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 podagratia* L. (Ground elder) is a shade-tolerant...
An 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. schachelinis* (F. Schmidt) Nakai (Giant knotweed) and their hybrid *R. × bohemia* Chrtrek 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 can 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 herbicidal 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 (Tørrøsen 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 re-fill 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\textsubscript{min} of different species occur. The following hypotheses were tested: (1) under adequate light levels, CR DW\textsubscript{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\textsubscript{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\textsubscript{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\textsuperscript{−2} s\textsuperscript{−1} with a photoperiod of 16 hr. However, Reynoutria spp. were only grown under 250 μmol m\textsuperscript{−2} s\textsuperscript{−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/m\textsuperscript{3} (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\textsuperscript{−2} s\textsuperscript{−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\textsuperscript{−2} s\textsuperscript{−1} and low light 100 μmol m\textsuperscript{−2} s\textsuperscript{−1} |

| Light | Experimental year | E2013 | E2015 | E2016 | E2017 | Total | Plants excluded |
|-------|------------------|-------|-------|-------|-------|-------|-----------------|
|       |                  |       |       |       |       |       | Dead | Outliers |
| Elymus repens | High       | 25    | 35    | 40    | 100   | 250   | 6    | 3      |
|         | Low        | 25    | 35    | 40    | 100   | 250   | 5    | 1      |
| Sonchus arvensis | High   | 25    | 35    | 40    | 100   | 250   | 1    | 1      |
|           | Low       | 25    | 35    | 40    | 100   | 250   | 2    | 2      |
| Aegopodium podagraria | High | 35    | 40    | 75    | 29    | 150   | 29   | 2      |
|           | Low       | 35    | 40    | 75    | 24    | 150   | 24   |        |
| Reynoutria sachalinensis | High | 22    | 40    | 62    | 6     | 130   | 6    | 1      |
| Reynoutria japonica | Low       | 40    | 40    | 40    | 3     | 160   | 3    |        |
| Reynoutria × bohemica | High    | 40    | 40    |        | 5     | 80    | 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 ≥1mm, 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 µmol m⁻² s⁻¹ treatment) were omitted from the analyses (Table 1).

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 log₁₀ 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 DWmin 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 α = 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 µmol m⁻² s⁻¹ had 35% lower total biomass than those grown at 250 µmol m⁻² 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 µmol m⁻² s⁻¹ and 0.49 with 100 µmol m⁻² s⁻¹ (Figure 2).

There was no interaction between leaf 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 µmol m⁻² s⁻¹ (Figure 1, Table 2) and they developed new rhizomes later. 23%, 59% and 100% of plants grown at 250 µmol m⁻² s⁻¹ had new rhizomes by leaf stages 2–4, while only one plant grown at 100 µmol m⁻² 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 µmol m⁻² 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 µmol m⁻² 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 leaf 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 µmol m\(^{-2}\) s\(^{-1}\)) and three Reynoutria spp. grown at one light level (250 µmol m\(^{-2}\) 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 µmol m$^{-2}$s$^{-1}$) and three *Reynoutria* spp. grown at one light level (250 µmol m$^{-2}$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 ≥ 0.05, ** = p ≥ 0.01, *** = p ≥ 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 |
|------------------|----|--------|--------|----------------|-----------|-----------|----------|----------|-------------|-----|----------------------|
| **Elymus repens**|    |        |        |                |           |           |          |          |              |     |                      |
| 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**                  |
| **Sonchus arvensis** | 10 | 10***  | 16***  | 67***         | 46***     | 150***    | 28***    | 43***    | 94***       | 72***| 11***                |
| Leaf#            | 1  | 28***  | 1      | 24***         | 6*        | 2         | 22***    | 30***    | 19***       | 16***| 12***                |
| Light            | 10 | 3**    | 0      | 1             | 1         | 1         | 1        | 0        | 2*          | 2   |                      |
| Leaf stage*Light | 1  | 121*** | 0      | 11**          | 6*        | 7*        | 52***    | 39***    | 36***       | 11** | 7**                  |
| Planted FW       | 1  | 67***  | 1      | 0             | 0         | 51***     | 28***    | 17***    | 11**        |     | 1                    |
| **Aegopodium podagraria** | 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 µmol m$^{-2}$s$^{-1}$ had 25% lower total biomass than those grown at 250 µmol m$^{-2}$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 µmol m$^{-2}$s$^{-1}$ and 0.44 under 250 µmol m$^{-2}$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 µmol m$^{-2}$s$^{-1}$ and 63%, 40%, 83% and 90% for plants grown under 250 µmol m$^{-2}$s$^{-1}$. The contribution of the new regenerative root biomass was still very limited until leaf stage 7 under 250 µmol m$^{-2}$s$^{-1}$ and until leaf stage 8 under 100 µmol m$^{-2}$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 µmol m$^{-2}$s$^{-1}$ had 29% lower total biomass than those at 250 µmol m$^{-2}$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 µmol m$^{-2}$s$^{-1}$, but not at 100 µmol m$^{-2}$s$^{-1}$ (Figure 1). At 250 µmol m$^{-2}$s$^{-1}$, root and
aboveground shoot production expanded quickly at leaf stage ≥ 2, but not at 100 μmol m⁻² s⁻¹. As a result, the total biomass was significantly higher at leaf stage 2 than at leaf stage 0 at 250 μmol m⁻² s⁻¹, but not until leaf stage 3 at 100 μmol m⁻² s⁻¹ (Figure 3).

At leaf stage 3, eleven out of eighteen (61%) plants grown at 250 μmol m⁻² s⁻¹ and eight out of fifteen (53%) plants grown at 100 μmol m⁻² 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 μmol m⁻² 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
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).

**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.

**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
\(DW_{\text{min}}\) 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 \(DW_{\text{min}}\) 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 \(DW_{\text{min}}\) to S. arvensis than E. repens was, while the
CR \(DW_{\text{min}}\) 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 \(DW_{\text{min}}\) 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 \(DW_{\text{min}}\) 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 start-
ing to appear at leaf stage 2. Thus, both the CR \(DW_{\text{min}}\) and the initi-
ation 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 dif-
f erences 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 \(DW_{\text{min}}\) than the agricultural weeds. Under adequate light con-
ditions (250 \(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\)), A. podagraria had its lowest total rhizome
biomass at around leaf stage 1–2, but there was a strong interac-
tion with light level (refer to section 4.2). The three Reynoutria spec-
ies 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 \(DW_{\text{min}}\)
of R. sachalinensis occurred very early, perhaps even at leaf stage
0 (i.e. before the plant has even produced one leaf with lamina
\(\geq 4 \text{ cm}\)), 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 \(DW_{\text{min}}\) 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 \(DW_{\text{min}}\) in E.
repens, but not S. arvensis; and had a very different effect in A.
podagraria. At 100 \(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\), A. podagraria behaved similarly
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 \(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\),
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 \(DW_{\text{min}}\): the reduction in reserves/storage weight in the 250 \(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\) 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 \(\mu\text{mol}\) treat-
ment, 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. ja-
ponica and R. x bohemica compared to R. sachalinensis was be-
cause 250 \(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\) 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 \(DW_{\text{min}}\), 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 in-
crease 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 refill 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 \(^{14}\text{C}\) 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\(_{\text{min}}\) as it maximises the loss of stored energy (Håkansson, 2003). Based on the current study, the CR DW\(_{\text{min}}\) occurred around leaf stage 0 (i.e. before the plant has even produced one leaf with lamina \(\geq 4\) cm) in R. sachaliensis, 1–2 leaves in A. podagraria and E. repens, and 4 leaves in S. arvensis, R. japonica and R. \(\times\) 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\(_{\text{min}}\) 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\(_{\text{min}}\) 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. \(\times\) 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. \(\times\) 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\(_{\text{min}}\) 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

1. When light was not a limited factor, CR DW\(_{\text{min}}\) 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. \(\times\) bohemica.

2. A reduction in light level delayed the CR DW\(_{\text{min}}\) in E. repens to leaf stage 3, but did not delay it in S. arvensis. At a lower light level, the CR DW\(_{\text{min}}\) occurred very early (<0 leaf with lamina \(\geq 2\) cm) in A. podagraria as it prevented it from initiating a growth spurt at the cost of additional CR resources.

3. Beyond CR DW\(_{\text{min}}\), the old CR became resource sinks in the three Reynoutria species, while production of new CR was seemingly prevalent in the other species.

4. 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.

PEER REVIEW

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REFERENCES

Brandsæter, L.O., Mangerud, K., Helgeheim, M. & Berge, T.W. (2017) Control of perennial weeds in spring cereals through stubble cultivation and mouldboard ploughing during autumn or spring. Crop Protection, 98, 16–23.

Clements, D.R., Larsen, T. & Grenz, J. (2016) Knotweed management strategies in North America with the advent of widespread hybrid Bohemian knotweed, regional differences, and the potential for bio-control via the psyllid Aplhalara itadori Shinji. Invasive Plant Science and Management, 9, 60–70.

D’Hertefeldt, T., Eneström, J.M. & Pettersson, L.B. (2014) Geographic and habitat origin influence biomass production and storage translocation in the clonal plant Agropodium podagraria. PLoS One, 9, e85407.

Elemans, M. (2004) Light, nutrients and the growth of herbaceous forest species. Acta Oecologica, 26, 197–202.

Gustavsson, A.D. (1997) Growth and generative capacity of plants of Cirsium arvense. Weed Research, 37, 229–236.

Håkansson, S. (1969a) Experiments with Sonchus arvensis. In: Proceedings of Clone-2000 - Ecology and Evolutionary Biology of Clonal Plants, Presented at the Clone-2000. Springer, Obergurigl, Austria, pp. 125–140.

Ringselle, B., Bergkvist, G., Aronsson, H. & Andersson, L. (2016) Importance of timing and repetition of stubble cultivation for post-harvest control of Elymus repens. Weed Research, 56, 41–49.

Tavaziva, V.J. (2012) Effects of competition on compensation point and phenological development in Sonchus arvensis. Acta Oecologica, 35, 35–95.

Tavaziva, V., Lundkvist, A. & Verwijst, T. (2018) Assessment of the chemical and non-chemical management of couch grass (Elymus repens). Agronomy, 10, 1178.

Thomsen, M., Mangerud, K., Riley, H. & Brandsæter, L.O. (2015) Method, timing and duration of bare fallow for the control of Cirsium arvense and other creeping perennials. Crop Protection, 77, 31–37.

Tørrøsen, K.S., Fykse, H., Rafoss, T. & Gerowitt, B. (2020) Autumn growth of three perennial weeds at high latitude benefits from climate change. Global Change Biology, 26, 2561–2572.

van Evert, F.K., Cockburn, M., Beniers, J.E. & Latsch, R. (2020) Weekly defoliation controls, but does not kill broad-leaved dock ( Rumex obtusifolius ). Weed Research, 60, 161–170.

Vermij, T., Tavaziva, V. & Lundkvist, A. (2018) Assessment of the compensation point of Cirsium arvense and effects of competition, root weight and burial depth on below-ground dry weight-leaf stage trajectories. Weed Research, 58, 292–303.

Westra, P. & Wyse, D. (1981) Growth and development of quackgrass (Agropyron repens) biotypes. Weed science, 29, 44–52.

White, A.C., Rogers, A., Rees, M. & Osborne, C.P. (2016) How can we make plants grow faster? A source–sink perspective on growth rate. Journal of Experimental Botany, 67, 31–45.

Neuteboom, J.H. (1981) Effect of different mowing regimes on the growth and development of four clones of couch (Elytrigia repens (L.) Desv., syn. Agropyron repens (L.) Beauv.) in monocultures and in mixtures with perennial ryegrass ( Lolium perenne (L.) Lam.). Landbouwhogeschool Wageningen, 81, 1–26.

Nkurunziza, L. & Streibig, J.C. (2011) Carbohydrate dynamics in roots and rhizomes of Cirsium arvense and Tussilago farfara. Weed Research, 51, 461–468.

Price, E.A., Gamble, R., Williams, G.G. & Marshall, C. (2002) Seasonal patterns of partitioning and remobilization of 14 C in the invasive rhizomatous perennial Japanese knotweed ( Fallopia japonica (Houtt.) Ronse Decraene). In: Proceedings of Clone-2000 - Ecology and Evolutionary Biology of Clonal Plants, Presented at the Clone-2000. Springer, Obergurigl, Austria, pp. 125–140.

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