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**Temporal patterns of seed germination in early spring-flowering temperate woodland geophytes are modified by warming**

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Climate change and seed germination

## ABSTRACT

- **Background and Aims** Understorey species in temperate deciduous woodlands such as wild daffodil (*Narcissus pseudonarcissus*) and common snowdrop (*Galanthus nivalis*) have complex dormancy: seeds shed in late spring require warm summer temperatures for embryo elongation and dormancy alleviation, but then cooler temperatures for germination in autumn. As seasons warm and tree canopies alter, how will different seasonal temperature sequences affect these complex dormancy responses?
- **Methods** Different sequences of warmer (+5°C), current, or cooler (-5°C) seasons (summer to spring) on seed germination patterns over seven successive seasons were investigated with all sequences combined factorially to determine the consequences of differential seasonal temperature change for the temporal pattern of germination (and so seedling recruitment).
- **Key Results** Little (<1%, *G. nivalis*) or no (*N. pseudonarcissus*) seed germination occurred during the first summer in any treatment. Germination of *N. pseudonarcissus* in the first autumn was considerable and greatest at the average (15°C) temperature, irrespective of the preceding summer temperature; germination was also substantial in winter after a warmer autumn. Germination in *G. nivalis* was greatest in the warmest first autumn and influenced by preceding summer temperature (average > warmer > cooler); the majority of seeds that germinated over the whole study did so during the two autumns but also in year 2's cooler summer after a warm spring.
- **Conclusions** Warmer autumns and winters delay first autumn germination of *N. pseudonarcissus* to winter but advance it in *G. nivalis*; overall, warming will deplete these species' soil seed bank making annual seed influx increasingly important for recruitment and persistence. This study provides a comprehensive account of the effects of temperature changes in different seasons on seed germination in these early spring-flowering geophytes

and consequently informs how these and other temperate woodland species with complex seed dormancy may respond to future climate change.

**Keywords:** Amaryllidaceae, climate change, daffodil, factorial experiment, *Galanthus nivalis* L., geophyte, morphophysiological dormancy, *Narcissus pseudonarcissus* L., seed germination. snowdrop, temperature, temperate woodland

## INTRODUCTION

Biodiversity is under direct and indirect pressure from an increasing human population, through habitat loss, invasive alien species, over-exploitation of species, pollution and global climate change (Millennium Ecosystem Assessment, 2005). Warming due to anthropogenic emissions of greenhouse gases is anticipated to continue throughout the remainder of the 21<sup>st</sup> century, with more frequent high temperature extremes and more variable rainfall (IPCC, 2013). The weakening of the Atlantic Meridional Overturning Circulation (AMOC) during the 20<sup>th</sup> century (Rahmstorf *et al.*, 2015) is also likely to continue in the 21<sup>st</sup> century (IPCC, 2013). Even though a collapse of the AMOC in the 21<sup>st</sup> century is very unlikely (IPCC, 2013); if it were to occur, the whole of the Northern Hemisphere would cool and further warming would be delayed (Drijfhout, 2015), as has occurred in the past (Henry *et al.*, 2016).

The phenology (timing of leaf appearance, flowering, fruiting) of many plant species has been advanced by warming (Parmesan and Hanley, 2015), with earlier spring species being more sensitive (Menzel *et al.*, 2006). British plant species, on average, flower earlier than previously and flowering is especially sensitive to temperature in the previous month, providing a strong biological signal of climate change (Fitter and Fitter, 2002). Early spring-flowering plants, which exploit the narrow time period prior to canopy closure, would have the greatest evolutionary pressure to respond to a changing climate by commencing development at the earliest opportunity (McEwan *et al.*, 2011). Acclimation to climate change within a habitat is necessary for forest understorey plants that cannot quickly alter their range of distribution to enable them to persist (De Frenne *et al.*, 2011). Woodland geophytes such as the wild daffodil (*Narcissus pseudonarcissus*) and common snowdrop (*Galanthus nivalis*) occupy a very distinct seasonal niche whereby they flower and shed seeds as the woodland canopy develops during the period from winter through to late spring

(Newton *et al.*, 2013). This narrow temporal window might provide a severe constraint to such species in the future under climate change, especially since future warming is unlikely to be uniform across regions and seasons (Christensen *et al.*, 2007). In *Narcissus*, earlier-flowering cultivars have been shown to be advanced most by warming (Bock *et al.*, 2015); similarly, flowering in *G. nivalis* was also advanced by warming (Maak and von Storch, 1997; Sparks *et al.*, 2000).

Whilst the effects of global warming involve responses to environmental change across a range of plant life history processes, those which impact recruitment are especially important (Cochrane *et al.*, 2019). Temperatures during seed development and maturation, as well as post-harvest environmental conditions, influence seed dormancy (Cochrane, 2019).

Temperature also directly affects seed dormancy, survival and germination (Roberts, 1988; Batlla and Benech-Arnold, 2015) and is an important factor in plant establishment by seed-seedling cycles (Woodward, 1988; Cochrane, 2016). Thus, there is a high risk that seed germination in the soil seed bank will be compromised by climate change (Cochrane *et al.*, 2015), particularly by extreme events such as heatwaves or summer drought (Giménez-Benavides *et al.*, 2018).

Seeds are not necessarily shed at the best time for seedling establishment (Murdoch and Ellis, 2000). Seed dormancy, the inability of a seed to germinate in a specified period of time in environments usually favourable for germination (JM Baskin and CC Baskin, 2004), has therefore evolved to prevent germination in environments favourable for germination when the probability of seedling establishment, growth and survival is low (Vleeshouwers *et al.*, 1995; Fenner and Thompson, 2005). Seed dormancy is a risk spreading mechanism which may be compromised by climate change and so affect population dynamics and species

persistence (Ooi *et al.*, 2009). In contrast to the general advancement of flowering, the consequences of warming for loss of dormancy and germination within single habitats are species-specific and can differ considerably (Hoyle *et al.*, 2013; Orsenigo *et al.*, 2015).

When investigating seed dormancy and ecology, fresh seeds are vital to ensure that it is the ecology of seed dormancy on shedding which is considered (CC Baskin *et al.*, 2006). This is because physiological changes may occur during storage which reduce seed dormancy and alter germination requirements (Benech-Arnold and Sánchez, 1995; Egli, 1995; Vertucci and Farrant, 1995; Probert, 2000). This is particularly so in the moist temperate woodland species *N. pseudonarcissus* (daffodil) and *G. nivalis* (snowdrop). The seeds of these amaryllids are shed during spring or early summer at high moisture content (Newton *et al.*, 2013). Seeds of Amaryllidaceae have linear embryos (Martin, 1946; Werker, 1997), with embryo elongation occurring within the seed after shedding and prior to germination. This phenomenon is termed morphological dormancy (CC Baskin and JM Baskin, 2014). Yet, as there is no arrest in embryo elongation, an alternative view is that dormancy, if present, is physiological (Newton *et al.*, 2015; Vandeloos *et al.*, 2019). Nonetheless, “morphophysiological” dormancy, where embryo elongation occurs and a physiological block must be overcome prior to germination, has been widely reported in *Narcissus* seeds (e.g. Vandeloos and van Assche, 2008; Herranz *et al.*, 2013a, b; Newton *et al.*, 2013; E Copete *et al.*, 2014; Herranz *et al.*, 2015). Seeds with morphophysiological dormancy typically have quite precise and often differing temperature requirements for embryo elongation, loss of dormancy, and then germination (CC Baskin and JM Baskin, 2014). In *G. nivalis* and *N. pseudonarcissus*, embryo elongation after the seeds are shed can continue for more than a year (Newton *et al.*, 2013). Warm summer temperatures followed by cooler autumns promote germination in the



first, second, or even third and fourth autumn-winter periods after shedding (Newton *et al.*, 2015).

Different responses to warming amongst species are driven by seed dormancy traits, such that those with deep seed dormancy are the most affected because reduced primary and secondary dormancy result in greater likelihood of germination soon after dispersal (Bernareggi *et al.*, 2016). Earlier or later germination are each theoretically possible with warmer temperatures in species with complex dormancy breaking patterns because it will depend upon which season's response is the most affected by warming (Walck *et al.*, 2011). The response may also depend upon which season experiences the greater increase in temperature. Moreover, the effect of warmer or cooler temperature in one season might not be detected until a later season in seeds with complex temperature requirements for dormancy alleviation and germination.

The impacts of anthropogenic climate change on seeds and seedlings, and consequently plant establishment, are understudied phases in the life cycle of plants (Parmesan and Hanley 2015). The response of seed dormancy and germination, the crux of the transition from seed to seedling and so plant recruitment, of the woodland geophytes *N. pseudonarcissus* and *G. nivalis* was not known. Sustainable forest management can reduce (and indeed sometimes reverse) land degradation and can support mitigation and adaptation to climate change, slowing biodiversity decline (IPCC, 2019). These geophytes represent an important component of temperate woodland understorey species, typified by complex seed dormancy breaking requirements and poor ability to disperse to and colonize new habitats, and are thus a focal constituent of temperate woodland ecology (Blandino, 2017). Our aim was to

determine the responses of germination to contrasting climate change scenarios, season by season, from shedding onwards in these exemplars. We tested the hypotheses within each species that (i) seasonal temperature affects germination in that season; (ii) seasonal temperature affects germination in subsequent seasons; and (iii) the most suitable temperatures for promotion of germination vary amongst the four seasons.

## MATERIALS AND METHODS

### *Study sites and seed collection*

Capsules from at least 30 individual plants of each species were collected on 31 May 2007 from a wild population of *N. pseudonarcissus* in the Loder Valley Nature Reserve (51°03'29''N, 00°05'33''W); or on 19 May 2008 from an introduced population of *G. nivalis* at Wakehurst (51°04'07''N, 00°05'16''W). Capsules were held at 75% RH and 15°C, representative of mean ambient study site conditions (Newton *et al.*, 2013), until the germination test treatments began on 4 June 2007 for *N. pseudonarcissus*. Germination tests began on the day of collection for *G. nivalis*. Only fully formed seeds from naturally-dehiscing capsules were selected for study.

### *Experimental design and selected temperature regimes*

A factorial experimental design was adopted to explore the potential effects of different seasonal (summer, autumn, winter, spring) warming and/or cooling on seed germination. The average experimental temperature for each season was that value closest (in 5°C increments from 0°C) to the average of the mean daily temperature weighted by photoperiod ("seasonal mean") between 1 December and 28/29 February for winter, 1 March and 31 May for spring, 1 June and 31 August for summer, and 1 September and 30 November for autumn.

The mean weighted daily temperature ( $T_w$ , °C) was calculated from Wakehurst temperature (T) records over a two-year period (2004 – 2005) as:

$$T_w = [(maximum\ T \times light\ hours) + (minimum\ T \times dark\ hours)] / 24.$$

The average treatment temperatures selected were: summer 20°C (seasonal mean = 19.2°C); autumn 10°C (seasonal mean = 11.6°C); winter 5°C (seasonal mean = 4.6°C); spring 10°C (seasonal mean = 11.0°C). The year of four average seasons was thus 20-10-5-10°C.

Seasonal temperatures were average, 5°C warmer (25, 15, 10 and 15°C, respectively) or 5°C cooler (15, 5, 0 and 5°C, respectively), providing a total of 81 different annual sequences of seasonal temperature. As the study sites (Loder Valley Nature Reserve and Wakehurst) are approximately 1 km apart, the same weighted temperatures were used for each population. Seeds of both species were placed directly into summer temperatures (as they were shed in late-May). Every possible factorial combination of seasonal temperature was provided in a complete “year” with the number of treatments ( $nt$ ) = 81 ( $3 \times 3 \times 3 \times 3$ ), each represented by a single 50-seed germination test. Hence, the first summer’s three treatments were represented by 27 replicate germination tests each, the first autumn’s nine treatments by nine replicate germination tests each, the first winter’s 27 treatments by three replicate germination tests each, and the first spring’s 81 different treatments (and all subsequent seasons in later years) were each represented by a single test. The inter-seasonal temperature changes imposed were broadly compatible with temperature variation measured near the collection sites: variation in daily temperature over 1- to 5-day periods included 1 to 8 swings of >10°C each year between 2004 and 2015 (Supplementary Data Fig. S1). Treatments were cycled through seven 84-day seasons, except for the second autumn for *N. pseudonarcissus* which was 112 days.

### *Germination tests*

Seeds were sown on 1% distilled water agar (Microbiological Agar Granules, Merck, Darmstadt, Germany) in 10 mm-diameter wells of Nunc multiwell dishes (Scientific Laboratory Supplies Ltd., Nottingham, UK). The latter were used to avoid fungal spread between seeds during long test periods (Newton *et al.*, 2013). Dishes were placed in clear plastic bags in cooled incubators (LMS Ltd., Sevenoaks, UK) at the specified temperatures with lighting provided by 30W cool white fluorescent bulbs. Based on known seed dispersal ecology and germination requirements for these species (Newton *et al.*, 2015), germination tests for *N. pseudonarcissus* were conducted in the light (8 h day<sup>-1</sup>) and for *G. nivalis* in the dark, by double-wrapping dishes in aluminium foil prior to incubation. Seeds of *N. pseudonarcissus* were checked for germination at regular intervals under a flow hood (Capitarhood, Bigneat Ltd., Waterloooville, UK) and seeds of *G. nivalis* in a dark room under a dim, safe, green light comprising 15 – 20 W cool white fluorescent tubes covered with three layers of no. 39 (primary green) Cinemoid film (Probert and Smith, 1986). Seeds were transferred to fresh agar at least every 84 days. They were scored as germinated when the radicle was at least 2 mm long. At the end of the experiment, ungerminated seeds were dissected to determine whether they remained white and firm, and so probably viable.

### *Data analysis*

Analyses were carried out in Genstat for Windows Eleventh Edition (VSN International Ltd, Hemel Hempstead, UK). Logistic regression analysis (i.e. fitting of a generalized linear model with logit link function and binomial error distribution) was used to determine the significance of the effect of different temperatures within each season on the final proportion of seeds that germinated at the end of each season.

## RESULTS

### *Narcissus pseudonarcissus*

No seeds of *N. pseudonarcissus* germinated during the first summer in any regime (Figs 1 and 2). Overall, the principal effect of the autumn temperature on cumulative germination throughout the 616-day study was striking (Fig. 1): under cold autumns of 5°C (left-side columns, Fig. 1) total germination was lower than average (10°C) (central columns, Fig. 1) or warm (15°C) autumns (right-side columns, Fig. 1). Almost all germination occurred during 5 and 10°C autumn periods, or 5 and 10°C winter periods following 15°C in autumn, in both years. Logistic regression analysis of final germination confirmed that the effects of summer, autumn and winter temperatures were significant (Supplementary Data Table S1). Autumn was the most influential, followed by winter, then summer, with spring having the least effect on germination (compare residual deviances, Supplementary Data Table S1).

Final germination in the average seasonal treatment (20-10-5-10°C) after 616 days was 88% (nt = 1) (Fig. 1) when almost all seeds capable of germinating had done so: only 4% of ungerminated seeds remained viable (Supplementary Data Table S2). Only 16 temperature combinations resulted in germination greater than the average seasonal treatment, eight of which combined a warm autumn (15°C) with a warm winter (10°C) (Fig. 1). In contrast, a cool winter (0°C) after a warm autumn (15°C) preceded by a cool summer (15°C) had the lowest final germination ( $10 \pm 4.2\%$ , nt = 3), with  $83 \pm 1.3\%$  (nt = 3) viable seeds remaining (Supplementary Data Table S2). Final germination in treatments with a cool autumn (5°C) was also comparatively low ( $30 \pm 3.0\%$ , nt = 27) (Fig. 1), with  $61 \pm 3.1\%$  (nt = 27) viable seeds remaining (Supplementary Data Table S2). Treatments with a cool winter (0°C) following an average summer (20°C) and a warm autumn (15°C) similarly resulted in the

germination of less than half of the population ( $43 \pm 3.7\%$ ,  $nt = 3$ ) (Fig. 1), with  $49 \pm 3.3\%$  ( $nt = 3$ ) viable seeds left (Supplementary Data Table S2).

Figure 2 summarises the cumulative results after the first three seasons. Germination during the first autumn was greatest at the average  $10^\circ\text{C}$  ( $62 \pm 1.3\%$ ,  $nt = 27$ ), irrespective of the preceding summer temperature (logistic regression,  $nt = 27$ ,  $P = 0.240$ ) which had negligible effect (Fig. 2). Germination at the cooler autumn of  $5^\circ\text{C}$  was negligible ( $3 \pm 0.4\%$ ,  $nt = 27$ ), and germination was also not affected by summer temperature (logistic regression,  $nt = 27$ ,  $P = 0.907$ ). At the warmer autumn of  $15^\circ\text{C}$ , however, germination was affected significantly by preceding summer temperature (logistic regression,  $nt = 27$ ,  $P < 0.001$ ), with greater germination the warmer the summer:  $43\%$  ( $\pm 3.8\%$ ,  $nt = 9$ ) after  $25^\circ\text{C}$ ;  $15\%$  ( $\pm 3.0\%$ ,  $nt = 9$ ) after  $20^\circ\text{C}$ ; and only  $2\%$  ( $\pm 0.6\%$ ,  $nt = 9$ ) after  $15^\circ\text{C}$  (Fig. 2).

Further germination occurred during winter at  $10^\circ\text{C}$  (warm) or  $5^\circ\text{C}$  (average) after a warm autumn of  $15^\circ\text{C}$  (Figs 1 and 2), but little in any other winter treatment. Cumulative germination at the end of the first winter was greatest in all treatments in which a warm winter of  $10^\circ\text{C}$  followed a warm autumn of  $15^\circ\text{C}$  ( $88 \pm 0.7\%$ ,  $nt = 9$ ) (Figs 1 and 2).

Negligible germination occurred in spring ( $<1\%$ ,  $nt = 81$ ) or the second summer ( $<1\%$ ,  $nt = 81$ ), irrespective of treatment (Fig. 1). During the second autumn, further germination ( $20 \pm 1.4\%$ ,  $nt = 27$ ) occurred in the average autumn ( $10^\circ\text{C}$ ) and in the cool autumn ( $5^\circ\text{C}$ ,  $26 \pm 3.0\%$ ,  $nt = 27$ ), but not thereafter (Fig. 1). With a warm second autumn ( $15^\circ\text{C}$ ), however, germination occurred then and/or in the subsequent winter, in a similar pattern to the previous year.

In the first autumn, most germination was observed at 10°C and least at 5°C, irrespective of the summer to autumn drop in temperature, whereas germination in 15°C autumns increased as this seasonal drop increased from 0 to 10°C (Fig. 2, Table 1). These patterns (as a percentage of seeds remaining) were repeated in the second autumn, with the exception of 5°C which provided 24% higher, on average, germination than the first autumn (Table 1). The patterns for winter germination were similar across years with the biggest drop in temperature providing greatest germination of remaining seeds within every winter regime (Table 1).

Of the seeds that germinated, most germination (58%) occurred at 10°C, followed by 26% at 5°C, 16% at 15°C and <1% at 0°C, with no germination at either 20 or 25°C. Overall,  $62 \pm 3.3\%$  of seeds germinated, with  $29 \pm 3.3\%$  viable and  $9 \pm 0.3\%$  non-viable on dissection at the end of the investigation (nt = 81, Supplementary Data Table S2). The number of non-viable seeds may have been influenced by summer temperature (logistic regression, nt = 81,  $P = 0.061$ ), with the possibility of slightly more non-viable seeds the warmer the summer.

### *Galanthus nivalis*

There was negligible germination during the first summer in *G. nivalis*. The majority of seeds that germinated did so during the autumn (years 1 and 2), but with some summer germination in year 2 at the (cool) 15°C after the (warm) spring of 15°C. Almost no seeds germinated (<1%, nt = 81) during the first summer in any treatment (Figs 3 and 4), and few over the entire 588-day study in regimes combining cold autumns (5°C) with cool and

average springs (5, 10°C, left-side columns, Fig. 3a, b). Cumulative germination benefitted from a warm autumn (15°C, Fig. 3).

Logistic regression analysis of final germination for *G. nivalis* confirmed that autumn temperature affected germination (Supplementary Data Table S3) and had the most influence, followed by spring, then summer, with winter having the least effect (compare residual deviances, Supplementary Data Table S3). Germination was greatest at a warm autumn of 15°C ( $32 \pm 3.0\%$ ,  $nt = 27$ , year 1), but was influenced by the preceding summer temperature (logistic regression,  $nt = 27$ ,  $P < 0.001$ ): greatest ( $47 \pm 2.6\%$ ,  $nt = 9$ ) when preceded by an average summer (20°C), less ( $36 \pm 2.0\%$ ,  $nt = 9$ ) when preceded by a warm summer (25°C), and least ( $13 \pm 1.9\%$ ,  $nt = 9$ ) after a cool summer (15°C; Fig. 4, Table 2). Negligible germination occurred during the following winter ( $<1\%$ ,  $nt = 81$ ) or spring ( $<1\%$ ,  $nt = 81$ ), irrespective of temperature regime (Fig. 3). In the second autumn, some further germination occurred at warm (15°C,  $6 \pm 1.2\%$ ,  $nt = 27$ ), average (10°C,  $8 \pm 1.0\%$ ,  $nt = 27$ ) and cool (5°C,  $3 \pm 0.8\%$ ,  $nt = 27$ ) seasonal temperatures, with little ( $<1\%$ ,  $nt = 81$ ) during the second winter.

Final germination in average seasons (20-10-5-10°C) after 588 days was 26% ( $nt = 1$ ) (Fig. 3), with 20% of the remaining seeds viable (Supplementary Data Table S4). Germination in treatments with warm (15°C) autumns (with the exception of 15-15-5-15°C with 24% germination) was equal to or greater than average seasons (Fig. 3), with only  $3 \pm 1.0\%$  ( $nt = 27$ ) viable seeds remaining (Supplementary Data Table S4). In contrast, germination in a cool autumn of 5°C was lower than average seasons (Fig. 3) (with the exception of 15-5-5-15°C with 26% germination) with  $41 \pm 3.1\%$  ( $nt = 27$ ) viable seed remaining (Supplementary Data Table S4).



Of the seeds that germinated, most germination (66%) occurred at 15°C, followed by 25% at 10°C, 7% at 5°C, 1% at 20°C and <1% at both 0 and 25°C. Overall,  $21 \pm 1.9\%$  of seeds germinated, with  $24 \pm 2.2\%$  viable and a much larger percentage non-viable ( $55 \pm 1.4\%$ ) at the end of the investigation (nt = 81, Supplementary Data Table S4). The non-viable proportion was not influenced by summer temperature (logistic regression, nt = 81,  $P = 0.442$ ).

## DISCUSSION

The periods required for germination were considerable in both *N. pseudonarcissus* and *G. nivalis*, extending to over 500 days in some individual seeds, whilst the temporal pattern of germination and the final value were affected greatly by the sequence of temperatures amongst seasons (Figs 1 and 3). The seeds' failure to germinate in the first summer (Figs 1 and 3) and their considerable germination, more in *N. pseudonarcissus* than *G. nivalis*, with the drop in temperature in the first autumn and winter (Tables 1 and 2) matched the expected response given their complex temperature requirements for germination (Newton *et al.*, 2013, 2015).

Although shared selection pressures can result in the convergence of germination syndromes in temperate forest herbs from different families (Vandelook *et al.*, 2019), the responses of seed germination in species with similar germination syndromes to warming can vary. For example, the temperate forest understorey plants *Anemone nemorosa* and *Milium effusum* showed contrasting responses of seed germination to warming: germination was consistently enhanced in *A. nemorosa* but not in *M. effusum* (De Frenne *et al.*, 2011). Warming increased

the germination of both *N. pseudonarcissus* and *G. nivalis* in the current study, but with key seasonal differences due to different optimal temperatures for seed germination for each species. Warming in autumn improved germination in that season in *G. nivalis* (optimum germination temperature of 15°C, Fig. 4) but reduced it in *N. pseudonarcissus* (optimum germination temperature of 10°C, Fig. 2), whilst warming in the winter promoted germination in *N. pseudonarcissus* but not in *G. nivalis*. Warming across all three seasons would be expected to extend the period of germination in *N. pseudonarcissus* from the autumn alone towards autumn and winter whilst in *G. nivalis*, germination would likely occur earlier in the autumn of the first year (Fig. 4).

Seasonal changes in the dormancy of seeds in soil have been well documented in temperate plants (e.g. CC Baskin and JM Baskin, 1988). Dormancy in winter annuals and perennials can be induced by cold temperatures whilst dormancy is relieved during warm summer temperatures before germination in autumn (CC Baskin and JM Baskin, 1988; JM Baskin and CC Baskin, 1994; CC Baskin *et al.*, 2002; MÁ Copete *et al.*, 2009; Newton *et al.*, 2015). After the winter treatments, little (*G. nivalis*) or no (*N. pseudonarcissus*) germination occurred in the spring or second summer (Figs 1, 3), which was also found in germination studies in *N. pseudonarcissus* seeds collected from both different populations and in different years and *G. nivalis* seeds collected in different years (Newton *et al.*, 2015). The failure to germinate in the first summer with a flush of germination in the autumn can be attributed to primary dormancy at shedding and its subsequent alleviation by warm temperatures. Whilst seeds that did not germinate may have retained primary dormancy throughout the first four seasons, the cold winter temperatures are more likely to have induced secondary dormancy as seeds failed to germinate at temperatures suitable for germination in the spring (Newton *et al.*, 2015). This consequently delayed germination until the second autumn (with secondary

dormancy lost in the second summer). Induction of secondary dormancy by low temperatures has been observed in *Papaver rhoeas* (CC Baskin *et al.*, 2002) and *N. hispanicus* (E Copete *et al.*, 2011), for example.

In both species, on average, viable seeds remained at the end of the study in most treatments, as also observed by Newton *et al.* (2015) in studies with different populations (*N. pseudonarcissus*) and from different years (*N. pseudonarcissus* and *G. nivalis*), with the notable exception of the warmest summer-autumn-winter combination (25-15-10-X°C). In general, seed germination in *N. pseudonarcissus* tended to be greater overall (Figs 1-4), with considerably more dead seeds at the end of the study in *G. nivalis* (Tables S2, S4). The results imply that soil seed bank depletion would be driven rather more by germination in *N. pseudonarcissus* and rather more by seed death in *G. nivalis*. Warming will deplete these species' medium-term presence in the soil seed bank and so annual seed influx will become yet more important in the future to these species' persistence. Thompson and Cox (1978) similarly predicted a reduction in the reserve population of dormant seeds in the soil in response to higher summer temperatures in *Hyacinthoides non-scripta*, a later spring flowering temperate woodland geophyte. A reduction in the soil seed bank in response to a warmer climate has also been observed in alpine plants (Mondoni *et al.*, 2015).

According to Christensen *et al.* (2007), the risk of summer drought is likely to become more severe in central Europe and the Mediterranean with climate change, posing a greater threat of desiccation to both *N. pseudonarcissus* and *G. nivalis* seeds. This is not likely to affect *N. pseudonarcissus* seed viability, as seeds are tolerant to drying throughout the warm summer period (Newton *et al.*, 2015). However, as *G. nivalis* seeds are sensitive to drying (Newton *et al.*, 2015), increased risk of summer drought may negatively impact seed viability and hence

the species' ability to regenerate from seed. Drying near the end of the summer period had the effect of preventing germination in a proportion of *N. pseudonarcissus* seeds in the subsequent autumn – likely by inducing dormancy – which was alleviated by warm temperatures in the second summer season, resulting in germination in the second autumn (Newton *et al.*, 2015). This may function to counteract the increased germination predicted to occur in *N. pseudonarcissus* during the first autumn due to warmer temperatures. Dormancy cycling through the imposition and alleviation of secondary dormancy within buried seeds of *Echinocactus platyacanthus* was similarly observed by Aragón-Gastélum *et al.* (2018) whilst studying the impact of warming in the Chihuahuan Desert.

*Narcissus pseudonarcissus* is native to the Western Mediterranean and all southern Europe and was likely introduced to Britain in the fourth and fifth centuries by the Romans (Church, 1908). *Galanthus nivalis* is native to southern, central and western Europe, introduced into the United Kingdom where it has naturalised extensively from escaped garden plants (Church, 1908). The origin of both species from regions with warmer climates than the United Kingdom may explain why best germination is observed at warmer rather than average autumn temperatures for West Sussex. Either the environment in West Sussex has not been sufficiently stressful to drive selection for germination at appreciably cooler temperatures than the Mediterranean, or the populations have not existed in this environment for long enough for adaptation to have taken place. However, even though their origin and germination temperature optima may suggest that these species will fare better in future, given that the soil seed bank is likely to be depleted more quickly, they may be at greater risk of one-off events such as extreme rainfall.

Extrapolation from single population studies to the possible behaviour of the species requires caution, as population size, as well as temperature and precipitation during seed development, for example, can affect seed dormancy and dormancy release (Simpson, 1990; Gutterman, 1992, 1997). Although this specific experimental design with all factorial permutations was not repeated on different populations of the same species, similar partial factorial temperature conditions during germination tests with seeds collected both in different years and from different populations (Newton *et al.*, 2015) showed broadly similar germination response patterns as reported here.

Other studies that have considered how future changes in average temperatures might influence germination have often focused on temperature optima for germination and involved germinating non-dormant seeds on temperature gradient plates (e.g. Fernández-Pascual *et al.*, 2015; Cochrane, 2016). However, this approach is too basic for species with complex dormancy, where seasonal changes in temperature may influence subsequent germination and recruitment.

## CONCLUSIONS

The experimental approach devised here enabled the effect of different permutations of seasonal warming and/or cooling to be ascertained, not simply warming (or cooling) over the whole year. This is pertinent: at high latitudes, greater warming is likely in winter than other seasons (IPCC, 2013); and whilst warmer temperatures on average promoted germination of seeds of these two woodland geophytes eventually, the effect varied considerably with the particular season(s) of warming, species, and cumulative number of seasons. Warming over the summer alone had little effect on seed germination in the first autumn for *N.*

*pseudonarcissus* but reduced it in *G. nivalis*; warming in autumn alone reduced germination in the first autumn in *N. pseudonarcissus* but increased it in *G. nivalis*; warming in winter alone had little or no effect on germination in either species in the first year. Whilst the response of seed dormancy and germination to warming in these temperate woodland geophytes was both temporally and species dependent, warming by 5°C did not expose a tipping point in the regulation of dormancy loss and germination by temperature. Hence, these two species' responses to anthropogenic climate change would be considered conventional rather than aberrant by Parmesan and Hanley (2015).

## SUPPLEMENTARY DATA

Supplementary data consist of the following. Table S1: results from logistic regression analyses, showing the relative importance of seasons on germination of *Narcissus pseudonarcissus* seeds. Table S2: the cumulative number of germinated *Narcissus pseudonarcissus* seeds and condition of non-germinated seeds at the end of the investigation, determined by dissection. Table S3: results from logistic regression analyses, showing the relative importance of seasons on germination of *Galanthus nivalis* seeds. Table S4: the cumulative number of germinated *Galanthus nivalis* seeds and condition of non-germinated seeds at the end of the investigation, determined by dissection. Fig. S1: Mean daily temperatures (weighted for photoperiod) at Wakehurst, West Sussex, from 2004 to 2015.

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## FIGURE CAPTIONS

FIG. 1. Cumulative seed germination (% , n = 50 seeds) of *Narcissus pseudonarcissus* under a (a) cool (5°C), (b) average (10°C) or (c) warm (15°C) spring with all possible combinations of summer [cool (15°C), average (20°C) or warm (25°C)], autumn [cool (5°C), average (10°C) or warm (15°C)], and winter [cool (0°C), average (5°C) or warm (10°C)] temperatures over 616 days with 84- or 112-day duration seasons. See materials and methods for durations of the seasons. Year 1 (1 in column) comprised four seasonal periods from summer onwards; year 2 (2 in column) ended at the end of winter. The control (average seasonal temperatures) result is highlighted by light grey shading (centre of figure).

FIG. 2. Cumulative seed germination (%) of *Narcissus pseudonarcissus* at the end of summer (nt = 27 treatments), autumn (nt = 9 treatments), and winter (nt = 3 treatments) in year 1 for average (A), cooler (C) or warmer (W) seasonal temperatures (T). Data from Figure 1. Germination was not significantly different (•) or was significantly lower (–) or higher (+) than the control (logistic regression,  $P = 0.05$ ). The control (average seasonal temperatures) result is highlighted by light grey shading (centre of figure).

FIG. 3. Cumulative seed germination (% , n = 50 seeds) of *Galanthus nivalis* under a (a) cool (5°C), (b) average (10°C) or (c) warm (15°C) spring with all possible combinations of summer [cool (15°C), average (20°C) or warm (25°C)], autumn [cool (5°C), average (10°C) or warm (15°C)], and winter [cool (0°C), average (5°C) or warm (10°C)] temperatures over 588 days with 84-day duration seasons. Note restricted y-axis. Year 1 (1 in column) comprised four seasonal periods from summer onwards; year 2 (2 in column) ended at the

end of winter. The control (average seasonal temperatures) result is highlighted by light grey shading (centre of figure).

FIG. 4. Cumulative seed germination (%) of *Galanthus nivalis* at the end of summer (nt = 27 treatments), autumn (nt = 9 treatments), and winter (nt = 3 treatments) for average (A), cooler (C), or warmer (W) seasonal temperatures (T). Data from Figure 3. Germination was not significantly different (●) or was significantly lower (–) or higher (+) than the control (logistic regression,  $P = 0.05$ ). The control (average seasonal temperatures) result is highlighted by light grey shading (centre of figure).

#### SUPPLEMENTARY FIGURE CAPTION

FIG. S1. Mean daily temperatures (weighted for photoperiod, see text) at Wakehurst, West Sussex, from 2004 to 2015.



TABLE 1. Effect of autumn or winter temperature and drop in temperature from the previous season (summer in the case of autumn, autumn in the case of winter) on the germination of *Narcissus pseudonarcissus* seeds during autumn (year) 1, winter 1, autumn 2 and winter 2, calculated as a percentage (mean  $\pm$  s.e., nt = 9) of those seeds that had not germinated at the start of each season.

Temperature drop (°C)	Germination temperature (°C)					
	Autumn 1			Autumn 2		
	5	10	15	5	10	15
0	—	—	2 $\pm$ 0.6	—	—	1 $\pm$ 1.2
5	—	59 $\pm$ 2.4	15 $\pm$ 3.0	—	41 $\pm$ 5.5	17 $\pm$ 4.5
10	3 $\pm$ 0.8	64 $\pm$ 1.8	43 $\pm$ 3.8	31 $\pm$ 6.8	57 $\pm$ 3.9	36 $\pm$ 8.1
15	3 $\pm$ 0.7	62 $\pm$ 2.3	—	23 $\pm$ 4.3	63 $\pm$ 4.5	—
20	3 $\pm$ 0.8	—	—	27 $\pm$ 5.0	—	—
	Winter 1			Winter 2		
	0	5	10	0	5	10
0	—	1 $\pm$ 0.3	0 $\pm$ 0.0	—	0 $\pm$ 0.0	1 $\pm$ 0.5
5	0 $\pm$ 0.0	0 $\pm$ 0.0	84 $\pm$ 2.0	0 $\pm$ 0.0	0 $\pm$ 0.0	30 $\pm$ 9.0
10	0 $\pm$ 0.0	42 $\pm$ 3.7	—	0 $\pm$ 0.0	54 $\pm$ 6.1	—
15	3 $\pm$ 1.8	—	—	1 $\pm$ 0.4	—	—

TABLE 2. Effect of autumn or winter temperature and drop in temperature from the previous season (summer in the case of autumn, autumn in the case of winter) on the germination of *Galanthus nivalis* seeds during autumn (year) 1, winter 1, autumn 2 and winter 2, calculated as a percentage (mean  $\pm$  s.e., nt = 9) of those seeds that had not germinated at the start of each season.

Temperature drop (°C)	Germination temperature (°C)					
	Autumn 1			Autumn 2		
	5	10	15	5	10	15
0	—	—	13 $\pm$ 1.9	—	—	15 $\pm$ 2.4
5	—	8 $\pm$ 1.3	47 $\pm$ 2.6	—	9 $\pm$ 2.4	1 $\pm$ 0.5
10	1 $\pm$ 0.4	11 $\pm$ 1.2	36 $\pm$ 2.0	3 $\pm$ 0.8	10 $\pm$ 1.8	7 $\pm$ 1.3
15	1 $\pm$ 0.5	2 $\pm$ 0.6	—	2 $\pm$ 1.2	8 $\pm$ 2.1	—
20	0 $\pm$ 0.0	—	—	3 $\pm$ 2.0	—	—
	Winter 1			Winter 2		
	0	5	10	0	5	10
0	—	0 $\pm$ 0.0	0 $\pm$ 0.0	—	0 $\pm$ 0.0	0 $\pm$ 0.0
5	0 $\pm$ 0.0	0 $\pm$ 0.3	3 $\pm$ 1.2	0 $\pm$ 0.0	1 $\pm$ 0.5	1 $\pm$ 0.6
10	0 $\pm$ 0.0	2 $\pm$ 0.7	—	0 $\pm$ 0.0	0 $\pm$ 0.3	—
15	1 $\pm$ 0.4	—	—	1 $\pm$ 0.7	—	—

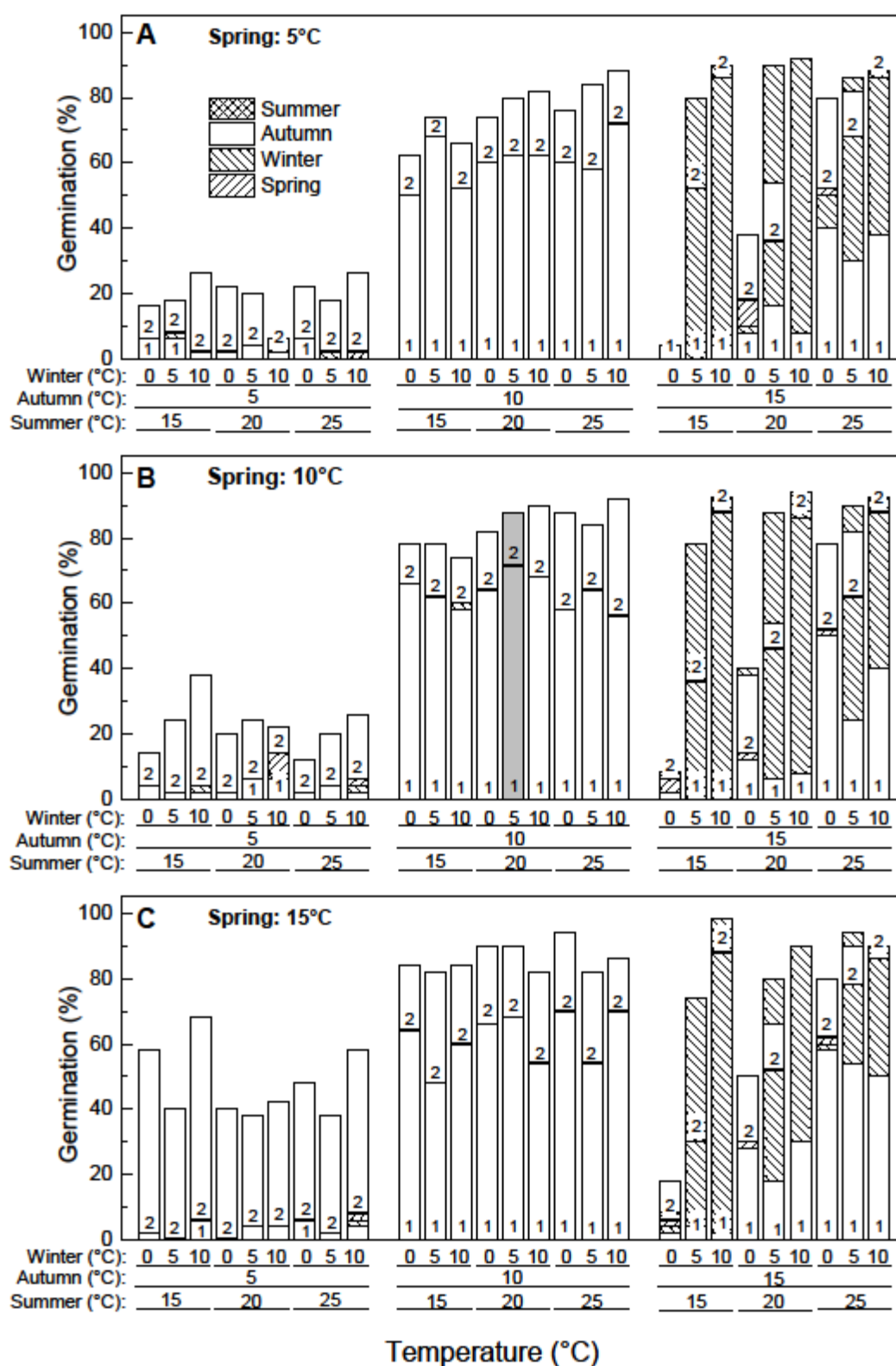


Figure 1

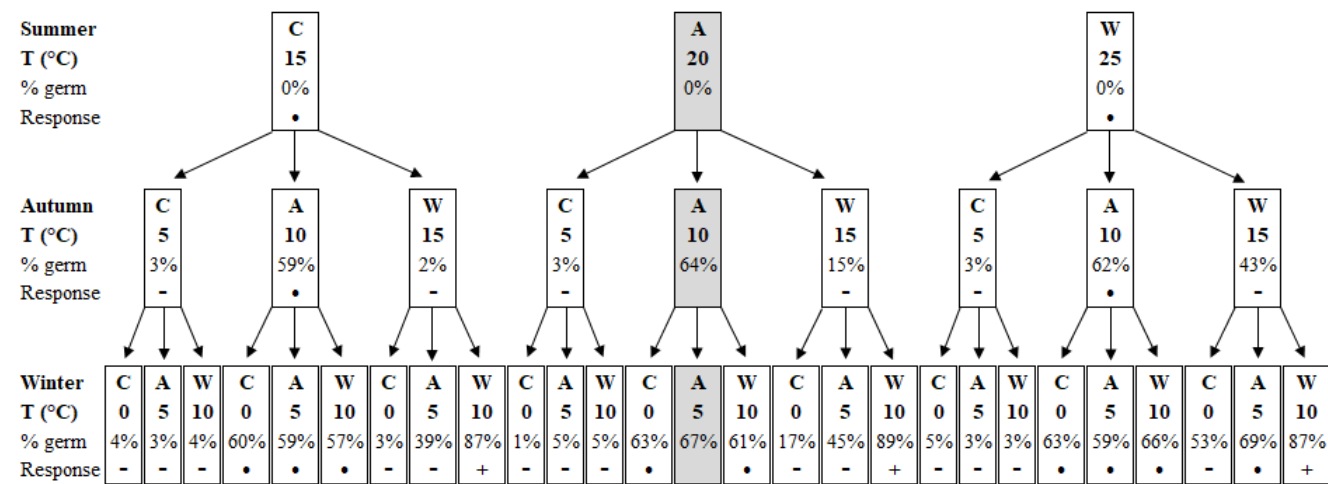


Figure 2

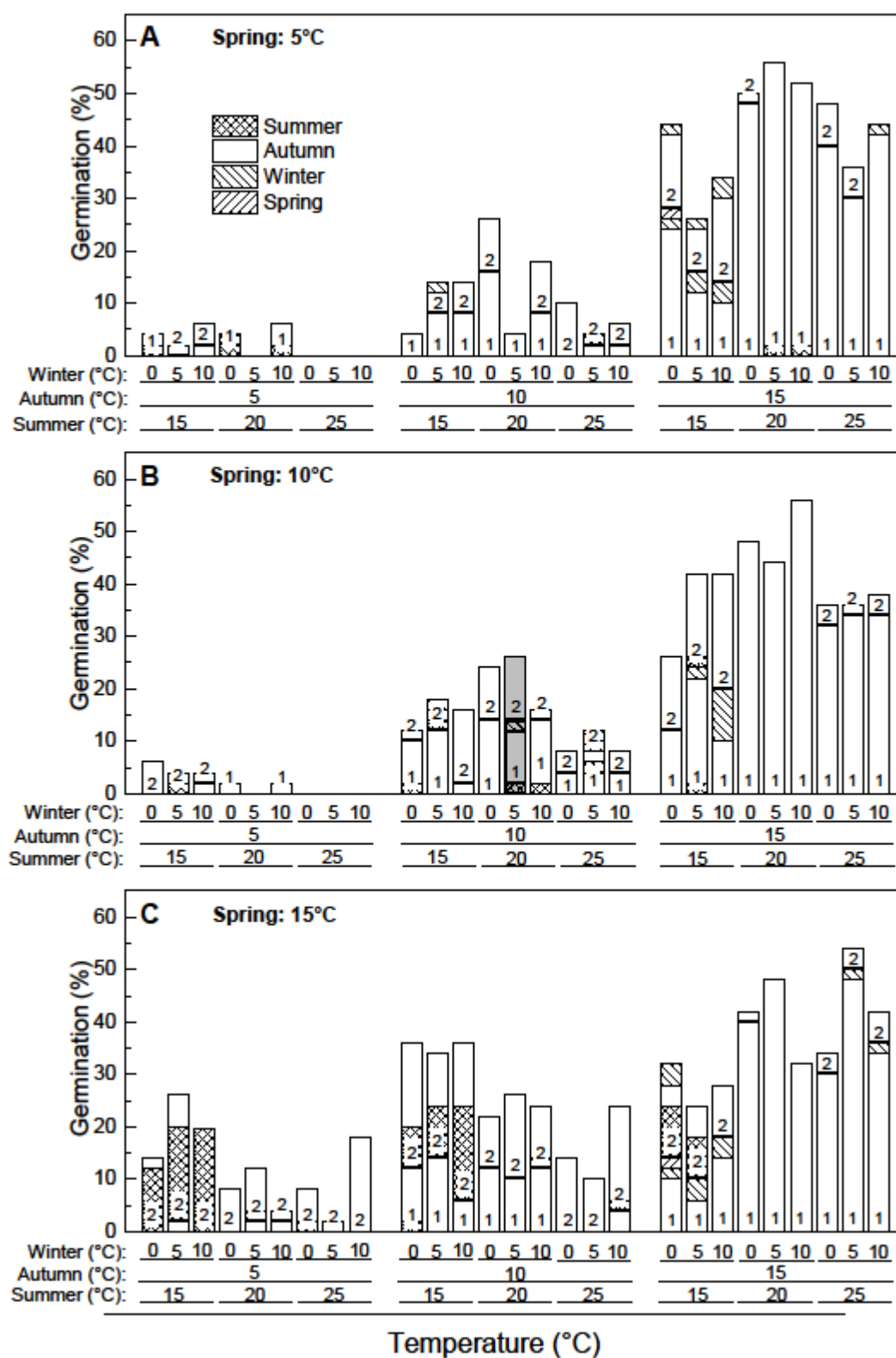


Figure 3

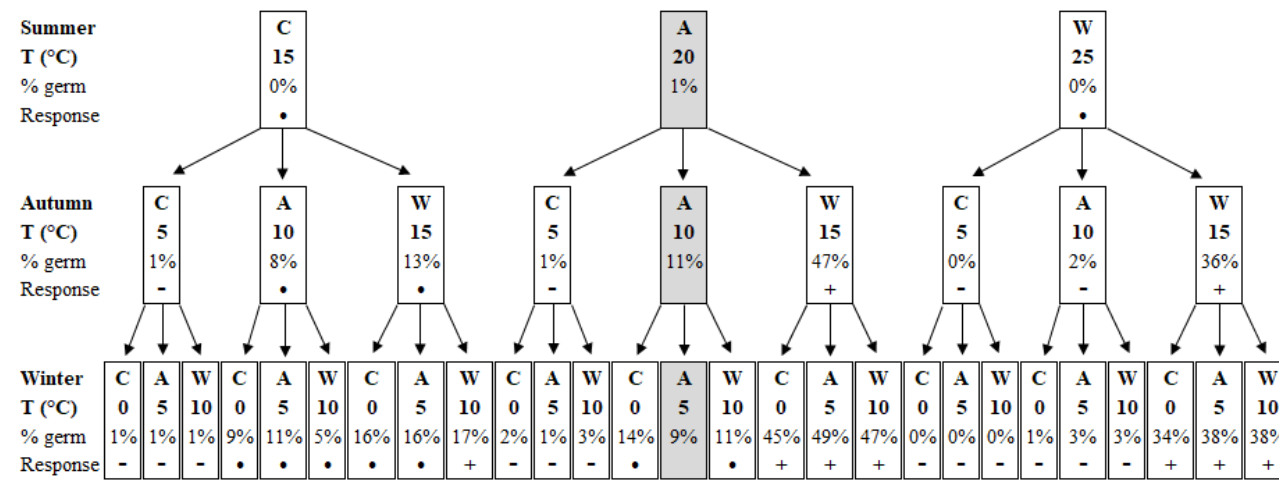


Figure 4

## SUPPLEMENTARY TABLES

TABLE S1. Results from logistic regression analyses, showing the relative importance of seasons (compare residual deviances) on germination of *Narcissus pseudonarcissus* seeds, and outcomes from the regression model as to whether or not terms could be dropped from the model.

Output of final germination data fitting summer, autumn and winter (spring excluded)					
Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	26	1427.4	54.900	54.90	< 0.001
Residual	54	167.7	3.106		
Total	80	1595.1	19.939		
Chosen action: no change					
Output of final germination data fitting summer, autumn and spring (winter excluded)					
Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	26	1140.8	43.879	43.88	< 0.001
Residual	54	454.3	8.413		
Total	80	1595.1	19.939		
Chosen action: dropping summer.autumn.spring.					
Output of final germination data fitting summer, winter and spring (autumn excluded)					
Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	26	253	9.73	9.73	< 0.001
Residual	54	1342	24.85		
Total	80	1595	19.94		
Chosen action: dropping summer.winter.spring.					
Output of final germination data fitting autumn, winter and spring (summer excluded)					
Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	26	1356.4	52.168	52.17	< 0.001
Residual	54	238.8	4.422		
Total	80	1595.1	19.939		
Chosen action: dropping autumn.winter.spring.					

TABLE S2. The cumulative number of germinated *Narcissus pseudonarcissus* seeds (of 50 tested) and condition of non-germinated seeds at the end of the investigation, determined by dissection: seeds white and firm on dissection were classified as viable; those discoloured or soft as non-viable. The average seasonal treatment (i.e. 20-10-5-10°C) is emboldened.

Temperature sequence (Summer-Autumn-Winter-Spring)	Number germinated	Number viable	Number non-viable
15-15-10-10°C	46	0	4
15-10-10-10°C	37	9	4
15-5-10-10°C	19	27	4
15-15-5-10°C	39	7	4
15-10-5-10°C	39	7	4
15-5-5-10°C	12	33	5
15-15-0-10°C	4	42	4
15-10-0-10°C	39	5	6
15-5-0-10°C	7	40	3
20-15-10-10°C	47	0	3
20-10-10-10°C	45	3	2
20-5-10-10°C	11	34	5
20-15-5-10°C	44	2	4
<b>20-10-5-10°C</b>	<b>44</b>	<b>2</b>	<b>4</b>
20-5-5-10°C	12	33	5
20-15-0-10°C	20	26	4
20-10-0-10°C	41	6	3
20-5-0-10°C	10	35	5
25-15-10-10°C	46	0	4
25-10-10-10°C	46	1	3
25-5-10-10°C	13	32	5
25-15-5-10°C	45	0	5
25-10-5-10°C	42	2	6
25-5-5-10°C	10	35	5
25-15-0-10°C	39	5	6
25-10-0-10°C	44	1	5
25-5-0-10°C	6	40	4
15-15-10-5°C	45	0	5
15-10-10-5°C	33	13	4
15-5-10-5°C	13	32	5
15-15-5-5°C	40	5	5
15-10-5-5°C	37	7	6
15-5-5-5°C	9	40	1
15-15-0-5°C	2	42	6
15-10-0-5°C	31	15	4
15-5-0-5°C	8	40	2



20-15-10-5°C	46	0	4
20-10-10-5°C	41	4	5
20-5-10-5°C	3	41	6
20-15-5-5°C	45	2	3
20-10-5-5°C	40	6	4
20-5-5-5°C	10	36	4
20-15-0-5°C	19	26	5
20-10-0-5°C	37	9	4
20-5-0-5°C	11	33	6
25-15-10-5°C	44	0	6
25-10-10-5°C	44	2	4
25-5-10-5°C	13	33	4
25-15-5-5°C	43	1	6
25-10-5-5°C	42	3	5
25-5-5-5°C	9	37	4
25-15-0-5°C	40	5	5
25-10-0-5°C	38	2	10
25-5-0-5°C	11	35	4
15-15-10-15°C	49	0	1
15-10-10-15°C	42	4	4
15-5-10-15°C	34	13	3
15-15-5-15°C	37	7	6
15-10-5-15°C	41	7	2
15-5-5-15°C	20	26	4
15-15-0-15°C	9	40	1
15-10-0-15°C	42	3	5
15-5-0-15°C	29	17	4
20-15-10-15°C	45	1	4
20-10-10-15°C	41	3	6
20-5-10-15°C	21	21	8
20-15-5-15°C	40	5	5
20-10-5-15°C	45	0	5
20-5-5-15°C	19	28	3
20-15-0-15°C	25	21	4
20-10-0-15°C	45	2	3
20-5-0-15°C	20	25	5
25-15-10-15°C	45	0	5
25-10-10-15°C	43	3	4
25-5-10-15°C	29	16	5
25-15-5-15°C	47	0	3
25-10-5-15°C	41	1	8
25-5-5-15°C	19	25	6
25-15-0-15°C	40	2	8
25-10-0-15°C	47	0	3
25-5-0-15°C	24	18	8

TABLE S3. Results from logistic regression analyses, showing the relative importance of seasons (compare residual deviances) on germination of *Galanthus nivalis* seeds, and outcomes from the regression model as to whether or not terms could be dropped from the model.

Output of final germination data fitting summer, autumn and winter (spring excluded)					
Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	26	602.6	23.176	23.18	< 0.001
Residual	54	165.3	3.061		
Total	80	767.9	9.598		
Chosen action: no change					
Output of final germination data fitting summer, autumn and spring (winter excluded)					
Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	26	705.88	27.149	27.15	< 0.001
Residual	54	61.98	1.148		
Total	80	767.86	9.598		
Chosen action: no change					
Output of final germination data fitting summer, winter and spring (autumn excluded)					
Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	26	57.8	2.22	2.22	< 0.001
Residual	54	710.1	13.15		
Total	80	767.9	9.60		
Chosen action: dropping summer.winter.spring.					
Output of final germination data fitting autumn, winter and spring (summer excluded)					
Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	26	637.3	24.510	24.51	< 0.001
Residual	54	130.6	2.418		
Total	80	767.9	9.598		
Chosen action: no change					

TABLE S4. The cumulative number of germinated *Galanthus nivalis* seeds (of 50 tested) and condition of non-germinated seeds at the end of the investigation, determined by dissection: seeds white and firm on dissection were classified as viable; those discoloured or soft as non-viable. The average seasonal treatment (i.e. 20-10-5-10°C) is emboldened.

Temperature sequence (Summer-Autumn-Winter-Spring)	Number germinated	Number viable	Number non-viable
15-15-10-10°C	21	5	24
15-10-10-10°C	8	23	19
15-5-10-10°C	2	12	36
15-15-5-10°C	21	7	22
15-10-5-10°C	9	11	30
15-5-5-10°C	2	26	22
15-15-0-10°C	13	9	28
15-10-0-10°C	6	17	27
15-5-0-10°C	3	20	27
20-15-10-10°C	28	0	22
20-10-10-10°C	8	15	27
20-5-10-10°C	1	29	20
20-15-5-10°C	22	1	27
<b>20-10-5-10°C</b>	<b>13</b>	<b>10</b>	<b>27</b>
20-5-5-10°C	0	20	30
20-15-0-10°C	24	0	26
20-10-0-10°C	12	12	26
20-5-0-10°C	1	28	21
25-15-10-10°C	19	0	31
25-10-10-10°C	4	15	31
25-5-10-10°C	0	26	24
25-15-5-10°C	18	0	32
25-10-5-10°C	6	21	23
25-5-5-10°C	0	25	25
25-15-0-10°C	18	0	32
25-10-0-10°C	4	16	30
25-5-0-10°C	0	34	16
15-15-10-5°C	17	4	29
15-10-10-5°C	7	27	16
15-5-10-5°C	3	20	27
15-15-5-5°C	13	7	30
15-10-5-5°C	7	18	25
15-5-5-5°C	1	34	15
15-15-0-5°C	22	4	24
15-10-0-5°C	2	16	32
15-5-0-5°C	2	27	21

20-15-10-5°C	26	0	24
20-10-10-5°C	9	16	25
20-5-10-5°C	3	28	19
20-15-5-5°C	28	0	22
20-10-5-5°C	2	20	28
20-5-5-5°C	0	19	31
20-15-0-5°C	25	1	24
20-10-0-5°C	13	23	14
20-5-0-5°C	2	25	23
25-15-10-5°C	22	0	28
25-10-10-5°C	3	17	30
25-5-10-5°C	0	16	34
25-15-5-5°C	18	0	32
25-10-5-5°C	2	14	34
25-5-5-5°C	0	22	28
25-15-0-5°C	24	0	26
25-10-0-5°C	5	15	30
25-5-0-5°C	0	22	28
15-15-10-15°C	14	4	32
15-10-10-15°C	18	8	24
15-5-10-15°C	10	6	34
15-15-5-15°C	12	3	35
15-10-5-15°C	17	2	31
15-5-5-15°C	13	4	33
15-15-0-15°C	16	1	33
15-10-0-15°C	18	7	25
15-5-0-15°C	7	7	36
20-15-10-15°C	16	0	34
20-10-10-15°C	12	5	33
20-5-10-15°C	2	13	35
20-15-5-15°C	24	0	26
20-10-5-15°C	13	7	30
20-5-5-15°C	6	9	35
20-15-0-15°C	21	0	29
20-10-0-15°C	11	5	34
20-5-0-15°C	4	16	30
25-15-10-15°C	21	0	29
25-10-10-15°C	12	8	30
25-5-10-15°C	9	20	21
25-15-5-15°C	27	0	23
25-10-5-15°C	5	14	31
25-5-5-15°C	1	22	27
25-15-0-15°C	17	0	33
25-10-0-15°C	7	18	25
25-5-0-15°C	4	24	22

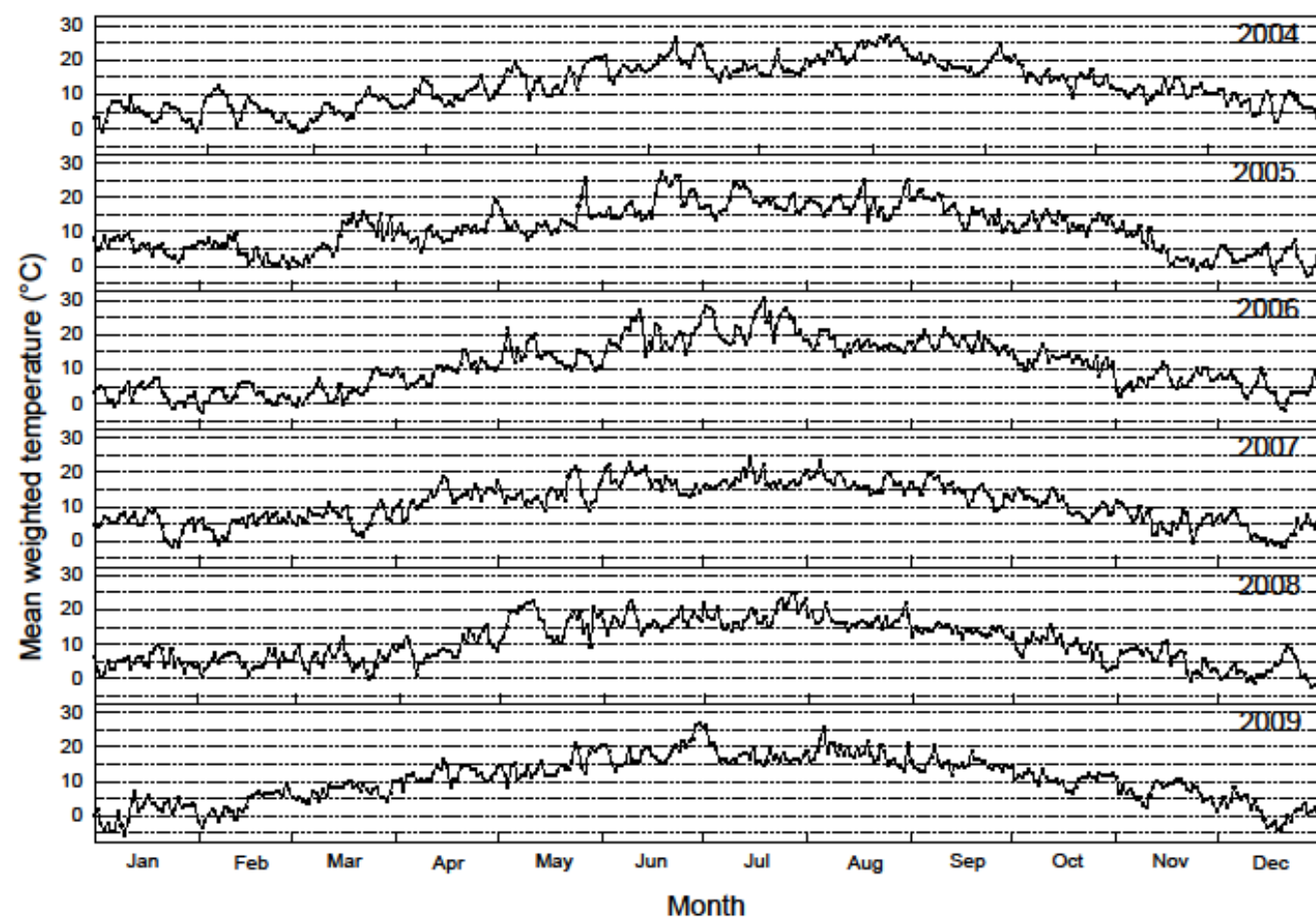
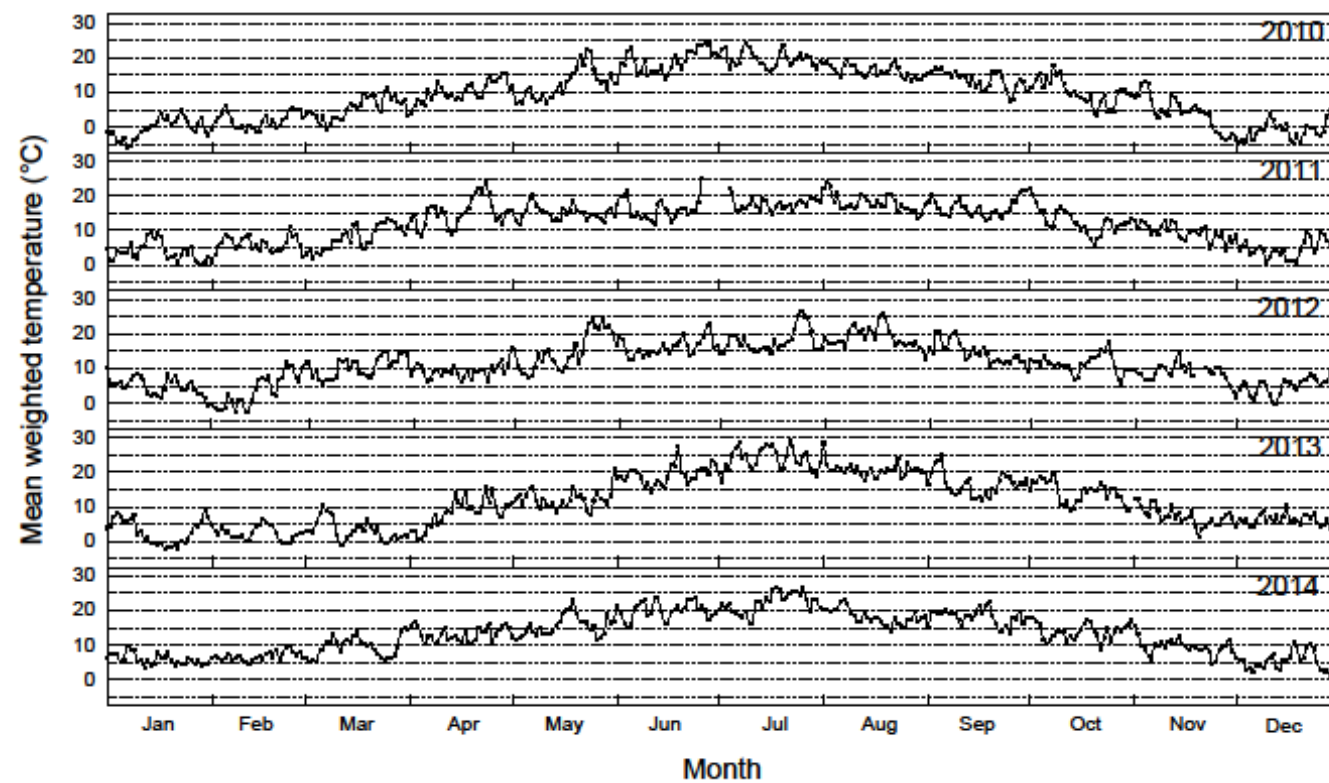


Figure S1



(continued)

Figure S1