

RESEARCH ARTICLE

Spring emergence and canopy development strategies in miscanthus hybrids in Mediterranean, continental and maritime European climates

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Abstract

Due to its versatility and storability, biomass is an important resource for renewable materials and energy. Miscanthus hybrids combine high yield potential, low input demand, tolerance of certain marginal land types and several ecosystem benefits. To date, miscanthus breeding has focussed on increasing yield potential by maximising radiation interception through: (1) selection for early emergence, (2) increasing the growth rate to reach canopy closure as fast as possible, and (3) delayed flowering and senescence. The objective of this study is to compare early season re-growth in miscanthus hybrids cultivated across Europe. Determination of differences in early canopy development on end-of-year yield traits is required to provide information for breeding decisions to improve future crop performance. For this purpose, a trial was planted with four miscanthus hybrids (two novel seed-based hybrids *M. sinensis* × *sinensis* [*M sin* × *sin*] and *M. sacchariflorus* × *sinensis* [*M sac* × *sin*], a novel rhizome-based *M sac* × *sin* and a standard *Miscanthus* × *giganteus* [*M* × *g*] clone) in the UK, Germany, Croatia and Italy, and was monitored in the third and fourth growing season. We determined differences between the hybrids in base temperature, frost sensitivity and emergence strategy. *M* × *g* and *M sac* × *sin* mainly emerged from belowground plant organs, producing fewer but thicker shoots at the beginning of the growing season but these shoots were susceptible to air frosts (determined by recording 0°C 2 m above ground surface). By contrast, *M sin* × *sin* emerged 10 days earlier, avoiding damage by late spring frosts and producing a high number of thinner shoots from aboveground shoots. Therefore, we recommend cultivating *M sac* × *sin* at locations with low risk and *M sin* × *sin* at locations with higher risk of late spring frosts. Selecting miscanthus hybrids that produce shoots throughout

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the vegetation period is an effective strategy to limit the risk of late frost damage and avoid reduction in yield from a shortened growing season.

KEYWORDS

base temperature, late spring frost, miscanthus, number of shoots, perennial rhizomatous grass, shoot sprouting, thermal time

1 | INTRODUCTION

In order to counteract anthropogenic global warming, the European Union increased the share of renewables in its energy mix from 9.6% in 2004 to 22.1% in 2020 (Eurostat, 2022). Biomass is one of many diverse resources available to meet global energy demands. Its main advantages are its storability and versatility (fuel, heat, and electricity), making it one of the most important renewable energies. Biomass already accounts for 60% of the EU's renewable energy supplies (European Union, 2019) and is additionally used as a feedstock for material applications. However, increasing demands and limited land availability make it necessary to use available land productively and simultaneously reduce the negative environmental impacts of the agricultural ecosystem. High-yielding biomass plants can play an important role here. Miscanthus, a perennial C_4 grass, is a promising biomass crop due to its high yield potential, low input demand (McCalmont et al., 2017) and diverse environmental benefits (Ferrarini et al., 2017; Lask et al., 2020; McCalmont et al., 2017). Although the initial investment is relatively high, once established, miscanthus offers simplified crop management with no need for soil cultivation and minimal fertiliser and herbicide requirements in the establishment phase due to rapid canopy closure (Clifton-Brown et al., 2007). Miscanthus can grow on contaminated (Nebeská et al., 2021; Rusinowski et al., 2019; Wang et al., 2021) and low-productivity soils (Awty-Carroll et al., 2022; Kalinina et al., 2017). When cultivated as a biomass crop on marginal land, it offers economic and environmental benefits and, at the same time, minimises conflicts with other land-use (Shepherd et al., 2023) options. Its suitability for cultivation as a commercial biomass crop in Europe has been investigated in several studies (Awty-Carroll et al., 2022; Kalinina et al., 2017; Lewandowski et al., 2000). This, and the development of global productivity models for miscanthus (Shepherd et al., 2020, 2023), have highlighted its potential and driven breeding programmes. A further understanding of genotype-by-environment ($G \times E$) interactions and the exploitation of natural genetic variation will become increasingly important in maximising productivity. One major determinant of the yield of miscanthus is the length of time between canopy closure and

aboveground dieback (Clifton-Brown et al., 2019; Robson, Farrar, et al., 2013). Until now, breeding programmes have focused on maximising the length of time of radiation interception through manipulating the growth start date (emergence, defined as the time of the first visible aboveground shoot), increasing the growth rate in the early vegetation period to reach canopy closure as fast as possible, and extending the vegetation period by delayed senescence (Robson, Farrar, et al., 2013). This breeding strategy aims to maximise the active growth period (period in which crop biomass increases) within the vegetation period. The active growth period is defined by miscanthus biomass models as either: (1) the period between the last day of frost in spring and the first frost event in autumn (Clifton-Brown et al., 2000; Hastings et al., 2009), or (2) days with an average daily temperature above 10°C (Kobayashi & Yokoi, 2017; Kotrla & Prcik, 2013, 2020). However, early emergence is only an effective strategy if the new shoots are not killed by late spring frosts (frost occurring at the beginning of the vegetation period). Frost and freezing damage plant tissue irreversibly through osmotic shock, dehydration stress and/or the formation of ice crystals. Damage by late frost events is mainly caused by a radiation freeze, which occurs during calm, clear nights with a dry atmosphere (Hocevar & Martsof, 1971; Leuning, 1988; Mukhopadhyay & Roychoudhury, 2018; Nobel, 2020). Conversely, freezing injuries occur during an advective freeze, where horizontal air movement at temperatures below freezing causes the formation of ice crystals in plant tissues (Mukhopadhyay & Roychoudhury, 2018). In this study, frost and freeze damage is referred to collectively as “frost damage”.

The vegetation period in Europe is currently extending as climate warming decreases the total number of days with temperatures below freezing (Menzel & Fabian, 1999; Myneni et al., 1997). However, these higher temperatures are also stimulating an earlier start to the growing season that in turn increases the risk of frost damage at the beginning of the growing season (Kreyling, 2013; Liu et al., 2018). In Europe, the number of frost days in the vegetation period increased significantly from 1982 to 2012, primarily in spring (Liu et al., 2018). For this reason, a better understanding of the early development of miscanthus hybrids, their vulnerability to late frost events

and the different strategies of the plants to overcome frost damage is crucial for the breeding of site-adapted hybrids and choice of site-specific hybrids.

The objective of this research is to assess the early plant growth of a set of four different miscanthus hybrids cultivated at multiple locations in Europe by identifying differences in leaf development in early spring and their effects on end-of-year yield parameters. The identification of strategies used to cope with frost damage can provide important information for the selection of the miscanthus hybrid best suited for each location and support selection for further breeding. The hybrids assessed were: (1) a standard rhizome-based clone of *Miscanthus* \times *giganteus* (*M* \times *g*); (2) a novel seed-based *M. sinensis* \times *sinensis* (*M sin* \times *sin*) hybrid; (3) a novel seed-based *M. sacchariflorus* \times *sinensis* (*M sac* \times *sin*) hybrid; and (4) a novel rhizome-based *M. sacchariflorus* \times *sinensis* (*M sac* \times *sin*) clone. They were cultivated in a multi-location trial at sites in the UK, Germany, Croatia and Italy. This study assesses the genetic and environmental factors determining the emergence of the miscanthus hybrids. It also analyses the effect of late spring frosts on the newly emerging shoots and the end-of-year yield parameters.

2 | MATERIALS AND METHODS

The field trials on which this study is based are part of the BBI JU demonstration project GRACE (www.grace-bbi.eu) and are described in detail by Awty-Carroll et al. (2022).

The four trial sites are located:

- at Trawsgoed (TWS; 52°20'12.48, 3°56'52.1556, 72 m a.s.l.) near Aberystwyth in Wales, United Kingdom.
- at Oberer Lindenhof (OLI; 48°28'45.42, 9°18'42.67, 706 m a.s.l.) on the Swabian Alb near Stuttgart, Germany.
- near Zagreb (ZAG; 45°85'05.55, 16°17'77.76, 119 m a.s.l.), Croatia, and
- near Piacenza (PAC; 45°00'30.33, 9°70'98.49, 70 m a.s.l.), Italy.

They were planted in 2018 by hand in a randomised complete block design with four replicates and 14 hybrids. Each plot was 9 m \times 9.68 m with a row distance of 0.75 m. From the first year after planting, the biomass in the measurement area at each site was harvested annually in spring (date depended on local weather conditions) by hand at a cutting height of 10 cm. The remaining border area was machine-harvested at the same time. Details of the four miscanthus hybrids investigated in this study and the different planting densities are given in Table 1.

Weather data, including hourly average, minimum and maximum temperatures, were recorded at each site by a WS-GP1 Compact Weather Station (Delta-T Devices Ltd) connected to the Delta-T cloud server. In addition, temperature loggers recorded soil temperature (Thermochron iButton, iButtonLink Technology) at a depth of 5 cm during the 2018/2019 winter at all sites and the 2019/2020 winter at OLI. Long-term average daily temperature and total monthly precipitation from 2009 to 2019 were obtained from the nearest weather station (TWS: adjacent to the TWS site; OLI: located on the field station; ZAG: at Zagreb-Pleso approximately 15 km from the field station; PAC: located on the field station).

2.1 | Field measurements

In spring 2020 and 2021, measurements of crop height, frost damage, and light interception were taken on a weekly basis at each site from five designated plants within the measurement area of each plot (see Awty-Carroll et al., 2022). The crop height was measured from the same side of the plant from the soil surface to the tip of the tallest shoot (Figure 1). Where frost had caused tissue damage, shoot height was measured up to the visible green tip. The extent of frost damage was assessed in the week following a frost, quantified using a 0–9 score. A score of 0 was given to plants with no visible frost damage. Plants with a score of 1 had mild scorching to the tips of leaves, and up to four had shrivelled or pulpous leaf tips. Plants

TABLE 1 Description of miscanthus hybrids used in the multi-location trials.

Hybrid	Species	Propagation method	Origin	Planting density
GRC 3	<i>Miscanthus sinensis</i> \times <i>sinensis</i> (<i>M sin</i> \times <i>sin</i>)	Seed-based hybrids	Wageningen University breeding programme	3 plants m ⁻²
GRC 9	<i>M.</i> \times <i>giganteus</i> (<i>M</i> \times <i>g</i>)	Rhizome-based clone	Terravesta	1.5 plants m ⁻²
GRC 14	<i>M. sacchariflorus</i> \times <i>sinensis</i> (<i>M sac</i> \times <i>sin</i>)	Seed-based hybrids	Aberystwyth University breeding programme	1.5 plants m ⁻²
GRC 15	<i>M. sacchariflorus</i> \times <i>sinensis</i> (<i>M sac</i> \times <i>sin</i>)	Rhizome-based clone	Terravesta	1.5 plants m ⁻²



FIGURE 1 Height measurement from the ground to the top of the tallest living shoot.



FIGURE 2 Two light measurements were taken: (1) incident light measured above the crop or outside the plot if the crop was too high; and (2) transmitted light within the plant 10 cm above ground level.

with a score of 9 were severely damaged, with all above-ground shoots (leaves and stems) killed by the frost. In this case, regrowth is only possible from the belowground

TABLE 2 Categorisation of frost events.

Categorisation of the frost event	Hourly minimum temperature
Extreme	$x \leq -9.3^{\circ}\text{C}$
Severe	$-9.3^{\circ}\text{C} < x \leq -6^{\circ}\text{C}$
Moderate	$-6^{\circ}\text{C} < x \leq -3^{\circ}\text{C}$
Light	$-3^{\circ}\text{C} < x \leq 0^{\circ}\text{C}$

rhizome meristems. At the end of each growing season, the number of shoots taller than 60% of the plant's height were counted.

The date of canopy closure (defined as 85% light interception) was determined by regular measurements of the light interception using a LightIntercepta (Awty-Carroll, 2020) in manner similar to that described in Robson, Farrar, et al. (2013). The LightIntercepta was assembled as suggested by Salter et al. (2019). It measures the relative light over 1 m using six pairs of photodiodes. The photodiodes are covered by 3 mm opal acrylic sheet to diffuse the light and provide a water-free internal environment (Salter et al., 2019). The light intercepted by the canopy was measured by positioning the LightIntercepta horizontally at an angle of 45° to the plant row (Figure 2) through the middle of a plant at a height of 10 cm above soil level. An additional reference measurement was taken with no light interception, either above the crop or outside the plot if the crop was too high. In total, five measurements per plot were taken.

2.2 | Data analysis

This study defines emergence as the time when visible aboveground shoots can be detected. The day of emergence was identified by a back-correlation from up to four steadily increasing shoot height measurements (Nunn et al., 2017). Due to frost damage however, a reverse correlation was not always possible. In such cases, the mean value between the measurements immediately before and after emergence was taken as the emergence day. In the rare cases where no measurement was taken before bud-break, the day of the first height measurement was taken as emergence day. In this study, the growth period is defined as the time between emergence and canopy closure. We categorised the frost events occurring at the beginning of the growing season into light, medium, severe, and extreme frosts based on air temperatures measured at 2 m above soil level by the WS-GP1 weather station (Table 2) according to Farrell et al. (2006).

A number of approaches were applied to calculate the soil temperature from the air temperature. Each approach

was validated by exploring the correlation coefficient between the calculated and the actual soil data measured at each location in 2018/2019. The best fit was found for the correlation. A model was set up for each location, including an additional model for snow days, if snow occurred. The base temperature was calculated according to Trybula et al. (2015) by a 1-week moving average of the daily air temperature in conjunction with the observed emergence dates.

The data were statistically analysed using the following linear model:

$$y_{ijkl} = \mu + g_i + s_j + t_l + r_{jkl} + (gs)_{ij} + (st)_{jl} + (gt)_{il} + (gst)_{ijl} + e_{ijkl},$$

where μ is the intercept; g_i the i th hybrid effect; s_j the j th site effect; t_l the l th year of measurement effect; r_{jkl} the effect of the k th field replicate at site j and in year l ; $(gs)_{ij}$ and $(gt)_{il}$ the interaction effects of the i th hybrid with the j th site and l th year, respectively; $(rst)_{jkl}$ the interaction effect of the k th field replicate with the j th site and the l th year of measurement; $(gst)_{ijl}$ the interaction effect of the i th hybrid effect with the j th site and the l th year of measurement effect; and e_{ijkl} the residual error term corresponding to y_{ijkl} . All effects except the error were assumed to be fixed. The final models were selected via Akaike information criterion. Where significant differences via global F test were found, a Fishers LSD test was performed for multiple comparisons. The least-square means were estimated and are presented with their standard deviation or a corresponding letter display in the results section. Pre-requirements of homogeneous variance and normal distribution of residuals (despite the heterogeneity already accounted for by the model) were checked graphically using residual plots. In the cases of deviations from the assumptions, the data were square-root or logarithmically transformed. In such cases, means and standard deviations were back-transformed.

The statistical analysis was performed using the PROC MIXED procedure of Statistical Analysis Software SAS version 9.4 (SAS Institute Inc.). Figures were produced with the R program (R Core Team, 2019) package ggplot2 (Wickham, 2009).

3 | RESULTS

3.1 | Weather conditions

On average between 2009 and 2019, the coldest location of the four trial sites was OLI, followed by TWS, ZAG and PAC (Table 3). The site at OLI, located in south Germany, had minimum winter temperatures as low as -20°C due to the high altitude. Late spring frosts occurred frequently but in summer, temperatures exceeded those at the most northern site TWS, which has a more moderate temperate climate due to its close proximity to the sea. The climate at ZAG and PAC is Mediterranean, with slightly higher temperatures at PAC. Several touches of frost occurred over winter at both locations but the risk of late spring frost is relatively low due to a more rapid temperature increase in spring. At TWS and ZAG, the monthly average air temperature between January and July in the two study years were in a similar range to the average of the previous 10 years. At PAC by contrast, both years were colder. At OLI, January and February 2020 were relatively mild but April and May 2021 were comparably cold. However, at all four sites, the 10-year average between 2009 and 2019 was relatively warm compared to previous decades due to the effect of global warming.

At all four sites, January to June was warmer in 2020 than in 2021 (Figure 3). Late spring frosts occurred at all locations in both years but with variations in magnitude and frequency. At TWS and OLI, they occurred in mid-May in 2020, while in 2021, more frosts occurred between January and April. TWS was frost-free from the end of April and OLI from the beginning of May. At ZAG, the latest frost occurred in April in both years. At PAC the latest frost was in early April in 2020 and mid-April in 2021.

Between 2009 and 2019, the lowest total annual precipitation was recorded at PAC, followed by OLI, TWS and ZAG (Table 3). At TWS and OLI, precipitation during the growth period (April–October) 2020 and 2021 was similar to that of the previous 10 years. PAC and ZAG however were extremely dry during the growth period in 2021.

TABLE 3 Mean temperature and total precipitation for year and vegetation period (1 April–31 October) for the field sites (TWS: Trawsgoed, UK; OLI: Oberer Lindenhof, Germany; ZAG: Zagreb, Croatia; and PAC: Piacenza, Italy).

	Mean temperature ($^{\circ}\text{C}$)						Total precipitation (mm)					
	Year			Vegetation period			Year			Vegetation period		
	2020	2021	2009–2019	2020	2021	2009–2019	2020	2021	10-year average	2020	2021	2009–2019
TWS	10.7	11.0	10.2	13.3	14.1	12.9	1484	1265	1245	744	613	653
OLI	8.6	7.5	8.1	12.7	11.7	12.8	801	878	899	549	683	632
ZAG	11.7	11.3	11.9	16.8	16.2	17.6	765	637	1409	581	388	874
PAC	13.9	13.4	15.9	19.6	19.1	22.0	740	160	820	418	38	453

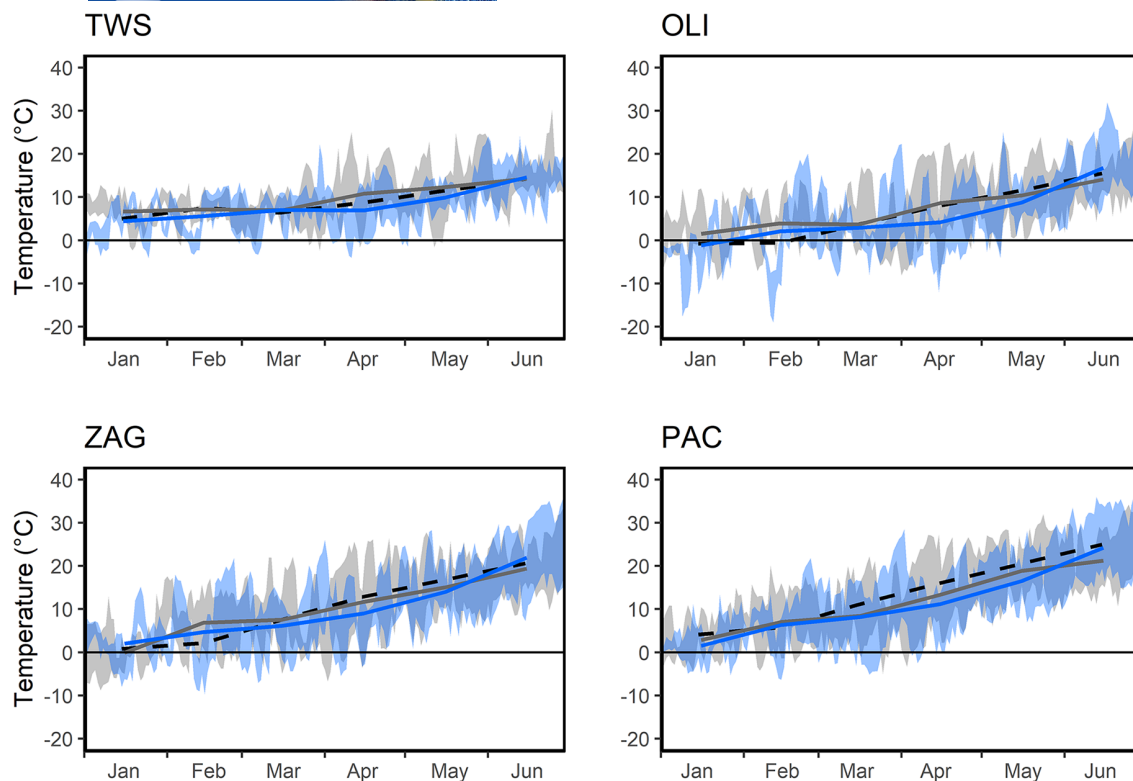


FIGURE 3 Temperatures at the four trial sites (TWS: Trawsgoed, UK; OLI: Oberer Lindenhof, Germany; ZAG: Zagreb, Croatia and PAC: Piacenza, Italy) between January and June. Grey line: average monthly temperatures in 2020; blue line: average monthly temperatures in 2021; dashed line: average monthly temperature from 2009 to 2019; grey area: daily minimum and maximum temperatures in 2020; blue area: daily minimum and maximum temperatures in 2021.

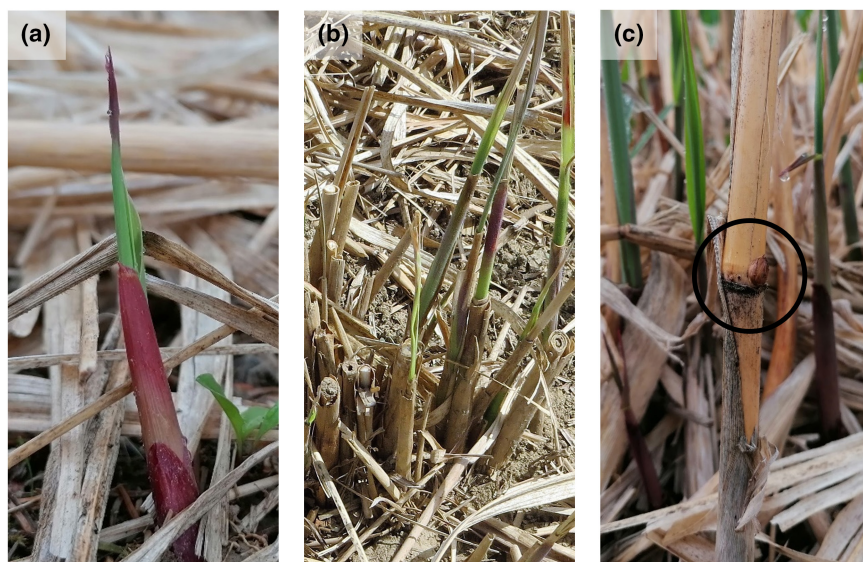


FIGURE 4 Miscanthus emergence types: from below the ground (a); and from previous year's aboveground stubble: inside the previous year's shoot (b), and an axillary nodal meristem (c).

3.2 | Emergence, growth period and canopy closure

New miscanthus shoots emerge from belowground plant organs (emergence type 1, Figure 4a) or from overwintered, unharvested aboveground shoot parts (emergence type 2), either a previous year's shoot (Figure 4b) or an

axillary bud (Figure 4c). However, the latter only occurs if the aboveground biomass does not die off over winter due to low temperatures without an isolating layer of snow. We observed that GRC 3 emerged mainly from aboveground unharvested shoots parts but also to a lesser extent from belowground crop organs, whereas GRC 9, 14 and 15 emerged from belowground organs only. Re-growth from

axillary nodal meristems was not observed in these trials, however, it has been previously observed for $M \times g$ in Aberystwyth, UK following a particularly mild winter. In our trials, GRC 9, 14 and 15 produced new shoots mainly at the beginning of the vegetation period whereas for $M \sin \times \sin$ emergence occurred throughout the vegetation period.

Due to the warmer temperatures in 2020, the hybrids emerged 20 days earlier than in 2021 (Figures 5 and 6). The largest difference between years was found for GRC 14 at PAC (41 days) and the lowest for GRC 14 at TWS (1 day). The lower temperatures in 2021 delayed emergence and reduced differences in emergence time between locations and hybrids. In both years, the hybrids emerged the earliest at the warmest site (PAC in 2020 and TWS in 2021) and latest at OLI, shortly after ZAG.

In 2020, GRC 3 was the first hybrid to emerge at all sites, presumably due to re-growth from aboveground overwintered shoots. The other hybrids emerged significantly later, except for GRC 14 at PAC. The latest hybrid to emerge in 2020 was GRC 14 at TWS where the crop was heavily damaged by late spring frosts the previous year. By contrast, in 2021, it was the first hybrid to emerge. At ZAG and OLI, emergence occurred relatively synchronously in both years. A comparison of the four miscanthus hybrids

showed that GRC 14 had the highest variation between emergence time, GRC 3 was generally the first hybrid to emerge, and GRC 9, GRC 14 and GRC 15 emerged late.

The base air temperature (7-day average of the air temperature 2 m above soil level at the time of emergence) is presented in Table 4. It was significantly affected by location ($p < 0.001$) and year ($p < 0.001$). The 7-day soil temperature curve (5 cm below ground level) from the beginning of the year until emergence shows that the soil base temperature had already been reached on several occasions before re-growth occurred (Appendix S1).

The later emergence in 2021 than 2020 did not lead to later canopy closure (Figure 5). The hybrids at PAC closed the canopy around the same time in both years. At TWS and ZAG, canopy closure was even reached earlier in 2021 than in 2020 (33 and 6 days earlier, respectively). However, at OLI, canopy closure was reached 15 days later in 2021 than 2020.

The growth period in 2021 was 30% shorter than in 2020 with the greatest decline observed at PAC (40%). OLI was the only site where the later emergence in 2021 also led to a later canopy closure. However, this only led to a longer growth period in GRC 14. Late frost damage in spring 2021 (Figures 3 and 6) caused a significant reduction in the number of shoots compared to the previous

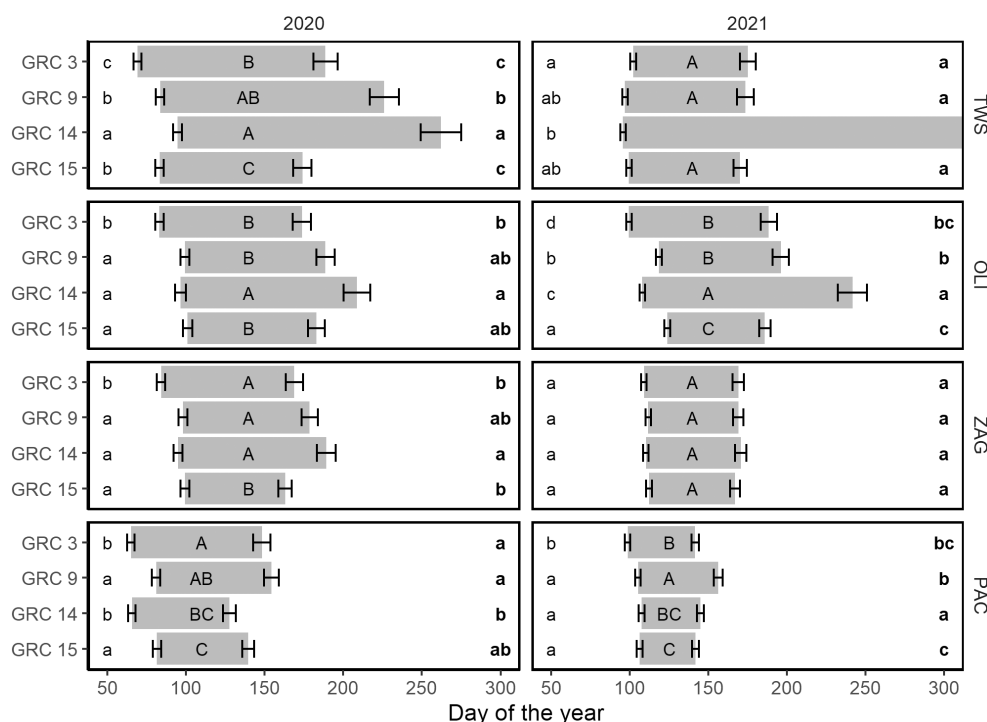


FIGURE 5 Growth period (grey bar, defined as the period between emergence and canopy closure) for each year and hybrid (GRC 3: *Miscanthus sinensis* \times *sinensis*; GRC 9: *M. x giganteus*; GRC 14 and GRC 15: *M. sacchariflorus* \times *sinensis*) and location (TWS: Trawsgoed, UK; OLI: Oberer Lindenhof, Germany; ZAG: Zagreb, Croatia and PAC: Piacenza, Italy). Identical letters indicate no significant difference (significance level $\alpha = 0.05$) between emergence day (lowercase letters), growth period (uppercase letters) or day of canopy closure (bold, lowercase letters) for each location and year separately.

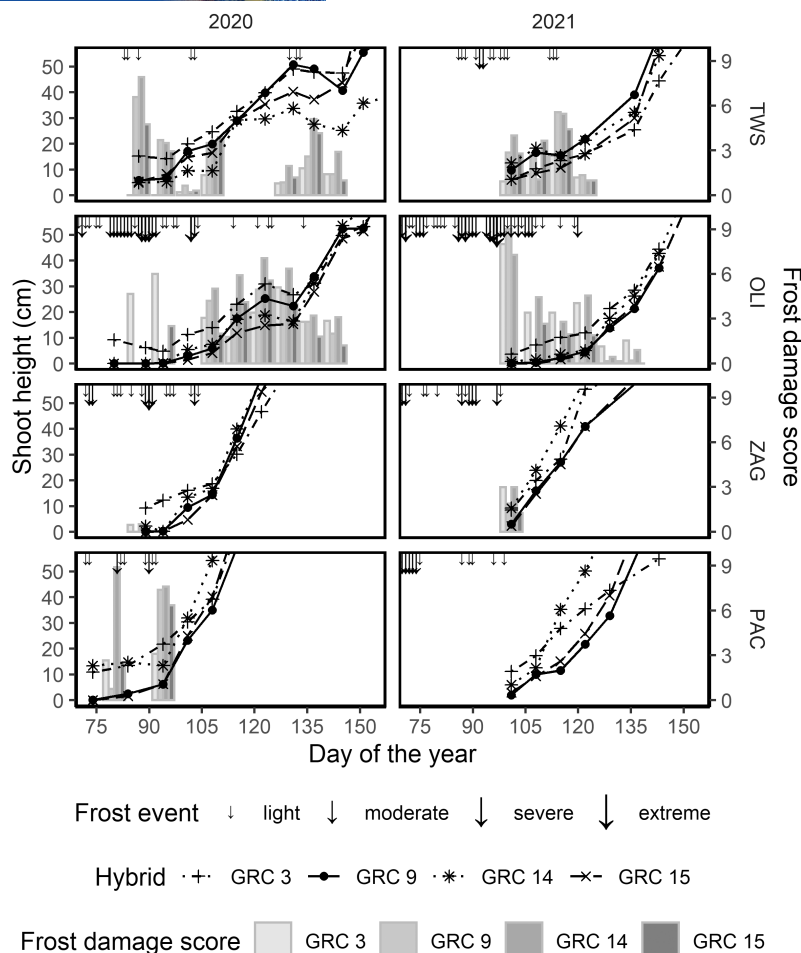


FIGURE 6 Average shoot height and frost damage scores per hybrid (GRC 3: *Miscanthus sinensis* × *sinensis* and GRC 14: *M. sacchariflorus* × *sinensis*, two novel seed-based miscanthus hybrids; GRC 15, a novel clone of *M. sacchariflorus* × *sinensis*, and GRC 9, a standard clone of *M. × giganteus*) at the four field sites (TWS: Trawsgoed, UK; OLI: Oberer Lindenhof, Germany; ZAG: Zagreb, Croatia and PAC: Piacenza, Italy) at the beginning of the growth period 2020 and 2021.

TABLE 4 Average base air temperature for shoot emergence (7-day average at emergence in °C) in 2020 and 2021 for each hybrid (GRC 3: *Miscanthus sinensis* × *sinensis*; GRC 9: *M. × giganteus*; GRC 14 and GRC 15: *M. sacchariflorus* × *sinensis*) and location (TWS: Trawsgoed, UK; OLI: Oberer Lindenhof, Germany; ZAG: Zagreb, Croatia and PAC: Piacenza, Italy). Identical letters indicate no significant difference ($\alpha = 0.05$) between base temperatures for each hybrid separately.

Location	GRC 3	GRC 9	GRC 14	GRC 15
TWS	6.7 a	6.3 c	7.7 b	6.2 c
OLI	2.4 b	8.3 bc	4.9 c	7.6 b
ZAG	7.4 a	11.0 a	8.6 a	11.4 a
PAC	7.4 a	9.1 b	7.8 ab	9.1 b
Average	6.0	8.7	7.2	8.6

year (Figure 7), resulting in 85% canopy closure in only three of the four plots. The same effect also occurred at TWS, where the number of shoots decreased significantly

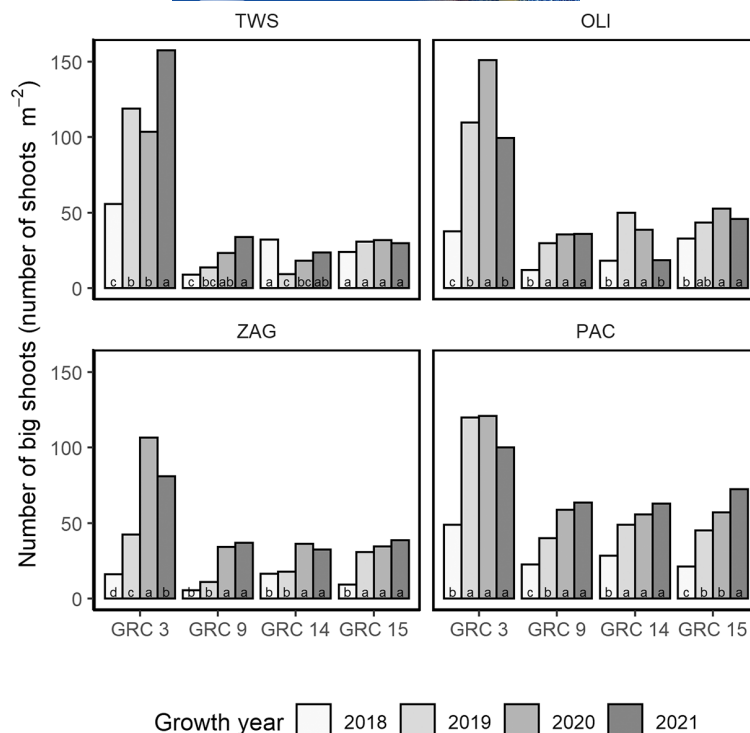
from 2018 to 2019 growing season, and in the 2021 growing season the number of shoots was even lower than in 2018 (Figure 7).

3.3 | Frost damage

The differing climatic conditions at the four sites (Figure 3) led to different effects of late frosts after the emergence of the hybrids (Figure 6). The measurements during the 2 years of the study showed variation in frost tolerance both between hybrids and between locations. Frost damage was visible with a delay of about a week after the frost event. The same frost damaged GRC 3 less severely than GRC 9 and 15, while GRC 14 was most severely damaged (Figure 6).

At PAC, on average the warmest site, the last frost (-3.3°C) was at the beginning of April in 2020 but on 17 April in 2021 (-0.1°C). GRC 3 and 14 emerged 1 month earlier, GRC 9 and 15 half a month earlier in 2020 than 2021. GRC 9, 14 and 15 were severely frost-damaged,

FIGURE 7 Number of big shoots at the end of growth year from the first (2018) until the fourth (2021) growth year (TWS: Trawsgoed, UK; OLI: Oberer Lindenhof, Germany; ZAG: Zagreb, Croatia and PAC: Piacenza, Italy; GRC 3: *Miscanthus sinensis* × *sinensis* and GRC 14: *M. sacchariflorus* × *sinensis*, two novel seed-based miscanthus hybrids; GRC 15, a novel clone of *M. sacchariflorus* × *sinensis*, and GRC 9, a standard clone of *M. × giganteus*). Identical letters for the same hybrid and location indicate no significant difference (significance level $\alpha = 0.05$) in number of shoots between years.



while GRC 3 had only mild damage. In 2021, the last frost did not cause any damage as only GRC 3 had emerged and was able to tolerate the mild frost unharmed. At ZAG, the last frost occurred in mid-April in both years. In 2021, the hybrids did not emerge until shortly after that frost, so no damage occurred. However, in 2020, the hybrids had emerged by the beginning of April, and several moderate frosts killed the first shoots of GRC 14 and led to much lower growth rates during the first weeks after emergence in 2020 than 2021. The sites most affected by frost were TWS and OLI. After hybrid emergence, OLI experienced temperatures as low as -8°C (2 m above ground level), while at TWS, only minor frosts occurred. Here, the last frost was recorded in mid-May in 2020 and at the beginning of May in 2021; the hybrids had emerged by mid-March and early April, respectively. At OLI, the hybrids emerged between late March and mid-April in 2020, and in mid-April in 2021. Field trial observations indicate that the hybrids were less severely affected by frost at the beginning of the growth period than by similar later occurring frosts.

3.4 | Number and height of shoots at end of growth period

In general, the shoot number increased from the first to the fourth growing season (Figure 7). However, a decrease compared to the previous year was found at some locations in some years. Significant decreases were found for

GRC 3 and GRC 14. Additionally, GRC 14 was the only hybrid with a lower number of shoots in the fourth than in the first growth year at the two coldest sites, TWS and OLI. At TWS, the number of GRC 14 shoots decreased by 60% from 2018 to 2019 due to an early spring followed by cold temperatures in 2019. This led to early emergence, followed by shoot dieback after frost. At OLI—the site with the latest spring frosts—the number of GRC 14 shoots decreased gradually from the second to the fourth growth year. At that site, the number of shoots decreased by 26% from 2020 to 2021 in all hybrids. In addition to GRC 14 (−50%), the reduction in shoot count was also significant for GRC 3 (−37%). For GRC 3, the hybrid planted at double the planting density of the other hybrids, a reduction in shoot number compared to the previous year was found at all four sites in either one growth year or the other. However, reduced shoot number was observed at TWS a year earlier than at the other three sites. Interestingly, this reduction also occurred at locations without late spring frosts. At those locations (PAC and ZAG), a more severe drought occurred in 2021.

At the end of the growth period, shoot height was generally lower in 2021 than 2020 (ZAG −26%, PAC −25%, OLI −10%), except for TWS (+11%) (Figure 8). It was significantly lower for each hybrid at each location except for GRC 3 and 15 at TWS and GRC 9 at OLI. This may have been caused by late spring frosts and growth rate limitations during the growth period. The considerable height decreases from 2020 to 2021 at ZAG and PAC were most likely due to the lower rainfall (33% and 81%, respectively) during the

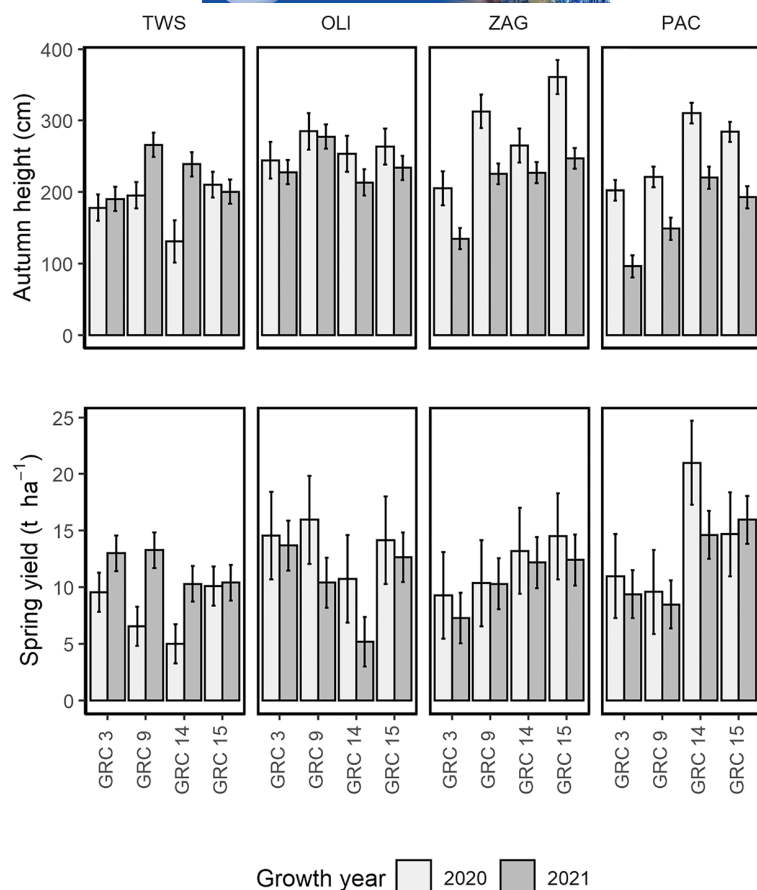


FIGURE 8 End-of-growth-year height and harvestable spring yield of the four miscanthus hybrids (GRC 3: *Miscanthus sinensis* × *sinensis* and GRC 14: *M. sacchariflorus* × *sinensis*, two novel seed-based miscanthus hybrids; GRC 15, a novel clone of *M. sacchariflorus* × *sinensis*, and GRC 9, a standard clone of *M. × giganteus*) at each location (TWS: Trawsgoed, UK; OLI: Oberer Lindenhof, Germany; ZAG: Zagreb, Croatia and PAC: Piacenza, Italy) in the growth years 2020 and 2021. Error bars indicate standard deviation.

growth period from April to October (Table 3). As a result of these interactions we did not observe that a more extended growth period leads to an increased shoot height at the end of the growing season. We did not find a significant effect of emergence day, time of canopy closure, or growth period on the end-of-season crop height at any location, including those not affected by drought (TWS and OLI), although there were other environmental effects (e.g. temperature and precipitation) at play during the growing period. As expected, the harvestable yield in spring following overwinter ripening generally correlated with shoot height in autumn, except where extreme environmental impacts occurred over winter, which had a hybrid-specific effect.

4 | DISCUSSION

This study assessed the impact of spring temperatures on the early development of *M* × *g* and three new miscanthus hybrids, and the resulting end-of-year height, shoot number and spring yield. The aim of breeding is to achieve high yields for the provision of biomass for the bioeconomy, using available land as productively as possible. It endeavours to extend the period between canopy closure and end of the growth period, as this is the time of maximal biomass accumulation (Robson, Jensen, et al., 2013).

An early canopy closure can be achieved by either a high planting density, a rapid early growth rate, or early emergence. However, at sites with late spring frosts, early emergence comes with a high risk of damaging or killing the first shoots, if frost tolerance is not sufficient (Farrell et al., 2006). In this multi-location and multi-year experiment, we found that emergence times, frost sensitivity and subsequent development vary between hybrids and locations.

4.1 | Emergence and canopy closure

A colder spring not only led to later emergence (as expected) but also reduced differences in the time of emergence between hybrids and locations. In 2020, the four hybrids analysed emerged between 5 March (DOY 65) and 10 April (DOY 101). In 2021, emergence was on average 20 days later, between 6 April (DOY 96) and 4 May (DOY 124). Other studies have reported shoot emergence in central Europe between March and April (Christian et al., 2008; Zub, Rambaud, et al., 2012) but in early May at a colder location in Canada (Friesen et al., 2014). In our study, the anticipated south-to-north gradient in emergence date between the four analysed European sites (Italy, Croatia, Germany and the UK) was not

substantiated. When comparing the hybrids, it was found that *M sin* × *sin* emerged earlier than the other three hybrids at all sites. In 2020, it was on average 15 days earlier but in 2021 only 5 days earlier. Interestingly, Farrell et al. (2006) and Clifton-Brown et al. (2002) determined diverse emergence patterns between different miscanthus hybrids, even though both studies were conducted at the same location in south Germany. While Farrell et al. (2006) reported an approximately 1-month earlier emergence of *M sin*, Clifton-Brown et al. (2002) identified a concurrent emergence of *M sin* and *M* × *g* and a two-week later emergence of *M sac*. Our results correspond to the findings of Farrell et al. (2006) but with substantially shorter times between emergence dates. This different observations could be due to differences in regrowth type. Type 1 emerges aboveground, from unharvested plant parts and is typical of *M sin*. *M sin* × *sin* (GRC 3) emerges partly from aboveground stubbles when these are not killed over winter, otherwise from belowground meristems. Type 2 emerges from belowground rhizomes, and is typical of *M sac*. These different types of resprouting impact the signalling and the timing of resprouting, and are considered to be predominantly controlled by air and soil temperature.

Base temperature (7-day average air temperature on the day of emergence) is a parameter widely used in modelling. For the four miscanthus hybrids analysed in this study, it ranged between 6 and 9°C. The lowest mean base temperature was found for *M sin* × *sin* (GRC 3: 6.0°C), followed by the seed-based *M sac* × *sin* (GRC 14: 7.2°C), the rhizome-based *M sac* × *sin* (GRC 15: 8.6°C) and the highest for *M* × *g* (GRC 9: 8.7°C). In the literature, base temperatures in a similar range were found for *M sin* (6.0–7.6°C), *M sac* (8.6°C) and *M* × *g* (8.0–8.6°C) (Farrell et al., 2006; Koike et al., 1975 cited by Stewart et al., 2009; Trybula et al., 2015). We found higher base temperatures for the warmer of the four analysed locations. One reason could be the weekly measurement interval being too coarse to capture these dynamics sufficiently accurately when spring temperatures are rising fast. In the Mediterranean climate (PAC and ZAG) a week's difference has a substantial temperature effect. Monitoring emergence at shorter intervals would therefore be advisable in future field assessments of miscanthus. This could increase the precision and may reduce the estimated base temperatures. As air temperatures fluctuate greatly for type 2 emergence hybrids, soil temperatures at a depth of ~5 cm are likely to be a better predictor of emergence time. For this reason, we estimated the soil temperature at a depth of 5 cm based on the air temperature (2 m above ground) at each location. This gave a soil base temperature of 3.1°C for *M sin* × *sin* (GRC 3), 4.2°C for the seed-based *M sac* × *sin* (GRC 14), and 5.2°C for both the rhizome-based *M sac* × *sin* (GRC

15) and *M* × *g* (GRC 9) hybrids. However, climate chamber studies have found that higher temperatures are required for rhizomes to sprout (Farrell et al., 2006). The reason for this could be that it is not the average soil temperature but the diurnal temperature variations, which are decisive for the time of emergence. Soil temperatures above the base temperature lead to shoot elongation, those below base temperature cause stagnation, and frosts below −3.5°C kill belowground shoots (Clifton-Brown & Lewandowski, 2000). To further explore these correlations, the soil temperature needs to be measured at the same time and same position (below- or above-ground, depending on the emergence type) as the growing shoot meristems. Additionally, the effects of soil moisture content, the litter layer and the rhizome-soil ratio on soil temperature need to be analysed.

Both air and estimated soil base temperatures were reached before emergence at each location and, considering the temperature curve, it seems reasonable that either a specific thermal time needs to be reached, or that multiple factors trigger emergence. In general, thermal time is a reliable indicator for the emergence of individual annual crops (Steinmaus et al., 2000). In our study, taking a mean thermal time over the four trial sites with a base temperature of 0°C gave 400 degree days for *M sin* × *sin* (GRC 3), 440 degree days for the seed-based *M sac* × *sin* (GRC 14), and 460 degree days for both the rhizome-based *M sac* × *sin* (GRC 15) and *M* × *g* (GRC 9). Increasing the base temperature for the calculation of degree days reduces the differences between the hybrids. However, more in-depth investigation of the thermal time is required, as perennial crops are not sown annually and, as such, the starting point for calculating thermal time needs to be determined. It may transpire that the thermal time of measured soil temperature is better for predicting the emergence of miscanthus hybrids than air temperature. Complex genetic processes such as a requirement for cold (vernalisation) also need to be considered. In this study, we found that, in addition to temperature, the photoperiod also significantly affects emergence. One reason for this could be the correlation with increased variation in diurnal soil temperature. Winter dormancy in temperate herbaceous perennials generally differs considerably between and within species and is induced by G × E interaction (Brummer et al., 2000; Muthoni et al., 2014). Photoperiod and temperature are probably central environmental factors for flowering and dormancy control of rhizomes. These two processes are linked in winter dormant herbaceous perennials because of several shared genes (Horvath, 2009; Sarath et al., 2014). Factors implicated in breaking winter dormancy are extended periods of cold temperatures (Chao et al., 2007; Horvath, 2009), increasing photoperiod (Heide, 2001; Sønsteby & Heide, 2006), extreme

temperatures, and intra-day fluctuation of temperature (Muthoni et al., 2014). Other factors may also influence the emergence of miscanthus including the age of the stand, as younger crops are more vulnerable to soil temperatures, probably due to the lower amount of rhizome mass (Jørgensen, 1995; Pude et al., 1997) or the time or method of harvest. The onset of emergence in annual crops is controlled by the time of sowing. However, for perennials, the only management option is the time of harvest. The soil temperature rises at a faster rate when the previous year's biomass is harvested earlier, so the biomass does not function as an insulation layer. Strullu et al. (2011) found a 4-day difference between early and late harvests due to the mulch layer of dead leaves, which delayed the soil temperature rise. Zub, Rambaud, et al. (2012) however did not identify an effect of harvest time on emergence. One reason for this could be that these differences disappear within a few days. In a separate field trial at the same location as the German site, no differences were noticeable between autumn and spring harvest after 8 days (data not shown). In conclusion, our results show a significant effect of temperature on emergence with differences between hybrids, and this corresponds to the findings of other studies (Clifton-Brown et al., 2011; Clifton-Brown & Jones, 1997; Farrell et al., 2006). Soil thermal time is probably the best predictor for the estimation of emergence but this needs to be proven by further measurement of growth and temperature at and just below the soil surface where the meristems responsible for spring regrowth are located.

In 2021, emergence was generally 20 days later but canopy closure was reached 36 days earlier than in 2020. This indicates a 'catch-up effect' but the stand age may also play a role. The catch-up effect can be explained by thermal time. To achieve the same thermal time, more than twice as many calendar days are required in spring than in summer. This suggests that later emergence does not substantially affect biomass yield, since the main biomass formation occurs during summer. Early canopy closure increases the light interception (Clifton-Brown & Jones, 1997; Sage et al., 2015); however, in this study, autumn shoot height—which according to Robson, Farrar, et al. (2013) correlates strongly with biomass yield—did not correlate with the length of the growth season. Biomass accumulation was most likely influenced by the weather patterns over these particular summer growing seasons and further observations in other years and locations are needed to test the generality of the emergence and growth projections. In 2021, the sites in Italy and Croatia were affected by a severe drought, which had a strong influence on crop height. Similarly to Zub, Rambaud, et al. (2012), we did not find the increase in yield due to a longer canopy duration reported by Robson, Farrar, et al. (2013). In contrast to emergence, which seems to be mainly

temperature-driven, canopy closure appears to be defined by the photoperiod and is thus independent of emergence time.

4.2 | Frost tolerance of miscanthus hybrids

In general, the killing of shoots by late spring frosts reduces the total shoot number and shortens the growth period in the respective year. We found that *M sin* × *sin* (GRC 3) tolerated more severe frost than *M* × *g* (GRC 9), and the most frost-affected hybrid was the seed-based *M sac* × *sin* (GRC 14). A single frost was enough to damage the crop severely and frost tolerance decreased with increasing shoot height. Earlier studies (Clifton-Brown et al., 2002; Farrell et al., 2006; Fonteyne et al., 2016) have also identified *M sac* to be less frost-tolerant than *M* × *g* and *M sin*. Emerging shoots of *M* × *g* have been found to be less cold-tolerant than some *M sin* hybrids (Farrell et al., 2006; Friesen et al., 2014; Jones et al., 2001; Zub, Arnoult, et al., 2012; Zub, Rambaud, et al., 2012). The results of our study indicate that the frost tolerance of miscanthus shoots decreases within the first weeks of emergence. This corresponds with the results of Zub, Arnoult, et al. (2012), who identified higher frost tolerance at the 3- to 5-leaf stage than at the 6- to 7-leaf stage. We classified the severity of frost events according to air temperature 2 m above ground level. However, when the air temperature at this height is around 0°C, the temperature at ground level can fall well below 0°C, causing a ground or grass frost (Geiger et al., 1995). To validate the results of this study, future research should additionally measure air temperature at soil level. In summary, late spring frost damage depends on the minimum temperature, exposure to sunlight at dawn, and frost tolerance and development stage of the hybrid.

4.3 | Emergence strategies of miscanthus hybrids

Despite its early emergence, *M sin* × *sin* (GRC 3) copes well with late spring frost due to its high frost tolerance, increased number of shoots and the production of new shoots during the growth period. By contrast, *M* × *g* (GRC 9) and *M sac* × *sin* (GRC 14 and 15) have low frost tolerance and a low number of shoots which are produced mainly at the beginning of the growing season. This increases the likelihood in *M* × *g* and *M sac* × *sin* that a higher proportion of the total shoot meristems emerge and then are damaged by late spring frosts. We found that *M sin* × *sin*

(GRC 3) has a more effective strategy for avoiding damage from late spring frosts: firstly, later emergence and secondly, higher frost tolerance. This could help explain why *M sin × sin* achieves higher yields than *M × g* and *M sac × sin* at colder locations (Awty-Carroll et al., 2022; Magenau et al., 2022).

Analysis of end-of-year shoot number showed a general increase from the first to the fourth growth year for all hybrids and locations. However, decreases from 1 year to the next did occur: for *M sac × sin* (GRC 14) in years with late spring frosts, and for *M sin × sin* (GRC 3) mainly from the third to the fourth growth year. *M × g* (GRC 9) had the lowest shoot number of all four hybrids, which increased continuously at all four locations from an average of 8.9 (4.4–16.8) shoots per plant in the first year to 32.6 (23.2–42.5) in the fourth year. The novel seed-based *M sac × sin* (GRC 14) emerged early in 2020 and 2021 at both the German and the UK site and was damaged by successive spring frosts. Accumulative damage appears to have caused a continuous decrease in shoot number, presumably by depletion of both active meristems and the reserves within the rhizome (Christian & Haase, 2001; Schwarz et al., 1994). Indeed, in 2021, *M sac × sin* (GRC 14) failed to reach the threshold for canopy closure (85% light interception) at TWS, and was also very late at OLI. The effects of late spring frosts on shoot reduction in the following growth years needs to be further analysed but such analyses can be confounded by the increasing interplant competition for light, which results in a self-thinning effect in mature *M × g* stands. Interplant competition was not observed in this study because the trials were less than 4 years old. In older trials however, reductions in shoot numbers would be expected, especially at the high planting density of 3 plants m⁻² used for the *M sin × sin* (GRC 3) hybrid.

4.4 | Conclusion and outlook

With continued climate change, the occurrence of frosts after the onset of emergence is predicted to increase. Thus frost tolerance of the newly emerged shoots remains an important trait impacting yield potential. Hybrids with particularly early emergence can only benefit from the extended growing season if lethal frosts do not occur after emergence. The field data presented here shows how severe late frosts after emergence weaken the plants, reducing the number of shoots for canopy, and thus yield formation. In extreme cases with multiple frost events between March and May, this can even result in plant death. Dead plants leave gaps in stands with negative consequences for yield. We found slightly higher frost tolerance in the *M sin × sin* hybrid than in

the *M × g* and *M sac × sin* hybrids. The *M sin × sin* hybrid also produced successive new shoots from the plant base throughout the growing season and we hypothesise that this may be an effective strategy for recovery from late spring frosts. Based solely on the results of these trials, we recommend *M sin × sin* for locations with higher risk of late spring frosts and *M sac × sin* for locations with low risk of late spring frosts. Drawing on results from earlier trials, we recommend extending the pool of *M sac* parents for the development of new *M sac × sin* hybrids by including germplasm collected at colder sites with higher latitudes and altitudes. ‘Within-population genetic improvement’ can be achieved through recurrent selection. This should result in hybrids with more frost tolerance—either by delayed emergence (avoidance) or increased frost resistance to late spring frosts. Such hybrids already exist as clones but the challenge is now to transfer this knowledge to seed-based hybrids that can be upscaled to large areas. This is necessary to generate the large quantities of biomass needed for products and energy from a range of marginal land types, whilst delivering a spectrum of ecosystem benefits.


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SUPPORTING INFORMATION

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