

Dry weight minimum in the underground storage and proliferation organs of six creeping perennial weeds

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Abstract

Many herbaceous perennial plant species gain significant competitive advantages from their underground creeping storage and proliferation organs (CR), making them more likely to become successful weeds or invasive plants. To develop efficient control methods against such invasive or weedy creeping perennial plants, it is necessary to identify when the dry weight minimum of their CR ($CR\ DW_{min}$) occurs. Moreover, it is of interest to determine how the timing of $CR\ DW_{min}$ differs in species with different light requirements at different light levels. The $CR\ DW_{min}$ of *Aegopodium podagraria*, *Elymus repens* and *Sonchus arvensis* were studied in climate chambers under two light levels (100 and 250 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$), and *Reynoutria japonica*, *R. sachaliensis* and *R. × bohemica* under one light level (250 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$). Under 250 $\mu\text{mol m}^{-2}\ \text{s}^{-1}$, the $CR\ DW_{min}$ occurred before one fully developed leaf in *R. sachaliensis*, around 1–2 leaves in *A. podagraria* and *E. repens* and around four leaves in *S. arvensis*, *R. japonica* and *R. × bohemica*. In addition to reducing growth in all species, less light resulted in a higher shoot mass fraction in *E. repens* and *S. arvensis*, but not *A. podagraria*; and it delayed the $CR\ DW_{min}$ in *E. repens*, but not *S. arvensis*. Only 65% of planted *A. podagraria* rhizomes produced shoots. Beyond the $CR\ DW_{min}$, *Reynoutria* spp. reinvested in their old CR, while the other species primarily produced new CR. We conclude that *A. podagraria*, *R. sachaliensis* and *E. repens* are vulnerable to control efforts at an earlier developmental stage than *S. arvensis*, *R. japonica* and *R. × bohemica*.

KEY WORDS

Aegopodium podagraria, Alien invasive plants, *bohemica*, *Elymus repens*, *Elytrigia repens*, *Fallopia*, *japonica*, Light, *Reynoutria*, Rhizomes, *sachaliensis*, *Sonchus arvensis*

1 | INTRODUCTION

Due to their rapid proliferation, persistence and tolerance of disturbance, some creeping perennial herbs have become invasive and harmful weeds in natural and semi-natural ecosystems, as well as in production systems. The grass *Elymus repens* (L.)

Gould. (Couch grass) and the dicotyledonous *Sonchus arvensis* L. (Perennial sow-thistle) are serious agricultural weeds in the temperate climate zones of the world, especially in organic farming (Ringselle et al., 2020; Salonen et al., 2011). As agricultural weeds, they primarily grow in light-rich environments. The dicotyledonous *Aegopodium podagraria* L. (Ground elder) is a shade-tolerant

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ornamental plant that has become a problematic garden weed in Scandinavia and invasive in North America (D'Hertefeldt et al., 2014). The tall dicotyledonous *Reynoutria japonica* Houtt. (Japanese knotweed), *R. sachalinensis* (F.Schmidt) Nakai (Giant knotweed) and their hybrid *R. × bohemica* Chrtek and Chrtková (Bohemian knotweed) are all shade-tolerant invasive species that smother native plants beneath a thick canopy (Clements et al., 2016; Jones et al., 2018).

Creeping perennials derive a major competitive advantage from their underground storage and proliferation organs: rhizomes in *E. repens*, *A. podagraria* and *Reynoutria* spp.; and thickened roots in *S. arvensis*, hereafter collectively named CR (creeping rhizomes or (thickened) roots). Firstly, CR enable asexual reproduction by creating clonal plants as they grow away from the mother plant. Secondly, as long as the clonal network is intact, it can increase the competitive ability of the clones through the sharing of resources and information (Liu et al., 2016). Thirdly, CR function as exploration organs for finding unexploited resources (Kleijn and Van Groenendaal, 1999). Fourthly, CR store a large proportion of the energy and nutrients captured by the plant and they can, therefore, produce new shoots when the old ones die (e.g. due to winter, ploughing etc.).

Because of the vast energy resources in their CR, it is generally not sufficient to simply destroy the aboveground biomass of perennial weeds (van Evert et al., 2020). Once these species are established, regular herbicide spraying or intensive tillage/cutting is often required to manage them (Håkansson, 2003). Systemic herbicides (e.g. glyphosate) is the most common chemical control method against perennial weeds as they can be transferred down to the CR and consequently has the potential to kill the whole plant. In the absence of herbicides, a common control method in agriculture is to use repeated tillage operations to force the CR to re-sprout over and over, hence starving them of resources (Brandsæter et al., 2017; Ringselle et al., 2016). The CR can also be starved by repeatedly cutting away the aboveground biomass, but the efficacy of this method vary greatly between perennial weed species (Thomsen et al., 2015). For instance, it is not considered effective against established stands of *R. japonica* (Jones et al., 2020). To make control measure as effective and resource efficient as possible, it is essential to determine when these weedy plants are most susceptible to different control measures.

Perennial weeds are considered to be at their most vulnerable to disturbance when their CR are at their dry weight minimum (CR DW_{min}). This occurs after the loss of their shoot biomass (e.g. by winter or mowing). Until the plants return to the compensation point (i.e. when the photosynthetic production is equal to the respiration loss), respiration and root exudation losses will expend CR resources (Verwijst et al., 2018). The overall plant dry weight will continuously decrease until the plants have reached the compensation point and then continuously increase. In comparison, the dry weight of the storage organs may increase, stabilise or continue to decrease after the compensation point depending on whether the plant prioritises growth or storage. Mechanical control measures (e.g. tillage or cutting) are usually recommended to be performed no later than at the CR DW_{min} to maximise the starvation effect and prevent the build-up of resources in the storage

organs (Håkansson, 2003). In comparison, systemic herbicides are recommended to be applied once the plant is passed CR DW_{min}, since at earlier stages resources are not being transferred to the storage organs, and consequently herbicides are not either (Hunter, 1995).

To be useful to end users (e.g. farmers, gardeners and landscapers), studies on arable weeds have generally sought to discover correlations between CR DW_{min} and easily identifiable developmental stages, such as the number of leaves of the main shoot during the early vegetative phase (cf. the BBCH scale by Lancashire et al., 1991). For instance, CR DW_{min} have been estimated to be just before 3–4 leaves in *E. repens* (Håkansson, 1967), at 5–7 leaves in *S. arvensis* (Håkansson, 1969a) and at 4–7 (Gustavsson, 1997) or 8 leaves in *Cirsium arvense* (L.) Scop. (Creeping thistle) (Nkurunziza and Streibig, 2011). However, more recent studies have placed CR DW_{min} for *S. arvensis* at four leaves (Tavaziva, 2012) and *C. arvense* at 3–4 leaves (Verwijst et al., 2018). The discrepancy between different estimates illustrates the importance of both revisiting old CR DW_{min} estimates and determining CR DW_{min} of unstudied species.

One reason CR DW_{min} estimates differ is that the allocation of resources is influenced by environment, in particular resource availability (Poorter et al., 2012), temperature (Tørresen et al., 2020) and biotic factors, such as competition. For example, Håkansson (1969b) found that a lower light level delayed CR DW_{min} for *E. repens*, and Tavaziva (2012) found that inter-specific competition delayed CR DW_{min} for *S. arvensis*. Thus, light availability plays a significant role in deciding at which developmental stage CR DW_{min} occurs. However, relatively little is known about how the same change in light availability affect CR DW_{min} of different creeping perennials, in particular species with different light requirements such as light-demanding agricultural weeds compared to more shade-tolerant species.

The concept of resource sinks and sources can explain many aspects of plant growth in different plant species (White et al., 2016). Exploiting phenological changes in CR source–sink relationships increases the efficacy of control treatments (Jones et al., 2018). Beyond the CR DW_{min}, creeping perennials can use excess resources to refill their old CR or form new ones, that is let their old CR become resource sinks rather than stay as resource sources. Which strategy prevails appears to differ greatly between species. For instance, *E. repens* tend to invest in new rhizomes as its rhizomes are relatively short-lived (usually not surviving longer than one to three years) and older rhizomes are more vulnerable to disturbance, for example tillage (Majek et al., 1984). *Sonchus arvensis* thickened roots are similarly short-lived as *E. repens* (Håkansson, 1969a). However, environmental factors may also play a part. Ringselle et al., (2017) observed that *E. repens* slightly increased its biomass allocation to old rhizomes over new rhizomes when grown under lower nutrient availability. In species with relatively long-lived CR like *Reynoutria* spp. (Price et al., 2002) and *A. podagraria* (Meyer and Hellwig, 1997), the old CR are more likely to become sinks beyond the CR DW_{min}.

Our aim was to study how CR DW_{min} differs in terms of developmental stage between different creeping perennial plants, some that have been studied before (*E. repens* and *S. arvensis*) and some that have

not (*A. podagraria*, *R. japonica*, *R. sachalinensis* and their hybrid *R. × bohemica*). Moreover, we wished to study how light levels affect when the CR DW_{min} of different species occur. The following hypotheses were tested: (1) under adequate light levels, CR DW_{min} will occur at a later developmental stage in the agricultural weeds (*E. repens* and *S. arvensis*) than in the shade-tolerant invasive species (*A. podagraria* and *Reynoutria* spp.); (2) a reduction in light supply will cause the CR DW_{min} to occur at a later developmental stage in *E. repens* and *S. arvensis*, but not in *A. podagraria* (since it is relatively shade-tolerant); and (3) the species with long-lived CR (*A. podagraria* and *Reynoutria* spp.) are expected to primarily refill their old CR beyond the CR DW_{min} while the species with short-lived CR (*E. repens* and *S. arvensis*) will primarily create new CR.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Growth chamber experiments were conducted in 2013, 2015, 2016 and 2017, designated as E2013, E2015, E2016 and E2017 respectively. In each experiment, CR of perennial plants were planted, placed in growth chambers and harvested at different developmental stages. Not all species were included in all experiments, refer Table 1 for a complete list. The perennial species were grown under light level 250 or 100 µmol m⁻² s⁻¹ with a photoperiod of 16 hr. However, *Reynoutria* spp. were only grown under 250 µmol m⁻² s⁻¹ as insufficient planting material was collected prior to the experiments to grow it under both light levels.

2.2 | Experimental setup

CR were collected just before the soil was frozen in autumn, late-October to late-November before the experimental years, in a nearby cereal field (*E. repens* and *S. arvensis*) or along roadsides and

building sites (*A. podagraria*, *R. japonica*, *R. sachalinensis* and *R. × bohemica*). After collection, the CR were stored in buckets with soil in a cooling chamber at 2–4°C until the start of the experiments. The buckets were irrigated as needed.

The day before starting the experiments, rhizomes were cut so they had two nodes, and regenerative roots so they were 5 cm long. One CR piece was planted per pot in all experiments except in E2017. There, two pieces were placed in each pot and one removed if both produced aerial shoots. Prior to planting, the CR pieces were weighed. To estimate their water content and initial dry weight, 10 additional CR pieces of each species were weighed before and after being dried for 72 hr at 60°C. In E2013, a sand-peat mixture (33% sand and 66% peat) was used, and a 100% peat-soil in the other experiments. The peat type was a limed peat enriched with nutrients [Tjerbo Torvfabrikk 'Plantejord', containing 80% (volume per cent) sphagnum peat, 10% composted bark and 10% fine sand. Each unit (50 litre) enriched with limestone flour (6 g) and 2 kg fertiliser (NPK 12-4-18), pH 5.5–6.5 and density 360 kg/m³ (applied volume)].

The pots used were plastic and square-shaped (10 × 10 cm) and placed on plastic trays. The growth chambers used high-pressure mercury lamps (Osram Powerstar HQI-T 250W/D) with a maximum light capacity of 250 µmol m⁻² s⁻¹. Day and night cycles were set to 16 hr day and 8 hr night with temperatures at 18°C and 12°C respectively. Humidity was set to 75%. Abiotic conditions inside chambers were automatically recorded every 15 min.

The growth chambers were 166 cm × 99 cm and could thus fit a maximum of 82 pots each. In E2013 and E2015, *E. repens* and *S. arvensis* shared the same growth chambers. The other species in E2015 (*A. podagraria* and *R. sachalinensis*) and the *Reynoutria* species in E2016 had their own growth chambers. In E2017, all four species were distributed among the chambers so that there were 20 pots of each species in each chamber. The pots were rotated twice a week in the chambers to compensate for variations in light levels at different spatial positions.

TABLE 1 Number of pots in each experiment (E2013, E2015, E2016 and E2017), in total and how many were excluded from analyses either due to the rhizome/root not producing any shoots by harvest (dead) or because they advanced to a leaf stage that had too few representatives and was thus not relevant enough to include in the analysis by grouping leaf stages (outliers). High light is 250 µmol m⁻² s⁻¹ and low light 100 µmol m⁻² s⁻¹

	Light	Experimental year				Total	Plants excluded	
		E2013	E2015	E2016	E2017		Dead	Outliers
<i>Elymus repens</i>	High	25	35		40	100	6	3
	Low	25	35		40	100	5	1
<i>Sonchus arvensis</i>	High	25	35		40	100	1	1
	Low	25	35		40	100	2	2
<i>Aegopodium podagraria</i>	High		35		40	75	29	2
	Low		35		40	75	24	
<i>Reynoutria sachalinensis</i>	High		22	40		62	6	1
<i>Reynoutria japonica</i>	Low			40		40		3
<i>Reynoutria × bohemica</i>	High			40		40		5

Pots were watered from below (i.e. water was poured onto the trays). During the first 3 weeks, the pots were irrigated with only tap water and afterwards fertilised with a complete nutrient solution.

2.3 | Harvests

The experiments started 8 February (E2013), 13 February (E2015), 5 February (E2016) and 17 February (E2017) and ended after approximately six weeks, except for E2016 which ended after nine weeks. Assessments and destructive harvests started two weeks after planting and were then carried out at a weekly interval until all pots had been harvested. At harvest, the number of leaves on the largest shoot with lamina ≥ 4 cm were counted on all species except *A. podagraria*. In *A. podagraria*, we counted the number of compound leaves where the middle subleaf had a lamina of ≥ 2 cm.

To the extent possible, all plant biomass was collected during harvesting. The biomass in each pot was divided into the following parts: (a) planted CR fragment, (b) new CR (defined as new developed CR with a diameter of ≥ 1 mm, measured by a digital caliper), (c) non-regenerative roots, (d) aboveground shoots, and (e) belowground shoots (i.e. plant crown and aerial shoots that have not emerged yet). All harvested plant parts were dried for 72 hr at 60°C before being weighed.

2.4 | Statistical analysis

Pots where the CR had not produced shoots were removed prior to analyses (Table 1). The remaining pots were divided into groups based on the number of leaves on the largest shoot in the pot. If there were fewer than three plants/pots in the group, they were generally combined into a larger group for the analyses of that species (Figure 1). If only one or two plants of the same species had produced a higher number of leaves than most other plants, they were considered outliers and omitted from the analyses (Table 1). For example, one plant with four leaves and one with five leaves (both in the 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ treatment) were omitted from the *A. podagraria* analyses, as all other plants had ≤ 3 leaves.

The shoot mass fraction (SMF) was calculated by dividing the total shoot biomass (above + belowground shoots) with the total plant biomass. The relative change in CR was calculated by dividing the total CR dry weight (old + new CR) by the initial planted CR dry weight. The initial planted CR dry weight was in turn calculated by the initial planted CR fresh weight times the dry weight percentage extracted from the 10 extra CR pieces that were weighed at the start of each experiment. All variables except new CR, SMF and CR relative reduction were transformed to \log_e prior to analyses.

Data were analysed with the GLIMMIX package in SAS version 9.4 (SAS Institute Inc.). Response variables were old CR, new CR, total CR, non-regenerative roots, belowground shoots, aboveground shoots, total belowground biomass, total plant biomass, SMF and CR relative reduction. Fixed factors were: (a) leaf stage when

destructively harvested, (b) light level (except for *Reynoutria* spp.), (c) species (only *Reynoutria* spp.) and interactions. Interactions between leaf stage and light or species were included to determine whether the CR DW_{min} and other important stages occurred at different leaf stages in different species or at different light levels. Experimental year (except for *Reynoutria* spp.) was treated as a random factor. Planted CR fresh weight was used as a covariate as this was a likely source of variation. Tukey-Kramer groupings at $\alpha = 0.05$ were used for determining significant differences between treatments.

3 | RESULTS

3.1 | *Elymus repens*

The light level significantly affected *E. repens* biomass production, and the difference increased with increasing leaf stage (Table 2, Figure 1). On average across all leaf stages, *E. repens* plants grown at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ had 35% lower total biomass than those grown at 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The effect was greater on new rhizome (-68%) and root weight (-48%) than old rhizome (-24%) and aboveground shoot weight (-22%). There was no significant interaction between leaf stage and light on SMF (Table 2). On average across all leaf stages, the SMF was 0.44 for plants grown with a light level of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 0.49 with 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 2).

There was no interaction between light level and leaf stage for *E. repens* old rhizome weight (Table 2). The old rhizome biomass was significantly lower at all leaf stages compared to plants at leaf stage 0 (i.e. before the plant has even produced one leaf with lamina ≥ 4 cm), except leaf stage 4 and 10-11 (Figure 1, Table 2). New rhizome biomass gradually increased with increasing leaf stage, but the increase was lower in plants grown at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1, Table 2) and they developed new rhizomes later. 23%, 59% and 100% of plants grown at 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ had new rhizomes by leaf stages 2-4, while only one plant grown at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ had new rhizomes by leaf stage 2, none by leaf stage 3, but 73% by leaf stage 4. For total rhizome (old + new) biomass, there was a significant interaction between leaf stage and light (Table 2). Plants grown under 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ had their lowest total rhizome biomass at leaf stages 1 and 2, which were both significantly lower than plants at leaf stage ≥ 4 (Figures 1 and 3). When grown in 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, plants had their lowest total rhizome biomass at leaf stage 3 and it was not until leaf stages 10-11 that total rhizome biomass was significantly higher than at leaf stage 1-5 (Figures 1 and 3). There was no interaction between light level and leaf stage for total biomass (Table 2). On average across both light levels, plants at leaf stage 2 had already amassed a significantly larger total biomass than those at leaf stages 0 and 1.

3.2 | *Sonchus arvensis*

The light level significantly affected *S. arvensis* total biomass production, but not all biomass fractions (Table 2). On average across

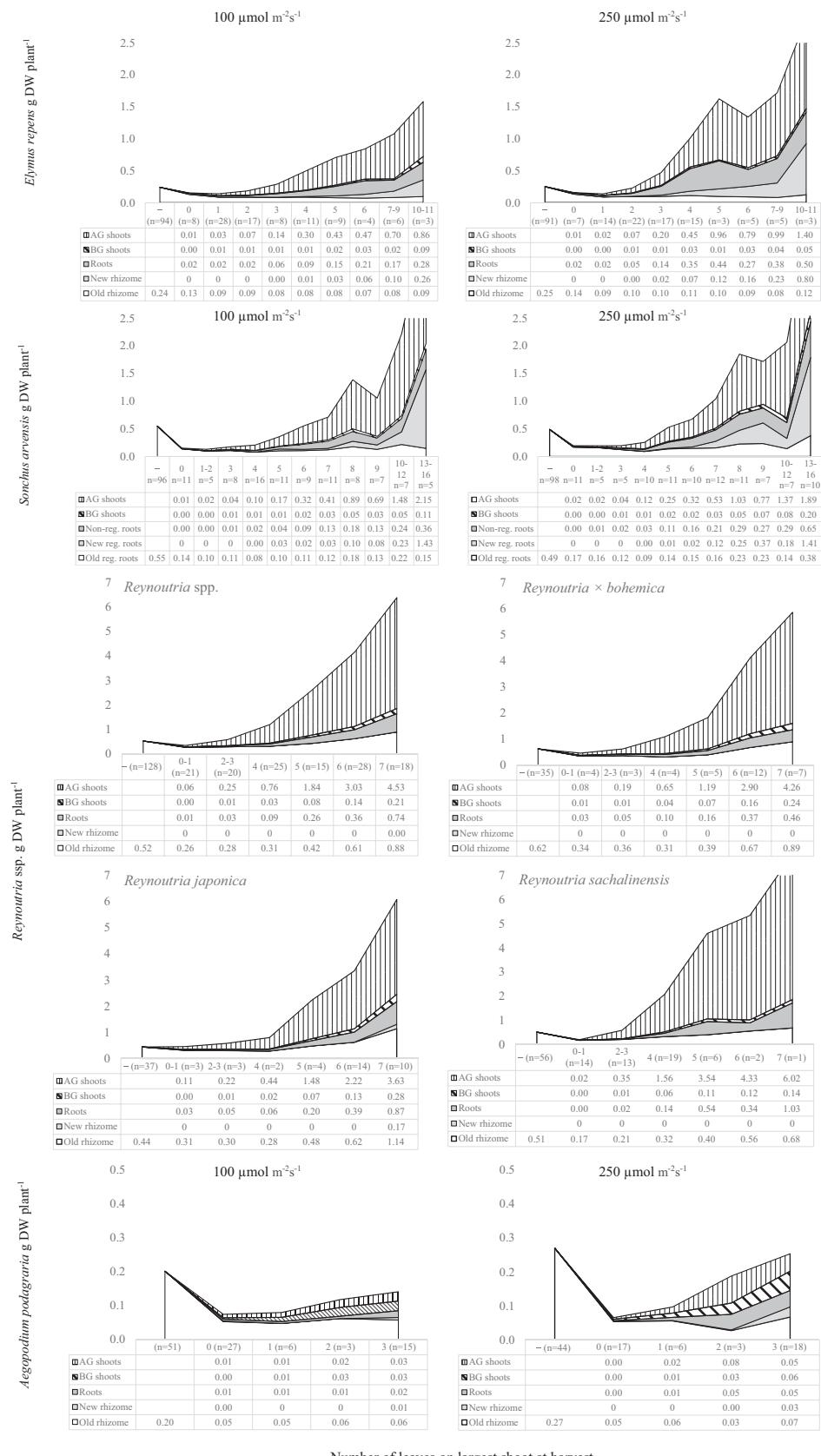


FIGURE 1 Shows the LS means of dry weight (DW) of aboveground (AG) and belowground (BG) shoots, non-regenerative roots and old and new rhizomes/regenerative roots (CR) of *Elymus repens*, *Sonchus arvensis*, *Aegopodium podagraria*, grown at two light levels (100 or 250 $\mu\text{mol m}^{-2}\text{s}^{-1}$) and three *Reynoutria* spp. grown at one light level (250 $\mu\text{mol m}^{-2}\text{s}^{-1}$), and harvested at different leaf stages. 0 indicates that there was no DW at harvest, while 0.00 indicates that DW at harvest weighed less than 0.00. n shows the number of plants harvested per leaf stage. Please note the difference in x- and y-scale

TABLE 2 ANOVA-table showing the F-values and significance level of the analyses of total plant biomass, total belowground (BG) biomass, aboveground (AG) and BG shoots, non-regenerative roots and old and new rhizomes/regenerative roots (CR), shoot mass fraction (SMF) and CR relative reduction of *Elymus repens*, *Sonchus arvensis*, *Aegopodium podagraria*, grown at two light levels (100 or $250 \mu\text{mol m}^{-2}\text{s}^{-1}$) and three *Reynoutria* spp. grown at one light level ($250 \mu\text{mol m}^{-2}\text{s}^{-1}$), and harvested at different leaf stages. Bold designates p-values ≤ 0.05 , with symbols showing the significance level (no symbol = $p < 0.1$, * = $p < 0.05$, ** = $p \geq 0.05$, *** = $p \geq 0.01$, **** = $p \geq 0.001$). Planted CR fresh weight (FW) was used as a covariate. Df = degrees of freedom

	df	Old CR	New CR	Non-reg. roots	BG shoots	AG shoots	Total CR	Total BG	Total plant	SMF	CR relative reduction
<i>Elymus repens</i>											
Leaf stage	8	4***	37***	60***	26***	120***	20***	56***	108***	64***	13***
Light	1	7**	53***	31***	3	6*	41***	61***	38***	8**	52***
Leaf stage*Light	8	1	9***	2*	2'	1	3**	5***	3**	1	5***
Planted FW	1	176***	0	14***	1	10**	87***	56***	42***	5*	7**
<i>Sonchus arvensis</i>											
Leaf#	10	10***	16***	67***	46***	150***	28***	43***	94***	72***	11***
Light	1	28***	1	24***	6*	2	22***	30***	19***	16***	12***
Leaf stage*Light	10	3**	0	1	1	1	1	1	0	2'	2*
Planted FW	1	121***	0	11**	6*	7*	52***	39***	36***	11**	7**
<i>Aegopodium podagraria</i>											
Leaf stage	3	2	3'	11***	40***	15***	4*	25***	28***	51***	5**
Light	1	1	1	1	1	1	0	6*	7*	2	0
Leaf stage*Light	3	3'	1	5**	1	3*	2	2'	4*	1	0
Planted FW	1	67***	1	0	0	0	51***	28***	17***	11**	1
<i>Reynoutria</i> spp.											
Leaf stage	5	9***	1	29***	25***	74***	9***	15***	44***	70***	7***
Species	2	2	0	0	1	2	2	0	1	9***	0
Leaf stage*Species	10	1	2	3**	1	5***	1	1	3**	3**	1
Planted FW	1	93***	1	3'	2	1	93***	39***	16***	41***	4*

all leaf stages, *S. arvensis* plants grown at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ had 25% lower total biomass than those grown at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$, primarily affecting the non-regenerative root biomass (~43%), old regenerative roots (~25%) and belowground shoots (~25%). The difference in new regenerative roots or aboveground shoots between light levels was not significant (Table 2). The SMF was on average 0.5 under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 0.44 under $250 \mu\text{mol m}^{-2} \text{s}^{-1}$, with a close to significant interaction with leaf stage (Table 2) as the SMF appeared similar at leaf stages 4 and 10–12 (Figure 2).

There was a significant interaction between leaf stage and light level for old regenerative roots (Table 2). However, the old regenerative root weight was still lowest at leaf stage 4, at both light levels (Figure 1), where it was significantly lower than plants at leaf stage 0 and ≥ 7 . At leaf stage 4, only two plants (one from each light level; 8% of all plants at leaf stage 4) had developed new regenerative roots. At leaf stages 5–8, the percentages of plants with new regenerative roots were 18%, 44%, 27% and 75% for plants grown under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 63%, 40%, 83% and 90% for plants grown under $250 \mu\text{mol m}^{-2} \text{s}^{-1}$. The contribution of the new regenerative root biomass was still very limited until leaf stage 7 under $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ and until leaf stage 8 under

$100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1). Since old regenerative root biomass was lowest at leaf stage 4 and new regenerative root biomass did not truly begin being produced until leaf stage ≥ 5 , the total regenerative root weight (old + new) was significantly higher for plants at leaf stage ≥ 5 than at leaf stage 4, under both light levels (Figures 1 and 3). Similarly, the total biomass was significantly higher in plants at leaf stage ≥ 5 than those at leaf stage ≤ 4 (Figures 1 and 3).

3.3 | *Aegopodium podagraria*

Only 65% of *A. podagraria* rhizomes produced shoots (Table 1). On average across all leaf stages, *A. podagraria* plants grown at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ had 29% lower total biomass than those at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1; Table 2), but the SMF was not significantly affected by the light level (Figure 2). There were significant or almost significant interactions between leaf stage and light level for old rhizomes, roots and aboveground shoots (Table 2). For old rhizomes, this was due to a lower old rhizome biomass at leaf stage 2 compared to leaf stage 3 at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$, but not at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1). At $250 \mu\text{mol m}^{-2} \text{s}^{-1}$, root and

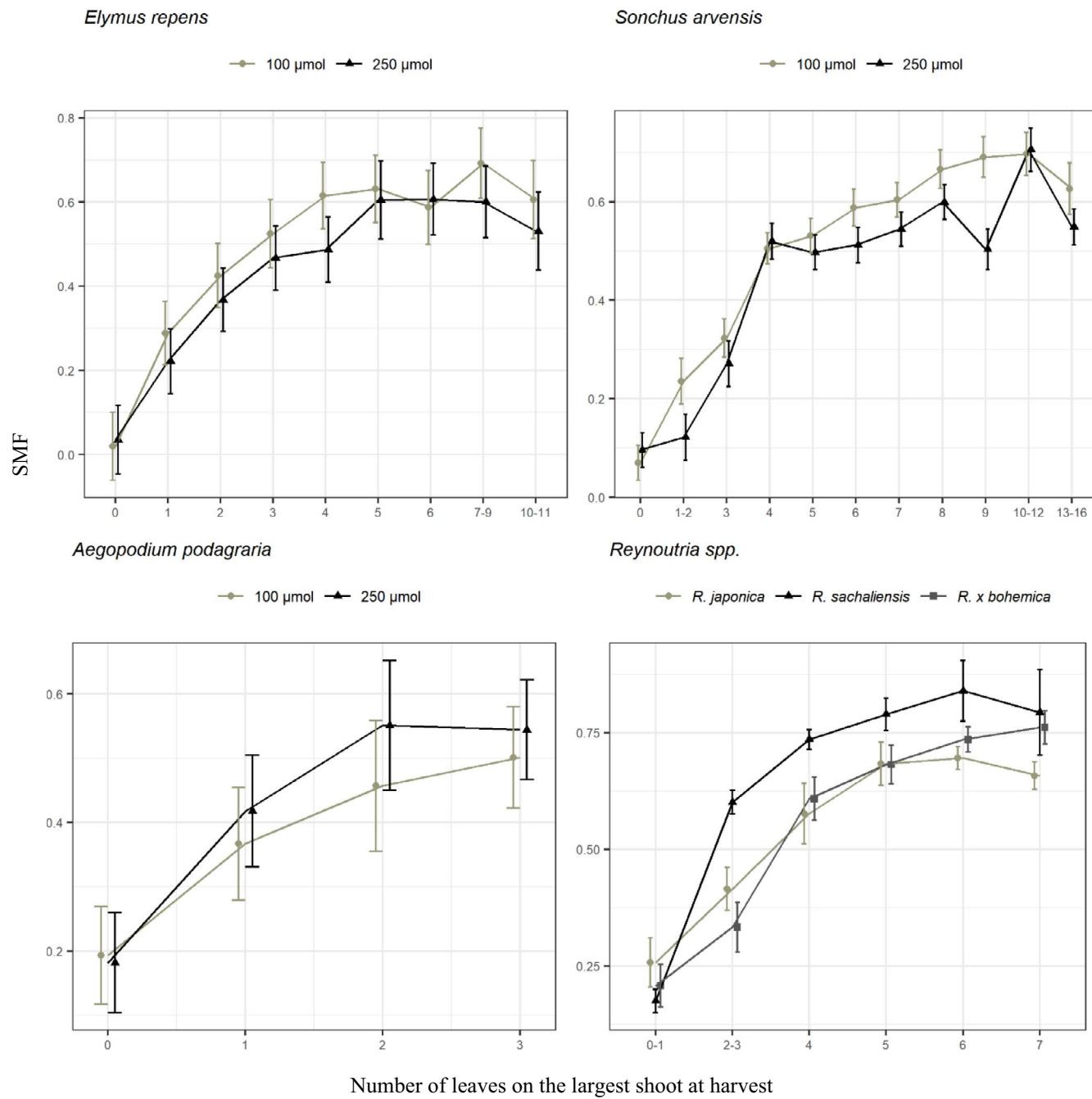


FIGURE 2 The LS means of the shoot mass fraction (SMF) of *Elymus repens*, *Sonchus arvensis*, *Aegopodium podagraria* and three *Reynoutria* spp. at different leaf stages. Error bars show the standard error. Please note the difference in the x- and y-scale between species. To improve visibility, the means and error bars have been jittered

aboveground shoot production expanded quickly at leaf stage ≥ 2 , but not at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$. As a result, the total biomass was significantly higher at leaf stage 2 than at leaf stage 0 at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$, but not until leaf stage 3 at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 3).

At leaf stage 3, eleven out of eighteen (61%) plants grown at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ and eight out of fifteen (53%) plants grown at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ had produced new rhizomes, compared to only one plant of each at leaf stage ≤ 2 . As a result, the total rhizome biomass was significantly higher at leaf stage 3 than at leaf stage 2 (Figure 3).

3.4 | *Reynoutria* spp

The *Reynoutria* spp. were only grown at $250 \mu\text{mol m}^{-2} \text{s}^{-1}$. There were significant interactions between the three *Reynoutria* species and leaf stage for total biomass, root biomass, aboveground shoot biomass and SMF (Table 2). These interactions are likely because *R. sachalinensis* had a large relative change in rhizome weight early on (Figure 3) and rapidly expanded its root and shoot biomass, particularly around leaf stage 4 and 5. However, the increase was relatively high in the shoot biomass as the SMF of *R. sachalinensis* was

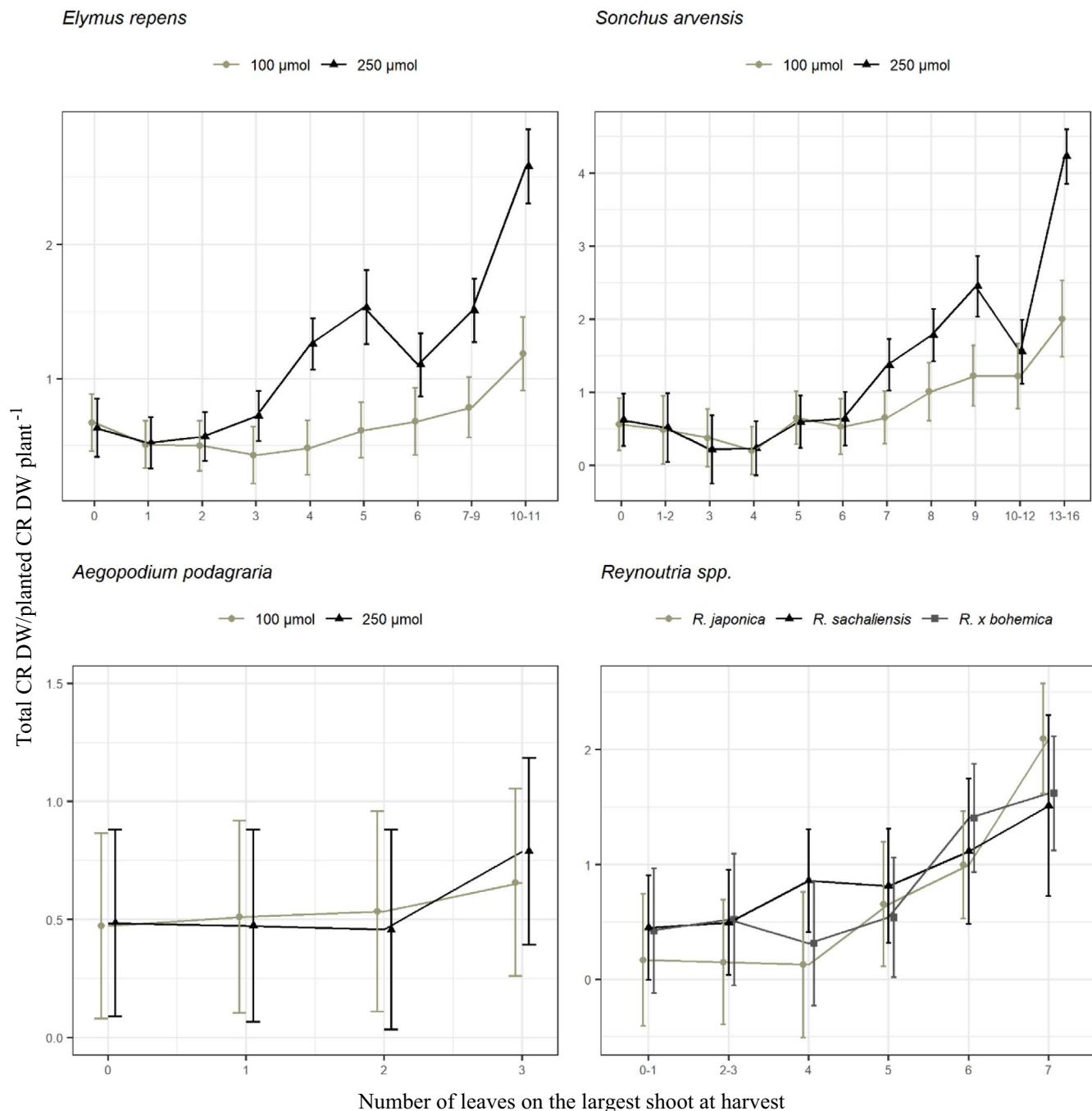


FIGURE 3 Shows the LS means of old+new rhizome/regenerative roots (CR) dry weight (DW) at harvest in relation to the planted CR DW of *Elymus repens*, *Sonchus arvensis*, *Aegopodium podagraria*, and *Reynoutria* spp. Error bars show the standard error. Please note the difference in the x- and y-scale between species. To improve visibility, the means and error bars have been jittered

significantly higher than the other *Reynoutria* species; on average 0.7 compared to 0.56 in both *R. japonica* and the hybrid (Figure 2).

At leaf stage 7, and only in *R. japonica*, one-third of the plants started producing new rhizomes, but the produced biomass was low. Thus, old rhizome weight and total rhizome weight were essentially the same. In *R. sachalinensis*, the total rhizome biomass increased with increasing leaf stage. In *R. japonica* and the hybrid, the total rhizome biomass was lowest at leaf stage 4 and increased from leaf stage 5 (Figures 1 and 3).

4 | DISCUSSION

4.1 | Developmental stage for CR DW_{min} of different creeping perennials

Exact comparisons between leaf stages of different plants species is difficult, especially as *E. repens*, *S. arvensis*, *Reynoutria* spp. and *A. podagraria* display very different leaf morphology. Thus, the definition of what constitutes a full leaf, and consequently, the developmental

stage of the plant, may have influenced the results. However, using the number of leaves to describe plant developmental stages is meant to function more as a guide than as a rule.

The first hypothesis stated that under adequate light levels, CR DW_{min} will occur at a later developmental stage in the agricultural weeds (*E. repens* and *S. arvensis*) than in the shade-tolerant invasive species (*A. podagraria* and *Reynoutria* spp.). This hypothesis was not supported as the species did not diverge in their CR DW_{min} along the lines of agricultural weeds versus shade-tolerant species. Instead, two of the *Reynoutria* species (*R. japonica* and *R. x bohemica*) were closer in their CR DW_{min} to *S. arvensis* than *E. repens* was, while the CR DW_{min} occurred early in *A. podagraria* and perhaps earliest of all in the third *Reynoutria* species, *R. sachalinensis*.

Of the two agricultural weeds, the results of the study matched previous estimates of CR DW_{min} for *S. arvensis*, but not for *E. repens*. At leaf stage 4, *S. arvensis* old regenerative root biomass was at its lowest, while new regenerative root biomass was negligible. In comparison, the total regenerative root biomass had significantly increased by leaf stage 5. Thus, the results of our study support the findings of Tavaziva (2012) that CR DW_{min} occurs at leaf stage 4 in *S. arvensis*. In *E. repens*, the old and total rhizome biomass were both at their lowest point at leaf stage 1–2, with new rhizome biomass starting to appear at leaf stage 2. Thus, both the CR DW_{min} and the initiation of new rhizome production occurred at an earlier stage than in Håkansson (1967). However, the difference between leaf stages 1–2 and 'just before leaf stage 3–4' is not large and could be due to differences in experimental setups (e.g. Håkansson, 1967's experiment was conducted in pots outside), leaf stage determination or clonal variation (e.g. Neuteboom, 1981; Westra and Wyse, 1981).

The shade-tolerant species did not have a consistently lower CR DW_{min} than the agricultural weeds. Under adequate light conditions (250 µmol m⁻² s⁻¹), *A. podagraria* had its lowest total rhizome biomass at around leaf stage 1–2, but there was a strong interaction with light level (refer to section 4.2). The three *Reynoutria* species differed in that *R. sachalinensis* had a strong early reduction in rhizome biomass (Figure 3) accompanied by a quick expansion in shoot and root biomass (Figure 1), leading to a higher SMF than the other *Reynoutria* species (Figure 2). The total rhizome weight of *R. sachalinensis* then consistently increased with increasing leaf stage. *Reynoutria japonica* and *R. x bohemica* had a comparatively small initial reduction in rhizome weight, followed by a relatively stable old rhizome weight with a slight dip at leaf stage 4 before starting to increase. One could therefore argue that the CR DW_{min} of *R. sachalinensis* occurred very early, perhaps even at leaf stage 0 (i.e. before the plant has even produced one leaf with lamina ≥4 cm), while the other *Reynoutria* species reached it around the same time as *S. arvensis*, leaf stage 4.

4.2 | Effect of light level

Elymus repens, *S. arvensis* and *A. podagraria* all had a slower and more gradual biomass build-up when grown in less light. In

particular, the refilling of their old CR and/or creating new CR were greatly delayed by a reduction in light level (Figure 3). One would expect the SMF to increase with less light (Poorter et al., 2012; Ringselle et al., 2017), and this was the case for *E. repens* and *S. arvensis*, but not for *A. podagraria* (Figure 2). This can be contrasted with Elemans (2004) who found that a reduction in light caused a reduction in SMF in *A. podagraria*. However, Elemans (2004) were comparing relatively low light levels (2 and 8% vs. 66% of full light in a greenhouse).

The second hypothesis stated that a reduction in light level would cause the CR DW_{min} to occur at a later leaf stage in *E. repens* and *S. arvensis*, but not in *A. podagraria*. This hypothesis was not supported as reduced light delayed the CR DW_{min} in *E. repens*, but not *S. arvensis*; and had a very different effect in *A. podagraria*. At 100 µmol m⁻² s⁻¹, *A. podagraria* behaved similarly to *R. japonica* and *R. x bohemica*, displaying a relatively stable old rhizome weight from leaf stages 0 to 3 while gradually building up root and shoot biomass. In plants grown at 250 µmol m⁻² s⁻¹, however, there was a dip in old rhizome biomass at leaf stage 2, accompanied by a strong expansion in root and shoot biomass. This pattern illustrates the difference between the compensation point and CR DW_{min}; the reduction in reserves/storage weight in the 250 µmol m⁻² s⁻¹ treatment is more likely to be due to an investment in growth than because the plant had not yet reached the compensation point. If the compensation point of *A. podagraria* was at leaf stage 2, it would be expected that plants in both light treatments would lose old rhizome weight until at least that stage. As this did not occur in the 100 µmol treatment, it seems more likely that the higher light level caused *A. podagraria* to invest more of its reserves into creating roots and shoots. This explanation is in line with *A. podagraria*'s strategy of fast expansion in spring (Meyer and Hellwig, 1997). However, this raises the question of whether the slow expansion of *R. japonica* and *R. x bohemica* compared to *R. sachalinensis* was because 250 µmol m⁻² s⁻¹ was sufficiently bright for *R. sachalinensis* to invest heavily in growth, but not bright enough for the other *Reynoutria* species.

4.3 | Old vs. new CR

The third hypothesis stated that *A. podagraria* and *Reynoutria* spp. would primarily focus on refilling their old CR beyond the CR DW_{min}, while *E. repens* and *S. arvensis* would focus on creating new CR. In support of the hypothesis, *E. repens* and *S. arvensis* did indeed focus on new CR and *Reynoutria* spp. focused almost exclusively on their old CR. For *A. podagraria*, the study cannot conclusively say one way or the other, because while there was some production of new rhizomes, there was no considerable increase in old rhizome weight.

One factor that has not been considered is the influence of the quality, age and origin of the sampling material. First, the age of the CR were not determined before the study. Age can

reduce CR viability (e.g. Majek et al., 1984). The age may also affect whether plants invest in new CR or refills their old, as older storage organs have a shorter life-expectancy. Since CR age has been shown to affect *E. repens* vitality despite the short life of their rhizomes (e.g. Majek et al., 1984), the effect may be even greater for species with long-lived CR. Secondly, the origin of the CR may also influence the development and growth of the plants. For instance, Håkansson and Wallgren (1976) found that *E. repens* clones from Northern Sweden emerged earlier in the year and produced a higher dry weight of rhizomes during the year than clones from Central and Southern Sweden. Similarly, D'Hertefeldt et al., (2014) found that *A. podagraria* clones from Northern Sweden produced more biomass than clones from Southern Sweden and that plants from rhizomes collected in forests translocated more ^{14}C to the rhizomes than plants from rhizomes collected from open areas (as *A. podagraria* rhizomes were in the current study).

4.4 | Implications for management

Mechanical control is arguably most effective around the CR DW_{min} as it maximises the loss of stored energy (Håkansson, 2003). Based on the current study, the CR DW_{min} occurred around leaf stage 0 (i.e. before the plant has even produced one leaf with lamina ≥ 4 cm) in *R. sachaliensis*, 1–2 leaves in *A. podagraria* and *E. repens*, and 4 leaves in *S. arvensis*, *R. japonica* and *R. × bohemica*. Håkansson (2003) also showed that it was more effective to control *E. repens* at leaf stage 2 than 3–4. However, he also illustrated that conducting control at leaf stage 3–4 does not result in a substantial increase in *E. repens* compared to conducting it at leaf stage 2, and may therefore be more resource efficient since it reduces the number of treatments per season. Our results support this view as it generally took most of the species several leaf stages beyond the compensation point and CR DW_{min} to accumulate significantly more CR biomass than they had at leaf stage 0, not to mention to recoup the initial weight of their storage organs.

A low light level further reduced the plants' ability to start refilling their old storage organs and creating new ones. For mechanical control, this primarily means that it is riskier to leave perennial weeds uncontrolled in high light environment, such as a stubble field, than under low light environments. For systemic herbicides, this means that spraying may need to be delayed under low light conditions to make sure that the storage organs are acting as resource sinks. Another argument for waiting until at least one leaf stage past the CR DW_{min}, regardless of light level, is that the experiments showed that a higher light level can lead to a reduction in stored resources, most likely due to an induced growth spurt. However, larger plant size can also reduce the efficacy of some herbicides.

Reynoutria sachaliensis is considered a less invasive plant than *R. japonica* and *R. × bohemica* (Herpigny et al., 2012). The larger initial depletion of rhizome resources to invest in roots and

shoots should in theory give it a larger competitive advantage than the other *Reynoutria* species, but also make it more vulnerable to control methods. Tillage, cutting and chemical control should all be more effective from an early stage for *R. sachaliensis*, while *R. japonica* and *R. × bohemica* are likely more resistant to control measures in the early stages of growth. However, in the case of *Reynoutria* spp., it is important to divine whether more effective is effective enough. Compared to *E. repens*, *S. arvensis* and *A. podagraria*, established stands of *Reynoutria* spp. have comparatively large CR and their resources can take many years to deplete (Jones et al., 2018). As a result, Jones et al., (2020) strongly warns against mowing and other mechanical control of *R. japonica* as it requires too many resources/treatment times and risks spreading the infestation further. In conclusion, while applying control efforts correctly in relation to the CR DW_{min} may potentially increase the control efficacy against *Reynoutria* spp., only field trials testing this hypothesis could determine if the effect is significantly enough to recommend mechanical control against these species.

That so few *A. podagraria* rhizome pieces produced shoots illustrates how vulnerable the species is to tillage.

5 | CONCLUSIONS

- When light was not a limited factor, CR DW_{min} occurred before one fully developed leaf in *R. sachaliensis*, around one to two leaves in *E. repens* and *A. podagraria* and around four leaves in *S. arvensis*, *R. japonica* and *R. × bohemica*.
- A reduction in light level delayed the CR DW_{min} in *E. repens* to leaf stage 3, but did not delay it in *S. arvensis*. At a lower light level, the CR DW_{min} occurred very early (<0 leaf with lamina ≥ 2 cm) in *A. podagraria* as it prevented it from initiating a growth spurt at the cost of additional CR resources.
- Beyond CR DW_{min}, the old CR became resource sinks in the three *Reynoutria* species, while production of new CR was seemingly prevalent in the other species.
- Aegopodium podagraria* and *R. sachaliensis* are likely to be vulnerable to control methods very early during sprouting compared to the other species, followed by *E. repens*.

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CONFLICT OF INTEREST

There is no conflict of interest.

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