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Phenology and morphology of the invasive legume *Lupinus polyphyllus* along a latitudinal gradient in Europe

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Abstract

Plant phenology, i. e. the timing of life cycle events, is related to individual fitness and species distribution ranges. Temperature is one of the most important drivers of plant phenology together with day length. The adaptation of their phenology may be important for the success of invasive plant species. The present study aims at understanding how the performance and the phenology of the invasive legume *Lupinus polyphyllus* vary with latitude. We sampled data across a >2000 km latitudinal gradient from Central to Northern Europe. We quantified variation in phenology of flowering and fruiting of *L. polyphyllus* using >1600 digital photos of inflorescences from 220 individual plants observed weekly at 22 sites. The day of the year at which different phenological phases were reached, increased 1.3–1.8 days per degree latitude, whereas the growing degree days (gdd) required for these phenological phases decreased 5–16 gdd per degree latitude. However, this difference disappeared, when the day length of each day included in the calculation of gdd was considered. The day of the year of the earliest and the latest climatic zone to reach any of the three studied phenological phases differed by 23–30 days and temperature requirements to reach these stages differed between 62 and 236 gdd. Probably, the invasion of this species will further increase in the northern part of Europe over the next decades due to climate warming. For invasive species control, our results suggest that in countries with a large latitudinal extent, the mowing date should shift by ca. one week per 500 km at sites with similar elevations.

Keywords

Flowering phenology, invasive plant, latitudinal gradient, legume, *Lupinus polyphyllus*, photoperiod

Introduction

Plant phenology is the timing of seasonal events, such as budburst, greening, flowering, and fruit ripening (Lieth 1974). Phenology influences the fitness of individual plants (Volis 2007; Anderson et al. 2012), controls species distribution ranges (Chuine and Beaubien 2001) and may have cascading effects on communities and ecosystems (Fargione and Tilman 2005; McKinney et al. 2012). Temperature is one of the most important drivers of plant phenology (Lieth 1974; Diekmann 1996) together with day length (e.g., Adole et al. 2019). In a long-term dataset, for example, between-year differences in average temperatures often corresponded to variation in the dates of first flowering (Fitter et al. 1995; Fitter and Fitter 2002). Furthermore, phenology studies have presented consistent evidence for the effects of climate change on organisms due to temperature shifts (Walther et al. 2002; Parmesan and Yohe 2003; Menzel et al. 2006; Cleland et al. 2007).

The potential for adaptation of phenology may also be key to understanding the success of invasive plant species (Wolkovich and Cleland 2011), which are predicted to benefit from ongoing global change (Hellmann et al. 2008; Willis et al. 2010). A large proportion of introduced terrestrial plants have shown evidence of climatic niche shifts (Atwater et al. 2018). Analyses of long-term data on first flowering dates showed that non-native species were better able to track inter-annual variation in temperature and tended to flower earlier than native plants, owing to higher climate sensitivity (Wolkovich et al. 2013). The invader plasticity model, which links phenology to plant invasions (Richards et al. 2006; Wolkovich and Cleland 2011) states that species with flexible phenology may be more successful invaders outside their native ranges, because their phenology will co-vary with weather conditions (Matesanz et al. 2010). Furthermore, a species with flexible phenology due to large phenotypic plasticity (Anderson et al. 2012) has the potential to disperse successfully along latitudinal gradients in the non-native distribution range.

Latitudinal gradients provide the opportunity to study the effects of climate on plants in natural experiments (De Frenne et al. 2013). While biomass, height and seed mass of plants usually decrease with decreasing temperatures along latitudinal gradients (De Frenne et al. 2013), northern populations often require fewer accumulated growing degree days than southern populations to reach the same phenological state (Langvall and Ottosson Löfvenius 2021). At the same time, northern populations experience longer day lengths during summer in the northern hemisphere. Whether the day length can compensate for the overall lower temperatures was rarely studied, but Adole et al. (2019), from a study in the southern hemisphere, conclude that the day length (also called daytime or photoperiod) is an important factor in driving vegetation phenology. However, there is little information on species-specific patterns of flowering and fruiting phenology in relation to temperature and day length of invasive species across long geographic gradients.

Lupinus polyphyllus Lindl. (Fabaceae) is a perennial herbaceous hemicryptophyte originating from western North America that was introduced in Central Europe as an ornamental plant in the 19th century. From Central Europe and Scandinavia, the species spread very successfully to almost all parts of Europe, now ranging from the Pyrenees in the West to the Ural (and beyond) in the East (Eckstein et al., unpublished data). From North to South, *L. polyphyllus* is currently covering the full range of Europe, except for Mediterranean zones such as the Iberian Peninsula and Italy (GBIF 2022). This species typically flowers relatively early and invades different habitats ranging from road verges to forest understories and different types of mountain meadows (Wissman et al. 2015; Ramula 2017; Klinger et al. 2019). Dominant stands of *L. polyphyllus* have significantly negative effects on local plant diversity (Valtonen et al. 2006; Hejda et al. 2009; Ramula and Pihlaja 2012), plant community composition (Hansen et al. 2021), and arthropod abundance (Valtonen et al. 2006). In contrast to other invasive neophytes in Europe, *L. polyphyllus* can, in particular, colonise nutrient-poor habitats of high nature conservation value (Hejda et al. 2009).

Our overall aim was to understand how the timing, temperature dependence of flowering and fruiting, and performance (canopy height, potential seed production and seed release height) of *L. polyphyllus* change along the latitudinal gradient from Central to Northern Europe. We tested differences between populations that were assigned to different climatic zones and quantified variation in phenology in relation to latitude. To our knowledge, the present study is the first attempt to quantify variation in phenology of an invasive plant across a large latitudinal gradient in the field. This information may help to develop more effective management schemes for this invasive plant. Generally, the species is managed by cutting at fixed calendar dates (Ramula 2017; Klinger et al. 2020). A phenology-based management, accounting for the timing of flowering and seed ripening may be key for optimal control of invasive species (Taylor et al. 2020). A strong positive correlation between the time of cutting and the germinability of seeds was found for *L. polyphyllus* in mountain meadows in Central Germany (Klinger et al. 2020). This suggests a trade-off between an early cut for avoiding ripe seeds and a late cut for avoiding a second flowering and therefore the need for another cut. However, plant phenology is rarely used for determining the timing of management practices and, further, it is poorly understood how to account for phenology in management (Ansquer et al. 2009; Taylor et al. 2020).

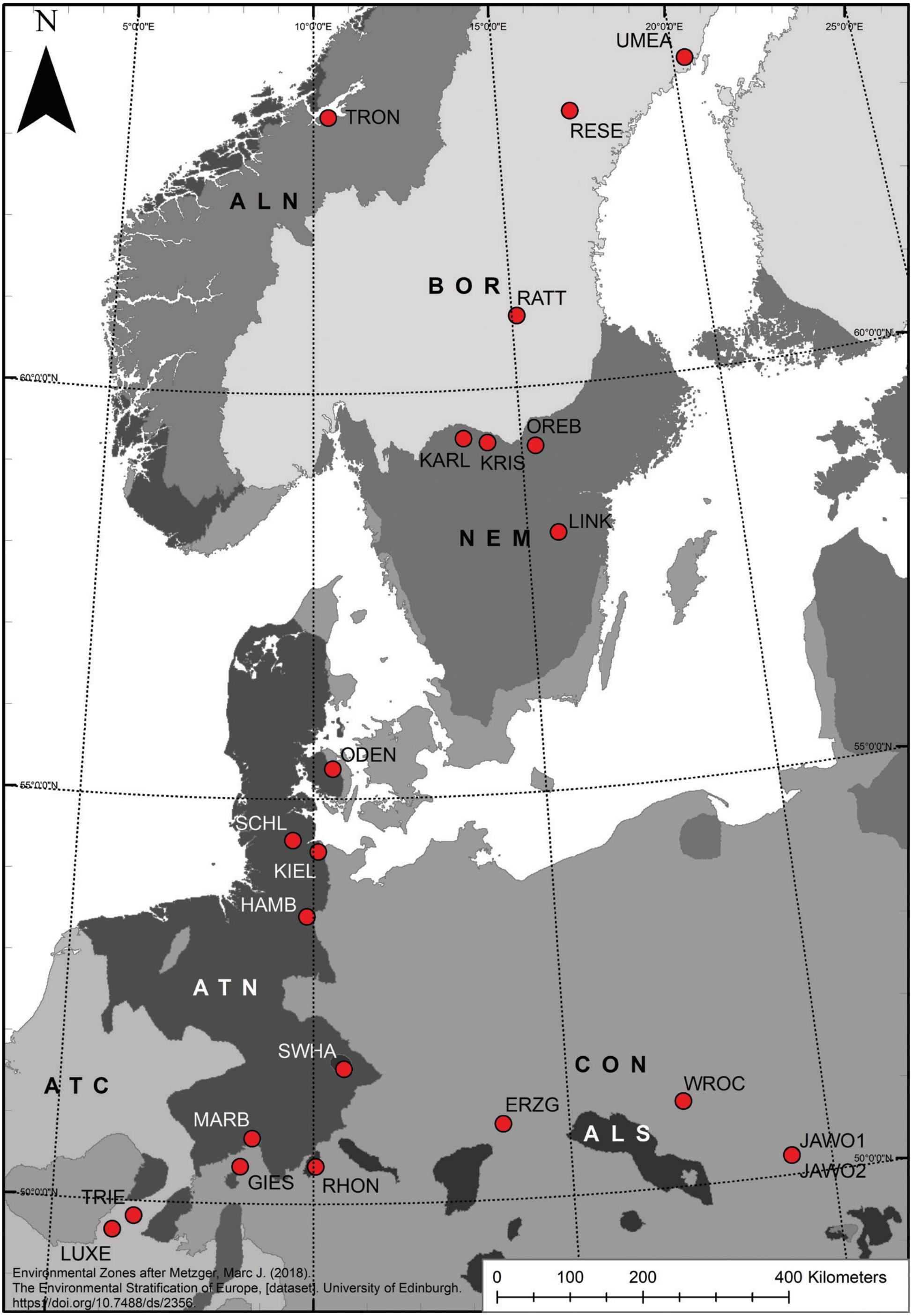
We wanted to test the following hypotheses:

1. Morphology is affected by latitude: canopy height, seed release height and the length of the inflorescence (as a proxy of potential seed production) decrease with decreasing temperatures; all three variables are significantly smaller in northern populations or populations in colder environments than in more southern populations in climatic zones with higher annual temperatures.
2. Reproduction is delayed with latitude: flowering and seed ripening are delayed in northern populations or populations in colder environments and there will be significant differences between populations of different climatic zones.
3. The number of accumulated growing degree days and accumulated growing degree day length (hours) required to reach flowering and seed ripening is negatively related to increasing latitude; accumulated growing degree days and growing degree day length (hours) to reach flowering and seed ripening vary significantly between climatic zones.

Methods

Study area

We analysed *Lupinus polyphyllus* populations along a latitudinal gradient ranging > 2000 km from Luxembourg, in Central Europe, to Umeå, in northern Sweden (49°38' – 63°49', Fig. 1). Twenty-two sites (Table 1) were included in the study with a minimum distance of 130 m (JAWO1 to JAWO2) and a maximum distance of 2196 km (LUXE to UMEA) between pairs of sites. We used the environmental stratification of Europe (Metzger et al. 2005; Metzger 2018) to assign each site to



Environmental Zones after Metzger, Marc J. (2018). The Environmental Stratification of Europe, [dataset]. University of Edinburgh. https://doi.org/10.7488/ds/2356.

Figure 1. Position of the 22 study sites (for site abbreviations, see Table 1). The background map depicts climatic zones according to the environmental stratification of Europe (Metzger et al. 2005; Metzger 2018). ATC – atlantic central, ATN – atlantic north; ALS – alpine south; CON – continental; NEM – nemoral; BOR – boreal; ALN – alpine north. For certain analyses, sites in BOR and ALN were pooled.

a climatic zone. The most south-westerly sites (LUXE, TRIE) are assigned to the **AT**lantic **C**entral climatic zone (ATC), most of the Central European sites (GIES, ERZG, JAWO1, JAWO2, WROC, ODEN) belong to the **CON**tinental climatic zone (CON), while four sites belong to the **AT**lantic **N**orth climatic zone (ATN: MARB, HAMB, KIEL, SCHL). Two sites at low latitudes (50–51°N) but at the highest elevations of the data set (SWHA: 508 m a.s.l., RHON: 767 m a.s.l.) are characterised by exceptionally cold climate for their latitude and thus belong to the **AL**pine **S**outh climatic zone (ALS). All sites in southern Sweden are part of the **NEM**oral climatic zone (NEM: LINK, OREB, KRIS, KARL), whereas the northern sites are assigned to the **BOR**eal climatic zone (BOR: RATT, RESE, UMEA). To avoid statistical problems due to climatic zones represented by a single site, we also included the TRON site to the BOR group although it has a more Atlantic climate and belongs to the alpine north zone (ALN) according to Metzger et al. (2005). However, TRON is most similar to the other sites in the boreal climatic zone with respect to mean annual temperatures, day length, and latitude (Table 1).

Table 1. Characteristics of the 22 study sites along the latitudinal gradient. For abbreviation of climatic zones, see Figure 1. Mean annual temperature (MAT) and mean annual sum of precipitation (MAP) for the study sites were derived from the nearest weather station. Distance (km) indicates the distance between the weather station and the respective study site.

Study site	Full site name	Coordinates (Latitude, Longitude)	Elevation (m a.s.l.)	Climatic zone	Weather station	Distance (km)	MAT (°C)	MAP (mm)
UMEA	Umeå	63.82961°N, 20.33164°E	38	BOR	Umeå Flygplats[1]	4.3	2.6	644
TRON	Trondheim	63.41364°N, 10.40789°E	41	ATN	Trondheim-Voll Plu[2]	2.3	4.7	925
RESE	Resele	63.34757°N, 17.00437°E	55	BOR	Forse[1]	22.4	2.5	536
RATT	Rättvik	60.87959°N, 15.12866°E	209	BOR	Leksand[1]	18.3	4.1	591
KARL	Karlstad	59.40300°N, 13.62328°E	78	NEM	Karlstad Flygplats[1]	17.0	5.7	635
KRIS	Kristinehamn	59.33775°N, 14.19258°E	139	NEM	Kristinehamn[1]	6.1	5.8	659
OREB	Örebro	59.26483°N, 15.33968°E	26	NEM	Örebro Flygplats[1]	17.3	5.8	586
LINK	Linköping	58.17554°N, 15.71404°E	99	NEM	Malexander A[1]	30.6	5.9	519
ODEN	Odense	55.36990°N, 10.42298°E	21	CON	Odense Lufthavn[3]	5.9	8.1	583
SCHL	Schleswig	54.48772°N, 9.56911°E	10	ATN	Schleswig[4]	4.0	8.0	926
KIEL	Kiel	54.34886°N, 10.10497°E	24	ATN	Kiel-Holtenau[4]	3.5	8.4	754
HAMB	Hamburg	53.54843°N, 9.86951°E	9	ATN	Hamburg-Fuhlsbüttel[4]	12.1	8.6	770
SWHA	Südwest-Harz	51.66625°N, 10.60720°E	508	ALS	Braunlage[4]	6.3	5.9	1263
WROC	Wrocław	51.04966°N, 17.25088°E	128	CON	Wroclaw-Strachowice[5]	25.3	8.4	588
ERZG	Erzgebirge	50.93647°N, 13.71082°E	432	CON	Dresden-Klotzsche[4]	20.2	8.9	667
MARB	Marburg	50.80591°N, 8.80855°E	332	ATN	Cölbe, Kr. Marburg-Biedenkopf[4]	5.6	8.9	756
RHON	Rhön	50.46310°N, 10.04884°E	767	ALS	Wasserkuppe[4]	8.4	4.8	1084
GIES	Gießen	50.45559°N, 8.58841°E	303	CON	Giessen-Wettenberg[4]	25.5	8.2	719
JAWO2	Jaworzno	50.23825°N, 19.22854°E	273	CON	Krakow -Balice[5]	43.3	7.8	679
JAWO1	Jaworzno	50.23744°N, 19.22739°E	275	CON	Krakow[5]	43.0	7.8	679
TRIE	Trier	49.81556°N, 6.57417°E	374	ATC	Trier-Petrisberg[4]	10.7	9.1	784
LUXE	Luxembourg	49.63619°N, 6.17952°E	365	ATC	Luxembourg/Luxembourg[4]	3.4	8.3	875

Sources for climatic data (1961–1990): [1]Swedish Meteorological and Hydrological Institute (SMHI), [2]Det Norske Meteorologiske Institutt (DNMI), [3]Danmarks Meteorologiske Institut (DMI), [4]Deutscher Wetterdienst (DWD), [5]Institute of Meteorology and Water Management – National Research Institute (IMGW – PIB).

Study design and data sampling

Each participant in the study selected a population of *L. polyphyllus* along a road verge or in close vicinity to a road, in an open, sunny locality. For each population we documented geographic coordinates, elevation, and climatic parameters from the nearest weather station (Table 1).

Ten randomly selected adult individuals, representative for each population, were marked early in the season in 2019. We focussed on the first developed central inflorescence of each of these marked individuals. Starting when the inflorescences were visible (mid-end April), we made digital photos of the inflorescence of each marked individual against a scale bar, usually a meter stick. We used photos for consistent measurements across sites and stored them for future analysis. The photos were sent to the project coordinator (RLE), who analysed all photos together with a student assistant. Additionally, all participants measured the maximum height from the ground to the top of the basal leaves (**canopy height**) and to the top of the inflorescence (**seed release height**) of the 10 marked individuals per measuring event on site. Usually, the photos and direct measurements were taken once per week until the first pods of the inflorescences were ripe. We obtained between five and twelve observations per site, resulting in 180 site × date combinations. Depending on the site location, observations ranged from 29 April to 4 August, with the majority of observations (160 site × date combinations) made between 6 May and 8 July.

In total, 1633 photos from 22 sites were analysed using the software ImageJ 1.52a (Schneider et al. 2012). We used the scale bar in each photo to calibrate distances before we estimated the following variables (Fig. 2a, b, Table 2) for each plant individual per measuring event:

1. **Total length of the inflorescence** from the lowermost flower bud, flower or flower scar to the top (A-C),
2. **Length of the inflorescence with open flowers** from the lowermost flower/flower scar to the uppermost open flower (A–B); open flowers were defined as flowers with unfolded standard, visible keel, and elongated pedicel (Fig. 2c).

Calculated variables

Using the photo measurements, we calculated the **relative length of the inflorescence with open flowers (*RLF*)** for each plant individual per measuring event (Table 2). *RLF* represents an indicator of progress of flowering phenology that can relatively easily be estimated in the field, e.g., for management purposes. Since *RLF* is bound between zero and one, we used these estimates (available for each individual per measuring event) to perform logistic regressions per site. The logistic regressions for *RLF* against the **day of the year (doy)** of the measuring events were significant ($p<0.05$). Using the parameters of all significant logistic regressions, we estimated the doy, at which half of the actual length of the inflorescence carried open flowers (doy.flow.half, Fig. 2d).

Table 2. Measured and calculated variables used in the manuscript.

Variable	Usage*	Description	Formula/Remarks
Canopy height	R	Maximum height from the ground to the top of the basal leaves (cm)	Measured in the field (usually weekly)
Seed release height	R	Maximum height from the ground to the top of the inflorescence (cm)	Measured in the field (usually weekly)
Total length of inflorescence	R	Length of inflorescence (cm) from the lowermost flower bud, flower or flower scar to the top (A–C in Fig. 2)	Determined via photos
Length of the inflorescence with open flowers	A	Length from the lowermost flower/flower scar to the uppermost open flower (A–B in Fig. 2);	Determined via photographs; open flowers were defined as flowers with unfolded standard, visible keel, and elongated pedicel
RLF	A	Relative length of the inflorescence with open flowers at each measuring event (t_x)	
doy	A/E/R	Day of the year	Day number (1st of January = day 1)
gdd	A/E/R	Number of growing degree days using a base temperature of 5 °C	$GDD=(T_{max} - T_{min})/2 - T_{Base}$, if $T_{Mean} > T_{Base}$
gdh	E	Cumulated day length: growing degree day length (hours), called growing day hours	GDH=GDD*Day length of each day included in calculation of GDD
Flow.half	R	Day of the year (doy.flow.half) or number of growing degree days (gdd.flow.half) when half of the actual length of the inflorescence carried open flowers	Estimated per site via RLF using logistic regressions
First.flow	R	Day of the year (doy.first.flow) or number of growing degree days (gdd.first.flow) when the first flower was formed	Determined via photographs
First.ripe	R	Day of the year (doy.first.ripe) or number of growing degree days (gdd.first.ripe) when the first black pod was formed	Determined via photographs

*Usage: A=Auxiliary (used to calculate other variables), E=Explanatory variable, R=Response variable.

Additionally, we recorded the doy of the measuring event at which the first open flower (doy.first.flow, Fig. 2c) and the first black pod (doy.first.ripe, Fig. 2e) were visible in the photographic documentation. This was not possible for the sites ODEN and RESE because the observation period did not include these phenological stages. For site RATT, no pods were black at the time of the second last observation date but all pods were black on the last observation date. However, as the last observation was done one month after the second last, the timing of this phase was clearly overestimated and we omitted this data point.

We obtained temperature measurements from meteorological stations located closest to the field sites (distances between 2.3 and 43.3 km from the studied sites (Table 1). Using the minimum and maximum daily temperatures, we calculated **growing degree days (gdd)** from January 1, using the standard base temperature of 5 °C (Table 2). Temperatures preceding a phenological phase (e.g., day of first flowering) are important drivers of phenology (e.g., Fitter et al. 1995) and the accumulation of gdd with the standard base temperature of 5 °C represents a simple but powerful proxy for predicting the flowering phenology of plants (e.g., Diekmann 1996; De Frenne et al. 2010). We then calculated the accumulated gdd for each observation date at each site from the temperature data of the meteorological stations (Table 2). Finally, we used the same logistic regression approach as above, but using accumulated gdd instead of doy to estimate gdd.flow.half. We also estimated the accumulated gdd until the first

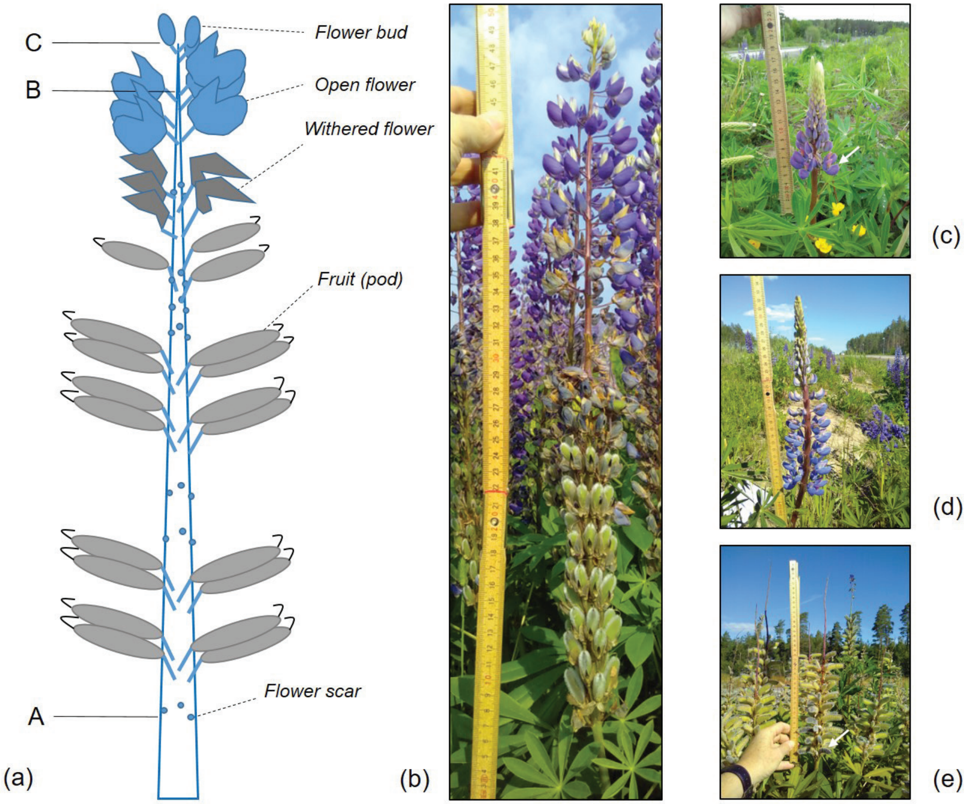


Figure 2. Measurements taken along the inflorescence of *Lupinus polyphyllus* (**a**): A – C: total length of inflorescence; A – B: length of inflorescence with open flowers; an example photo showing the flower development taken at the population KARL, on the 7th of June 2019 by Lutz Eckstein (**b**) and example photos showing the different stages first.flow, on 24th of May (**c**), flow.half, on 31st of May (**d**), and first.ripe, on 5th of July (**e**). White arrows show an open flower and a ripe pod, in (**c**) and (**e**), respectively.

open flower (gdd.first.flow) and the first black pod (gdd.first.ripe) were visible in the photographic documentation.

Since light is an important driver of phenology alongside temperature, and day length increases with latitude, we aimed at incorporating differences in day length in our analyses by creating a variable (**growing degree day length, in accumulated hours: gdh**) that combines growing degree days and day length. For each population and day of the year, the day length was calculated using the 'geosphere' package in R (Hijmans 2017). We then multiplied the respective gdd (with the base temperature of 5 °C) with day length hours of that day. In addition to growing degree days, the combined factor growing day hours was included for all populations. In the literature about phenology studies, day length is rarely accounted for, although it may be an important factor (Adole et al. 2019; Ettinger et al. 2021).

Statistical tests

For all variables, we used mean values from the ten measured individuals per site. We analysed the data in two different ways. Firstly, we calculated linear regressions of doy.first.flow, doy.flow.half, doy.first.ripe, gdd.first.flow, gdd.flow.half, and gdd.first.ripe against latitude (decimal degrees north) to quantify the rate of change in flowering, seed ripening, and seed shedding phenology with latitude. In these analyses, we excluded the two sites of the Alpine south climatic zone (SWHA, RHON), since these populations potentially experience much higher temperature selection due to a cold montane climate as compared to other sites at similar latitudes and would lead to confounding latitude and elevation. Secondly, we did one-way ANOVAs of all six dependent variables with the climatic zones according to Metzger et al. (2005) to test whether climatic zones differed significantly with respect to the timing (using doy) and energy dependence (using gdd and gdh) of phenology. Test assumptions were checked visually and homogeneity of variances tested using Levene's test. In case of violation of variance homogeneity, we did a White-adjusted ANOVA (White 1980) using the R-package *car* (Fox and Weisberg 2019). When we found significant differences using ANOVA, we performed a pair-wise comparison to determine significant differences among climatic zones using the Tukey HSD test of the R-package *agricolae* (Mendiburu 2015). We tested the residuals of all statistical models for spatial autocorrelation, using a global Moran`s I with the spdep package in R (Bivand and Wong 2018). Only in two of 18 tests, autocorrelation was detected, in both cases for the response variable first.ripe (Appendix 1: Table A1), which had no significant results in the ANOVA tests. Thus, we assume that spatial autocorrelation is not relevant in our analyses. All statistical analyses were done using R version 3.6.2 (R Core Team 2020).

Results

The maximum canopy height varied by a factor of 2.5 between sites (min: HAMB – 34.9 cm; max: JAWO2 – 88.0 cm). Averaged across sites of the same climatic zone (Fig. 3a), it varied by a factor of 1.4, ranging from the Atlantic north zone (ATN) with a mean canopy height of 55.2 cm to the continental zone (CON) with a mean canopy height of 76.9 cm. Overall, we detected a marginally-significant effect of climatic zone on canopy height ($F_{5,16} = 2.59$, $p = 0.0674$), but canopy height patterns were not consistent with latitudinal direction. We found a significant effect of climatic zone on seed release height ($F_{5,16} = 5.96$, $p = 0.0028$), but even here there was no consistent pattern with latitude or temperature (Fig. 3b). Seed release height varied by a factor of 1.9 between single sites (min: LINK – 74.5 cm; max: TRON – 141.2 cm). On average, it ranged between 90.3 cm (ALS) and 122.7 cm (BOR) among climatic zones. The length of the inflorescence varied by a factor of about 2 between sites (min: LINK – 24.9 cm; max: JAWO1 – 49.3 cm) and ranged between 36.0 cm (NEM) and 43.1 cm (ATN) among the climatic zones (Fig. 3c); there was neither a significant effect of climatic zone ($F_{5,16} = 1.03$, $p = 0.4317$) nor a latitudinal trend.

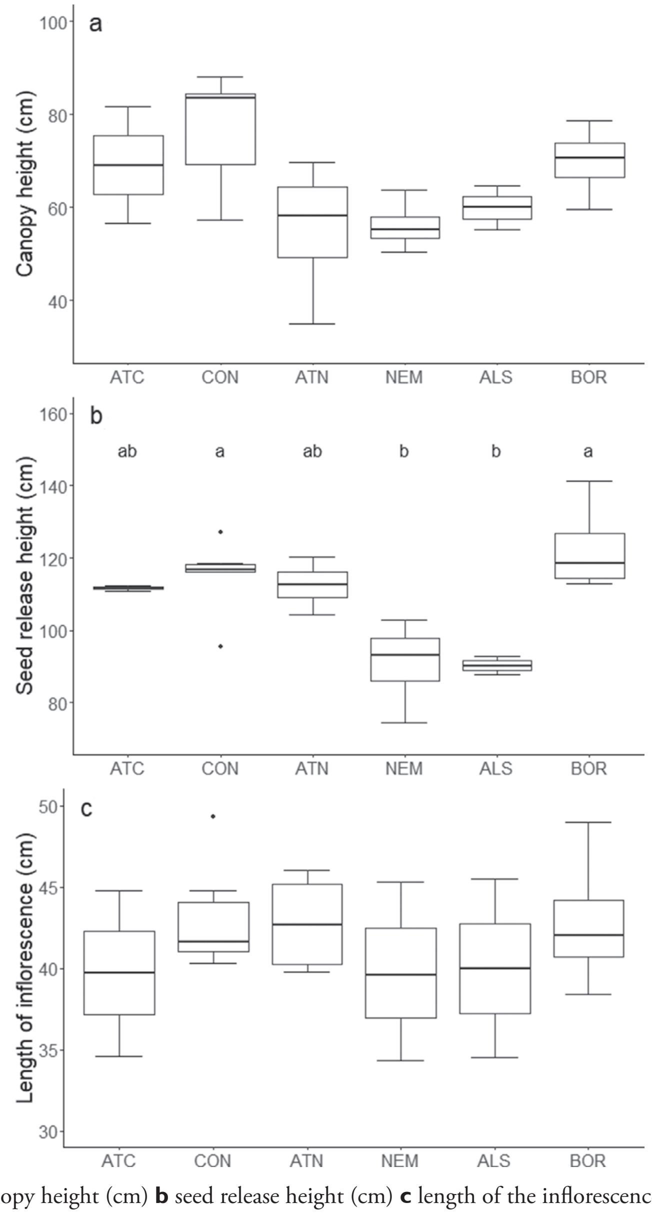


Figure 3. a canopy height (cm) **b** seed release height (cm) **c** length of the inflorescence (cm) during the time of biomass maximum for sites grouped according to climatic zones according to Metzger (2018). Climatic zones: ATC: atlantic central, CON: continental, ATN: atlantic north, NEM: nemoral, ALS: alpine south; BOR: boreal (includes the climate zone ALN, i.e. site TRON). Climatic zones are ordered according to decreasing mean annual temperatures. With the exception of ALS, the ordering corresponds to increasing northern latitude. Different letters indicate significant effects of climatic zone ($p < 0.05$).

The doy at which the inflorescences of *L. polyphyllus* reached first.flow, flow.half, and first.ripe increased with decreasing mean annual temperatures of the climatic zones (Fig. 4). The effects of climatic zone were highly significant for first.flow ($F_{5,16} = 22.06$, p <

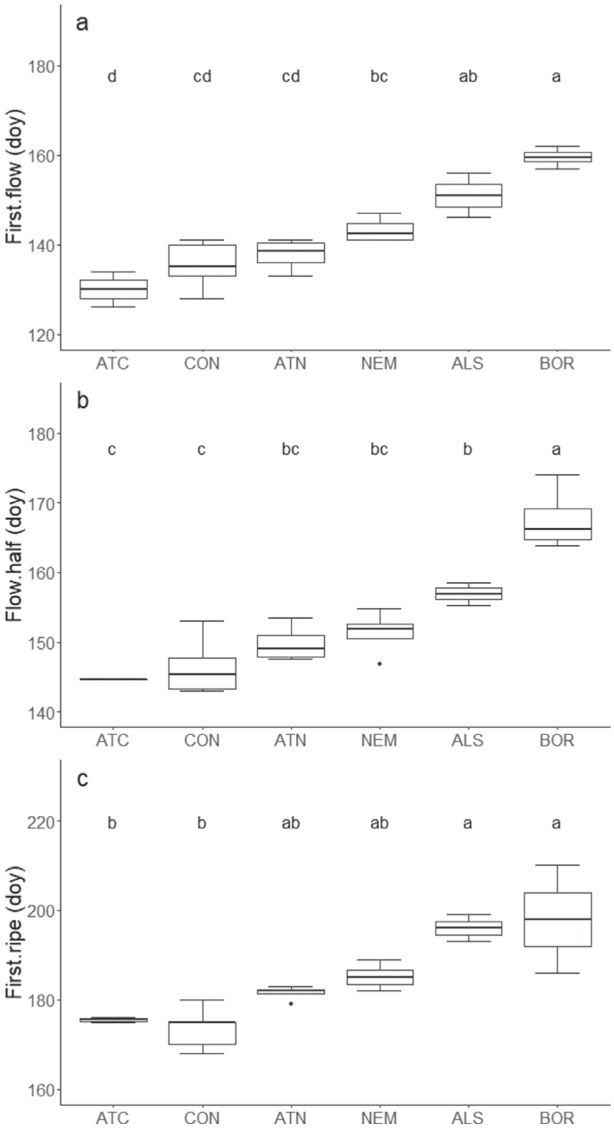


Figure 4. Day of year (doy), at which **a** the first open flower was observed (first.flow) **b** half of the length of the inflorescence bears open flowers (flow.half) **c** the first ripe (black) pods were observed (first.ripe) for each climatic zone (Metzger 2018). Climatic zones are ordered according to decreasing mean annual temperatures. With the exception of ALS, the ordering corresponds to increasing northern latitude. Different letters indicate significant effects of climatic zone ($p < 0.05$). For abbreviations of climatic zones, see Fig. 3.

0.001), flow.half ($F_{5,16} = 21.49$, $p < 0.001$), and first.ripe (White-adjusted ANOVA: $F_{5,13} = 12.98$, $p < 0.001$) and there was a monotonic increase of doy with latitude. Lupines in the boreal climatic zone (BOR) needed significantly more days to reach flow.half (doy = 168, 17th of June) than in all other climatic zones (Fig. 4b). Both, the populations in the BOR and in the alpine south climatic zone (ALS) needed significantly more days to reach first.

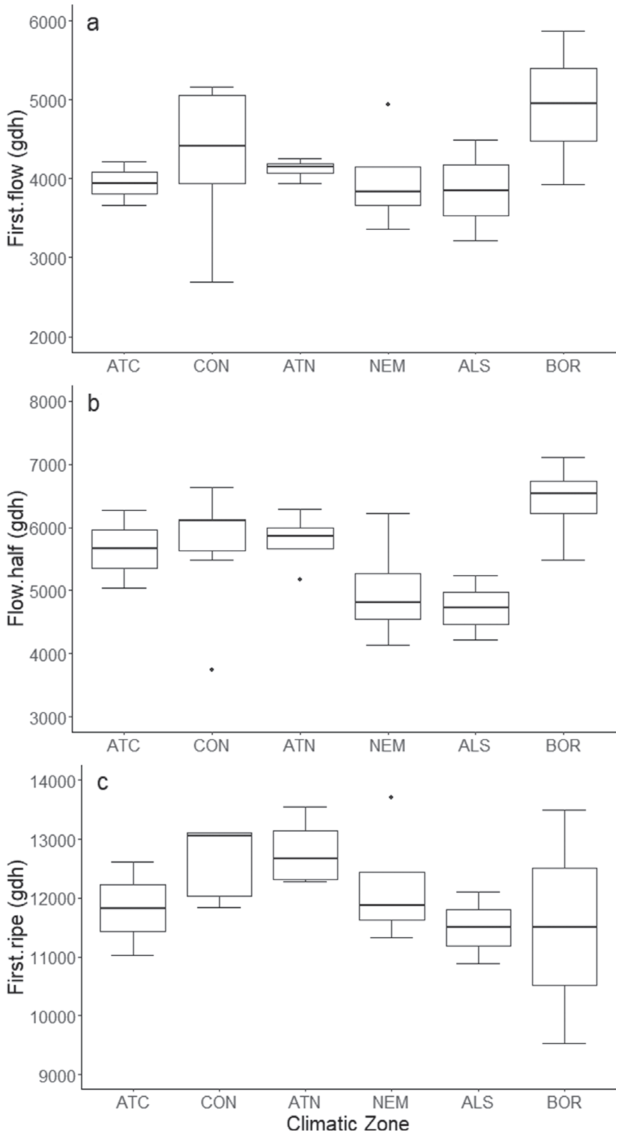


Figure 5. Growing day hours (gdh), at which **a** the first open flower was observed (first.flow) **b** half of the length of the inflorescence bears open flowers (flow.half) **c** the first ripe (black) pods were observed (first.ripe) for each climatic zone (Metzger 2018). Climatic zones are ordered according to decreasing mean annual temperatures. With the exception of ALS, the ordering corresponds to increasing northern latitude. For abbreviations of climatic zones, see Fig. 3.

flow (on 18th of June and 31st of May, respectively) than in the Atlantic north zone (ATN, 18th of May), in the continental zone (CON, 16th of May) or in the Atlantic central zone (ATC, 1th of May) (Fig. 4a). The same patterns as in first.flow and flow.half were visible for first.ripe (Fig. 4c). The difference in average number of days between the earliest and the latest climatic zone was 30 days for first.flow and ca. 23–24 days for flow.half and first.ripe.

The gdd at which the inflorescences reached first.flow did not differ between the climatic zones ($F_{5,16} = 0.96$, $p = 0.468$). For the next phenological phase, flow.half, the gdd tended to be affected by the climatic zone ($F_{5,16} = 2.48$, $p = 0.076$). Finally, the effects of climatic zone on gdd were significant for first.ripe ($F_{5,13} = 5.96$, $p = 0.0047$). More specific, the gdd at which the inflorescences reached flow.half and first.ripe tended to be lower for the zones NEM, ALS and BOR than for ATC, CON and ATN (data not shown). While these patterns were found for growing degree days (gdd), they disappeared when the day length was taken into account as growing day hours (gdh, Fig. 5). No significant effect of the climatic zones was found on the growing day hours that are needed to reach first.flow ($F_{5,16} = 0.964$, $p = 0.468$), flow.half ($F_{5,16} = 1.714$, $p = 0.189$), and first.ripe ($F_{5,13} = 0.729$, $p = 0.614$) (Fig. 5).

Our regression analyses showed that the day of the year, on which the first open flower was observed (first.flow), half of the inflorescence's length at each site had open flowers (flow.half), and the first ripe pod (first.ripe) was observed increased significantly ($p < 0.001$) with latitude (Appendix 1: Fig. A1). The day of the year on which first.flow was observed ranged between the 6th of May and the 11th of June across sites; for flow.half between the 23rd of May and the 23rd of June; and for first.ripe between the 17th of June and the 29th of July. The slope of the linear regression was 1.76 days per degree latitude for first.flow (Appendix 1: Fig. A1a, $F_{1,18} = 49.33$, $R^2 = 0.7178$), 1.42 days per degree latitude for flow.half (Appendix 1: Fig. A1b, $F_{1,18} = 35.36$, $R^2 = 0.6439$), and 1.53 days per degree latitude for first.ripe (Appendix 1: Fig. A1c, $F_{1,15} = 27.83$, $R^2 = 0.6264$).

The accumulated growing degree days (gdd) until the inflorescence at each site reaches first.flow, flow.half, and first.ripe, decreased significantly (all p-values < 0.05) with latitude (Appendix 1: Fig. A2a–c). The slope of the linear regression was -5.17 gdd per degree latitude for first.flow (Appendix 1: Fig. A2a, $F_{1,18} = 6.67$, $R^2 = 0.2298$), -8.26 gdd per degree latitude for flow.half (Appendix 1: Fig. A2b, $F_{1,18} = 13.53$, $R^2 = 0.3973$), and -16.14 gdd per degree latitude for first.ripe (Appendix 1: Fig. A2c, $F_{1,15} = 27.61$, $R^2 = 0.6245$). While the growing degree days (gdd) decreased significantly with latitude, this pattern disappeared when the day length was considered (Appendix 1: Fig. A2d–f).

Data accessibility statement

The data supporting the findings of this study are openly available in the repository dryad at https://doi.org/10.5061/dryad.stqjq2c3t (Ludewig et al. 2022).

Discussion

According to our findings, canopy height and the length of the inflorescence of *Lupinus polyphyllus* does not vary significantly among climatic zones. Seed release height shows significant variation among climatic zones but there is no consistent pattern with latitude. Consequently, we found no evidence for our first hypothesis that the latitudinal gradient affects these measures of performance of the invasive *L. polyphyllus*. Plant height and seed mass usually decrease with decreasing temperatures along latitudinal gradients (De Frenne et al. 2013), though some studies did find no latitudinal variation or even increased performance with latitude (De Frenne et al. 2013). Other factors, including the productivity of the habitat or the height of the competing vegetation, can additionally influence this relationship. Nevertheless, the absence of this relationship and the production of black pods show that although phenology is delayed at more northern latitudes, *L. polyphyllus* is able to produce mature seeds even in Northern Europe.

The day of year (doy) at which the first open flower was found, half of the length of the inflorescence had open flowers and the doy at which the first black, ripe pod was found, increased significantly with northern latitude. Populations in zones with a colder climate reach these phenological phases significantly later than populations in climatic zones with higher annual temperatures. Therefore, and as stated in our second hypothesis, the phenology of flowering and seed ripening is delayed in populations of *L. polyphyllus* in the northern part of the gradient. More specifically, all measured phenological parameters were delayed under colder climate conditions, i.e., at higher latitudes or elevations (boreal and alpine south zone). This is in contrast to studies, in which plant material from latitudinal gradients was collected and grown in common garden experiments (Olsson and Ågren 2002; Kollmann and Bañuelos 2004). For example, *Impatiens glandulifera* plants grown from seeds flowered faster when the seeds originated from northern populations compared to southern populations (Kollmann and Bañuelos 2004). This finding is not surprising and can be expected when plants at higher latitudes are adapted to flower earlier (relative to the beginning of the vegetation period) and their seeds are then transferred to lower latitudes in a study. Generally, temperature is an important driver for the timing of seasonal events (e.g., Lieth 1974; Diekmann 1996; De Frenne et al. 2013), but also day length plays an important role in our study about *L. polyphyllus*.

While the accumulated growing degree days (gdd) required to reach the different phenological phases decrease with latitude, suggesting that energy requirements for flowering and fruit ripening are lower at higher latitudes, this effect disappeared when day length was considered. This finding shows that longer day lengths may compensate for the fewer growing degree days at northern latitudes. As a result, the energy demands of *L. polyphyllus* to reach the studied phenological phases, measured as growing day hours (gdh), do not differ significantly along the latitudinal gradient. The first finding is in line with Langvall and Ottosson Löfvenius (2021) who showed that the leafing phenology of *Betula pubescens* and *Pinus sylvestris* is delayed in northern populations compared to southern populations in Sweden, but that northern populations need fewer growing degree days than southern populations to reach the same phenological phase. However, Langvall and Ottosson Löfvenius (2021) did not include the day length in their study, but identified day length as a possible underlying factor for their results. Overall, we found some evidence for our third hypothesis that the number of accumulated growing degree days required to reach certain flowering and seed ripening phases is negatively related to northern latitude, but this was not the case when day length was accounted for. For future studies on plant phenology across latitudinal gradients, we recommend including day length in the analysis.

Longer day length during summer allows *L. polyphyllus* to fulfil its life cycle relatively quickly in the investigated northern latitudes. Therefore, populations in the northern part of the gradient have probably not changed their climatic niche (Guisan et al. 2014; Atwater et al. 2018) and react plastically to the energy input they get. Similarly, two other species in the same genus show high phenotypic plasticity on small spatial scales. *Lupinus lepidus* responded with differing phenology between established (surviving) and newly colonized populations on lava fields after an eruption of Mount St. Helens (Bishop and Schemske 1998). The second species is the woody *Lupinus arboreus*, which shows small-scale differentiation in traits such as plant size, flowering phenology, or fecundity in subpopulations in dune and grassland habitats < 500 m apart from each other (Kittelson and Maron 2001). This high capability for plastic responses of *Lupinus* species may be advantageous in the light of climate change. We anticipate that the invasion of this species will further expand in the northern part of Europe over the next decades. This will be facilitated by a temperature increase that is predicted to be faster and higher in northern ecosystems (Hewitson et al. 2014). Also, we expect this species to do well in northern latitudes as the shorter growth season is at least partly compensated for by longer days.

For invasive species control, our results suggest that in countries with a large latitudinal extent, the timing of management (e.g. mowing date) should shift by ca. one week every 500 km, at least for sites at lower elevations. For example, in Germany (ca. 900 km south-north extent) or Sweden (ca. 1600 km south-north extent), the southernmost populations should be managed ca. 12 and 22 days earlier, respectively, than the northernmost populations. The variable flow.half may represent a good indicator for the optimal time for management since no viable seeds are present at this stage. In our study year, flow.half was reached in the southernmost populations in Germany and Sweden at the end of May and beginning of June, respectively. With later mowing the possibility of seed shedding increases and the potential to limit the spread of *L. polyphyllus* decreases. The practical planning of phenology-based control of invasive plants (Taylor et al. 2020) would benefit from an open large-scale phenology dataset providing information on the timing of flowering and seed ripening for plant species relevant for nature conservation management.

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Appendix 1

Table A1. Results of the global Moran´s I test for spatial autocorrelation in the statistical models.

Dependent	Independent	Figure	Moran's I standard deviate	p-value
Canopy height	Climatic zone	3a	0.08931	0.4644
Seed release height	Climatic zone	3b	-0.12235	0.5487
Length infl.	Climatic zone	3c	-0.11074	0.5441
First.flow(doy)	Climatic zone	4a	-0.21516	0.5852
Flow.half(doy)	Climatic zone	4b	-0.11702	0.5466
First.ripe(doy)	Climatic zone	4c	-0.57733	0.7181
First.flow(gdh)	Climatic zone	5a	-0.93938	0.8262
Flow.half(gdh)	Climatic zone	5b	0.26693	0.3948
First.ripe(gdh)	Climatic zone	5c	**2.1708**	**0.01497**
First.flow(doy)	Northern latitude	A1a	0.045778	0.4817
Flow.half(doy)	Northern latitude	A1b	0.34373	0.3655
First.ripe(doy)	Northern latitude	A1c	0.21317	0.4156
First.flow(gdd)	Northern latitude	A2a	0.87796	0.1900
Flow.half(gdd)	Northern latitude	A2b	-0.36804	0.6436
First.ripe(gdd)	Northern latitude	A2c	-0.78436	0.7836
First.flow(gdh)	Northern latitude	A2d	-0.0080847	0.5032
Flow.half(gdh)	Northern latitude	A2e	-0.0091361	0.5036
First.ripe(gdh)	Northern latitude	A2f	**2.2439**	**0.01242**

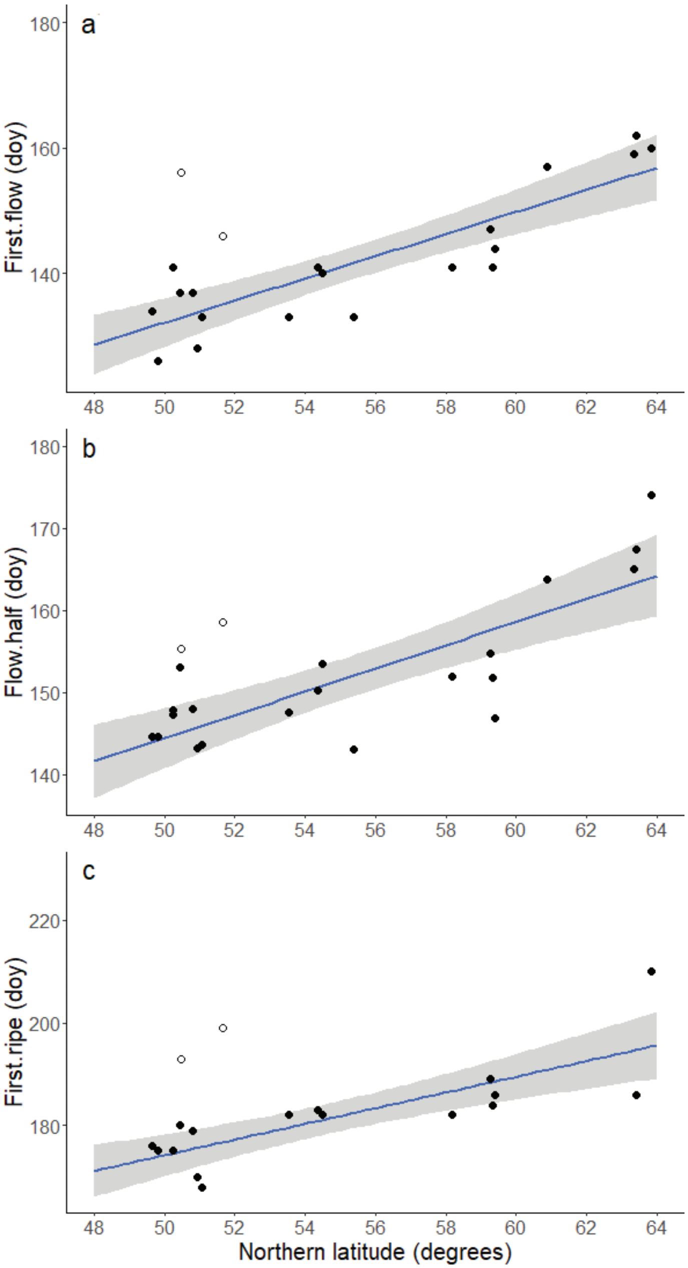

Figure A1. Linear regressions of the day of year (doy) for each site, at which **a** the first open flower was observed (first.flow) **b** half of the inflorescence's length bears open flowers (flow.half) **c** the first ripe (black) pods was observed (first.ripe), against latitude (°N). Only the sites in black were included into the model. White symbols are sites of the high altitude, alpine south climatic zone (RHON, SWHA) that were omitted from this analysis and only shown for comparison. Grey areas depict 95% confidence intervals.

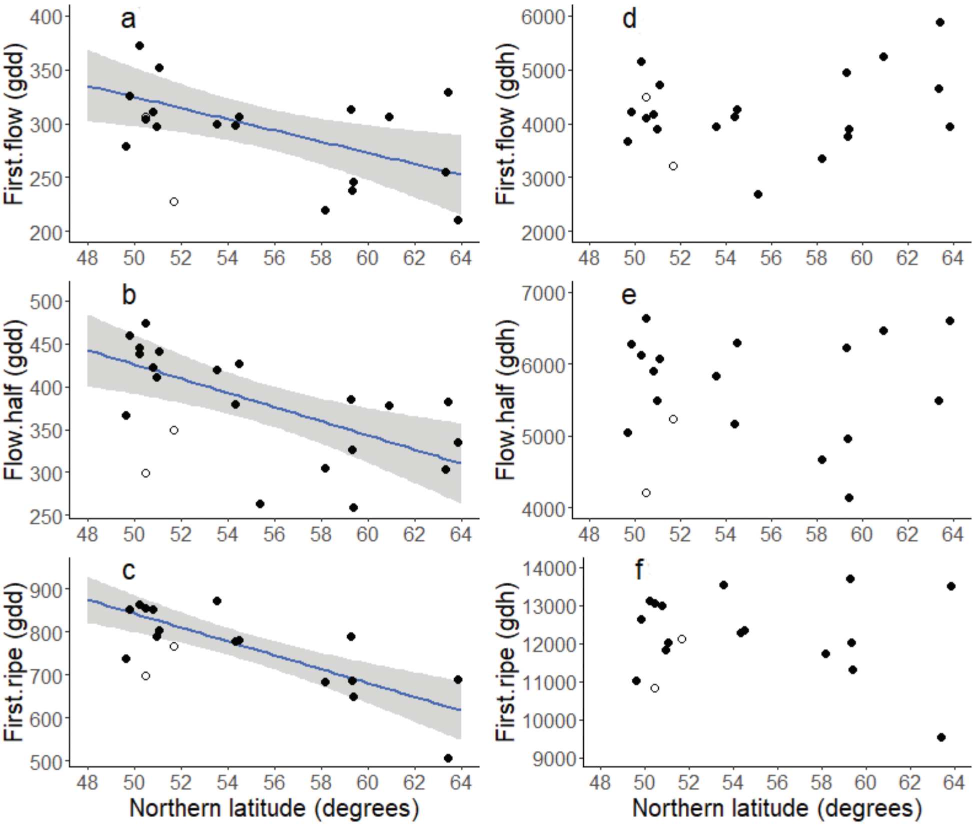

Figure A2. Linear regressions of the accumulated growing degree days (gdd; from January 1, base temperature: 5 °C from weather stations) for each site, until **a** the first open flower was observed (first.flow) **b** half of the inflorescence's length bears open flowers (flow.half) **c** the first ripe (black) pods were observed (first.ripe), against latitude (°N). Furthermore, linear regressions of the accumulated growing day hours (gdh) for each site, until **d** the first open flower was observed (first.flow) **e** half of the inflorescence´s length bears open flowers (flow.half) **f** the first ripe pod was observed (first.ripe) against latitude (°N). Only the sites in black were included into the model. White symbols are sites of the high altitude, alpine south climatic zone (RHON, SWHA) that were omitted from this analysis and only shown for comparison. Grey areas depict 95% confidence intervals.