# Variation in phenology traits in a common garden trial of *Prunus avium* L. seed sources in the Netherlands, Belgium and Germany

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## Abstract

*Prunus avium* (wild cherry) is considered as one of the candidate tree species to diversify forest stands. Due to climate change there is also more interest in using seed sources from warmer or dryer areas. The objective of the study is to investigate differences in budburst and senescence with a focus on seed orchard material.

For this, we established a *P. avium* common garden trial with sites in the Netherlands, Belgium and Germany. We planted material from 14 seed orchards, 3 seed stands and 1 source-identified seed source from five Western European countries. The repeated assessments of budburst and senescence were analysed for differences between seed sources by modelling spatial trends using the 'SpATS' R-package and using a multi-site approach.

We found small but significant differences between seed sources in both budburst and senescence. The largest difference between the average time of the appearance of first visible leaves between seed sources was approximately eight days. There was no clear geographical trend in budburst timing. Calculations of Finley-Wilkinson measures and Wricke's ecovalence $W^2$ indicate that most seed sources are stable over the different environments.

We therefore conclude, when only considering budburst and senescence, that the forest reproductive material from these seed sources show potential for future reforestation in the Netherlands, Belgium and northern Germany. Nevertheless, the small differences in budburst and senescence may be taken into account when selecting forest reproductive material for specific sites with higher risk of damage by late or early frost events.

Keywords: wild cherry, seed orchards, budburst, senescence, stability

## Introduction

Forest managers are diversifying forest stands with the aim of increasing resilience to climate change and upcoming pests and diseases (Bauhus et al. 2017). To achieve this, relatively rare native species like *Prunus avium* L. (Wild cherry) are increasingly implemented in temperate European forests due to its superior wood quality, fast growth and high litter quality (Turok et al. 1996, Wauer 2010, Ducci et al. 2013, De Avila et al. 2021). *P. avium* has a continuous natural range in Europe that extends from Spain to Georgia and from the South of Italy to central mid Norway. It also occurs naturally in North Africa and Western Asia (Welk et al. 2016). It is a light demanding relatively short-lived species that favours fertile slightly acidic soils that are well supplied with water (Russell 2003, Welk et al. 2016). Because of its high light demand it can be found more often in woodland edges. It is therefore seldom that *P. avium* occurs in continuous stands but rather as scattered individuals in between other deciduous species (Ducci et al. 2013). When established an individual can also maintain itself by resprouting or by using root suckers (Frascaria et al. 1993, Ducci et al. 2013).

For forestry purposes frequently local provenances have been recommended for planting with the idea that these populations are locally adapted (MCPFE 1993). With progressing climate change this assumption is being questioned and assisted migration of populations from warmer and dryer areas is seen as an alternative (De Avila et al. 2021). For several species, like common beech, it has been proven that southern or eastern provenances may grow faster compared to local provenances (Eilmann et al. 2014). This is most likely related to differences in spring and autumn phenology, where higher productivity can be linked with early flushing or late senescence, ensuring that more photosynthesis products are accumulated during a growing season. However, this higher

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productivity may come at a risk to be damaged by frost (Basler and Körner 2012, 2014, Vander Mijnsbrugge and Moreels 2020, Rosique-Esplugas et al. 2021). For species like *Fraxinus excelsior* more stem defects have been reported when early flushing provenances were planted (Rosique-Esplugas et al. 2021). Late spring frost in Northwestern Europe may occur till mid-May and can cause dieback of developing buds and leaves, where early flushing provenances are especially prone (Vitasse and Rebetez 2018, Zohner et al. 2020, Baumgarten et al. 2023). Furthermore, late leaf senescence carries the same trade-off between longer productivity and the risk of damage to buds and shoots if early frost leads to e.g. premature leaf fall (Estiarte and Peñuelas 2015).

Yet, for *P. avium* provenance differences in leaf phenology were, hardly studied, except for the study of differences in phenology between progenies from Sweden (Lobo et al. 2018). This is in contrast to flower phenology that has extensively been studied in cultivars of sweet cherry (Chmielewski et al. 2018, Kaya and Kose 2022). So far limited provenance research was conducted on *P. avium* with the aim to select productive provenances (Weißenbacher 2011, Ducci et al. 2013). Provenance research was hampered by the scarcity of genuine populations of *P. avium*, a consequence of the scattered distribution and by its predominantly clonal nature through root suckering (Frascaria et al. 1993, Ducci and Santi 1997). For this reason, research efforts have focused primarily on the selection of plus trees for the establishment of seed orchards, as well on the selection of individual clones and multi-clonal varieties (Ducci et al, 2013). Over the past decades, several seed orchards were established in multiple European countries (Franke et al. 1988, Kleinschmit et al. 2000, Ducci et al. 2013). However, since seed orchards are typically selected based on growth and form, limited information exists on leaf phenological traits—such as budburst and senescence—under varying environmental conditions. It is therefore pertinent to investigate whether registered seed orchard material can be recommended for afforestation across a broader geographical range than that for which it was originally developed.

In this study we use a common garden trial with sites in the Netherlands, Belgium and Germany to assess (i) differences and stability in budburst and (ii) differences in leaf senescence of in total fourteen seed orchards, three seed stands and one source-identified seed source originating from the Northwestern European part of the distribution range of *P. avium*. We expect that seed orchards containing plus trees from warmer regions (e.g. France) or more continental regions (eastern Germany) may exhibit earlier budburst and/or delayed senescence. However, given the synthetic nature of seed orchards —containing plus trees often selected from a larger geographic range — the environmental signal represented by a seed orchard may be much broader than of a single seed stand.

## Materials and methods

### *Trial sites*

In winter 2018/2019, we established a multi-site trial with *Prunus avium* L. in the Netherlands, Belgium and Germany with two sites per country. The Dutch sites had a single tree plot with block design with 17 seed sources, the Belgian sites were completely randomized single tree plots with 16 seed sources and the German sites had 9 seed sources in a completely randomized block design (appendix 1). All sites used a spacing of 2 m x 2 m between trees except Linciaux (BE) which has a spacing of 2.5 m x 2.5 m (appendix 1). Mean annual temperature ranged between 8.9 °C (Schafflund, DE) and 10.9 °C (Beersel, BE). Mean annual precipitation ranged between 681 mm (Hagenow, DE) and 960 mm (Linciaux, BE). Mean temperatures and precipitation were generated with the ClimateEU v5.00 software package (Mahony et al. 2022; Marchi et al. 2020) and were used to make Walter Lieth graphs in figure 1.

### *Seed sources*

In total, we used up to nineteen seed sources from five Western European countries (table 1 & figure 1). All seed sources are approved basic material, either seed orchards (category Qualified), seed stands (category Selected) or an ex situ seed source (category Source-identified). Seeds were collected in 2016 and 2017 and distributed between the countries. The seed lots were sown in plant beds at local nurseries. All trees were planted at 2 years old. Two entries were accidentally mixed during planting in the Belgium trials and therefore given a new number (504). Survival was generally high, and in the first two years dead trees were replaced. In Linciaux (BE) mortality was too high (18 %) to replace the trees with the original seed source. So another seed source 308 (DE) was used for replanting. The total overview of seed sources and the number of trees present at each site can be found in appendix 2.

Table 1: Overview of seed sources

| Code | Seed source name | Country | Code national register | Type of basic material (category of FRM) | Region of provenance | Origin of plus trees/ material |
|---|---|---|---|---|---|---|
| 101 | Helix | Belgium (BE) | 1VB8260.1 | Seed orchard (Q) | ten Noorden van Samber en Maas | North and west Belgium and North France |
| 102 | Mommedeel | Belgium (BE) | 1VB8260.2 | Seed orchard (Q) | ten Noorden van Samber en Maas | North and west Belgium and North France |
| 103 | Rattenberg | Belgium (BE) | 1VB6260.3 | Seed stand (S) | ten Noorden van Samber en Maas | South west Belgium and north of France |
| 104 | Bois d'Yves | Belgium (BE) | 7WB0254 | Seed stand (S) | Laag Maasplateau | East Belgium |
| 105 | Tournibus | Belgium (BE) | OWB0567 | Seed orchard (Q) | Belgium | unknown |
| 201 | Vaartbos-01 | Netherlands (NL) | NL.ZQ.8.3.02-01 | Seed orchard (Q) | Netherlands | Netherlands |
| 202 | Vaartbos-02 | Netherlands (NL) | NL.SI.8.3.02-02 | Seed source (SI) | Netherlands | East Netherlands |
| 301 | Gatersleben | Germany (DE) | 03 4 81404 002 3 | Seed orchard (Q) | West- und Süddeutsches Bergland sowie Alpen und Alpenvorland | South west Germany hilly area |
| 302 | Polle | Germany (DE) | 03 1 81404 002 3 | Seed orchard (Q) | West- und Süddeutsches Bergland sowie Alpen und Alpenvorland | North and west Germany |
| 303 | Fuhrberg | Germany (DE) | 03 1 81401 001 2 | Seed stand (S) | Norddeutsches Tiefland | unknown |
| 304 | Knechtsteden (Schnorrenberg) | Germany (DE) | 05 1 81401 001 3 | Seed orchard (Q) | Norddeutsches Tiefland | two forest areas Ville and Knechtsteden |
| 305 | Liebenburg | Germany (DE) | 03 4 81402 001 3 | Seed orchard (Q) | Mittel- und Ostdeutsches Tief- und Hügelland | unknown |
| 306 | Graupa | Germany (DE) | 14 1 81403 002 3 | Seed orchard (Q) | Südostdeutsches Hügel- und Bergland | Lowland and south east germany |
| 307 | Liliental I | Germany (DE) | 08 3 81404 001 3 | Seed orchard (Q) | West- und Süddeutsches Bergland sowie Alpen und Alpenvorland | West and south Germany |
| 308 | Neuhemsbach | Germany (DE) | 07 4 81404 001 3 | Seed orchard (Q) | West- und Süddeutsches Bergland sowie Alpen und Alpenvorland | unknown |
| 401 | Avessac-VG | France (FR) | PAV-VG-003 | Seed orchard (Q) | France (PAV901) | France |
| 501 | Southeast England | United Kingdom (UK) | pavOR2QU | Seed orchard (Q) | Southeast England (RoP30) | Southeast England |
| 502 | Southwest England and Wales | United Kingdom (UK) | pavOR5QU | Seed orchard (Q) | Southwest England and Wales (RoP40) | Southwest England and Wales |
| 504 | Mixed 501 and 502 | United Kingdom (UK) | pavOR2QU & pavOR-5QU | Seed orchard (Q) | - | - |

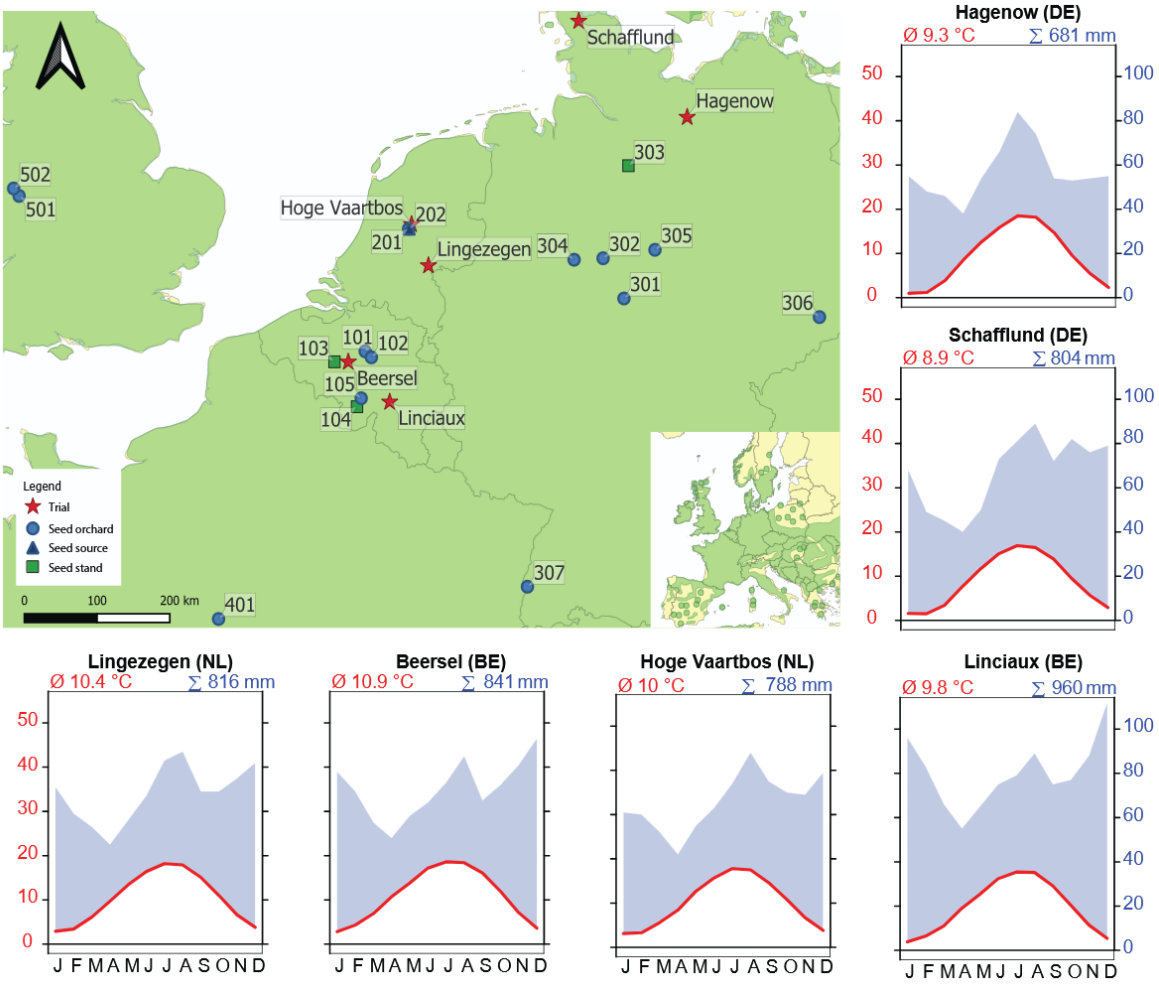


Figure 1: The location of trials (red stars), seed orchards (blue circles), seed stands (green squares) and seed source (dark blue triangle) along with their Walter-Lieth climate diagrams (1990-2019). Note that the coordinates of the seed orchards and the ex situ source identified seed source indicate the location of the seed orchard and not the origin of the plus trees. Seed orchard entries are synthetic populations formed from plus trees selected across broader regions. In green the geographical range of *P. avium* in Europe indicated as (green area), Caudullo et al. 2017).

## Observations and assessments

### *Budburst*

Budburst was scored in the years 2021, 2022 and 2023 at all sites using the same six stage protocol: 1 = dormant buds, scales brown and closed, 2 = buds expanding, scales still fully closed, 3 = scales start to separate, 4 = scales completely open, new leaves visible, 5 = leaves are flushing, 6 = leaves are fully expanded and new sprouts are in elongation (appendix 3). Only the apical bud was scored. If the apical bud was damaged or dead, this was recorded and the next highest bud was scored.

In 2021 the Belgian and Dutch sites were scored every week for eight or nine weeks. In 2022 the Dutch sites again were scored weekly for six or seven weeks. At the other sites and years budburst was scored once or twice when variation of budburst was highest (appendix 4).

### *Senescence and leaf fall*

In 2022 senescence was recorded at the German and Dutch sites using the following discolouring and leaf fall protocol: 1 = foliage still fully green, 2 = leaves begin to discolour, 3= beginning of leaf fall, 4 = 50 % of leaves discoloured or fallen, 5 = all leaves fallen. However, the protocol proved difficult to use, as the observed combination of leaf discolouring and fallen leaves did not always correspond with the predefined scoring categories. Therefore, at the Belgian sites it was decided to focus on leaf fall with a percentage based scoring system: 1 = 0 % shed leaves, 2 = between 1 – 25 %, 3 = 26 - 50 %, 4 = 51 – 75 %, 5 = 76 – 99 % and 6 = 100 % shed leaves. To be able to correlate the protocols, we used both protocols in 2023 at the Belgian and the Dutch sites (appendix 4). However, in 2023 we were not able to score senescence in Hoge Vaartbos (NL), since the leaves had fallen unexpectedly early. In Germany the same

protocol was used in 2022 and 2023. For an overview of what protocol was used in what site and year see appendix 4.

## Statistical analysis

First we excluded trees in the border rows, dead trees and trees with an unknown provenance. Entries 504 (UK) and 308 (DE) were only present in one site, Linciaux (BE). They were included in the spatial adjustment in SpATS to maximize the use of available information in estimation of the spatial trend in this site. Including all seed sources present in Linciaux (BE) improved the estimation of spatial heterogeneity in Linciaux (BE). However, as these two seed sources were present only in one site, they were excluded from further analysis comparing seed sources over multiple sites.

We selected a different subset of data based on the analysis and available data. A total overview of the number of seed sources, years and sites are included in each analysis can be found in appendix 4.

### *Spatial correction*

As different experimental designs were used in the trials, instead of a linear model such as ANOVA with block as a factor, R package SpATS (Rodríguez-Álvarez et al. 2018) was used to model spatial variation and adjust trait values for spatial noise. The model in SpATS was run using the PSANOVA algorithm. This is a semi-parametric linear mixed model, which includes seed source as a fixed effect, and smooth spatial surfaces as random effects over the row and column coordinates of the plots. SpATS models both fixed seed source effects and spatial trends simultaneously, and can be summarised as:

$$y=X\beta+X_s\beta_s+Z_s s+Z_u u+e \qquad (1)$$

where the vector $y$ contains the trait values per tree, $\beta$ is a vector of fixed terms including the intercept and seed source effects, and $X$ is the respective design matrix Fixed term $X_s\beta_s$ and random term $Z_s s$ together model the spatial variation as a smooth function of row and column coordinates. Finally, $Z_u u$ is a random component that accounts for discontinuous spatial variation. More details of the model are provided in the original publication (Rodríguez-Alvarez et al. 2018). Best Linear Unbiased Estimates (BLUEs) of the traits were obtained for each seed source, site and date corrected for spatial trends. These adjusted means were saved for further analysis.

### *Analysis of budburst across sites*

To test the effects of seed source and site on budburst, in a common moment in time, the following ANOVA model was fitted:

$$\text{Budburst}_{ij} = \text{Site}_i + \text{Seed source}_j + \text{error}_{ij} \qquad (2)$$

Where Budburst is the vector of adjusted seed source means per seed source and per site, on a similar date (between 21 and 23 April) in 2021. Site is a vector with six levels corresponding to each site, and Seed source is a vector with all 19 entries that were present in at least one of the six sites. Model (2) adjusts for Site effects using the common seed sources that are present in multiple sites and then seed source means adjusted for Site effects can be estimated from this model (2). Since the experiment was strongly unbalanced, it was not possible to test for interaction effects between Seed source and Site on the whole dataset. We conducted a formal interaction test on two balanced subsets of the data, with 9 seed sources present across all six sites and with 14 seed sources present across four sites, but seed source x site interaction was not significant (data not shown).

### *Stability of budburst*

In order to compare the stability among seed sources, we selected a single budburst observation moment per site and year. Whenever multiple budburst assessments were available for a given site-year combination, the assessment showing the most variation between entries was chosen. Since we wanted to compare variation between site-years, it was important to select entries present in all sites. Because only seed sources common at the sites could be included in the stability analysis, two datasets were used. First, a dataset comprising the 9 entries present at all six sites (18 site-year combinations) was analysed. Then, to include a larger number of entries, stability measures were calculated using data from the Belgium and Dutch sites only, which had 14 seed sources in common (12 site-year combinations). Stability measures were computed overall and separately for each year (2021–2023). We calculated the following classical stability measures, based on SpATS seed source means:

Finley Wilkinson Regression Coefficient (FW) (Finlay and Wilkinson 1963) is estimated as the β_i coefficient in the following linear model:

$$B_{ij} = \alpha_i + \beta_i \bar{B}_{.j} + \varepsilon_{ij} \qquad (3)$$

Where $B_{ij}$ is mean Budburst of seed source i in environment (Site-Year) j; $\bar{B}_{i.}$ is the mean Budburst in Environment j, over all seed sources. Coefficient $\beta_i$ is the estimated slope corresponding to seed source i. $\beta_i$ represents how a seed source's performance changes relative to the mean environment. In other words, FW compares the performance of each seed source with the mean performance of all seed sources per environment. A $\beta_i$ close to one indicates average stability. Values of $\beta_i$ <1 suggest that the seed source is less sensitive to environmental variation (i.e., it performs better than average in poor environments, but worse than average in good environments). Values of $\beta_i$ >1 indicate greater sensitivity of the seed source (it performs better than average in favorable environments but worse than average in poor environments).

*Wricke's Ecovalence* $W^2$ (Wricke 1962) quantifies the contribution of each seed source to the seed source-by-environment interaction. It is estimated as:

$$W^2 = \sum j \, (B_{ij} - B_{i.} - \bar{B}_{.j} + \bar{B}_{..})^2 \qquad (4)$$

where $\bar{B}_{i.}$ is the mean Budburst of Seed source i over all environments, and $\bar{B}_{..}$ is overall mean Budburst, over all environments and over all seed sources. $W^2$ quantifies how much a seed source deviates from what would be expected based on additive seed source and environment effects. Lower values of $W^2$ indicate lower seed source-by-environment interaction, and thus greater stability.

### *Analysis of budburst in a time series*

At four sites (two Dutch and two Belgian sites), we analysed the weekly development of budburst. For this the fourteen entries that were present in all four sites were selected. For each seed source the mean Julian date was calculated when stage 3 (BBCH growth stage 10) was reached. We chose stage 3, as this was the first stage that the leaves are exposed and likely vulnerable to damaging frosts. For this analysis, we used the adjusted means from SpATS.

### *Analysis of senescence*

Although the two protocols that were used differ methodologically, they are expected to be correlated. To verify this, we checked correlations in the year x site datasets for which both protocols were available. In 2023 both protocols were applied simultaneously in three sites: Lingezegen (NL), Beersel (BE) and Linciaux (BE). The seed source rankings of the two protocols showed a strong association with Spearman correlations r > 0.80 between protocols, justifying our approach to use rankings to combine measurements. The percentage protocol was selected at the Beersel (BE) and Linciaux (BE) sites in both 2022 and 2023, and at the Lingezegen (NL) site in 2023. The discolouring senescence protocol was selected at Hagenow and at Shafflund (DE) in 2022 and 2023, and at Hoge Vaartbos (NL) and at Lingezegen (NL) in 2022 (appendix 4). In order to compare senescence between all 19 seed sources, SpATS-derived means for each seed source were transformed into within-site-year rankings, ranging 1 to 19, where the lowest mean has a rank of 1, and the highest mean has a rank of 19. These rankings were used to test for differences between seed sources while accounting for repeated measures across years within sites. A linear mixed model was fitted, with seed source and year as fixed effects and site as a random effect:

$$Senescence_{ijk} = Seed\ source_i + Year_k + Site_j + error_{ijk} \qquad (5)$$

Where $Senescence_{ijk}$ is within Site-Year rankings of senescence, corresponding to Seed source i, Site j and Year k.

## Results

### *Spatial trends*

Spatial analysis using the SPATS model revealed substantial within-site heterogeneity across all six trial locations. For mean budburst (at 21-23 April 2021) the fitted spatial trends are shown in figure 2 a-f. At some sites, such as Hoge Vaartbos (NL) and Linciaux (BE), directional gradients were observed along one field axis, with higher fitted values along one side of the trial field and lower values at the opposite side. In contrast, sites such as Hagenow (DE) and Lingezegen (NL), exhibited more irregular and patchy spatial patterns. Following spatial correction, the distribution of genotypic BLUEs was more homogeneous than before correction. This confirmed that the SpATS model effectively accounted for environmental noise and improved the precision of the seed source mean estimates compared to blocking.

### *Budburst timing across all sites*

Differences in mean budburst, after spatial adjustment were significant for both Site effect (F= 397.49, p<0.000) and Seed source effect (F= 6.55, p<0.000). The Seed source expected marginal means adjusted for Site at a single point in time (21 – 23 April 2021) range between 4.11 and 4.84 (table 2). The Belgian seed stand 103 (BE) was flushing latest, while the German seed orchard 301 (DE) was the earliest. Most German seed sources (303, 305, 306, 307) were among the earliest to flush. On average budburst was late at Schafflund (DE) with an estimated marginal mean of 3.1 for the site and earliest at Beersel (BE) with an estimated marginal mean of 5.6.

### *Stability of budburst*

Regarding stability across environments including nine entries in common in all the six sites and three years, Finley-Wilkinson measures (FW) were between 0.85 and 1.07, indicating that all seed sources showed close to average budburst over environments. The seed orchards 101 (BE) and 102 (BE) had the lowest FW (< 1), an indication that these seed sources are less responsive. The ecovalence $W^2$ was low for most seed sources, except for 102 (BE) and 103 (BE). These seed sources showed the highest values, indicating lower stability. The seed orchards 304 (DE), 305 (DE) and 307 (DE) were the most stable since they have the lowest $W^2$. Overall, the $W^2$ values indicate that budburst is a rather stable trait for these 9 seed sources.

When calculating stability measures for 14 entries, for the four sites in the Netherlands and Belgium , FW and $W^2$ values again show similar results (table 3). Again, 103 (BE) shows the lowest stability and is most responsive to environmental change. Both seed sources 201 (NL) and 202 (NL) show high stability, together with the 101 (BE) as they have the lowest $W^2$. 401 (FR) has a moderate $W^2$ and FW close to zero, meaning it is reasonable stable. Also 104 (BE) and 105 (BE) show good overall stability across both measures.

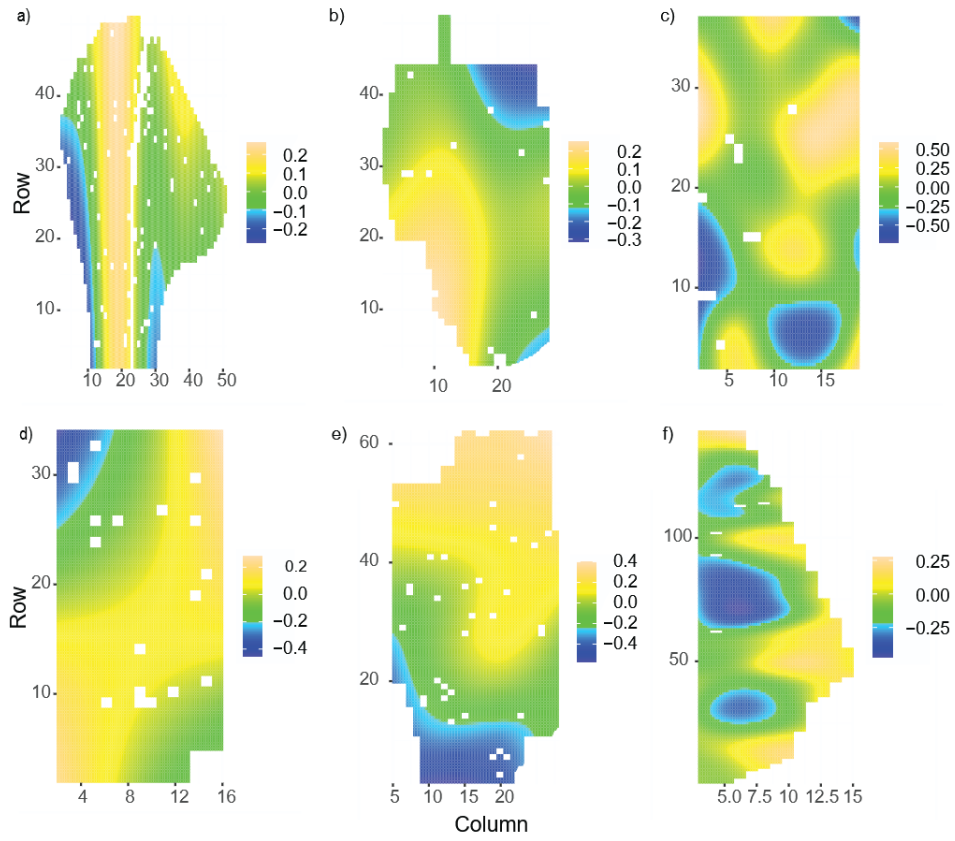


Figure 2: Fitted Spatial trends generated by SpATS for budburst data collected between 21 and 23 April 2021 at the six sites (a = Beersel (BE), b = Hagenow (DE), c = Hoge Vaartbos (NL), d = Linciaux (BE), e = Schafflund (DE), f= Lingezegen (NL))

Table 2: Estimated means per seed source, estimated over all sites. Emmean is the seed source estimated marginal mean of model (2) and lower.CL and upper.CL are the lower and upper 95 % confidence limits of the seed source mean).

| Seed source | Emmean | lower CL | Upper CL |
|---|---|---|---|
| 103 (BE) | 4.11 | 3.98 | 4.24 |
| 401 (FR) | 4.21 | 4.06 | 4.37 |
| 102 (BE) | 4.23 | 4.10 | 4.35 |
| 302 (DE) | 4.25 | 4.13 | 4.38 |
| 202 (NL) | 4.29 | 4.13 | 4.44 |
| 502 (UK) | 4.29 | 4.07 | 4.52 |
| 104 (BE) | 4.33 | 4.17 | 4.49 |
| 304 (DE) | 4.34 | 4.21 | 4.46 |
| 201 (NL) | 4.35 | 4.20 | 4.51 |
| 501 (UK) | 4.38 | 4.15 | 4.60 |
| 101 (BE) | 4.42 | 4.30 | 4.55 |
| 105 (BE) | 4.50 | 4.34 | 4.65 |
| 306 (DE) | 4.52 | 4.40 | 4.65 |
| 307 (DE) | 4.56 | 4.44 | 4.69 |
| 303 (DE) | 4.64 | 4.51 | 4.76 |
| 305 (DE) | 4.72 | 4.60 | 4.85 |
| 301 (DE) | 4.84 | 4.61 | 5.06 |

Table 3: Stability measures (Finley-Wilkinson regression coefficient b and Wricke's ecovalences) for 9 seed sources over six sites and three years and 14 seed sources over four sites and three years.

| Seed source | FW 6 sites | $W^2$ 6 sites | FW 4 sites | $W^2$ 4 sites |
|---|---|---|---|---|
| 101 (BE) | 0.92 | 0.50 | 1.01 | 0.09 |
| 102 (BE) | 0.85 | 1.13 | 0.77 | 0.95 |
| 103 (BE) | 1.01 | 1.49 | 1.15 | 1.29 |
| 104 (BE) | - | - | 0.88 | 0.47 |
| 105 (BE) | - | - | 0.89 | 0.30 |
| 201 (NL) | - | - | 1.02 | 0.15 |
| 202 (NL) | - | - | 1.02 | 0.13 |
| 302 (DE) | 1.03 | 0.67 | 0.94 | 0.24 |
| 303 (DE) | 1.01 | 0.48 | 1.00 | 0.35 |
| 304 (DE) | 1.07 | 0.29 | 1.06 | 0.18 |
| 305 (DE) | 1.04 | 0.21 | 1.06 | 0.26 |
| 306 (DE) | 1.06 | 0.46 | 1.06 | 0.20 |
| 307 (DE) | 1.02 | 0.37 | 1.00 | 0.29 |
| 401 (FR) | - | - | 1.13 | 0.39 |

### *Budburst dynamics over time*

For the analysis of budburst dynamics over time, we used adjusted means from SpATS, corresponding to observations, repeated over time, of 14 seed sources in common at the Belgian (2021) and Dutch sites (2021 and 2022). Adjusted budburst mean per seed source were plotted against Julian days, separately for each site and year as shown in figure 3. The results indicate that in 2021 budburst occurred later at the Dutch sites (Hoge Vaartbos and Lingezegen) than at the Belgian sites (Beersel and Linciaux). For instance around Julian Day 90, the mean budburst stage remained closer to stage 2 at the Dutch sites, whereas most seed sources at the Belgium sites had already reached stage 3 or beyond.

Table 4 summarizes the average number of days required for each seed source to reach a mean budburst stage of ≥3 across the six site-year combinations. Most seed sources reached this stage between Julian Day 93 and 101. 305 (DE) was the earliest, attaining a mean budburst of 3.3 after an average of 93 days (corresponding to 2$^{nd}$ of April). In contrast, 302 (DE) was the latest, reaching a comparable mean budburst stage (3.3) after 101 days on average (corresponding to 11$^{th}$ of April). Hence, the largest difference between the average time to show first visible leaves between seed sources was approximately eight days.

### *Senescence*

Seed source effect for senescence is significant (Chisq = 67.04, $p<0.000$), but we did not find a year effect (Chisq = 0.02, $p=0.88$). The means based on rankings per seed source (table 5) indicate that the highest-ranking seed sources, corresponding to earlier senescence or leaf fall were 501 (UK), 303 (DE) and 306 (DE), whereas the lowest-ranking seed sources corresponding to later senescence or leaf fall were 101 (BE), 302 (DE), 102 (BE) and 401 (FR). Adjusted SpATS means per seed source for each trial and year are summarized in appendix 5. Differences in senescence timing among seed sources was limited, with the difference between the earliest and latest senescing seed source never exceeding one point on the fifth and six-point scoring scale in all trials and years.

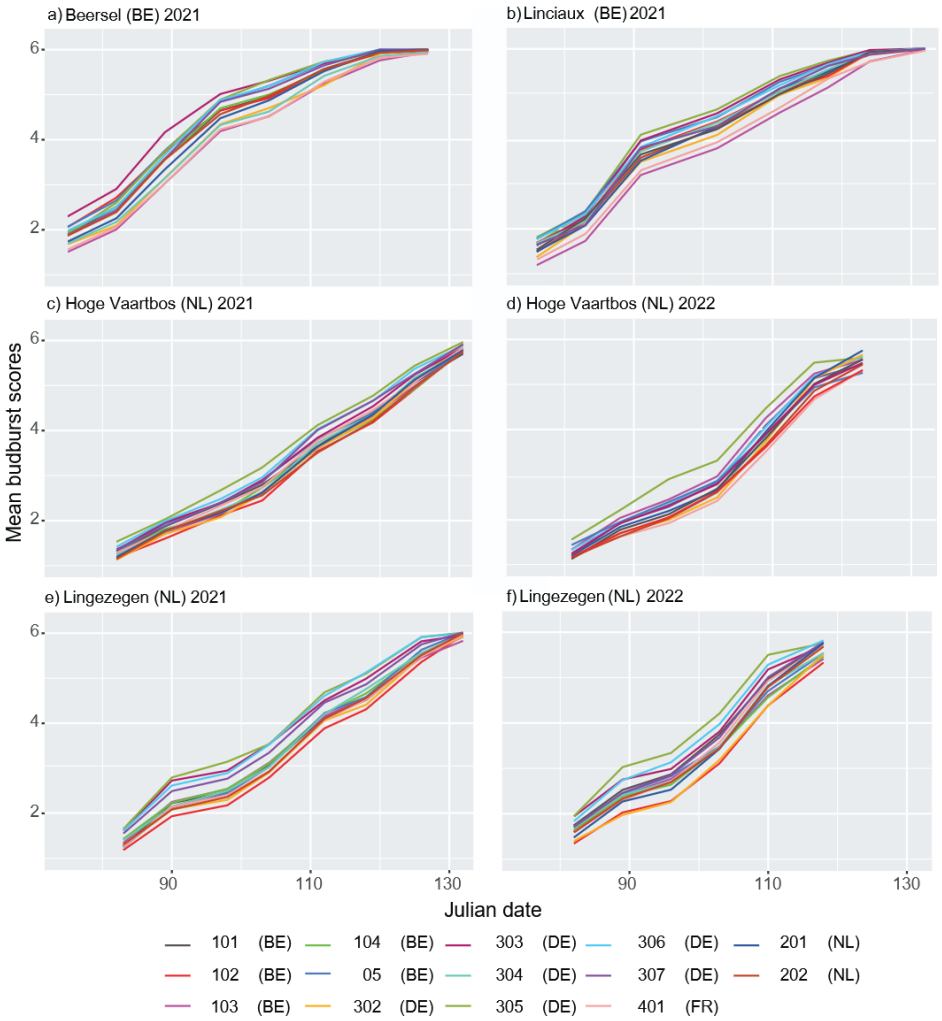


Figure: Adjusted mean budburst scores per seed source over time in the Dutch and Belgium sites. The x-axis is the time in Julian days. On the y-axis the mean budburst score from 1 (dormant buds) to 6 (leaves fully expanded) is given.

Table 4: Mean number of Julian days per seed source until reaching budburst stage ≥3, calculated over 2021 (Belgian and Dutch sites) and in 2022 (Dutch sites only) and its standard error.

| Seed source | mean budburst | mean julian date | se |
|---|---|---|---|
| 305 (DE) | 3.3 | 92.8 | 0.9 |
| 303 (DE) | 3.0 | 92.8 | 1.4 |
| 306 (DE) | 3.2 | 95.3 | 1.1 |
| 307 (DE) | 3.1 | 96.5 | 0.9 |
| 105 (BE) | 3.2 | 97.5 | 1.1 |
| 101 (BE) | 3.3 | 98.7 | 1.3 |
| 401 (FR) | 3.0 | 98.7 | 1.3 |
| 103 (BE) | 3.1 | 98.8 | 1.4 |
| 104 (BE) | 3.4 | 99.8 | 1.3 |
| 304 (DE) | 3.4 | 100.0 | 1.6 |
| 102 (BE) | 3.3 | 100.0 | 1.9 |
| 201 (NL) | 3.5 | 101.0 | 1.5 |
| 202 (NL) | 3.4 | 101.0 | 1.5 |
| 302 (DE) | 3.3 | 101.0 | 1.5 |

Table 5: Estimated marginal means of senescence rankings, over 6 sites and 2 years.

| Seed source | Emmean | lower CL | Upper CL |
|---|---|---|---|
| 101 (BE) | 2.3 | 1 | 4.9 |
| 302 (DE) | 5.4 | 2.7 | 8.0 |
| 102 (BE) | 5.5 | 2.8 | 8.1 |
| 401 (FR) | 5.5 | 2.3 | 8.6 |
| 103 (BE) | 6.0 | 3.4 | 8.6 |
| 105 (BE) | 6.0 | 2.9 | 9.2 |
| 201 (NL) | 6.7 | 3.6 | 9.9 |
| 104 (BE) | 7.2 | 4.0 | 10.3 |
| 301 (DE) | 7.2 | 2.6 | 11.7 |
| 502 (UK) | 7.5 | 2.9 | 12.1 |
| 305 (DE) | 8.0 | 5.4 | 10.6 |
| 307 (DE) | 8.1 | 5.5 | 10.7 |
| 202 (NL) | 9.0 | 5.9 | 12.2 |
| 304 (DE) | 9.3 | 6.7 | 11.9 |
| 306 (DE) | 10.2 | 7.6 | 12.8 |
| 303 (DE) | 11.0 | 8.4 | 13.6 |
| 501 (UK) | 13.8 | 9.2 | 17 |

## Discussion

### *Seed source effect on leaf phenology*

We found a small but significant seed source effect on budburst. Budburst observations from the Dutch and Belgian sites showed that the maximum difference among seed sources in reaching leaf-unfolding stage 3 did not exceed eight days. There was also no clear geographical trend between seed sources in budburst timing. This contrasts our expectation that more southern and eastern seed sources, for example seed orchards with plus trees collected in France or Eastern Germany would exhibit earlier budburst. For example, the French Avessac-VG seed orchard includes plus trees collected from across France (15 different departments) and represent high genetic diversity (Ricodeau et al. 2020). Similarly, plus trees of the UK seed orchards 501 and 502 were collected across the South-West of Great Brittain (Region of provenance 30) and the lowlands of England (RoP 40) respectively. German seed orchards likewise were collected from their respective region, namely: 'Norddeutsches Tiefland' in the North-west, the 'Mittel- und Ostdeutsches Tief- und Hügelland', 'Südostdeutsches Hügel- und Bergland' in the East and a more Southwestern Region of provenance of 'West- und Süddeutsches Bergland sowie Alpen und Alpenvorland'. Although, many German seed sources (301, 303, 305, 306, 307) were among the earliest to flush, other German seed sources from the same regions were late flushing and thus, attributing this pattern directly to region is difficult. Also the budburst of the UK seed orchards were similar to German seed sources and although 501 (southeast England) and 502 (South-west England and Wales) are both suitable across the majority of Great Britain, 501 is deemed more suitable for maritime conditions (Future tree trust 2022). Thus, the environmental variation encompassed by the seed sources in this study covers a substantial portion of the species' European range, yet evidence for earlier budburst in eastern compared with western seed sources is weak. Moreover, the most Southern French seed source did not flush earlier than the northern Belgian and Dutch seed sources.

Studies on leaf phenology in *P. avium* are limited and to the best of our knowledge this is the the first to document seed source effects on both budburst and senescence. Lobo et al. (2018) studied spring phenology in the northern part of the species' distribution and reported high within-genetic diversity but no clear differentiation among populations in budburst timing, nor any correlation between budburst and climate, latitude, or longitude of the original population sites. Although the study by Lobo et al (2018) covered a small (relative to ours) geographic area (Southern Sweden), their findings that populations differences were small, corresponds to our findings. We observed a small but significant seed source effect on senescence in *P. avium*. We expected seed sources from colder climates to senesce earlier southernmost seed orchard from warmer climates. However, no consistent pattern emerged across all entries, although the most Southern seed orchard, the French Avessac-VG (401), was among the latest to senesce in our ranking analysis.

Compared with other temperate deciduous trees, *P. avium* appears relatively uniform in both budburst timing and leaf senescence. A recent review by Zeng and Wolkovich (2024) found weak evidence of provenance effects in spring

phenology across Europe and North America, noting that spring events show much weaker clines than autumn events. Similarly, reviews by Alberto et al. (2013) and Aitken and Bemmels (2016) reported stronger genetic differentiation along clines for autumn than for spring phenology. Zeng and Wolkovich (2024) also documented important variation between species in spring phenology: angiosperms tended to budburst earlier in provenances that were from warmer and more southern regions, while gymnosperms often showed the opposite direction. Examples of angiosperm tree species in which populations from warmer environments flush earliest in common garden trials include common ash (Rosique-Esplugas et al. 2021), sessile oak (Girard et al. 2022) and common beech (Schueler and Liesebach 2015).

### *Site effect and stability*

We observed a large site-effect on budburst timing in our study. Timing of budburst is known to be controlled by multiple factors, mainly spring temperature (forcing) and temperature in winter (chilling) and to a minor extent day length (Schueler and Liesebach 2015). The mean annual temperature differs up to 2 °C between Schafflund (DE) and Beersel (BE). However, temperature contrasts are even more pronounced in spring. According to ClimateEU (Marchi et al. 2020) the mean temperature in April for Schafflund (DE) is 7.7 °C and in Beersel (BE) it is 10.7 °C. These climatic differences correspond well with our observations, as trees in Schafflund (DE) exhibited the latest budburst, whereas those in Beersel (BE) flushed the earliest.

The analysis of $W^2$ values enables the identification of seed sources that contribute most to genotype-by-environment interactions. The low $W^2$ values found for nearly all seed sources, except the Belgian 102 and 103, indicate that budburst is stable across sites and thus that there is no strong genotype x environment interaction (GxE) for budburst.

In general, genotype x environment interactions for growth traits tend to be small (e.g. Whittet et al. 2019. For phenological traits, only a limited number of studies have reported on provenance by site interactions. Examples include common ash, with significant genotype x environment interaction (Clark 2013), and pedunculate oak and black alder for which Baliuckas and Pliūra (2008) found significant genotype x environment effect for flushing. Rosique-Esplugas et al. (2021) however, reported non-significant genotype x environment effects.

Ma et al. (2019) and Chamberlain et al. (2021) have predicted the occurrence and severity of late frost events can also increase as winters are expected to become more mild and more variable. On the other hand, both studies also agree that increased frost damage risk is not universal for all species and provenances. When a predictable response is a priority, the Belgian seed sources 102 (BE) and 103 (BE) may be less suitable as their budburst is less stable across environments. It should also be noted that *P. avium* is a relatively frost-tolerant species (Baumgarten et al. 2023). Petrokas (2010) demonstrated that unfolding leaves, a developmental stage at which the species is expected to be most sensitive to frost, can withstand temperatures as low as -8 °C. Whether the earlier flushing seed sources are therefore more at risk of frost damage should be further studied.

## Conclusion

Stability of phenology can serve as an important criterion for selecting seed sources. Based on the high stability of budburst in our study, we conclude - when only considering budburst as trait - that forest reproductive material from the studied seed sources, show potential for future (re)forestation in the Netherlands, Belgium and northern Germany. The small differences in budburst and stability of budburst may be considered when selecting seed sources for sites where frost damage in spring is to be expected. For such sites late flushing seed sources as 201 (NL), 101 (BE), 304 (DE), and 302 (DE) or reasonable stable (e.g. 401, FR) might be advantageous. Where late frost risk is not considered as important, seed sources such as 305 (DE), 303 (DE), 306 (DE) and 307 (DE), characterized by early and stable budburst might benefit from longer growing seasons to enhance productivity. Although these same seed sources were also among the earliest to senesce, which possibly negates the effect on growing season length.

In order to provide deployment recommendations for the studied seed sources, traits like survival, productivity and stem form need to be taken into account.

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# Appendices

## Appendix 1: Trial details

| Trial name | Country | Latitude | Longitude | Altitude (m.a.s.l.) | Soil type | Design | Spacing (m) |
|---|---|---|---|---|---|---|---|
| Hoge Vaartbos | Netherlands (NL) | 52.39 N | 5.54 E | 1 | Light clay | Completely Randomized Block | 2 x 2 |
| Lingezegen | Netherlands (NL) | 51.90 N | 5.88 E | 9 | Clayey sand | Completely Randomized Block | 2 x 2 |
| Hagenow | Germany (DE) | 53.52 N | 11.28 E | 55 | sandy loam | Randomized Block | 2 x 2 |
| Schafflund | Germany (DE) | 54.75 N | 9.13 E | 9 | loamy sand | Randomized Block | 2 x 2 |
| Beersel | Belgium (BE) | 50.75 N | 4.30 E | 103 | - | Completely Randomized | 2 x 2 |
| Linciaux | Belgium (BE) | 50.28 N | 5.11 E | 279 | - | Completely Randomized | 2.5 x 2.5 |

## Appendix 2: Seed sources and number of trees in present in each of the sites

| Code | Seed source name | Country | Beersel (BE) | Linciaux (BE) | Hagenow (DE) | Schafflund (DE) | Hogevaart (NL) | Lingezegen (NL) |
|---|---|---|---|---|---|---|---|---|
| 101 | Helix | Belgium (BE) | 242 | 62 | 72 | 54 | 77 | 80 |
| 102 | Mommedeel | Belgium (BE) | 157 | 51 | 72 | 54 | 78 | 77 |
| 103 | Rattenberg | Belgium (BE) | 258 | 61 | 71 | 53 | 80 | 77 |
| 104 | Bois d'Yves | Belgium (BE) | 169 | 52 | - | - | 78 | 79 |
| 105 | Tournibus | Belgium (BE) | 186 | 53 | - | - | 77 | 79 |
| 201 | Vaartbos-01 | Netherlands (NL) | 75 | 52 | - | - | 80 | 82 |
| 202 | Vaartbos-02 | Netherlands (NL) | 90 | 52 | - | - | 79 | 78 |
| 301 | Gatersleben | Germany (DE) | - | - | - | - | 22 | 25 |
| 302 | Polle | Germany (DE) | 28 | 27 | 71 | 54 | 80 | 82 |
| 303 | Fuhrberg | Germany (DE) | 15 | 49 | 72 | 54 | 80 | 82 |
| 304 | Knechtsteden (Schnorrenberg) | Germany (DE) | 35 | 57 | 72 | 54 | 66 | 63 |
| 305 | Liebenburg | Germany (DE) | 12 | 55 | 70 | 54 | 34 | 34 |
| 306 | Graupa | Germany (DE) | 45 | 48 | 69 | 53 | 79 | 79 |
| 307 | Liliental I | Germany (DE) | 42 | 33 | 71 | 52 | 80 | 82 |
| 308 | Neuhemsbach | Germany (DE) | - | 181 | - | - | - | - |
| 401 | Avessac-VG | France (FR) | 126 | 56 | - | - | 80 | 79 |
| 501 | Southeast England | United Kingdom (UK) | - | - | - | - | 78 | 78 |
| 502 | Southwest England and Wales | United Kingdom (UK) | - | - | - | - | 79 | 77 |
| 504 | Mixed 501 and 502 | United Kingdom (UK) | 2 | 54 | - | - | - | - |

Appendix 3: Visual illustration of the categories that were used for scoring budburst


Photos and scoring system developed by Bart De Cuyper.

Appendix 4: Number of seed sources, sites and years used per analysis

| | Beersel (BE) | | | Linciaux (BE) | | | Hagenow (DE) | | | Schafflund (DE) | | | Hoge Vaartbos (NL) | | | Lingezegen (NL) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| analysis | 21 | 22 | 23 | 21 | 22 | 23 | 21 | 22 | 23 | 21 | 22 | 23 | 21 | 22 | 23 | 21 | 22 | 23 |
| Budburst between sites, 2021 | 15 | | | 16 | | | 9 | | | 9 | | | 17 | | | 17 | | |
| Budburst in a time series | 14 | | | 14 | | | | | | | | | 14 | 14 | | 14 | 14 | |
| Budburst stability six sites | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Budburst stability four sites | 14 | 14 | 14 | 14 | 14 | 14 | | | | | | | 14 | 14 | 14 | 14 | 14 | 14 |
| Senescence ranking between sites and years | | 15* | 15* | | 16* | 16* | | 9** | 9** | | | 9** | | 17* | | | 17** | 17* |

* % of leaffall protocol

** discolouring and leaffall protocol

## Appendix 5: Adjusted SpATS means of senescence per seed source, trial and year

| Seed source | Beersel (BE) 2022 | Beersel (BE) 2023 | BE Linciaux (BE) 2022 | BE Linciaux (BE) 2023 | Lingezegen (NL) 2022 | Lingezegen (NL) 2023 | Hoge Vaartbos (NL) 2022 | Hagenow (DE) 2022 | Hagenow (DE) 2023 | Schafflund (DE) 2022 | Schafflund (DE) 2023 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 101 (BE) | 3,3 | 4,2 | 2,8 | 5,5 | 2,2 | 2,2 | 2,8 | 2,7 | 3,7 | 4,0 | 3,2 |
| 102 (BE) | 3,4 | 4,5 | 3,0 | 5,6 | 2,5 | 2,5 | 3,0 | 2,8 | 3,9 | 4,0 | 3,7 |
| 103 (BE) | 3,1 | 4,3 | 3,6 | 5,7 | 2,6 | 2,6 | 3,1 | 2,8 | 3,6 | 3,8 | 3,5 |
| 104 (BE) | 3,3 | 4,4 | 3,2 | 5,6 | 2,6 | 2,6 | 3,3 | - | - | - | - |
| 105 (BE) | 3,4 | 4,3 | 3,2 | 5,6 | 2,4 | 2,6 | 3,3 | - | - | - | - |
| 201 (NL) | 3,2 | 4,4 | 3,2 | 5,8 | 2,5 | 2,5 | 3,3 | - | - | - | - |
| 202 (NL) | 3,5 | 4,4 | 3,1 | 5,7 | 2,6 | 2,6 | 3,4 | - | - | - | - |
| 301 (DE) | - | - | - | - | 2,5 | 2,5 | 3,5 | - | - | - | - |
| 302 (DE) | 3,7 | 4,3 | 3,0 | 5,7 | 2,5 | 2,4 | 3,2 | 2,9 | 3,9 | 3,7 | 3,3 |
| 303 (DE) | 3,3 | 4,4 | 3,3 | 5,8 | 3,0 | 2,8 | 3,4 | 2,9 | 4,1 | 4,1 | 3,9 |
| 304 (DE) | 3,5 | 4,6 | 3,0 | 5,7 | 2,9 | 2,8 | 3,3 | 2,7 | 4,1 | 4,6 | 4,0 |
| 305 (DE) | 3,2 | 4,4 | 3,6 | 5,9 | 2,7 | 2,4 | 3,3 | 3,1 | 3,7 | 4,2 | 3,6 |
| 306 (DE) | 3,7 | 4,6 | 3,3 | 5,9 | 2,9 | 2,7 | 3,2 | 2,7 | 3,8 | 3,8 | 3,7 |
| 307 (DE) | 3,5 | 4,5 | 2,9 | 5,8 | 2,8 | 2,7 | 3,1 | 2,7 | 3,7 | 4,1 | 4,1 |
| 401 (FR) | 2,9 | 3,7 | 2,8 | 5,6 | 2,6 | 2,7 | 3,4 | - | - | - | - |
| 501 (UK) | - | - | - | - | 2,8 | 2,9 | 3,4 | - | - | - | - |
| 502 (UK) | - | - | - | - | 2,5 | 2,5 | 3,4 | - | - | - | - |