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Optimizing seed-based *Miscanthus* plug plant production with supplemental heat and light, compost type and volume

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[Correction added on 16-Apr-2022, after first online publication: Chris Ashman was designated as corresponding author.]

Abstract

To help meet greenhouse gas mitigation targets perennial biomass crops will need to be planted at large scales and at a much greater pace over the coming decades. *Miscanthus* is a leading biomass crop but rapid upscaling is technically challenging due to costly and time-consuming clonal propagation. Seed-based hybrids are considered a viable route to rapid upscaling, but direct sowing has not been found feasible under temperate climate conditions due to high thermal requirements for germination and slow early plant development compared with larger seeded annuals. Seed-based plug plants, initially propagated in greenhouses, provide a suitable route to improve field establishment. Here, we describe an input optimization experiment for seeded *Miscanthus* plugs raised for spring planting in a naturally lit greenhouse with the following treatments: supplemental heat to maintain a minimum of 15°C, supplemental predawn light from modern LEDs at PPFD 300–400 µmol m^{-2} s^{-1}, two proprietary types of compost (known as 50k and 70k), and two compost volumes (35 and 15 cm^3). Our results showed that variations in all four factors had significant effects on above- and belowground biomass: (i) supplemental heat increased root-to-shoot ratio, (ii) supplemental light increased total biomass and root-to-shoot ratio, (iii) compost type affected total biomass and (iv) compost volume was positively correlated with total biomass and stem base diameter. No factor had a significant effect on axillary shoot formation. We recommend nurseries in the United Kingdom use LEDs as predawn supplemental light but no supplemental heat, compost that has both good water-holding capacity and aeration, and a larger compost volume, which provided optimized cost-plug resilience for spring-sown, seed-based *Miscanthus* plug production.

KEYWORDS
biomass, bioenergy, bioeconomy, glasshouse propagation, transplants, seed-based hybrid, resilience, compost, marginal land

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1 | INTRODUCTION

Miscanthus is among the most promising lignocellulosic biofuel crops to mitigate climate change (Clifton-Brown et al., 2019; Hastings et al., 2008) and is targeted to marginal lands that are less suitable for food crops (Winkler et al., 2020). The crop sequesters carbon and produces energy at 20 to 30 times lower carbon cost than fossil fuels (Hastings et al., 2008). Current commercial production mostly involves the cultivation of a high-yielding clone *Miscanthus × giganteus* (*M × g*) genotype *Greef et Deu* (Hodkinson & Renvoize, 2001), a sterile triploid that must be propagated vegetatively. However, low clonal propagation rates and high establishment costs impede progress in *Miscanthus* biomass production (Ashman et al., 2018; Winkler et al., 2020; Witzel & Finger, 2016). Propagation through seed is considered the best alternative (Clifton-Brown et al., 2017; Hastings et al., 2017; Lewandowski et al., 2016; Xue et al., 2015), and several seed-based *Miscanthus* hybrids have been bred as a more upscalable alternative to *M × g* (Clifton-Brown et al., 2017; Lewandowski et al., 2016).

The techniques required to optimize the seed-based establishment of *Miscanthus* are not yet fully determined. Two main factors to consider are a good germination rate and robust seedling growth to maximize the survival of the first winter. Germination of *Miscanthus* requires high moisture and temperature (Clifton-Brown et al., 2011); and early spring direct sowing field trials have shown this is not viable under natural conditions in temperate zones. Furthermore, if sowing is delayed until warmer weather arrives, the growing season may not be long enough to sufficiently establish the seedlings by winter (Ashman et al., 2018). In addition, direct-sown seedlings are prone to desiccation under dry warm conditions (Christian et al., 2005). To improve the establishment of direct-sown *Miscanthus*, Ashman et al., 2018 trialed seed priming, mulch film, and different sowing dates at field sites in the East and West of the United Kingdom. In the East of the United Kingdom, moisture is often limited, and in the West temperature. Even with mulch films that increase temperature and can improve hydraulic contact direct sown plants were much smaller than greenhouse-raised plugs entering the first winter after establishment (C. Ashman, unpublished). Planting smaller plants with less-developed rhizomes/stem bases that are needed for overwintering, results in higher risks of over-winter losses (Ashman et al., 2018). Slower plant development in the first year also increases the number of years to reach sufficient rhizome maturity to produce profitable yields. Hence, to date, seeded *Miscanthus* production trials for commercial upscaling have focused on sowing and raising seed-based plugs with methods similar to vegetable crops (Gruda et al., 2019). Current plug propagation techniques enable 85%–95% establishment rates at two-thirds the economic cost but threefold greenhouse gas cost of rhizome propagation (Hastings et al., 2017). Such an increase in greenhouse gas emissions is due to the high energy input required for growth in late winter and spring. With the need for a considerable expansion in *Miscanthus* cultivation, it is urgent to develop seed-based establishment techniques that are both economically and environmentally sound while guaranteeing crop establishment success.

Supplemental light and heat in glasshouses during early spring compensates for the low temperature and short day length in temperate climates, facilitating photosynthesis and seedling growth (Aguirre-Becerra et al., 2020; Bowman & Albrecht, 2021). Among currently available artificial light sources, light-emitting diodes (LEDs) provide higher light intensity per unit energy input, lower heat release, and are versatile in terms of spectrum and control of irradiance (Bantis et al., 2018; Velasco & Mattsson, 2019; Wei et al., 2020). LEDs are, therefore, widely used in both seedling nurseries and scientific research. The effects of supplemental LED light on seedling growth under various light conditions have been extensively studied using vegetable, fruit, and forest crop transplants (Aguirre-Becerra et al., 2020; Bantis et al., 2018; Bowman & Albrecht, 2021; Velasco & Mattsson, 2019; Viršilė et al., 2017) but not yet using *Miscanthus*. Considering most commercial nurseries have fixed LED modules, we were interested in optimizing the timing of lighting and light intensity and not increasing the number of lights. Nighttime supplemental light increased light use efficiency and, therefore, cost-efficiency (Tewolde et al., 2016), inasmuch as supplementing light at predawn resulted in 65% higher photosynthetic light use efficiency (PLUE) than at the end of the day (Paponov et al., 2020). The lower PLUE at the end of the day is due to excessive photoassimilate accumulation, resulting in sink-limitation and feedback inhibition of photosynthesis (Paponov et al., 2020). An additional 2-h supplemental light before sunrise could effectively alter stomatal characteristics and promote seedling growth, regardless of light quality (Wei et al., 2020). Different irradiances are used with different efficiencies by plants; in *Miscanthus* photosynthetic rate is light-limited below 140 μmol m^{-2} s^{-1} PPFD, and is close to light-saturation at 500 μmol m^{-2} s^{-1} PPFD (Collison et al., 2020; Pignon et al., 2017).

C_4 plants are in general more chilling sensitive than C_3 plants (Osborne et al., 2008). The light-saturated CO_2 assimilation rate is 15% higher in *Miscanthus* leaves grown at 14°C than at 10°C (Farage et al., 2006). This is because when grown at 10°C, *Miscanthus* leaves respond to chilling stress by decreasing chlorophyll while accumulating other photopigments such as zeaxanthin. However, the base temperature for *Miscanthus* shoot growth ranges

from 5 to 8°C (Farrell et al., 2006), which is approximately the average spring temperature at the main *Miscanthus* production sites in the United Kingdom. Shoot growth exhibits a strong linear relationship with temperature from 7 to 20°C (Clifton-Brown & Jones, 1997, 1999). These data suggest that supplemental heat to raise temperature to above 10°C would significantly enhance *Miscanthus* seedling growth.

Different potting composts vary for a range of factors including water-holding capacity, nutrient availability, bulk density, and aeration porosity. Compost compression increases both shoot and root dry biomass of pepper transplants (Grazia et al., 2002). Compost type affects germination rate, stem base diameter, short shoot formation, and plug plant biomass in perennial grasses including *Arundo donax* and several *Miscanthus* species (Lee et al., 2005; Romero-Munar et al., 2018; Zheng et al., 2021). Although no effect on overwinter survival rate or field establishment rate was observed, the nutrient levels in the compost could affect transplant growth up to 1 year after establishment (Lee et al., 2005; Meyer & Cunliffe, 2004; Romero-Munar et al., 2018; Zheng et al., 2021).

The compost volume in trays determines the amount of compost, fertilizer, nursery space, and nursery time required per seedling and, therefore, is an important economic consideration for production optimization. Biologically, compost volume determines competition for light and rooting volume. The optimal volume will depend on the size the plug plants are grown to (Poorter, Bühler, et al., 2012). Larger volumes increase seedling growth rate and produce seedlings greater in height, stem base diameter, and total biomass (Bernaola-Paucar et al., 2018; Dominguez-Lerena et al., 2006; Hanson et al., 2017; Jacobs et al., 2020; Meyer & Cunliffe, 2004; Pinto et al., 2012, 2015). Under favorable field conditions, the initial size of transplants does not affect the survival rate (Dominguez-Lerena et al., 2006; Hanson et al., 2017; Pinto et al., 2015; Romero-Munar et al., 2018). However, the size advantage of transplants from larger compost volumes is maintained up to at least 3 years after the establishment in tree species (Dominguez-Lerena et al., 2006; Pinto et al., 2015) and 2 years in *Arundo donax* (Romero-Munar et al., 2018). For *Pinus* species, larger compost volumes increase survival rate in semi-dry climates (Bernaola-Paucar et al., 2018) or under drought stress induced by weed competition (Pinto et al., 2012). This may be because greater initial root biomass is related to better root growth after transplanting (Grossnickle, 2012). Because *Miscanthus* cultivation is targeted to marginal lands, compost volume may be a decisive factor for establishment success.

The objective of this experiment was to evaluate the effects of combinations of factors that are expected to contribute to the economic and biological optimization

of seedling growth prior to field transplanting in spring. The factors chosen are appropriate and relevant to commercial seed-based *Miscanthus* plug production including supplemental heat, supplemental predawn light, compost type, and compost volume. We hypothesize that (i) elevated temperature and extended photoperiod at predawn will efficiently speed up plug growth and increase the production of axillary buds and secondary shoots and (ii) that compost type and compost volume will impact root-to-shoot ratios improving establishment rates. These results will help to find the optimum between nursery input that determines costs and plug resilience that determines field establishment.

2 | MATERIALS AND METHODS

2.1 | Plant material and site selection

The plant material used in this experiment was a commercial hybrid of Chinese *M. sacchariflorus* × Japanese *M. sinensis* (GRC-14; Clifton-Brown et al., 2019). This hybrid is high-yielding and has high seed multiplication rates of up to 5000 and is, therefore, well suited for seed-based propagation. Seeds were coated with turquoise colorant by Elsoms Seeds Ltd. to add a clear visual distinction between the seeds sown and the compost. The coating contained no germination stimulant but physically blocked pathogens at germination.

A glasshouse located at the Institute of Biology, Environmental and Rural Sciences (IBERS), Aberystwyth University, Gogerddan, Aberystwyth, United Kingdom (52°25′54.8″N, 4°01′07.3″W), was used to grow the seedlings in plugs. The 10-year average minimum and maximum temperatures in Aberystwyth from March to May were 2 and 16°C, respectively. Average hours of sunshine per day were 4.3, 6.1, and 7.0 in March, April, and May, respectively. The glasshouse was a plant material quarantine house, and the completely isolated compartments allowed different heat and light treatment combinations to be tested.

2.2 | Glasshouse experimental design and environmental control

A schematic view of the setup within the glasshouse is in Figure 1. The experiment was a 2^4 factorial design within a randomized complete block design. The four factors and their levels were as follows: with/without supplemental heat; with/without supplemental light; large/small compost volume; and two proprietary types of compost (Table 1).
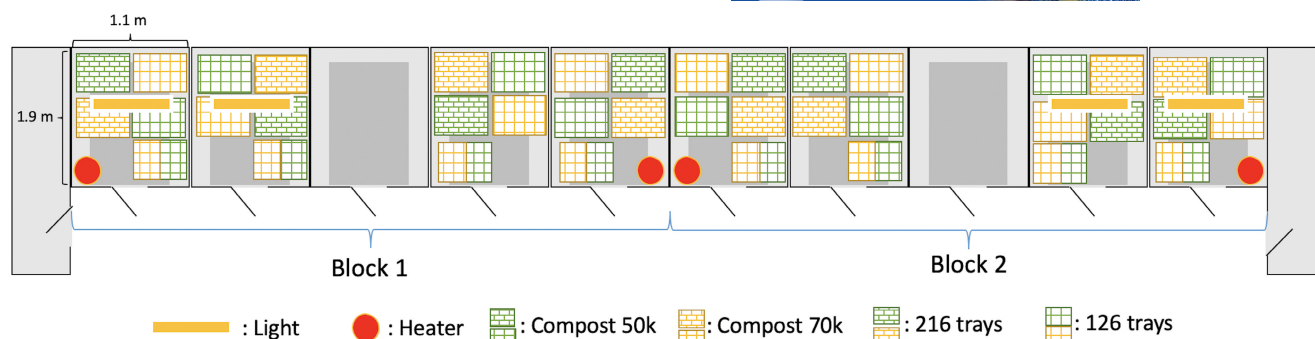
FIGURE 1 A schematic diagram of the experimental design

TABLE 1 The compost composition and volumetric container capacities of the two proprietary types of compost

Compost	Nitrogen (% w/w)	Phosphate-P (ppm)	Potassium (meq%)	Organic matter (%)	Container capacity (% v/v)
50k	1.06	150.1	2.33	86.8	84.8
70k	1.18	106.8	2.67	88.1	79.9

Temperatures varied with ambient air temperature, but supplemental heat maintained the air temperature in heated cabinets above 15°C, whereas the air temperature in all cabinets was maintained below 30°C by cooling fans. Inkbird ITC-308 (Inkbird Productions Ltd.) temperature controllers were used for temperature control. Supplemental light was provided between 3:00 and 8:00 hours because Paponov et al. (2020) had demonstrated that plants have higher PLUE during the predawn period than at other times of the day. Kroptek HiKROP-240 (Kroptek Ltd.) LED lights were placed 1 m above ground level to provide a PPFD of 300–400 μmol m^{-2} s^{-1} at plant height. Cabinets with supplemental light and without supplemental light were separated by one empty cabinet to avoid light spill between treatments. Compost type and compost volume treatments were nested under the environmental treatments of heat and light. The composition and volumetric "container capacity" (Cassel & Nielsen, 2018) of the two composts are described in Table 1. The trays with 126 plug cells per tray, herein referred to as the "126 trays," had a larger compost volume of 35 cm^3 per plug cell than the "216 trays" containing 216 plug cells per tray with a smaller compost volume of 15 cm^3 per plug cell. Each cabinet accommodated five trays (Figures 1 and 2b), three containing the larger volume cells in the "126 trays" and two containing the smaller volume cells of the "216 trays" to produce sufficient and similar numbers of plugs per treatment. The glasshouse was split into two blocks: the east and the west side of the glasshouse. The 16 treatments were replicated in the two blocks to make a complete randomized block design with two replicates (Figure 1).

Air temperature and photosynthetically active radiation (PAR) were monitored using a Campbell CR1000 data logger (Campbell Scientific Ltd.). A Type T thermocouple (copper/constantan) was installed in every cabinet and outside, and a quantum sensor was installed in two of the cabinets with LEDs and two without LEDs. Both the thermocouples and the quantum sensors were placed in the same position of the cabinet at plant height and were connected to the data logger (Figure 2b,c). Temperature and PAR readings were collected every 30 s and 10-min averages were recorded. Data from the same environmental treatment (heat and light) were grouped and averaged. Total growing degree days (GDDs) were calculated based on a base temperature of 10°C (Clifton-Brown et al., 2000, 2004; Khanna et al., 2008; McVicker, 1946).

2.3 | Germination conditions and germination test

Experience has shown that *Miscanthus* plug plants produce better biomass and root-to-shoot ratios on compact soil (Wilson, 2019). Consequently, the two supplied compost types were compressed to 2.3 times their loose volume by packing them down with a wooden peg on top of a thin layer of rockwool preventing any compost leakage from the plug drainage holes. Each tray of the same size was filled with the same weight of compost to the nearest gram to ensure consistency. The compost-filled trays were wetted up by dipping the plug trays into shallow water on a flat surface for about a minute and allowing water uptake by capillary rise through the drain holes
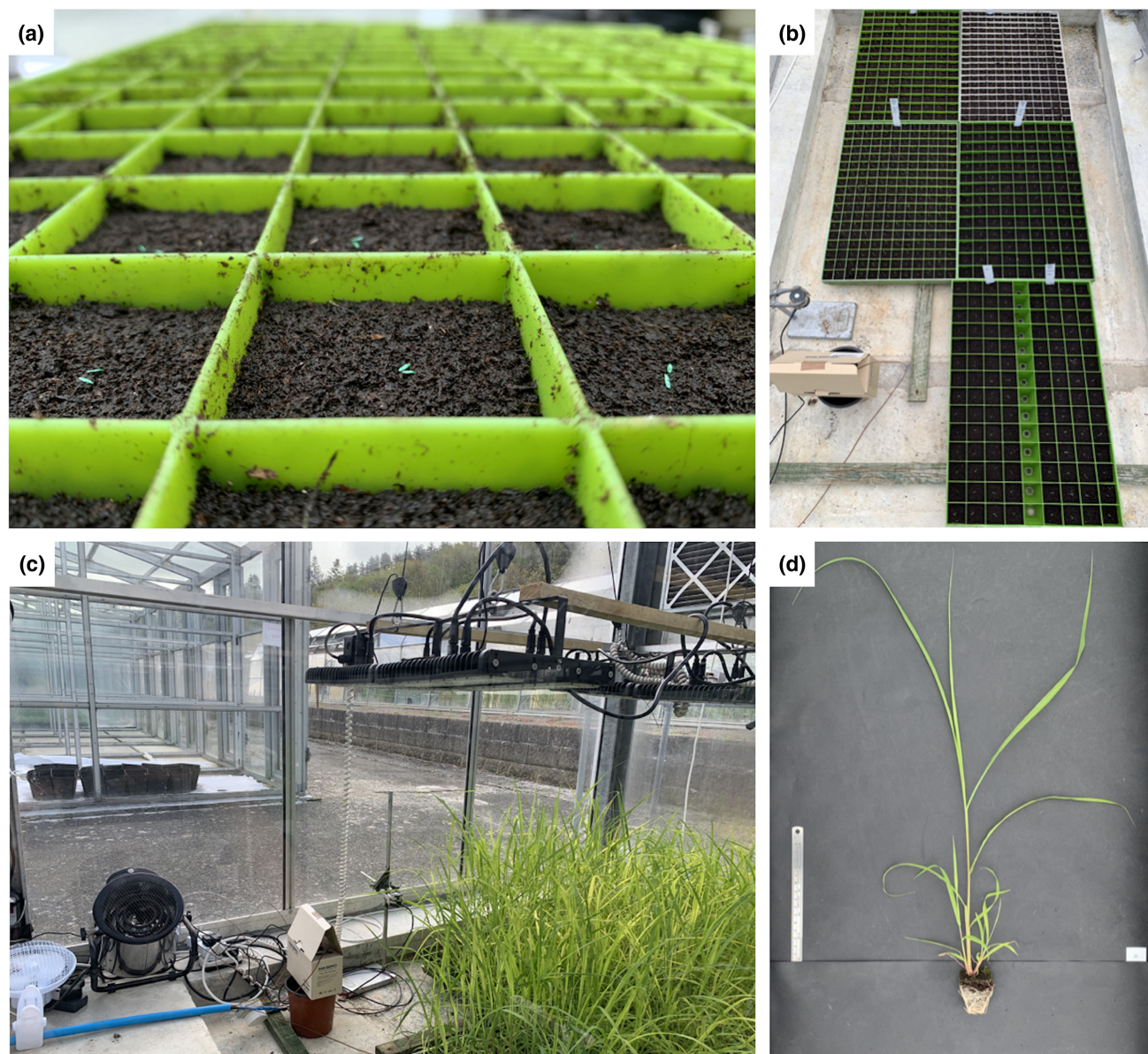
FIGURE 2 (a) Two seeds per plug cell (here the "126 tray") were sown on compressed compost. (b) Each cabinet had one and a half "126 trays" and one "216 tray" of each compost type. Datalogged thermocouples and the controller thermistors were shaded from direct radiation by cardboard. (c) The LED lights were positioned one 90 cm above the seedling canopy height and the fan heater and desk fan maintained air circulation. (d) Three plugs per tray were randomly harvested and imaged before morphological trait and biomass measurement

until a surface compost darkening indicated saturation. The trays were then allowed to free drain to container capacity and put onto capillary matting. To reduce what are known commercially as "blind plugs" where no seedling grows in a plug two seeds were sown per plug cell and in line with nursery sowing best practice. Seeds were sown (depth = 0 cm) directly on the compost surface (Figure 2a) and germinated in a temperature-controlled germination room at 23°C on 8 March 2021. The trays were covered with plastic film to retain moisture. Meanwhile, four Petri dishes each containing 100 seeds sown on traditional Whatman™ filter paper (Cytiva) were distributed across

the germination room to test the maximum germination potential of the seed lot used.

Germination percentages on a per seed basis were calculated from visual counts 1 week after sowing on four randomly selected replicates of the "126 trays" from each compost type and the four distributed Petri dishes.

2.4 | Glasshouse agronomic practices

On 15 March 2021, 1 week after sowing, the trays were moved from the germination room with capillary matting

to the glasshouse. Replicates of compost volume and compost type treatments were randomly assigned to cabinets of heat/light treatments using R (R Core Team, 2019). The trays were not placed on capillary matting (Figure 2b) but were lifted up 2.5 cm from the concrete floor on wooden laths to facilitate air root pruning. Air root pruning, which is commercial best practice, prevents the potential for damage of roots grown into the capillary matting when seedlings were removed at the end of the nursery period. To ensure that each plug in a tray obtained the same amount of water, irrigation was provided using a version of the "flood and drain method" by dipping the trays in shallow water when almost daily tray weighing indicated compost moisture contents had fallen below ~65%–70% of container capacity. Miracle-Gro® NPK 24-8-16 fertilizer (Evergreen Garden Care Ltd.) was applied at the suggested dosage for container plants from 6 weeks after sowing. This was applied weekly instead of fortnightly, as suggested by the manufacturer because dilution of fertilizer would occur during the dipping-tray irrigation. Trays were rotated within each cabinet after every irrigation to reduce the possible impact of local environmental heterogeneity. To introduce air movement needed to induce shoot strengthening through lignification a table fan was installed in a corner of each cabinet (Figure 2c).

2.5 | Morphological traits and biomass measurement of plugs

At the end of the nursery period on 19 May 2021, three plugs per treatment per block were randomly sampled (Figure 2d). Each plug, not each seedling, was considered as an experimental unit. Compost was gently washed from the roots by water. The number of leaves, stretched length (the "stretched length" described by Cornelissen et al., 2003), stem length, stem base diameter, and the number of short shoots ≥1 mm (axillary buds at "stage 20.5" described by Tejera & Heaton, 2017) to the number of main shoots were measured. Plugs were then oven-dried at 80°C until constant weight. Each plug was separated into leaves, stem, and root to determine the aboveground, belowground and total dry biomass.

2.6 | Statistical analysis

The germination percentages from each compost type and on filter paper in Petri dishes were tested with a one-way ANOVA using R. This used 50k compost, 70k compost, and paper as three levels to test the effects of compost type on germination.

Morphological trait and biomass measurements were analyzed with linear mixed-effects models using lmer function in R "lme4" package (Bates et al., 2015) considering the nested structure of the experimental design. Supplemental heat, supplemental light, compost type, compost volume, and block were treated as fixed factors and the four environmental condition categories of the cabinets (the heat/light treatment interactions) were treated as random grouping factors. Significance of factors was determined using likelihood ratio tests for mixed models. Satterthwaite's degrees of freedom approximation and p-value calculation used the drop1 function in R "lmerTest" package. Three scenarios of models were compared in which heat and light treatments were two-level ordinal variables or continuous variables as GDDs and daily light integrals (Table S1). In fitted model 2, GDDs were calculated by the McVicker (1946) equation on daily maximums and minimums, whereas in fitted model 3, GDDs were integral total GDDs based on temperature data recorded every 10 min in the glasshouses. Both functional and integral GDDs were calculated using a base temperature of 10°C. The rest of the factors were consistent as described above across the three models. The Akaike information criterion (Akaike, 1998) was used to compare model fit.

2.7 | Cost analysis

To evaluate the cost-efficiency of supplemental light, heat, and increasing compost volume, the corresponding costs, and CO_2 emission were calculated based on the experimental setup and multiplied to commercial scale. The two types of compost used did not differ in price and, thus, were not compared with one another. Field planting density was estimated as 16,000 plants per hectare (Hastings et al., 2017; Xue et al., 2015). The costs include costs of electricity, trays, compost, and fertilizer. Nondomestic electricity price was 0.133 GBP according to DBEIS (2021a). Electricity emissions were 0.212 kg equivalent CO_2 per kWh DBEIS (2021b).

3 | RESULTS

3.1 | Glasshouse environmental conditions

The average daily ambient temperature during the period of the glasshouse treatments between 15 March to 19 May 2021 was 10.4°C, with an average daily minimum temperature of 5.3°C and an average daily maximum temperature of 16.7°C. The average temperatures at plant level in

cabinets with both heat and light, cabinets with heat but without light, cabinets without heat but with light, and cabinets without heat nor light were 19.3, 18.9, 18.3, and 17.5°C, respectively, with average minimum daily temperatures of 14.6, 14.4, 11.6, and 11.1°C and average daily maximum temperatures of 27.2, 26.5, 26.1, and 27.0°C, respectively. Heat emitted by the LED lights increased the temperature by approximately 3°C within 1 h of the lights being on. Supplemental heat provided an average of extra 114.0 (18.9%) functional total GDDs or 76.4 (15.3%) integral total GDDs. Supplemental light provided an average of extra 18.5 (3.1%) functional total GDDs when calculated conventionally from temperature maximum and minimum of 40.8 (8.2%) integral total GDDs when the temperature measurements throughout the day were integrated (Table 2).

The average daily light integral from natural light in the glasshouse was 13.8 mol m^{-2} day^{-1}. The supplemental LED lights added 5.3 mol m^{-2} day^{-1}, adding on average 38.4% to the daily light integral. Supplemental LEDs were especially impactful of daily light integral in the first 2 weeks of the nursery period, when the average daily light integral from natural light was only 6.0 mol m^{-2} day^{-1}.

3.2 | Seed germination

The percentage of seed that germinated was greater than 90% on all three media (Table 3). There was no significant difference ($p = 0.25$) in germination between seeds germinated on paper in Petri dishes, 50k compost, or 70k

TABLE 2 Extra functional and integral total growing degree days (GDDs °C-day) under different supplemental heat and light treatments

	Extra functional growing degree day	Extra integral growing degree day
Heat + Light	+132.5	+117.1
Heat, no light	+95.0	+88.8
Light, no heat	−0.5	+53.2
None	0.0 (602.5)	0.0 (498.0)

Note: In both cases, the base temperature was 10°C.

TABLE 3 Germination rates in different media

	Germination rate (%)
Petri dish	95.8 ± 1.71[a]
Compost 50k	94.4 ± 0.56[a]
Compost 70k	93.4 ± 2.74[a]

[a]There was no significant difference ($p = 0.25$) among the three treatments.

compost. In the trays, on average, 0.7%–1% of cells were empty where neither seed had germinated.

3.3 | Treatment effects on morphological traits and biomass

Block had no significant effect on any of the traits measured or biomass; therefore, it was removed from subsequent analyses, and further discussion. Supplemental heat to maintain a minimum temperature of 15°C significantly increased the root to shoot dry matter ratio by 9% (Table 4). Heat did not significantly affect any of the other traits measured (Table 5).

Supplemental predawn light increased aboveground biomass by 111% and belowground biomass by 155% (Table 4). Total biomass was 120% higher under supplemental light. It also increased the root-to-shoot ratio. The effects of supplemental light produced the greatest statistical significance compared with other factors that had significant effects on biomass accumulation (Table 5). Increases in stem base diameter were also associated with extra light, but this was not significant. Compost significantly affected total biomass but not shoot-to-root ratio. The 70k compost produced plugs with 42% more aboveground biomass and 46% more belowground biomass (Table 4) than the 50k variant. Similarly, compost volume significantly affected both the aboveground and belowground biomass but not shoot-to-root ratio. The larger compost volume produced plugs with 52% more aboveground biomass and 57% more belowground biomass (Table 4). Increasing compost volume increased total biomass by 2.7% per cm^3 rooting volume. Compost volume was the only factor that had a significant effect on stem base diameter in this experiment (Table 5). The larger compost volume increased stem base diameter by 16.7% (Table 4). There was a significant interaction between light and compost volume. The larger compost volume resulted in 72.1% greater total biomass and 33.5% greater in stretched length when grown with supplemental light than the smaller compost volume under the same lighting. Adding light to the larger compost volume resulted in 29.4% more total biomass per daily light integral. No treatment had a significant effect on the production of short axillary shoots.

3.4 | Fitted models

Among the three fitted models, model 1, in which the heat and light variables were both ordinal factors, has a global best fit to the data (Table S2). The results from the three models are mostly consistent except for slight differences

TABLE 4 The impact of different environmental conditions on the growth of Miscanthus seedlings growing as plug plants in a controlled environment glasshouse

Treatment	Shoot dry weight (mg)	Root dry weight (mg)	Root-to-shoot ratio	Stem base diameter (mm)	No. of short to main shoots
Heat					
Y	264.5 ± 178.0	87.1 ± 70.5	0.31 ± 0.07	4.0 ± 0.9	1.6 ± 1.3
N	240.2 ± 145.8	71.8 ± 51.3	0.29 ± 0.05	3.8 ± 0.8	1.2 ± 0.9
Light					
Y	345.7 ± 172.8	115.3 ± 68.5	0.33 ± 0.06	4.4 ± 0.8	2.0 ± 1.2
N	164.0 ± 83.4	45.3 ± 24.2	0.28 ± 0.05	3.4 ± 0.7	0.9 ± 0.9
Compost type					
70k	297.5 ± 183.7	94.6 ± 74.1	0.30 ± 0.06	4.0 ± 0.9	1.3 ± 1.2
50k	209.2 ± 125.6	64.7 ± 42.6	0.30 ± 0.06	3.8 ± 0.8	1.5 ± 1.2
Compost volume					
Large	302.3 ± 188.9	96.2 ± 74.6	0.30 ± 0.06	4.2 ± 0.9	1.5 ± 1.1
Small	198.3 ± 104.3	61.1 ± 36.3	0.30 ± 0.06	3.6 ± 0.7	1.3 ± 1.2

Note: The mean value ± 1 SE (standard error) is shown ($n = 6$).

TABLE 5 *P*-values of likelihood ratio tests for factor effects under the three model-fitting scenarios

Factor	Model	Shoot dry weight	Root dry weight	Root-to-shoot ratio	Stem base diameter	Number of short to main shoots
Heat	1	0.4133	0.2234	0.0427*	0.5971	0.4430
	2	0.4914	0.1238	0.0054**	0.2507	0.1187
	3	0.5050	0.2036	0.0180*	0.5215	0.2330
Light	1	0.0002***	0.0003***	0.0001***	0.1648	0.2210
	2	0.0002***	0.0002***	0.0002***	0.0897[†]	0.1658
	3	0.0007***	0.0013**	0.0088**	0.1440	0.3820
Compost type	1	0.0212*	0.0376*	0.8548	0.1675	0.6930
	2	0.0189*	0.0287*	0.9356	0.1495	0.7900
	3	0.0206*	0.0371*	0.8801	0.1608	0.7610
Compost volume	1	0.0162*	0.0354*	0.5045	0.0001***	0.6240
	2	0.0154*	0.0331*	0.4320	0.0002***	0.7208
	3	0.0162*	0.0378*	0.4800	0.0002***	0.6920

Note: In each model, there are a response variable, the measurement, and four fixed-effect variables: heat, light, compost type, and compost volume. Model 1 considered both heat and light variables as ordinal, two-level factors. Model 2 used functional total growing degree days as the heat variable and daily light integral as the light variable. Model 3 used integral total growing degree days as the heat variable and daily light integral as the light variable. Compost type and compost volume variables were seen as ordinal, two-level factors in all the models.

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

[†]$p < 0.1$.

in significance levels for root dry weight and root-to-shoot ratio (Table 5).

4 | DISCUSSION

Extensive field trials have shown that poor first-year establishment is common (Zimmermann et al., 2014), and as crops are grown on lower grade lands less suitable for food crops under more extreme weather associated with climate change that the challenge for early plant development intensifies. Agronomic methods need to be developed that not only can manage these risks but also ensure faster maturation of the rhizome, which allows the plant to withstand climate extremes. Further accelerating establishment is important to resist weed pressure and to reach maximum yield earlier (Clifton-Brown et al., 2017; Lewandowski et al., 2016). Our experiment aimed

to address the glasshouse growing conditions needed to grow stronger plug plants with optimum input using a novel seeded interspecific *Miscanthus* hybrid.

Germination in both composts was as good as that in Petri dishes (Table 3), suggesting that the germination method used allowed the seed lot to reach its maximum germination potential (96%) and no inhibitors were present.

We hypothesized that it would be more true to reality using total GDDs and daily light integral data from the datalogger as numeric independent variables in the mixed-effects models (Table S1). The common McVicker's (1946) GDD equation only takes account of daily maximum and minimum temperatures, thus the 5-h additional heat emitted by the LEDs could not be exhibited (Table 2). On the other hand, integral GDDs show a clear gradient of extra GDDs from both supplemental heat and light (Table 2). Yet, the three models with ordinal or numeric, functional/integral independent variables of heat and light did not differ in model fits (Table S2).

To maximize resource use efficiency, various aspects of propagation by seed-based *Miscanthus* plug were scrutinized and refined in this study. The use of LEDs instead of high-pressure sodium lamps saved 40% of energy consumption and thus CO_2 emission for lighting (Katzin et al., 2021). Providing light at predawn maximized light use efficiency (Paponov et al., 2020; Tewolde et al., 2016). The reusable, durable trays reduced costs as well as plastic waste compared with disposable trays.

Further experiments are needed to link growth in the glasshouse and performance in the field. However, common indices of plug resilience include aboveground and belowground dry biomass, root-to-shoot ratio, and stem base diameter (Bantis et al., 2016; Grazia et al., 2002; Meyer & Cunliffe, 2004; Poorter, Bühler, et al., 2012; Romero-Munar et al., 2018; Velasco & Mattsson, 2019; Zheng et al., 2021). In addition, we consider the ability to form new side shoots from axillary meristems that form on the nodes a key to *Miscanthus* plug resilience. New side shoot formation was hypothesized to be a possible factor in the survival and establishment success of bluestem, switchgrass (Smart et al., 2003), and two other grass species (Brown, 1982) but was not definitively proven. As *Miscanthus* seedlings mature the stem base develops a lignified node with a meristematic ring of small buds like in other grass species (Labanauskas, 1954). These axillary buds at stem base indicate regrowth ability (Withers, 2015). Once developed this increases the resilience to cold and desiccation after planting which reduces transplanting losses (Close et al., 2005; Withers, 2015). In the UK, field experiments are ongoing with transplanting to the field, the transplants are covered by mulch film that increases heat and reduces evaporative moisture loss (Zhao et al., 2012). Most of the initial aboveground growth will die and newly formed side shoots

become dominant and break through the mulch film. In this case, it might be more interesting to focus on factors that significantly affect belowground biomass, which is related to root growth capacity, and stem base diameter and short shoot formation, which are related to mechanical resistance and transplant growth. Our results show that supplemental heat, supplemental light, compost type, and compost volume each had significant effects on seed-based *Miscanthus* plug development.

4.1 | Supplemental heat

It was anticipated that supplemental heat would promote shoot extension and new shoot emergence by providing extra GDDs (Clifton-Brown & Jones, 1997, 1999; Farrell et al., 2006). Instead, supplemental heat increased the root-to-shoot ratio. No other expected significant effects of heat were found. In 33 out of the 66 days of nursery period, the daily minimum temperatures outside the glasshouse were below 5°C. Nevertheless, the glasshouse provided relatively mild temperatures during the nursery period; in a cabinet without heat nor light, the average temperature was 17.5°C, with an average minimum of 11.1°C. The base temperature for *Miscanthus* ranges from 6 to 10°C (Davey et al., 2017; Farrell et al., 2006), and there is significant genotypic variation in cold tolerance (Clifton-Brown & Lewandowski, 2000; Farrell et al., 2006) and thermal response of plant extension rate (Clifton-Brown & Jones, 1997). The base temperature of the specific hybrid used here remains to be determined. On the other hand, narrowing diurnal temperature amplitude negatively impacted seedling aboveground biomass of maize under warm and high-temperature conditions (mean daily temperature of 30 and 35°C) due to night respiration (Sunoj et al., 2016).

Contrary to our results, Poorter, Niklas, et al. (2012) reviewed studies on biomass allocation and pointed out a negative correlation between temperature and root-to-shoot ratio. This is because shoot growth is more sensitive to temperature than root growth (Engels & Marschner, 1990). While there was no significant difference in total biomass between the heat treatments in this experiment, the increase of root-to-shoot ratio in this experiment may be due to a greater water deficit experienced between watering under the heated treatment. The evapotranspiration rate in heated/lit cabinets was higher than in those without heat/light. All the trays were watered at the same time once any of the trays fell below the irrigation threshold (65%–70% container capacity); therefore, plugs in heated/lit cabinets experienced wider variations in water availability. Increased biomass allocation towards roots under short (≤4 days, Padilla et al., 2009) and long (10–28 days, Fleisher

et al., 2013) periodic drought cycles has been reported. This may point to the potential of introducing stress to plug plants to improve *Miscanthus* seedling establishment. Deficit irrigation is a common nursery pre-conditioning strategy for improved transplant survival under semi-arid to arid conditions (Franco et al., 2006; Sánchez-Blanco et al., 2019). Drought pre-conditioning was also found to be linked to improved freezing tolerance in some genotypes of *Lolium perenne* (Hoffman et al., 2012).

4.2 | Supplemental light

This study was the first to test the effects of supplemental light on seed-based *Miscanthus* plug quality in a commercial nursery-like environment. The results show significant positive effects of light on biomass accumulation and an increase in root-to-shoot ratio. Such results demonstrate the effectiveness of predawn/nighttime supplemental light on out-of-season growth in temperate growing conditions as previously reported by Paponov et al. (2020) and Tewolde et al. (2016). Earlier experiments with pot grown *Miscanthus* showed that at constant temperature, leaf extension rates were faster in the night than in day (Clifton-Brown & Jones, 1997). This was attributed to decreased evaporative demands at night, with a concomitant increase in turgor to driven cell expansion. An enhanced root pressure by nighttime LED lighting further facilitated water supply and subsequent aboveground growth (Paponov et al., 2020). However, stem base diameter and new axillary short shoot formation were not significantly increased. Boersma and Heaton (2012) found that a daily 8-h 507 μmol m^{-2} s^{-1} PPFD (14.6 mol m^{-2} day^{-1} daily light integral) illumination increased $M \times g$ axillary shoots initiation from lower node cuttings compared with complete dark treatment under a constant soil temperature of 30°C. This may indicate that axillary bud formation in *Miscanthus* plug involves both light and temperature and that the conditions in this experiment did not exceed the threshold.

A PPFD of 300–400 μmol m^{-2} s^{-1} used in this experiment was higher than in most published research on supplemental light for seedlings (Bantis et al., 2018; Viršilė et al., 2017). Light intensity was proportional to seedling dry weight and root growth capacity in shade-intolerant *Pinus* seedlings (Velasco & Mattsson, 2019) and root-to-shoot ratio in *M. sinensis* seedlings (Matlaga et al., 2012) but did not affect stem diameter in *Pinus* seedlings.

4.3 | Compost type

Previous studies demonstrated both compost-dependent (Lee et al., 2005; Zheng et al., 2021) and compost × genotype interaction-dependent (Zheng et al., 2021) variation in *Miscanthus* plug biomass. In Zheng et al. (2021), the three composts best for each *M. sinensis*, *M. lutarioriparius*, and *M. sinensis × M. lutarioriparius* genotype all shared similar physical properties of higher water holding capacity, air-filled porosity, and air-to-water ratio despite having distinct NPK components and were all peat-perlite based. In Lee et al. (2005), a peat:perlite 1:1 (v/v) compost resulted in the best seedling growth and new side shoot formation in *M. sacchariflorus* compared with other mixtures of peat, perlite, and vermiculite. We did not observe any change in new side shoot formation caused by compost, but one of the composts produced significantly more total biomass than the other. In our study, both composts were finely milled peat-based with high water holding capacity, differed slightly in nutrient levels (Table 1). Because all the plants were under the same fertilizer regime, initial nutrient levels should not have a significant impact. The compost 70k that had lower volumetric container capacity generated greater aboveground and belowground biomass. These suggest that water holding capacity and aeration are both important elements for *Miscanthus* plug growth. The peat-based composts in this study provided sufficient water availability but not necessarily aeration. A further increase in aeration could possibly improve plug biomass or lateral growth, which needs to be verified by future experiments.

We provided plugs of all treatments with the same amount of compost by weight. Since the two composts differed in water holding capacity, the actual dry matter of compost per plug differed by compost type, with 50k having less dry compost. This resulted in different water availability, firmness, and aeration. In addition, due to watering being determined by the trays that had the least water, the plants under the 50k compost treatment experienced less water deficit (data not shown). This experiment could not distinguish the effects of factors that confounded with compost type.

4.4 | Compost volume

The findings in this experiment on compost volume are consistent with previous studies (Bernaola-Paucar et al., 2018; Dominguez-Lerena et al., 2006; Hanson et al., 2017; Jacobs et al., 2020; Meyer & Cunliffe, 2004; Pinto et al., 2012, 2015). Larger compost volume here provided the seedlings better access to a range of resources: light (both natural and artificial), water, nutrients, and rooting volume. Compost volume was positively correlated with the size of plug plants produced, indicating that using larger compost volumes could effectively shorten the nursery time needed for seed-based *Miscanthus* plugs, as suggested

Heat	Light	Compost volume	Costs (GBP)	Extra biomass (mg/ pence)	Energy use (kWh)	CO$_2$ emission (kg)
		S	201	-	0	0
		L	414	68	0	0
	✓	S	449	113	1867	396
	✓	L	840	91	3200	679
✓		S	2544	6	17,618	3741
✓	✓	S	2792	11	19,485	4137
✓		L	4431	2	30,203	6413
✓	✓	L	4857	16	33,403	7092

TABLE 6 Economic costs, extra biomass per plug per extra pence spent (compared with no additional heat, light, and the smaller compost volume), energy use, and equivalent CO$_2$ emission by electricity of establishing one hectare of *Miscanthus* using different heat/light treatments and compost volumes to produce spring-sown plug plants

Note: The table is sorted by costs.

Abbreviations: L, large; S, small.

by Hwang et al. (2018). Moreover, plants grown in larger compost volume had a greater biomass increase under the supplemental light treatment. The larger compost volume provided 65% more surface area per plug than the smaller compost volume, allowing more light interception at lower canopy. Leaves forming the canopy are the main source of photoassimilates for the roots in many species (Wardlaw, 1990). A more abundant supply of photoassimilates to roots also enhanced water and nutrient supply from roots to shoots (Paponov et al., 2020).

The downsides of increasing compost volumes include more inputs such as compost, water, fertilizer, space, and electricity. It also will slow planting by requiring more frequent pauses for reloading trays for the same numbers of plants. Current *Miscanthus* plug nurseries generally use the "216 trays" with smaller compost volumes and would need to change the module of their automatic planting machine if shifting to larger compost volumes. Despite these downsides, compost volume could be worthy of investment since it significantly affected belowground biomass that is related to root growth capacity, and stem base diameter that is related to mechanical resistance (Close et al., 2005). The initial size of transplants resulted from compost volume had long-term effects on biomass yield in perennial crops (Dominguez-Lerena et al., 2006; Pinto et al., 2015; Romero-Munar et al., 2018) and would possibly influence the time required to reach maximum yield.

4.5 | Economic costs and greenhouse gas costs

Supplemental light alone resulted in the highest plug biomass gain per extra pence spent (113 mg/pence), 18.8 times of supplemental heat alone (6 mg/pence), and 1.7 times of larger compost volume alone (68 mg/pence, Table 6).
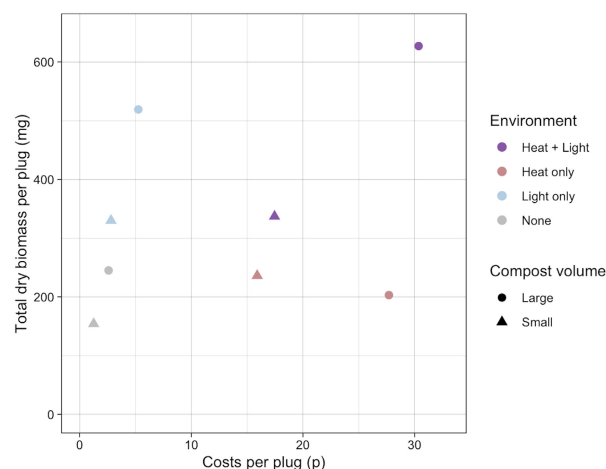

FIGURE 3 Average of total biomass per plug (mg) by compost volume, heat, and light treatment combinations and the corresponding costs per plug (pence). Each data point represents the average biomass of 12 plug samples. The two types of compost used did not differ in price and, thus, were not compared with one another. Costs include electricity, trays, compost, and fertilizer

Introducing supplemental heat would surge the costs yet had the lowest cost-efficiency (Figure 3, Table 6). A shift from the "216 trays" with a smaller compost volume to the "126 trays" with a larger compost volume nearly doubled the costs due to the extra nursery space needed but resulted in significant biomass increases per pence when without supplemental heat (Figure 3, Table 6).

The optimized plug biomass was generated under supplemental light and the larger compost volume (Figure 3) at an economic cost of 840 GBP per ha. This is similar to the estimation of seed-based plug production costs by Hastings et al. (2017), whereas the greenhouse gas emissions were almost halved (1264 kg CO$_2$ in Hastings et al., 2017). However, the reduction in greenhouse gas emissions was mainly because of the reduction in the conversion

factors of equivalent CO_2 emissions by electricity from 2016 (0.412 kg CO_2 per kWh generated; DBEIS, 2016) to 2021 (0.212 kg CO_2 per kWh generated; DBEIS, 2021b).

4.6 | Summary and conclusions

The use of supplemental light, the 70k compost, and larger compost volume maximized the cost-efficiency of input-biomass production while improving various complementary plug resilience indices: belowground biomass, which is related to root growth capacity; stem base diameter, which is related to mechanic resistance; and plug plant size, which is related to maturation rate and yield within the first few years of establishment. Therefore, we recommend nurseries in the United Kingdom use LEDs as predawn supplemental light but no supplemental heat, compost that has both good water holding capacity and aeration, and a larger compost volume for spring-sown seed-based *Miscanthus* plug production.

CONFLICT OF INTEREST
There is no conflict of interest for this article.

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