RESEARCH PAPER

Spring temperatures affect senescence and N uptake in autumn and N storage for winter in *Rhynchospora alba* (Cyperaceae)

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Abstract

Environmental and physiological factors underlying variation in timing of autumn senescence are not well known. We investigated how the time of the onset of the growth in spring affects senescence and its functional consequences for nitrogen (N) uptake in autumn and storage of N for the winter, in a species that each year develops its bulbils for storage and overwintering anew. *Rhynchospora alba* was grown outdoors with two treatments, identical except for a 3 week difference in the start of growth in May. Leaf and root growth and senescence, and N uptake were recorded from August to November. By August, late-starting plants had caught up in size and total N content, but had smaller bulbils. They had a higher δ
tsub{13}C, indicating a higher stomatal conductance during growth. Leaf and root senescence were delayed, extending 15N tracer uptake by 4 weeks. Nevertheless, after senescence, plants with an early start had 55% more N in their overwintering bulbils, due to earlier and more efficient remobilization. We conclude that timing of senescence in *R. alba* is a result of an interplay between the status of winter storage and cold temperatures, constrained by a trade-off between prolonged nutrient uptake and efficient remobilization of nutrients.

Keywords: Autumn, 15N enrichment, growing season length, leaf senescence, nutrient remobilization, nutrient storage, nutrient uptake, root senescence, spring temperature, wetland.

Introduction

Growing season length is an important factor in determining ecosystem productivity (Körner, 2006), and longer growing seasons are associated with increased gross primary production (Piao et al., 2007; Baptist and Choler, 2008). Annual variation in growing season length results from variation in the timing of budburst in spring and leaf senescence in autumn in response to variation in environmental conditions (Suni et al., 2003; Dunn et al., 2007). Variation in spring phenology is closely associated with variation in temperature, but factors underlying variation in autumn phenology are less investigated and more complex (Menzel et al., 2006; Gallinat et al., 2015; Delpierre et al., 2016). Warm spring temperatures usually result in an early onset of growth, but may also affect autumn senescence by either advancing or delaying it (Fu et al., 2018). Reasons for this ambiguity of autumn phenology are interactions with other environmental conditions such as drought (Fu et al., 2014; Liu et al., 2016), latitudinal variation (Estiarte and Penuelas, 2015), and interspecific differences (Körner and Basler, 2010; Tateno and Takeda, 2018). Some species, called periodic species (Sørensen, 1941), have a genetically determined fixed growing period regardless of environmental conditions, while others delay their senescence if growing conditions remain favorable for a longer time in the autumn (Wookey et al., 2009). For example, the arctic herbaceous species *Carex bigelowii* and
Rhiocneorpe vaginatum delay their senescence in response to warmer temperatures (Natali et al., 2012), while another arctic herb, Bistorta officinalis (formerly Polygonum bistorta), shows an advanced senescence in response to a warmer spring (Starr et al., 2000). The alpine sedge Carex foetida initially increased its productivity after a delayed spring, but showed no change in the time of senescence compared with plants with an early spring, resulting in an unchanged total productivity (Baptist et al., 2010).

Autumn senescence in a climate with harsh winters is an adaptation to pre-empt frost damage and avoid resource loss (Schippers et al., 2015). Late in the growing season, overwintering organs acquire hardiness to be able to survive the winter, and the plant remodelizes nutrients from the organs to be shed to storage organs (Keskitalo et al., 2005). Timing of these phenological processes is a trade-off between maximizing productivity by delaying senescence, and minimizing the risk of frost damage and nutrient losses by not delaying it for too long (Howe et al., 2000; Wipf et al., 2009). The resulting balance of acquired resources may be crucial for growth and survival in the following year in environments with short growing seasons and harsh winters (Weih and Karlsson, 1999). The importance of remobilization of nutrients from senescing organs in autumn for a plant’s nutrient economy has been mostly investigated for annual crop species and trees, while fewer data are available for herbaceous species of natural environments such as wetlands (Côté et al., 2002; Maillard et al., 2015). Although great progress has been made in understanding cues initiating autumn senescence, the exact mechanisms controlling the precise timing remain unknown (Havé et al., 2017; Michelson et al., 2018; Woo et al., 2018).

In the present study, we investigate how a delay in the onset of the growing season (i.e. with identical conditions except for timing of the onset of growth-enabling temperatures) affects the amount of resources a plant can store for the following winter. The focus will be on the role of nutrient uptake late in the season, and the timing of senescence. The study species is Rhynchospora alba, a small wetland graminoid characteristic of nutrient-poor wet environments in the Northern Hemisphere, often occurring on sites with few other vascular plant species (Ohlson and Malmer, 1990; Fontaine et al., 2007). The species is well suited to study nutrient conservation from one year to another because it has small compact bulbils as the only overwintering vegetative organs, and it is adapted to nutrient-poor habitats (Ohlson and Malmer, 1990). The bulbils are produced each year anew. In contrast to many other wetland species, roots of R. alba live only for one growing season, senescing in autumn (Nieman et al., 2018). Nutrient accumulation, allocation, and retention in R. alba have been thoroughly studied by Ohlson and Malmer (1990), who found that this species shows a considerable annual nutrient turnover for a species of such nutrient-poor environments. Based on its early senescence as observed by Ohlson and Malmer (1990), we assume that R. alba is a periodic species; that is, it has an inherently determined growing season length (Starr et al., 2000). The question arises of how variation in length of the climatic growing season affects this strategy. Starr et al. (2000) found hardly any physiological changes in the periodic species Bistorta officinalis in response to an earlier spring, except for a shift in phenology. However, we hypothesize that this may change if the season is shorter than the plant’s inherent cycle.

Most phenological studies on nutrient uptake focus on events in spring, as seasonal fluctuations in microbial activity lead to a flush of available nutrients at snowmelt (Brooks et al., 1998; Lipson et al., 1999; Edwards and Jeffries, 2010), and competition for nutrients by microbes is high in autumn (Jaeger et al., 1999). Nevertheless, nitrogen (N) uptake by plants late in the autumn can be substantial despite an even larger uptake by microbes (Grogan and Jonasson, 2003). Consequently, the timing of autumn senescence potentially influences the amount of nutrients stored for the winter in R. alba, as this species lacks any long-lived organs. Its sole nutrient reserves to start a new growing season are bulbils that have been produced the previous year. We hypothesize that a delay in the beginning of that previous growing season will reduce the amount of nutrients the plant can acquire in its nutrient-poor environment, potentially affecting the species’ performance. Our goal is to understand how variation in onset of the growing season contributes to nutrient balance of the species by the end of the season. This is of importance in the context of potential consequences with respect to year to year variation in temperatures, the warming climate, and the northern limits of distribution of the species.

Parallel to N uptake by R. alba in late summer and autumn, we follow the progress of leaf and root senescence, during which nutrient remobilization occurs (Hörtensteiner and Feller, 2002). Recent studies have shown that root production phenology is not synchronous with shoot production, lasting longer in the autumn compared with shoot production (Steinaker et al., 2010; Abramoff and Finzi, 2015). Our data will show for R. alba how root senescence and root function (i.e. nutrient uptake) relate to above-ground senescence in the autumn. Content of 15N will give information on stomatal conductance during growth.

Materials and methods

Experimental design

The study was conducted with Rhynchospora alba (L.) Vahl (Cyperaceae), a small perennial wetland graminoid of nutrient-poor wetlands in northern temperate and southern boreal regions around the Northern Hemisphere (Hultén and Fries, 1986), ranging in eastern North America from Georgia to Hudson Bay (Flora of North America Editorial Committee, 1993; Riley, 2003). As overwintering organs, this species forms winter buds as bulbils from a meristem located behind the basal sheaths of its leaves (Ohlson and Malmer, 1990). The plants used in the experiment originated from a floating fen 50 km northwest of Sudbury, and had been propagated in 10 litre pots on horticultural peat for 5 years. Growing season length in the region is on average 125–145 d, defined as the period between the last and first frost (http://www.omafra.gov.on.ca/english/crops/facts/climzoneveg.htm).

The experiment was conducted in an outdoor garden over 6 months in Sudbury, Ontario, Canada (46°36’N, 81°06’W). Bulbils of R. alba were collected on 19 April 2017 from pots in the garden where they had been overwintering at 0–2 °C under a cover of straw and snow. This temperature is comparable with the winter conditions the plants experience in snow-covered local wetlands (see Supplementary Fig. S1 at JXB online). After collection, the bulbils were stored in a refrigerator at 2–6 °C until 6 May, conditions that were similar to the overwintering conditions with
respect to temperature and light (Supplementary Fig. S1). The bulbils were stored in a refrigerator to prevent start of growth before the experimental treatments in case of warm temperatures outdoors in late April. The two treatments with different temperatures were started on 6 May to simulate different times of beginning of a growing season. On that day, plants in the warm May treatment were planted outdoors in 2 litre pots, while plants in the cold May treatment remained in the refrigerator for a further 3 weeks until they were planted outdoors. There were 75 bulbils in each treatment.

During the 3 weeks of treatment, the warm May plants were placed under a cold frame covered with a sheet of clear plastic to simulate an early spring. Air and substratum temperatures during this treatment were on average 18.1 °C and 16.4 °C, compared with 10.4 °C and 11.2 °C in the garden outside the cold frame. The warm May treatment temperatures were comparable with air and substratum temperatures to which all plants were exposed after the treatment, the averages of the first 3 weeks being 17.4 °C and 18.6 °C, respectively (Supplementary Fig. S1). Hence, the change in average temperatures from overwintering to growing season was similar for plants in both treatments, despite the difference of 3 weeks in time.

In the cold May treatment simulating a late spring, the bulbils remained in the refrigerator at 2–3 °C for a further 3 weeks compared with warm May treatment, planted into two 750 ml containers with the basin end of the bulbil stuck in wet substrate, in the same manner and the same substrate as bulbils potted in the warm May treatment. Previous observations have shown that R. alba bulbils do not grow under these temperatures, and we did not expect any root growth during the treatment. On 27 May 2017, these bulbils were planted in pots in the garden, in a similar manner to the warm May bulbils 3 weeks earlier. The cold May treatment had 57 °Cd growing degree days (GDD) with 0 °C base over the 21 d of treatment, while the warm May treatment had 359 °Cd over the same period (Fig. 1A). At the time of planting, the cold May bulbils in the pots, the warm May plants had already developed 2–3 leaves with an average length of ~12 cm (measured for 17 plants). At planting, the bulbils of cold May treatment did not show any signs of shoot or root growth. In summary, plants in the cold May treatment experienced until late May conditions that were similar to those to which they had been exposed during the winter, after which they were exposed to early summer temperatures. Plants in warm May treatment had this change in exposure 3 weeks earlier. The 3 week difference in time is within the observed natural year to year variation in the study area. For example, the last day of snow on the ground at Sudbury Airport in 2012 was 15 March, while in 2002 it was 13 April. In that year, the maximum air temperature the day after the last trace of snow had disappeared was 27.1 °C (http://climate.weather.gc.ca/historical_data/search_historic_data_e.html). Hence, a sudden change from close to zero temperatures to summer temperatures >20 °C can occur naturally at snowmelt for this species that overwinters as bulbils on wet peat under the snow.

After 27 May, all plants were grown in 2 litre CPVC pots in two uncovered pools, 25 cm deep, one plant per pot, with the water level just below the surface of the substrate. The plants of the two treatments were distributed equally across the two pools in an alternating pattern. Plants for each harvest and tracer addition were pre-selected to be distributed in the different parts of the two pools. The growth substrate was horticultural peat (pH 4.0), with 0.125% composted manure added in the top 5 cm to provide some nutrients (Fafard et Frères, Saint-Bonaventure, Québec, Canada). Nutrient availability in the growth substrate was measured using Plant Root Simulator (PRS) (Western Ag Innovations, Saskatoon, Canada) incubated for 4 weeks in July in four pots with no plants (Qian and Schoenau, 2002). Availability for NH₄-N, P, and K were 46, 58, and 1.5 μg 10 cm⁻² per 4 weeks, respectively. NO₃⁻N was below the detection limit. These values are comparable with values found in nutrient-poor wetlands in Canada, with the NH₄-N being relatively high (Wang et al., 2018). To weigh the peat-filled pots down in the pools, the base of each pot was filled with 0.25 litres of 2–3 cm quartz rocks. During the 3 weeks delay in planting the bulbils in the cold May treatment, pots of this treatment were kept underneath a sheet of clear plastic, in order to minimize differences in temperature and precipitation experienced by the substrate in pots for the two treatments.

Temperatures were measured throughout the experiment with iButton® DS1921G-F5# data loggers (Maxim Integrated, San Jose, CA, USA). Substrate temperature was measured in two pots with no plants, and air temperature was measured just above these pots. After the 3 weeks of different temperature treatments, air temperature varied mostly between 15 °C and 30 °C until the end of September, with the exception of ~2 weeks of slightly cooler temperatures in early September (Fig. 1A). Starting at the end of September, night temperatures frequently fell below 5 °C and, after 25 October, daytime temperatures did not rise much above that. Substrate temperatures showed a similar pattern, but with smaller diurnal fluctuations (Fig. 1B).

Tracer additions and measurement of plant traits and senescence

Plant growth and senescence were assessed with mostly biweekly destructive harvests from late summer before any visible signs of above-ground senescence until senescence was completed. Dates of the harvests were 5 and 25 August, 8 and 22 September, and 5 and 20 October. Harvest times
were chosen based on previous observations of the species to cover the entire senescing period. Three replicate plants were harvested on each occasion. The measured growth-related traits were dry masses of plant parts including senesced material (after drying for at least 24 h at 75 °C), the numbers of leaves, capitula in the inflorescence, and overwintering bulbils, and the total root length. Root length was measured using the grid-intersection method (Newman, 1966; Tennant, 1975). As variables to describe the progress of above- and below-ground senescence, the ratio of the number of dead leaves to total number of leaves, and the ratio of dead root length to total root length were calculated. Root vitality was evaluated by staining with a solution of 0.3% 2,3,5-triphenyltetrazolium chloride (TTC) and 10 mM glucose for at least 24 h at 30 °C; living roots turn red after reduction of TTC to formazan (Comas et al., 2000; Ryser and Kamminga, 2009). A random representative sample was collected of each root system, and infiltrated under near vacuum (7 kPa) to facilitate the penetration of the reagents into the roots (Ruf and Brunner, 2003). The percentage of living and dead root length was assessed from this sample by determining under a dissecting microscope the presence of red stain in at least 100 random root sections per sample.

At the time of each harvest, a solution containing the stable isotope \(^{15}\text{N}\) was added as a tracer to six other pots in each treatment to assess the importance of nutrient uptake at different times during the autumn for the conserved nutrient pool in overwintering organs. The tracer was added as 10 ml of a 0.5 mM solution of \(^{15}\text{NH}_4^{+}/^{15}\text{NO}_3^-\) (Sigma-Aldrich, St Louis, MO, USA; Ref. 366528), resulting in 10 µmol \(^{15}\text{N}\) per plant; half as ammonium and half as nitrate. To facilitate the penetration of the tracer into the rooting zone, the waterlogged pots were taken out of the pools to allow excess water to percolate for 5 min, after which the tracer was added. After another 5 min, 10 ml of water was added to push the tracer further down. As the rim of the pots was above the water surface, the tracer remained in the pots even when they were placed back in the pools. The amount of nitrogen added as tracer was considered to be insignificant for plant growth. After tracer addition, the plants were moved to an adjacent pool to avoid cross-contamination of the tracer to plants not having received it yet. Additionally, to investigate tracer uptake and allocation after the application, on 5 August tracer was added to four and five replicate plants in the warm and cold May treatments, respectively, to be harvested and analysed for \(^{15}\text{N}\) and total N 2 weeks later on 19 August. In this harvest, the above-mentioned growth- and senescence-related variables were measured as well, with the exception of root length and root mortality, and included in the data set of trait variation over time. Five plants in each temperature treatment were left without tracer addition as controls with a natural \(^{15}\text{N}\) content of the bulbils in November.

**Nutrient uptake and conservation**

On 3 November 2017, bulbils of the fully senesced plants were harvested. The bulbils were dried at 75 °C for 72 h, weighed, ground using a ball mill (Retsch GmbH, Haan, Germany), and analysed for concentration of total N and isotopic composition of \(^{15}\text{N}\) and \(^{13}\text{C}\) at the Stable Isotopes in Nature laboratory at the University of New Brunswick, Fredericton, NB, Canada (SINLAB). In addition to the bulbils harvested in November, all plant parts harvested on 19 August were analysed to assess uptake and allocation of N 2 weeks after the first addition of tracer.

**Statistical analyses**

Statistical analyses were conducted using SYSTAT 12.1 (Systat Software, San Jose, CA, USA). Data were tested for normality using a one-sample Kolmogorov–Smirnov test. To attain normality, data on bulbil dry mass were log-transformed, and data on leaf and root senescence arcsine-transformed. The growth- and senescence-related data were analysed using an ANOVA, with treatment and harvest as factors. Data of the special harvest on 19 August were included when applicable. For analysis of bulbil dry mass, data of the final harvest on 3 November were included as well, using the control plant data. Potential late-summer growth during 5 weeks after the first harvest was tested using a stepwise general linear model for data collected between 5 August and 8 September, with date of harvest as a continuous independent variable and treatment as an independent factor. For δ\(^{15}\text{N}\) in bulbils at the end of the growing season, a two-way ANOVA was conducted, with treatment and the time of tracer addition as independent factors. To test for temporal changes in tracer uptake within each treatment, differences in δ\(^{15}\text{N}\) among all times of addition and control plants were tested with Tukey’s HSD test after separate one-way ANOVAs for each treatment. Treatment effect on other variables of bulbils in November, and on variables measured in all organs at the harvest of 19 August were tested with one-way ANOVAs.

**Results**

**Growth and senescence**

An earlier start of the growing season resulted in a larger dry mass of bulbils during the entire harvesting period after 5 August, but plant dry mass excluding the bulbils did not significantly respond to the treatment during that time (Fig. 2A, B; Table 1). Over the period of 5 August to 8 September, total bulbil dry mass per plant increased significantly in both treatments, while there was no significant increase in plant dry mass excluding the bulbils (Fig. 2A, B; Table 2). The number of bulbils per plant was larger for warm May plants (Fig. 2C; Table 1). The numbers of bulbils for cold May plants increased until 8 September, while warm May plants did not produce new bulbils after the first harvest on 5 August, resulting in a significant treatment×date interaction (Fig. 2C, Table 2).

The number of inflorescence capitula per plant was slightly higher in the cold May treatment (Fig. 2D; Table 1). The general decrease of numbers of capitula in October was a result of them falling off after seed ripening. There was no significant treatment effect on the total number of leaves or on the total root length (Fig. 2E, F; Table 1). There was some indication that the numbers of capitula and leaves still may have increased after the first harvest (P<0.10; Table 2).

On 5 August, practically no external sign of senescence could be observed, but thereafter some leaves and roots started to die (Fig. 2G, H). While leaf senescence exceeded 10% already by 19 August, root senescence exceeded this value only 1 month later, on 22 September. For both the percentages of dead leaves and dead root length, there was a significant treatment×harvest interaction (Table 1). This was a result of the earlier senescence for warm May treatment plants (Fig. 2G, H). The main effect of the treatment was significant only for leaf senescence (Table 1).

**Plant size, and nitrogen content and uptake in late summer**

On 19 August, bulbil dry mass was significantly higher for warm May plants than for cold May plants, resulting in a higher total bulbil N content (Fig. 3A, B; Table 3). The bulbils contained 2.2 mg and 7.0 mg N in the cold and warm May treatments, respectively. There was no treatment effect on the dry mass or on N content of other plant parts, the total plant N content being 26.3 mg on average for both treatments. Average N concentration in roots, shoots, and bulbils was
higher for the cold May plants than for the warm May plants, but for bulbils the difference was not significant. Capitulum N concentration was significantly higher for warm May plants (Fig. 3C; Table 3). $^{15}$N-tracer had been added 2 weeks before that harvest, and $\delta^{15}$N values showed similar relationships between the treatments to those of the N concentrations, but without significant treatment effects (Fig. 3D; Table 3). $\delta^{13}$C in bulbils and shoots was higher in the warm May treatment than in the cold May treatment, but in capitula there was no significant difference (Fig. 3E). In roots, the $\delta^{13}$C values were clearly higher than in other plant parts, with no significant treatment effect.

Nitrogen uptake in autumn and storage for the winter

In early November, bulbils of warm May plants had on average 59% higher dry mass and 55% more N than those of cold May plants (Fig. 4A, B; Table 4). Bulbils in the cold May and warm May treatments contained 13.2 mg and 20.4 mg N, respectively. N concentration in bulbils was not significantly affected by the treatment (Fig. 4C; Table 4). $\delta^{15}$N in bulbils strongly declined with advancing date of tracer addition in both treatments (Fig. 5). The main effects of both treatment and time of addition were significant ($P<0.001$; ANOVA; $r^2=0.763$; $n=80$) with generally higher

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**Fig. 2.** Plant traits related to growth and senescence for *Rhynchospora alba* in the harvests from 5 August to 20 October in the two treatments, warm May (red circles) and cold May (blue squares), plotted against the ordinal date. Bulbil dry mass (A). Plant dry mass without bulbils (B). Number of bulbils per plant (C). Number of capitula per plant (D). Total number of leaves per plant (E). Total root length per plant (F). Percentage of leaves senesced (G). Percentage of root length senesced, determined by TTC staining (H). Mean values ±SE.
values for the cold May treatment. There also was a significant treatment×time of addition interaction (P=0.023), resulting from the decline of δ¹⁵N at earlier addition dates for the warm May treatment compared with the cold May treatment. Tukey tests between all times of addition within each treatment revealed that in the cold May treatment the δ¹⁵N in bulbils was significantly higher than in controls (no tracer added) until the second-last addition date on 5 October, but not any more on the last addition on 20 October (Fig. 5). Correspondingly, δ¹⁵N values were significantly lower in plants with tracer added on 20 October, compared with plants with earlier additions. In the warm May treatment, a higher δ¹⁵N compared with controls was observed only until the tracer addition on 8 September, but not thereafter. In this treatment, there were no significant differences in δ¹⁵N values among harvests after 8 September.

Bulbils of warm May plants had a higher (less negative) δ¹³C than bulbils of cold May plants (Fig. 4D; Table 4).

### Discussion

**Delay in start of the growing season delays senescence**

Photoperiod and temperature are central cues controlling plant activity—dormancy cycles in seasonal climates (Cooke et al., 2012; Estiarte and Peñuelas, 2015). Besides these environmental cues, plant-inherent factors—either genetic or phenotypic—contribute to seasonal changes in plant activity and modify phenological processes, such as the time of senescence in the autumn, potentially leading to different phenological responses to a warming climate (Junttila, 1982; Starr et al., 2000; Körner and Basler, 2010; Fu et al., 2014). Our data indicate that in the case of R. alba, resource accumulation in overwintering organs plays a major part in the timing of senescence. *Rhynchospora alba* is a perennial plant, as it overwinters as vegetative bulbils that are produced each year anew, but the rest of the plant completely senesces before winter (Ohlson and Malmer, 1990). Such behaviour makes it comparable with monocarpic annuals, in which remobilizing nutrients from other organs into the seeds leads to senescence (Thomas, 2013). The early senescence of *R. alba* plants with an early start of the growing season indicates that the species is a periodic species and the time of its senescence is not delayed in response to extended favourable conditions in the autumn. However, its compensatory responses to cold May also indicate that the delay in the start of the growing season did not merely result in a temporal shift in the phenology, but the plants with a delay were acclimating to the situation early on, trying to catch up.

**Acclimation after a late start**

The higher δ¹³C in both leaves and bulbils in mid-August, and in the overwintering bulbils in November, indicates a higher stomatal conductivity during growth (Farquhar et al.,...
When atmospheric water pressure deficit is high, wetland plants regulate their water loss by stomatal closure, even if water availability is not limited (Takagi et al., 1998). Our data indicate that with a late start to their growth, plants increased their stomatal conductance, and possibly their photosynthesis. In addition, Starr et al. (2000) found for Bistorta officinalis that...
an advancement of growing season in a field experiment led to lower water potentials and, to some extent, lower transpiration rates, although in that study this was probably caused by the drier soils associated with the early start of the season. In our experiment, water supply was not limiting plant growth. Potential cues for a photosynthetic response in our experiment could have been the slightly warmer soil temperatures, or the longer photoperiod in relation to the plant’s developmental stage (Starr et al., 2004; Bauerle et al., 2012). The high root δ13C values in our study may have been caused by respiratory fractionation (Bathellier et al., 2008). By mid-August, the late-starting plants had mostly caught up in growth, with no difference in plant dry mass or total N between the treatments, except for a 70% lower dry mass and N content of the still relatively undeveloped bulbils. After mid-August, the only observed significant growth was for bulbil mass. The late-starting plants also acclimated by producing a higher number of capitula. An increased investment in sexual reproduction may be an advantage because seed ripening is faster than bulbil ripening, although seedling establishment is probably more risky than regrowth from asexual bulbils (Ronsheim, 1994). In *Mimulus primuloides*, a shorter growing season along an elevation gradient leads to an increased contribution of sexual reproduction relative to vegetative reproduction (Douglas, 1981). On the other hand, a complete cessation of sexual reproduction has been shown towards the northern distribution limit of *Decodon verticillatus* (Dorken and Eckert, 2001).

**Table 4.** Results of one-way ANOVAs testing the effects of treatment (cold May, warm May) on dry mass, nitrogen contents and concentration, and δ13C of *Rhynchospora alba* bulbils on 3 November

|          | n  | r²  | F     | P     |
|----------|----|-----|-------|-------|
| Dry mass | 80 | 0.407 | 53.5 | <0.001|
| Total N  | 80 | 0.443 | 62.1 | <0.001|
| [N]      | 80 | 0.008 | 0.6  | 0.432 |
| δ13C     | 80 | 0.455 | 65.1 | <0.001|

**Fig. 4.** Dry mass (A), N content (B), N concentration (C), and δ13C (D) of *Rhynchospora alba* bulbils on 3 November. Lighter blue: cold May; darker red: warm May. Mean values ±SE. n=41 (cold May) or n=39 (warm May).

**Fig. 5.** δ15N in *Rhynchospora alba* bulbils on 3 November, with additions of 10 ml of 0.5 mM 15NH415NH3 tracer at six different times from 5 August to 20 October, resulting in 10 µmol 15N, and in control plants without tracer addition. Different lower case letters above the bars indicate significant differences (P<0.05) among times of tracer addition within each of the treatments, respectively (Tukey’s HSD; separate ANOVAs for each treatment). Lighter blue: cold May; darker red: warm May. Mean values ±SE. Six replicate plants, except for four for cold May 10–20 addition, and five for the control plant.

**Phenology of the senescence**

There was no major difference between the treatments in the time of onset of senescence, but, once started, it proceeded faster in plants that had started their growth earlier, indicating different cues for these processes. Initiation of senescence and speed of subsequent processes have previously been shown to respond to different cues in *Populus tremula* (Fracheboud et al., 2009). The lower N concentration in leaves and roots of the warm May R. alba in mid-August indicates an earlier start of nutrient remobilization in that treatment, and the higher N concentration in capitula of these plants at that time indicates a more advanced state of seed filling. The cold May plants continued to take up the added 15N tracer until shortly before full senescence in late October, while in the warm May plants significant uptake ceased 4 weeks earlier. The decrease in tracer uptake was more gradual in warm May plants, compared with the rather abrupt cessation of uptake in the cold May plants when...
temperatures reached close to zero. This indicates that in the cold May plants senescence was enforced by the cold temperatures, while in warm May plants it was driven more by inherent factors; that is, the status of bulbils. Root senescence started 5 weeks later than leaf senescence, indicating that asynchrony in leaf and root phenology can also be found with respect to senescence, in addition to asynchrony in production times that has previously been observed (Steinaker et al., 2010; Abramoff and Finzi, 2015). However, the timing of the decrease in tracer uptake in the two treatments better matched the temporal patterns of leaf senescence than those of root senescence, indicating that below-ground functions are connected with above-ground functions, possibly due to reduced nutrient uptake as a consequence of reduced transpiration of the senescing leaves (Constable and Rawson, 1980; McDonald et al., 2002) or due to less photosynthetic energy being available for the energy-demanding processes of N uptake (Bogard et al., 2011). This also shows that root function may decrease well before root senescence.

**Nutrient remobilization**

In August, plants in both treatments contained similar amounts of N in total. From August to November, cold May plants with their long-lasting uptake activity increased the N content in their bulbils by a factor of six. For warm May plants with more N in their bulbils in August, and with an earlier cessation of uptake, this increase was only by a factor of three. Nevertheless, the absolute increase in N content between August and November was 24% more for the warm May plants, which at the end of the season reached a 55% higher total N content. This clearly shows the importance of nutrient remobilization and its timely start for this species. The lower final δ15N values in the bulbils of warm May plants further emphasize the importance of N that was taken up early in the season. The amount of N in bulbils of the warm May plants in November was 78% of the amount found in the whole plant at the peak in August when senescence was just starting. This value is comparable with the highest remobilization values observed for leaves of many deciduous trees (Côté et al., 2002; Keskitalo et al., 2005), although the real value for retained nutrients is probably somewhat lower as the calculation does not include uptake after 19 August. For cold May plants, bulbils in November contained only 50% of the N in the whole plant in August, despite the extended period of uptake. This suggests that a late start of remobilization reduces the efficiency of nutrient retrieval. Ohlson and Malmer (1990) found a 55% remobilization for N in *R. alba*. Such a lower value may have been caused by the earlier senescence of their plants, possibly initiated by nutrient deficiency as the bulbils in their study were smaller than in our experiment. Senescence induced by stress, such as drought, is known to result in an inefficient resorption at senescence (Killingbeck et al., 1990; Estiarte and Peñuelas, 2015). The higher percentage of retained nutrients in the earlier senescing warm May *R. alba* in our experiment was probably a result of the senescence being initiated by inherent factors, rather than the external stress of low temperatures. Cold temperatures later in the season may further slow down the senescence kinetics of late-senescing plants (Bogard et al., 2011). A delayed start in growth, resulting in a delayed anthesis and reduced time available for senescence, is also known to reduce grain filling for annual crops such as corn or barley (Cirilo and Andrade, 1996; Mitchell et al., 1996), compared with which the perennial *R. alba* with its small annually produced propagules as the only vegetative overwintering organs has a certain functional similarity (Verburg and During, 1998).

**Distribution limits of *R. alba***

A 3 week delay in the start of the growing season resulted in a one-third reduction of the mass and N content of bulbils at the end of the season. This is bound to considerably affect the start-up potential for the next year, presumably of great importance in the nutrient-limited environment where the species grows. Our conclusion that this circumboreal species is limited by short or cool growing seasons is supported by the known northern limits for its distribution. *Rhynchospora alba* is widely spread across Northern Ontario, but is missing at the coasts of Hudson Bay and James Bay (Riley, 2003), an area which has strongly reduced GDDs due to the mass effect of this cool body of water (Rouse, 1991). In northern Europe, *R. alba* is limited to areas where the growing season starts in early May or earlier (Hultén and Fries, 1986; Tveito et al., 2001). Furthermore, characteristic habitats of *R. alba* are Sphagnum-dominated poor fens and boggy, open sites, often on floating mats (Flora of North America Editorial Committee, 1993) that have soils that warm up earlier in spring and have warmer temperatures in the summer, compared with wetlands with sedge or shrub cover (Glenn et al., 2006; Chong et al., 2012).

**Conclusions**

Our data contribute to understanding of the previously observed variation among plant species in response of autumn senescence to warming climate. Species such as *R. alba* need a certain length of growing season to produce storage organs for the winter. Senescence starts early so that the process of immobilization can be optimized, rather than extending the period of uptake of new nutrients. Such a behaviour may be an adaptation to environments with a poor nutrient availability in the autumn. In the case of a late onset of growing season, the species acclimates, possibly catching up by late summer in size and in total amount of acquired nutrients, but lagging behind in storage development. A delay in senescence increases nutrient uptake rate in the season, but reduces the efficiency of nutrient remobilization. The timing of senescence is determined by an interplay between the status of winter storage, and the onset of close to freezing temperatures. Longer growing seasons caused by a warming climate may not improve the performance of *R. alba* in southern areas, but would probably enable it to spread further north.

**Supplementary data**

Supplementary data are available at JXB online.

Fig. S1. Comparison of temperatures to which the experimental plants were exposed between 1 April 2017 and 27 May 2017 (days 91–147) in the different experimental situations.
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