfactory until the causes of the lower field germination are better understood. The model also remains inaccurate in non-soil conditions. A lag to germination for the lab-based experiments was noticeable; if the field death factor were not switched off the lab germination, would have also been too low, because the seed loss factor proves too extreme for a lab environment. This imprecise way of controlling the model’s behaviour based on conditions seems non-optimal but is practical and identifies a need to identify the factor or factors that are currently not accounted for in field experiments. This model should be seen as an experimental method for modelling establishment that will lead to better models, as has been the case in other *Miscanthus* models that have gone through several iterations (Hastings, Clifton-Brown, Wattenbach, Mitchell, & Smith, 2009).

This seed death/loss effect in the field is probably due to at least two factors: The hydraulic contact between the surface and the seed is not as good with soil as it is with paper in the lab, and there is a much lower average water potential (section 6.3d above) in the field from the lab or controlled environment. The lower hydraulic contact between seed and soil than with wet paper is also in effect for the controlled environment soil experiment. This possibly explains the result, because seeds with good water contact would germinate very quickly due to the optimal temperature, while seeds without it would quickly dry out due to the temperature.

The lag in the rate of germination in both the controlled environment soil and the lab may be caused by the humidity at the 25°C temperature. When the lab was compared to a 25°C based model, the lag disappeared. This may be because lower temperatures on the thermal gradient had slower germination not just due to thermal time but also the effect of the humidity surrounding the seed.

#### Future Improvements

Further investigation of field sowing methods as used in section 6.2c & 5 above could provide data that could be integrated as a germination curve with the existing model or as a modifier to the germination process if the model was updated.

The model original model by C. Dürr et al., (2001) used water potential to calculate whether the seed had a chance of germinating (only as a minimum that a seed with adequate thermal time to germinate must be above). This is a more accurate measure of water useable by the seed than percentage soil water; however, this is not directly measurable in the field without knowing the composition of the soil. The model for *Miscanthus* uses a soil water percentage (W/V) calculated from the ‘Soil Water Content’ (section 6.2d), as minimum water for germination. The model may be improved by utilising a more complicated equation, because the soil water experiment, conducted at 25°C (section 6.2d), did not have a sudden point at which no germination took place but a decrease in germination with soil water percentage. However, this effect may be due to the decreased chance of good soil to seed hydraulic contact and not a factor of the amount of water in the soil. This would require further investigation. The model could also take account of water potential and chance of good hydraulic contact where more detailed soil data was available.

Future improvements to the model should include a better growth model, which accounts for *Miscanthus* height in its secondary phase. A parametrised seed loss and seedling death model would be useful, because the current estimates are only estimations used to limit growth to more realistic field conditions. The model testing seems to suggest the seed loss/death estimation in the soil should be increased to account for the over estimation of germination in the controlled environment, while the seedling death chance should be decreased to account for the under estimation of surviving seeds in the field. The growth of a seedling around a clod should be better defined if below soil sowings are used in the future, as should the chance of a seedling making it through a crusty soil both of which are part of the original model. Eventually information from the time of year could be used for day length when the effect of diurnal cycles on *Miscanthus* germination and growth is better understood.

Factors that are using estimates currently are coded into the Python part of the model; these should be added to the user interface as data becomes available for them.

#### Mapping

Data from both trials was used in the ‘SimPlE’ Modal (Dürr et al., 2001) to produce results that could be used to better predict the effect of climate variation on *Miscanthus*. By using the model, the data collected can be expanded to tell a broader story about the use of film and priming in different climates within Britain. This would provide a much better idea of when to plant, and of what seed and sowing treatments may be necessary depending on other climates.

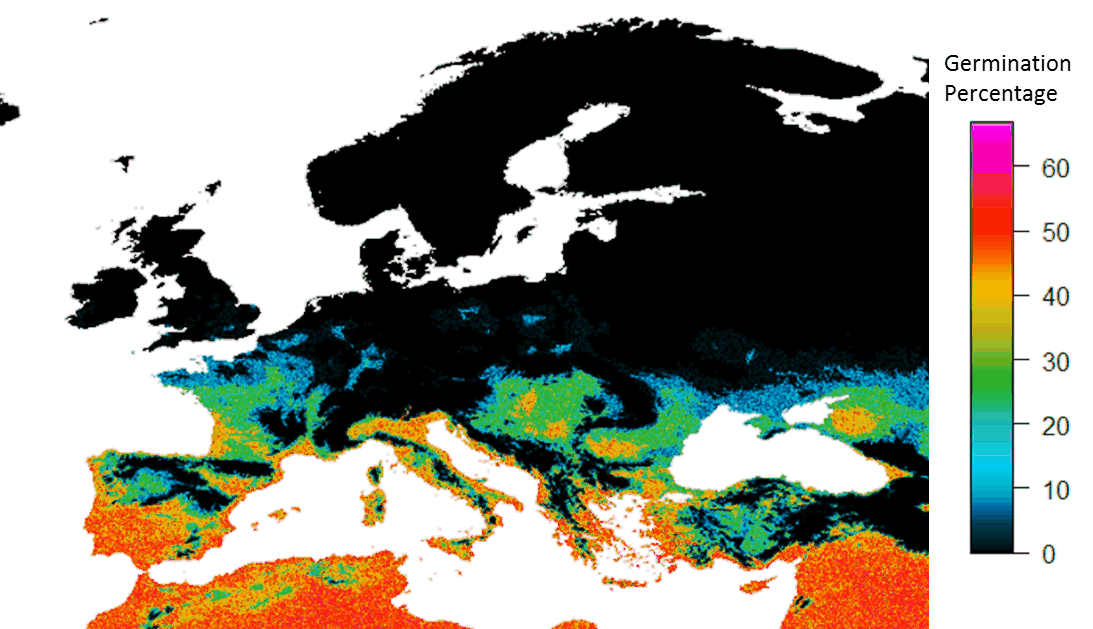


Figure 6‑52: Modelled map using April EU temperatures (Klein Tank et al., 2002). The percentage of seeds germinated in the month was calculated, by sowing 100 SYN55 seeds at each pixel and running the SimPlE model. Soil water data was not known so this was calculated as germination on wet soil.

In the future, the model could (with R package ‘raster’ (Hijmans, 2016)) be run over maps (e.g. Figure 6‑52), though this would require making assumptions about the soil temperature and water. This could be achieved by adding a simple soil water balance equation to the model so the rainfall can be modelled for soil water or with soil data water potential that the seed experiences.

The model was compared to germination on wet paper and soil within controlled environment and treated against field trial. Its performance at the three levels was mixed, it predicted germination percentage well on germination paper; however, the model overestimated emergence in the controlled environment soil after showing the correct rate of emergence early on, and the field models were low for many sowings. Real soil germination rates in soil, even in the wet controlled environment conditions, were lower than expected by the model, probably because of unaccounted variation in seed soil hydraulic contact. This was despite soil water parameterisation and a random death chance having been added into the model to limit emergence. More parameterisation may be needed to resolve these differences of scale fully. The field modelling also struggled with an environment with so many variables. Across all environments tested, the model was good at predicting germination/emergence.

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