Environmental and Experimental Botany 205 (2023) 105147

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

# Why is root sprouting not more common among plants? Phytohormonal clues and ecological correlates

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ARTICLE INFO

*Keywords:*
Auxin/cytokinin ratio
Buds
Carbohydrate storage
Disturbance
Growth
Phytohormones

ABSTRACT

Root sprouting (RS) species can regenerate from even small root fragments. The root buds are usually well protected against disturbance because they are deep in the soil, and injury oftentimes boosts root sprouting. Despite these obvious advantages, only 10% of plants exhibit RS ability. Are there specific ecophysiological barriers to RS ability? We set up a controlled experiment with ten congeneric pairs of herbs differing in RS ability and exposed them to severe aboveground biomass removal and assessed how RS and non-RS species differ in biomass production, root nitrogen and phosphorus content, and root tissue carbohydrate concentrations and whether phytohormone profiles explain variation in RS ability. No differences were observed in regenerated biomass three months after biomass removal, although RS species had lower root dry matter content, lower root nitrogen content, higher soluble sugar content, and a lower auxin-to-cytokinin ratio than non-RS species. RS and non-RS herbs differed in root tissue carbohydrate concentrations, which suggests that RS species, apart from having RS ability, might be better prepared for disturbance due to the availability of stored energy and carbon. Presumably, the key barrier to the more frequent occurrence of RS ability in the herbaceous plants studied here is a low auxin-to-cytokinin ratio, which is necessary to induce RS but is likely non-existent in most plants in order to avoid the risk of developmental deformities.

## 1. Introduction

Plant growth and related architecture are based either on the addition of new leafy axes to the existing plant structure (mostly in trees and shrubs or herbs from nonseasonal environments) or the replacement of old leafy axes with new ones (in herbs from seasonal environments) (Klimešová, 2018). The leafy axes predominantly grow from axillary buds on stems, and the patterns of their formation are so regular and predictable that they can be described by architectural models (Hallé et al., 1978; Krumbiegel, 1998; Klimešová and Klimeš, 2008). Alternatively, new leafy axes can be produced by roots, where they grow from adventitious buds; this ability is called root sprouting (RS) (Fig. 1) (Groff and Kaplan, 1988). These two basic methods of constructing a plant, namely, axillary bud sprouting and root sprouting, naturally occur at different frequencies. RS is much less common and evolutionarily independent from axillary bud sprouting (Herben and Klimešová, 2020; Bartušková et al., 2021).

The growth of leafy axes – new shoots – from either axillary or root buds and the localization of those buds along a plant are not only responsible for plant architecture, including a variety of growth forms from nonclonal to several clonal habits (Klimešová, 2018), but also determine vulnerability or resistance to disturbance (Pausas et al., 2018; Ott et al., 2019). RS species are considered better adapted to disturbance (Iwasa and Kubo, 1997; Suzuki and Stuffer, 1999; Vesk and Westoby, 2004; Ottaviani et al., 2020; Bartušková et al., 2021) because of their ability to regenerate from small fragments of the root, which is a typical feature of the most noxious weeds of arable land (Klimešová and Martínková, 2022). Additionally, root buds are usually located deeper in the soil than axillary buds on rhizome or stem bases, and RS species typically exhibit greater survival than non-RS species following disturbances that disrupt deeper soil profiles (Ott et al., 2019). Moreover, regeneration from adventitious root buds is more vigorous than that

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https://doi.org/10.1016/j.envexpbot.2022.105147
Received 14 July 2022; Received in revised form 25 October 2022; Accepted 14 November 2022
Available online 17 November 2022

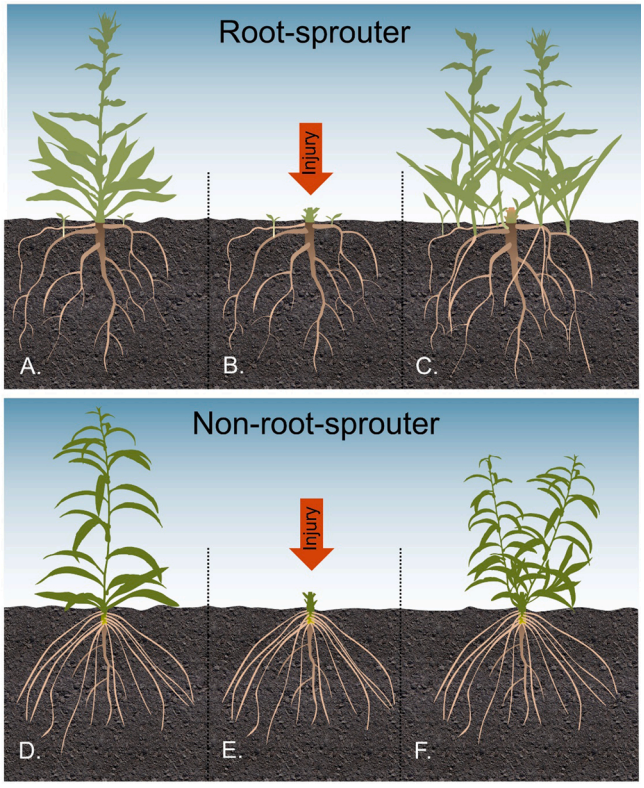


**Fig. 1.** Example of a plant with the ability of root sprouting – a root-sprouter (A, B, C) – and a plant without this ability: a non-root-sprouter (D, E, F). Root-sprouters can grow new shoots from both axillary buds on stems and from adventitious buds on roots, while non-root sprouters can grow only from axillary stem buds. After severe removal of aboveground biomass (B. and E.), root-sprouters can regenerate from both adventitious buds on roots and preserved axillary buds (C), while non-root-sprouters can only regenerate from preserved axillary buds (F.). Root-sprouters are considered better adapted to disturbance. First, root buds are usually located deeper in the soil than axillary buds and thus are better protected from disturbance. Second, root-sprouters are not limited by the number of buds available for regeneration because root buds can be formed anywhere on a root system and not only in nodes. Additionally, sprouters can regenerate even from small root fragments.

from axillary buds, and RS ability is frequently induced by biomass removal (Palacio et al., 2007; Martínková et al., 2016a; b; Bartušková et al., 2021). Despite the obvious advantages of RS under disturbance, RS ability is found in only approximately 10% of Central European plants (Bartušková et al., 2021), which is probably due to ecophysiological barriers to RS ability.

We can speculate that the evolution of these two architectures – based on axillary versus root buds – probably does not require complex genomic changes because even within one genus, close relatives differ in RS ability (Palacio et al., 2007; Martínková et al., 2016a; b; Bartušková et al., 2021). However, there are likely mechanisms that prevent RS ability in order to avoid plant growth deformities and ensure that a plant is branching according to the rules, i.e., from axillary buds on the stem. We hypothesize that such a simple but strictly controlled mechanism might be based on a phytohormonal imbalance that occurs either intrinsically or is induced by injury, and a low auxin-to-cytokinin ratio (aux/CK ratio) might be a trigger of RS ability (Winton, 1968; Wolter, 1968; Guo et al., 2017). The aux/CK ratio has long been known to govern the fate of callus cell cultures and to drive the differentiation of callus cells into plant organs, especially roots (Skoog and Miller, 1957; Schaller et al., 2015). A relatively narrow range of ratio values and total concentrations of auxin and cytokinin are responsible for plant organ differentiation (Wicaksono et al., 2021). A relatively high concentration of auxin leads to the differentiation of roots, a relatively high concentration of cytokinin leads to the differentiation of shoots, and intermediate values support the development of both roots and shoots, but only at certain absolute concentrations of phytohormones (Skoog and Miller, 1957; Schaller et al., 2015).

However, phytohormones affect the route of differentiation only in receptive tissues, such as callus tissue in cell cultures, and whether these results for the aux/CK ratio can also be applied to roots has not been determined. Two crucial conditions must be met to resolve this issue. First, root tissue must be similar to callus tissue, which is sensitive to phytohormone signals; and second, this tissue must possess a source of undifferentiated cells available for differentiation to shoots or roots. These two conditions might be fulfilled by the lateral cambium in a secondarily thickened root (Bartušková et al., 2021), pericycle (Kerstetter and Hake, 1997) or callus tissue formed on a wounded root (Rauh, 1937). The idea that lateral cambium is the most suitable receptive tissue is supported by the fact that most root-sprouting plants are eudicots capable of secondary thickening, while other taxonomical groups that do not have secondary growth largely lack this ability (Bartušková et al., 2021). Several researchers have suggested that a low aux/CK ratio might trigger RS ability (Winton, 1968; Wolter, 1968; Guo et al., 2017), although this trigger has been experimentally confirmed in only one study thus far. RS *Inula britannica* from the *Asteraceae* family was proven to have a lower aux/CK ratio than non-RS *Inula salicina,* and injury to the plant body further decreased the ratio; however, the injury was less important than intrinsic phytohormonal profiles alone (Martínková et al., 2022).

Plants with axillary bud-derived architecture (non-root-sprouting species, non-RS) and root-derived architecture (root-sprouting species, RS) visually resemble each other. Both species groups similarly protect their buds against disturbance by placing organs bearing them (i.e., roots, stem bases and rhizomes) belowground. This strategy increases the probability of survival and consequential successful regeneration after disturbance in both groups (Pausas et al., 2018; Ott et al., 2019). However, the species are also different in several aspects. First, unlike non-RS species, RS species are not limited by the number of buds available for regeneration because root buds can be formed anywhere on a root system, not only in nodes (Benot et al., 2010; Cornelissen et al., 2014; Herben and Klimešová, 2020). Second, nonclonal species with RS ability can regenerate even after the loss of all aboveground biomass or body fragmentation, while non-RS nonclonal species do not exhibit this capability (Martínková et al., 2006; Martínková and Klimešová, 2016a). A third difference is the ontogenetic forwardness of clonal RS species. While rhizomes are frequently formed during the second growing season (Klimešová and Klimeš, 2008), adventitious buds and root-borne shoots appear a few months after germination (Martínková et al., 2016b, 2021). Therefore, RS species can potentially survive severe disturbances that occur very early in life because they are not ontogenetically limited by bud formation (Martínková et al., 2021).

Although RS seems to be a very convenient ability under disturbance, the growth strategies and physiological responses of RS versus non-RS species to disturbance are largely unknown. For successful vegetative regeneration after disturbance, in addition to proper architecture and available buds, plants also need sufficient carbohydrate reserves for regrowth (Iwasa and Kubo, 1997; Klimešová, 2018; Lubbe et al., 2021). The more available reserves in storage organs, the higher the probability of survival. Similar to the abovementioned ontogenetic forwardness of root buds, storage in roots might develop earlier than storage in rhizomes because the root is already present while the rhizome is not yet formed. Therefore, not only the total content of reserves but also the ontogenetic limitations of carbohydrate storage formation might play a role in successful regeneration. The ontogenetic readiness to disturbance, which might provide a consequential competitive advantage in the form of biomass production, seems to favour RS species. However, the ability to survive disturbance might be influenced by the content of carbohydrates that are soluble in water because only soluble

carbohydrates are easily transportable from storage organs and provide quickly available energy for shoot growth (Lubbe et al., 2021). Regeneration might be further limited by nutrient availability because nitrogen and phosphorus are necessary for the construction of new biomass (Wise and Abrahamson, 2008). Therefore, it is important to determine whether carbohydrate and nutrient contents differ between RS and non-RS species and how these differences might contribute to their different life-history strategies.

We aimed to (i) describe the growth of RS species versus non-RS species in early ontogeny and their response to severe disturbance and (ii) analyse whether phytohormones might be responsible for the presence of RS in plants. We set up a pot experiment with ten congeneric pairs of closely related herbs differing in RS ability and exposed them to severe biomass removal. We asked how injury to RS and non-RS species affects biomass production and allocation, nitrogen and phosphorus content in root biomass, root tissue carbohydrate concentrations and phytohormone profiles. We expect higher regenerated biomass, a higher R:S ratio, higher nitrogen and phosphorus content, higher root tissue carbohydrate concentrations, and a lower aux/CK ratio in RS than in non-RS species.

## 2. Methods

### 2.1. Selection of species and germination

To test the effects of injury on biomass parameters, carbohydrate reserves, and phytohormonal profiles of root-sprouting (RS) versus non-root-sprouting (non-RS) herbs, we set up a pot greenhouse experiment in 2019. For the experiment, we acquired congeneric pairs of species, with one RS and one non-RS species from the same genus, to minimize the effects of phylogeny on the observed plant behaviour. Data on RS ability were obtained from the Clo-Pla database (Klimešová and de Bello, 2009). According to the seeds available from the commercial supplier Planta Naturalis (Markvartice u Sobotky, Czech Republic, 50.4286017 N, 15.1989097 E), we preselected 21 genera and 73 species of predominantly common Central European perennial dicotyledonous herbs.

Seeds of the preselected species were sown separately by species on sterilized wet sand in Petri dishes and were kept under wet–cold stratification in a refrigerator (dark, 3 °C) in March 2019. After one month of stratification, the Petri dishes were transferred to a growth chamber (day: 23 °C for 15 h, night: 16 °C for 9 h), where they germinated under high-pressure sodium lamps. After one week of germination, we selected 10 congeneric pairs (Table 1) that exhibited sufficient germination so that one RS and one non-RS species could be obtained for each genus.

In mid-April 2019, 5-day-old seedlings were transplanted from the Petri dishes to 2.2 L pots filled with sand and garden loam substrate at a 3:2 vol ratio. We planted one seedling per pot and used 27 pots per species. Immediately after transplantation, the 540 pots were placed in the unheated greenhouse without artificial light at the Institute of Botany, Třeboň, Czech Republic (49.0057336 N, 14.7724625 E).

### 2.2. Experimental setup

The pots in the greenhouse were randomly assigned to three groups with nine replicates per species. One group represented injury treatment (INJURY). Two other groups of pots represented nondisturbed controls, with the first harvested at the time of injury in the beginning of July 2019 (C. July) to quantify plant traits at the time of disturbance. The second control group was harvested at the same time as the injured group of plants between the end of September and the beginning of October 2019 (C. Sept), and this control was used for comparison with injured plants. The injury treatment represented the removal of all aboveground biomass 1 cm above the soil surface. Such severity of disturbance is common in human-affected habitats that are usually inhabited by RS species. Additionally, the injury severity was selected to mimic a severe disturbance that leaves axillary buds of non-RS species intact and allows their survival. A standard liquid NPK nutrition solution (0.5/0.1/0.07 g N, P, K per litre of substrate) was regularly added, and the plants were watered with tap water throughout the experiment. The level of nutrients was set to ensure that plants would not be limited by nutrients during their regrowth after the injury, similar to human-affected habitats.

**Table 1**
List of species used in the experiment. RS – root-sprouting species (in bold), non-RS – non-root-sprouting species (from Clo-Pla Database, Klimešová and de Bello, 2009); S – species spontaneously forming root buds and root-borne shoots, and R – species that form root buds and root-borne shoots only after aboveground biomass removal (from Bartušková et al., 2017), clonal – clonal species, non-clonal polycarpic – nonclonal perennial species (including root-splitters) reproducing more than once per life, biennial – biennial species forming vegetative rosettes during the first year of life and reproducing and dying the next year (from Clo-Pla Database, Klimešová and de Bello, 2009).

| Species name | Family | RS/ non-RS | Type of RS | Growth form |
|---|---|---|---|---|
| ***Inula britannica*** | ***Asteraceae*** | **RS** | **S** | **clonal** |
| *Inula salicina* | *Asteraceae* | non-RS | – | clonal |
| ***Achillea nobilis*** | ***Asteraceae*** | **RS** | **S** | **clonal** |
| *Achillea millefolium* | *Asteraceae* | non-RS | – | clonal |
| ***Artemisia campestris*** | ***Asteraceae*** | **RS** | **S** | **nonclonal polycarpic** |
| *Artemisia absinthium* | *Asteraceae* | non-RS | – | clonal |
| ***Senecio jacobaea*** | ***Asteraceae*** | **RS** | **S** | **clonal** |
| *Senecio erraticus* | *Asteraceae* | non-RS | – | biennial |
| ***Pilosella officinarum*** | ***Asteraceae*** | **RS** | **S** | **clonal** |
| *Pilosella lactucella* | *Asteraceae* | non-RS | – | clonal |
| ***Centaurea jacea*** | ***Asteraceae*** | **RS** | **S** | **clonal** |
| *Centaurea pseudophyringa* | *Asteraceae* | non-RS | – | clonal |
| ***Plantago media*** | ***Plantaginaceae*** | **RS** | **S** | **nonclonal polycarpic** |
| *Plantago maritima* | *Plantaginaceae* | non-RS | – | nonclonal polycarpic |
| ***Silene vulgaris*** | ***Caryophyllaceae*** | **RS** | **R** | **nonclonal polycarpic** |
| *Silene gallica* | *Caryophyllaceae* | non-RS | – | nonclonal polycarpic |
| ***Trifolium repens*** | ***Fabaceae*** | **RS** | **R** | **clonal** |
| *Trifolium pratense* | *Fabaceae* | non-RS | – | nonclonal polycarpic |
| ***Hypericum perforatum*** | ***Hypericaceae*** | **RS** | **S** | **clonal** |
| *Hypericum maculatum* | *Hypericaceae* | non-RS | – | clonal |

### 2.3. Biomass analysis

During harvests, the aboveground and belowground (root) biomass of plants was separated. The aboveground biomass of each plant was dried at 50 °C to constant weight and weighed. The belowground biomass of six replicates (from the total of nine replicates) per species and treatment was separated into three representative parts. One was used to analyse the carbohydrate concentrations (see the "Carbohydrate analysis" section), one was used to analyse root tissue nitrogen and phosphorus content, and one was used to determine root tissue dry matter content (RDMC, dried at 50 °C). The belowground biomass of the other three replicates (from a total of nine replicates) was used for the analysis of phytohormones (see the "Phytohormone analysis" section). The nitrogen (N) and phosphorus (P) contents in dry belowground biomass (dried at 50 °C) were analysed colorimetrically after acid

digestion in diluted samples by an automatic FIAstar 5010 Analyser (Tecator, Sweden; for all analytical details, see Adamec, 2002), and the N:P mass ratio (N:P ratio) was calculated. The dry belowground biomass of each representative part was summed per replication, the root-to-shoot ratio (R:S ratio) for each plant was calculated, and the dead aboveground biomass was excluded.

### 2.4. Carbohydrate analysis

Immediately after washing off the cultivation substrate, the belowground biomass for carbohydrate analysis was placed in cryovials and deep-frozen in liquid nitrogen. After several minutes in liquid nitrogen, the cryovials were transferred to a − 80 °C freezer, where they were stored for several days until lyophilization and tissue homogenization by an oscillating mill (Retsch MM 400). In some cases, samples per species and harvest were pooled from several individuals due to a low amount of root biomass. To determine the ethanol-soluble carbohydrates (mono-, di- and oligosaccharides, mainly glucose, fructose, saccharose, raffinose) and sugar alcohols (myo-inositol, sorbitol, mannitol), approximately 0.1 g of each homogenized tissue was taken as the ground biomass per sample. The samples were extracted with a boiling ethanol-water mixture (80:20, v/v) and centrifuged for 10 min at 3000 rpm. The extraction and centrifugation procedures were repeated two times. The collected supernatants were dried, dissolved in distilled water, filtered through a nylon microfilter (0.45 μm), and analysed. The ethanol-soluble carbohydrates were assessed using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD, Dionex ICS-3000 system) (Klimešová et al., 2019). For the separation of individual carbohydrates, we performed a gradient elution using 16 mM NaOH and 200 mM NaOH as the mobile phase and a Dionex CarboPac PA1 column (4 ×250 mm, 10 μm).

The sediments from the ethanol-soluble carbohydrate extractions and centrifugations were used for starch analysis (Klimešová et al., 2019). The starch content was determined using the total starch assay procedure, namely, the Association of Official Agriculture Chemists (AOAC) Method 996.11 and American Association of Cereal Chemists (AACC) Method 76–13.01 developed by Megazyme Ltd. (www.megazyme.com). The sediments were hydrolysed using thermostable α-amylase and amyloglucosidase. Glucose, as the product of enzymatic hydrolysis, was coloured using GOD/POD (glucose oxidase/peroxidase) reagent, and the absorbance (at 510 nm) was measured by spectrophotometry (Shimadzu UV-1800 Spectrophotometer).

Fructans were determined using the fructan assay procedure based on AOAC Method 999.03 and AACC Method 32–32.01 (www.megazyme.com). From a sample aliquot (0.1 g homogenized root tissue), fructans were extracted with boiling water. For the removal of sucrose, starch, and reducing sugars, specific enzymes and alkaline borohydride were applied. FOS (fructo-oligosaccharides), fructans, and reduced FOS were specifically hydrolysed by exo- and endo-inulinase and endo-levanase to glucose and fructose, which were measured using the PAHBAH (p-hydroxybenzoic acid hydrazide) reducing sugar method. The absorbance of the samples (at 410 nm) was measured by spectrophotometry.

In the statistical analyses (see “Statistical analyses” below), carbohydrate concentration, expressed in % of dry biomass, was analysed either as the sum of all carbohydrate concentrations (total carbohydrates) or as carbohydrate functional groups: transport sugars, starch, and other reserves, where individual carbohydrates were summed per functional group (for classification of carbohydrate functional groups, see Table S1).

### 2.5. Phytohormone analysis

Plant hormones were determined in roots as previously described (Prerostová et al., 2021). Three replicates per species and treatment were used for each analysis. Immediately after washing off the substrate, root samples were flash-frozen by immersion in liquid nitrogen and then lyophilized. Phytohormones were extracted from the lyophilized material (an aliquot of 10–20 mg) with 50 μL of cold extraction solvent (1 M formic acid), and isotopically labelled standards (10 pmol/sample) were added to each sample: [$^{13}C_6$]IAA, [$^{2}H_4$]OxIAA, and [$^{2}H_4$]OxIAA-GE (Cambridge Isotope Laboratories); [$^{2}H_4$]SA (Sigma–Aldrich); [$^{2}H_6$]ABA, [$^{2}H_3$]PA and [$^{2}H_3$]DPA (NRC-PBI); and [$^{2}H_2$]GA19, [$^{2}H_5$]JA, [$^{2}H_5$]*tZ*, [2 $H_5$]*tZR*, [$^{2}$H5]*tZRMP*, [$^{2}H_5$]*tZ7G*, [$^{2}$H5]*tZ9G*, [$^{2}H_5$]*tZOG*, [$^{2}H_5$]*tZROG*, [$^{15}N_4$]*cZ*, [$^{2}H_3$]DZ, [$^{2}H_3$]DZR, [$^{2}H_3$]DZ9G, [$^{2}H_3$]DZRMP, [$^{2}$H7]DZOG, [$^{2}H_6$]iP, [$^{2}H_6$]iPR, [$^{2}H_6$]iP7G, [$^{2}H_6$]iP9G, and [$^{2}H_6$]iPRMP (Olchemim). Samples were homogenized with zirconia beads (1.5 mm diameter) in a FastPrep-24TM 5 G Instrument (MP Biomedicals, Eswege, Germany) for 40 s at 6 m/s. After centrifugation (4 °C, 30,000 x *g*), the supernatants were collected and applied to SPE Oasis HLB 96-well column plates (10 mg/well; Waters, Milford, MA, USA) activated with 100 μL methanol and then eluted with 100 μL 50% acetonitrile using a Pressure+ 96 manifold (Biotage, Uppsala, Sweden). The pellets were re-extracted in 100 μL portions of 50% acetonitrile, centrifuged, and reapplied again to the column plates.

Phytohormones in each eluate (injected in technical duplicates) were separated on a Kinetex EVO C18 column (2.6 μm, 150 ×2.1 mm, Phenomenex, Torrance, CA, USA). The mobile phases consisted of A) 5 mM ammonium acetate in water and B) 95:5 acetonitrile/water (v/v). The following gradient program was applied: 5% B at 0 min, 7% B from 0.1 to 5 min, 10–35% from 5.1 to 12 min, 100% B from 13 to 14 min, and 5% B at 14.1 min. A hormone analysis was performed using an LC–MS system consisting of a UHPLC 1290 Infinity II (Agilent, Santa Clara, CA, USA) and 6495 Triple Quadrupole mass spectrometer (Agilent). Mass spectrometric analysis was performed in multiple reaction monitoring mode using the isotope dilution method. Data acquisition and processing were performed using Mass Hunter software B.08 (Agilent).

The phytohormone content was calculated as the content (in picomoles) per g dry weight of belowground biomass. For the statistical analyses, all individual contents of phytohormones analysed were combined into six functional groups: auxins, cytokinins, ABA-types, gibberellins, jasmonates, and phenolics (see Table S2 for classification of phytohormones into functional groups). The ratio of auxin to cytokinin was calculated as the ratio of the contents of the functional group of auxins and the functional group of cytokinins.

### 2.6. Statistical analysis

We used a Bayesian linear mixed-effect model to evaluate the response of root-sprouting and non-root-sprouting plants to injury in terms of mortality, biomass production and allocation, nitrogen and phosphorus content in root biomass, carbohydrate concentrations, and phytohormones.

(A) First, we analysed the main responses of species: the R:S ratio, N:P ratio, total carbohydrate concentrations (see the “Carbohydrate analysis” section), and aux/CK ratio (see the “Phytohormones analysis” section).

(B) Second, to obtain better insights into the response of experimental plants, we additionally explored the compounds of previously analysed ratios and constituents of total carbohydrates. Therefore, for biomass, we analysed the belowground and aboveground biomass, N and P content, and RDMC. For carbohydrates, we analysed the transport sugars, starch, and other carbohydrate functional groups (for classification of carbohydrate functional groups, see Table S1), and for phytohormones, we analysed the auxins, cytokinins, ABA, gibberellins, jasmonates, and phenolics functional groups (for the classification of phytohormones into functional groups, see Table S2).

Each of the traits was analysed separately. Treatments (C. July, C. Sept, INJURY), species root sprouting ability (RS versus non-RS), and treatment and species RS ability interactions were used as predictors. We controlled for the hierarchical structure of the data by adding the random effect of congeneric pairs. Therefore, each trait (*Resp*) was

modelled:

$$Resp \sim Normal(\alpha + \beta_1 * RS + \beta_2 * Treatment + \beta_3 * RS * Treatment + Pair, \sigma)$$

$$Pair \sim Normal(0, \varphi)$$

where *RS* is a binary variable denoting RS versus non-RS species, *Treatment* is a dummy variable coding the treatment, *Pair* denotes congeneric pairs of species, and $\alpha$, $\beta$, $\sigma$ and $\varphi$ are parameters of the model. All responses except RDMC were log-transformed before the analyses since they had highly skewed distributions. Responses were standardized to 0 mean and standard deviation 1 afterwards. All parameters had a normal prior distribution with a mean of 0 and standard deviation of 5. Zero-centered priors slightly reduce posterior distributions towards zero, thereby weakening multiple testing problems (Gelman and Tuerlinckx, 2000). The prior predictive check was performed to ensure that priors were only weakly informative for all treatments (Gelman et al., 2020). The model was fitted using a no-U-turn sampler (Hoffman and Gelman, 2014) with 4 chains, each with 4000 iterations, with half of them as a warm-up. Convergence was checked using R-hat statistics (lower than 1.01 in all cases). The model was written in Stan (Carpenter et al., 2017) using the package rstan (version 2.21.2; Stan Development Team, 2020) in R (version 4.1.0; R Core Team, 2021).

## 3. Results

Injury caused mortality in 40 individuals from eight of the twenty species. Sixteen individuals died among the three RS species. Twenty-four individuals died among the five non-RS species. In three species, injury caused mortality of all or nearly all individuals (RS *Pilosella officinarum,* non-RS *Pilosella lactucella* and non-RS *Silene gallica*). In the control noninjury treatments, mortality was low, and only five individuals did not survive in either noninjury treatment (Fig. 2).

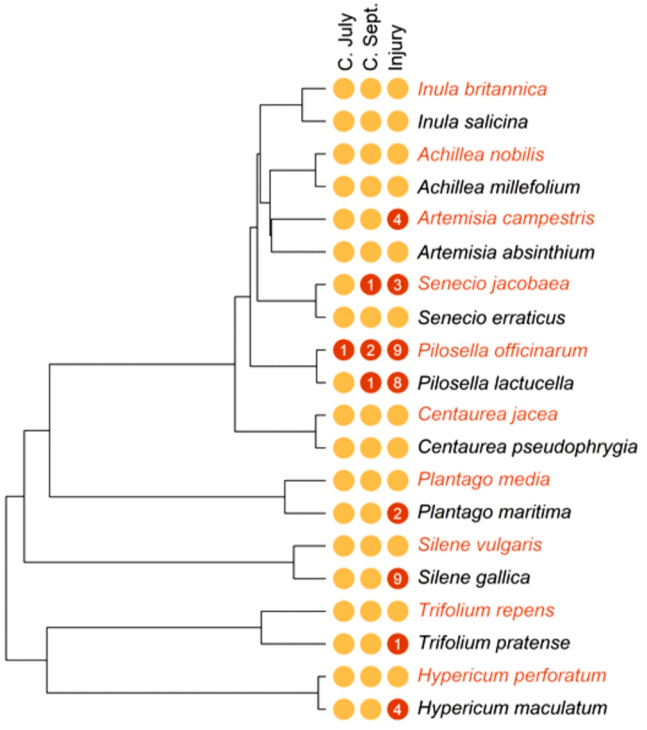


**Fig. 2.** Phylogenetic tree of the species used in the experiment (from Durka and Michalski, 2012). Root sprouters are shown in red, and non-root sprouters are in black. Numbers in circles denote the number of plants that died in each treatment (the total number of replicates per treatment was nine). Orange circles indicate no mortality. C. July – control plants in July, C. Sept. – control plants in September, Injury – injured plants (injury applied in July, plants analysed in September).

Next, we evaluated the effect of root sprouting ability and ontogeny on the measured traits. Early in ontogeny (at the time of the injury in July, Control July), RS plants had greater aboveground and belowground biomass, lower P content, higher starch and transport carbohydrate concentrations and a lower concentration of auxin than non-RS plants (Table 2, Figs. 3, 4, 5). At the end of the season (Control September, C. Sept), the RS plants had larger belowground biomass, lower nitrogen and starch concentrations, and higher concentrations of other carbohydrate reserves than non-RS plants (Table 2, Figs. 3, 4, 5). An interaction between the ability to resprout from roots and ontogeny was found for the concentration of carbohydrates (Table 3): starch and transport sugars accumulated faster in RS than in non-RS species (Fig. 5).

Second, the effects of root sprouting ability and injury on measured traits were evaluated three months after injury. Injured RS species had

**Table 2**
Results of the main (A.) and additional analyses (B.) of differences in trait values between root sprouting and non-root sprouting in the three treatments. C. July – control plants in July, C. Sept. – control plants in September, Injury – injured plants (injury applied in July, plants analysed in September). Positive values denote higher trait values in root-sprouters than in non-root-sprouters. The first column shows the values of parameter β1. The second column is calculated as β1 + β3_September, and the third is calculated as β1 + β3_Injury. For details on the model, see the Statistical methods. Posterior means and 95% credible intervals are shown. Intervals not overlapping zero (in bold) indicate the difference in trait values between root-sprouting and non-root-sprouting species. R:S ratio – belowground to aboveground biomass ratio; N:P ratio – nitrogen to phosphorus ratio; aux/CK – auxin-to-cytokinin ratio; RDMC – dry matter content of belowground (root) biomass.

| Trait | C. July | C. Sept. | Injury |
|---|---|---|---|
| A. Main analyses | | | |
| R:S ratio | 0.080 [-0.070, 0.228] | 0.104 [-0.044, 0.252] | 0.032 [-0.140, 0.199] |
| N:P ratio | 0.255 [-0.034, 0.545] | -0.098 [-0.389, 0.186] | -0.108 [-0.440, 0.221] |
| Carbohydrates | -0.156 [-0.406, 0.095] | -0.018 [-0.275, 0.232] | -0.250 [-0.529, 0.028] |
| Aux/CK ratio | -0.340 [-0.743, 0.066] | -0.315 [-0.689, 0.050] | **-0.480 [-0.931, -0.040]** |
| A. Additional analyses | | | |
| Biomass | | | |
| Aboveground biomass | **0.258 [0.064, 0.444]** | 0.184 [-0.011, 0.376] | 0.133 [-0.087, 0.348] |
| Belowground biomass | **0.236 [0.070, 0.405]** | **0.192 [0.025, 0.359]** | 0.128 [-0.065, 0.319] |
| RDMC | -0.065 [-0.244, 0.120] | 0.029 [-0.158, 0.211] | **-0.264 [-0.473, -0.049]** |
| N content | -0.007 [-0.243, 0.232] | **-0.287 [-0.526, -0.038]** | -0.112 [-0.389, 0.163] |
| P content | **-0.272 [-0.521, -0.019]** | -0.256 [-0.513, 0.002] | -0.023 [-0.321, 0.279] |
| Carbohydrates | | | |
| Transport sugars | **0.713 [0.476, 0.955]** | 0.228 [-0.013, 0.468] | 0.256 [-0.009, 0.509] |
| Starch | **0.685 [0.452, 0.920]** | **-0.277 [-0.520, -0.043]** | -0.207 [-0.466, 0.053] |
| Other reserves | -0.037 [-0.203, 0.129] | **0.173 [0.003, 0.339]** | -0.050 [-0.229, 0.130] |
| Phytohormones | | | |
| Auxins | **-0.583 [-1.107, −0.055]** | -0.204 [-0.697, 0.284] | -0.083 [-0.653, 0.507] |
| Cytokinins | -0.012 [-0.410, 0.397] | 0.206 [-0.147, 0.567] | **0.476 [0.042, 0.917]** |
| ABA-types | 0.119 [-0.361, 0.598] | 0.289 [-0.139, 0.722] | 0.067 [-0.451, 0.596] |
| Gibberellins | 0.125 [-0.431, 0.668] | 0.382 [-0.098, 0.867] | **0.630 [0.030, 1.213]** |
| Jasmonates | -0.143 [-0.614, 0.346] | 0.367 [-0.073, 0.792] | 0.118 [-0.404, 0.645] |
| Phenolics | -0.185 [-0.650, 0.283] | -0.182 [-0.595, 0.237] | -0.422 [-0.932, 0.095] |

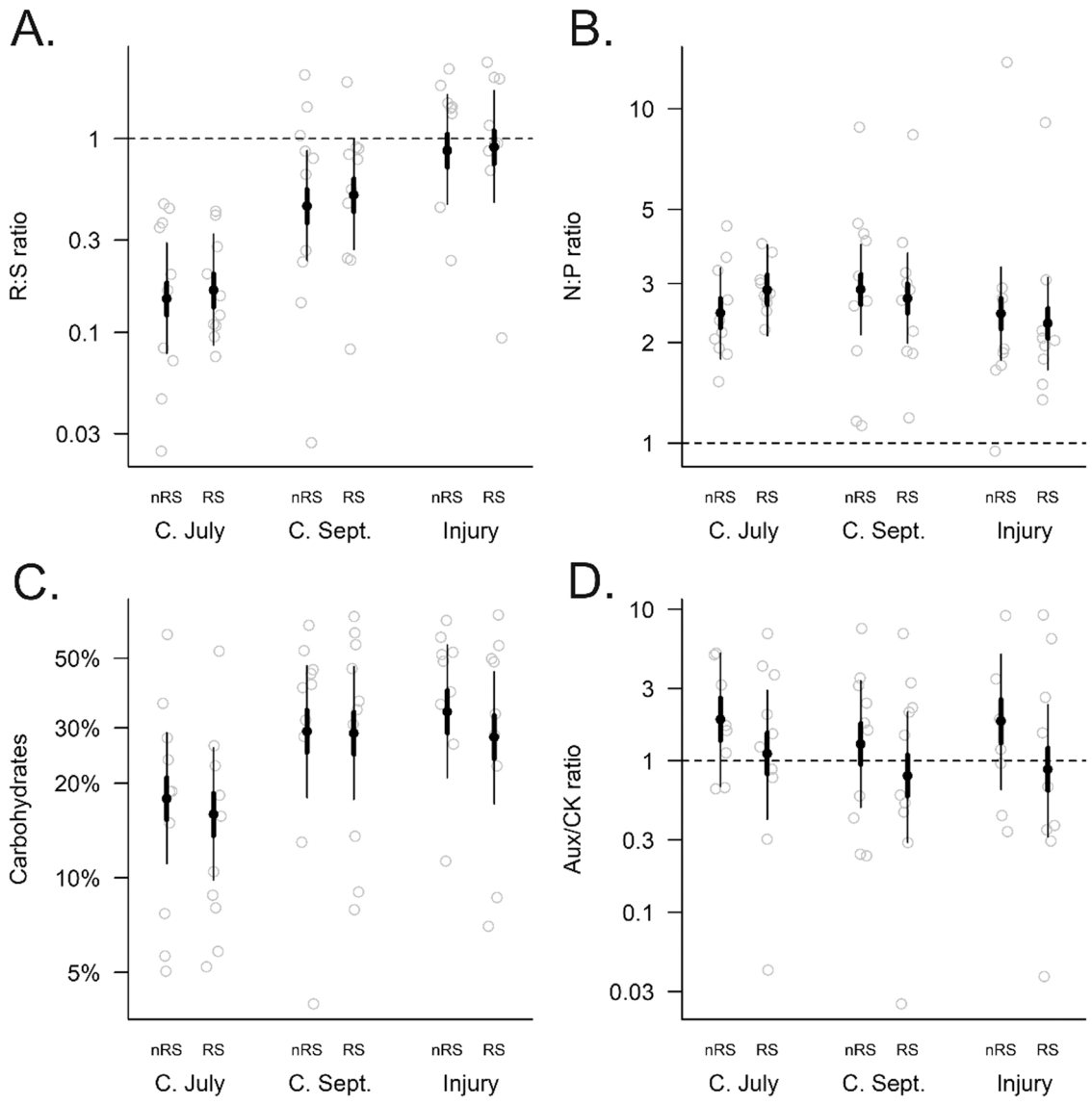


**Fig. 3.** Main growth, storage and phytohormone measures of the control and injured root-sprouter and non-root-sprouter plants. (A.) R:S ratio – belowground to aboveground biomass ratio; (B.) N:P ratio – nitrogen to phosphorus ratio; (C.) Carbohydrates – the total content of root tissue carbohydrate concentrations expressed as a percentage of belowground biomass weight; (D.) aux/CK – auxin-to-cytokinin ratio, RS – root-sprouting species, nRS – non-root-sprouting species, C. July – control plants in July, C. Sept. – control plants in September, Injury – injured plants (injury applied in July, and plants analysed in September). Thick lines denote 50% credible intervals, thin lines denote 95% intervals, and the midpoint is the posterior mean. Empty points show mean values per species. For the statistical results, see Tables 2 & 3.

lower root dry matter content (RDMC), lower aux/CK ratio and higher concentrations of cytokinins and gibberellins than non-RS plants (Table 2, Figs. 3, 4, 5, 6). Additionally, injury decreased root dry matter content (RDMC) more in RS than in non-RS plants (Table 3). Other measured traits were not affected.

## 4. Discussion

In the present experimental study analysing the potential advantage of the RS strategy under severe disturbance, we compared the mortality, biomass production, root tissue carbohydrate concentrations and nutrient contents of closely related herbs that differ in root sprouting ability. Mortality in our experiment was low overall and species specific, mostly occurring in injured plants and with similar prevalence among RS and non-RS species. RS species had larger concentrations of carbohydrates in roots as well as larger belowground biomass than non-RS species. Therefore, RS species are likely better prepared for injury at early ontogeny than non-RS species. Despite this advantage, the regeneration after injury measured as aboveground biomass did not differ between RS and non-RS species in our experiment. Concerning phytohormonal profiles of RS and non-RS plants, we have supported the idea that RS ability is facilitated by a low aux/CK ratio. Moreover, we found that gibberellin might also play a crucial role in sprout regrowth from root buds after injury.

Herbaceous species with more buds located belowground are considered to be adapted to more severe disturbances (Klimešová et al., 2018; Pausas et al., 2018; Ott et al., 2019.). Accordingly, we can align herbs into three groups from the least to best adapted: nonclonal non-root-sprouting, clonal with clonal growth organs of stem origin located belowground, e.g., rhizomatous herbs, and clonal and nonclonal root-sprouting herbs (Ott et al., 2019). In RS species, the number of buds is potentially unlimited, while in non-RS species, the number of buds potentially available for regeneration is defined by the number of stem nodes that are present belowground (Benot et al., 2010; Cornelissen

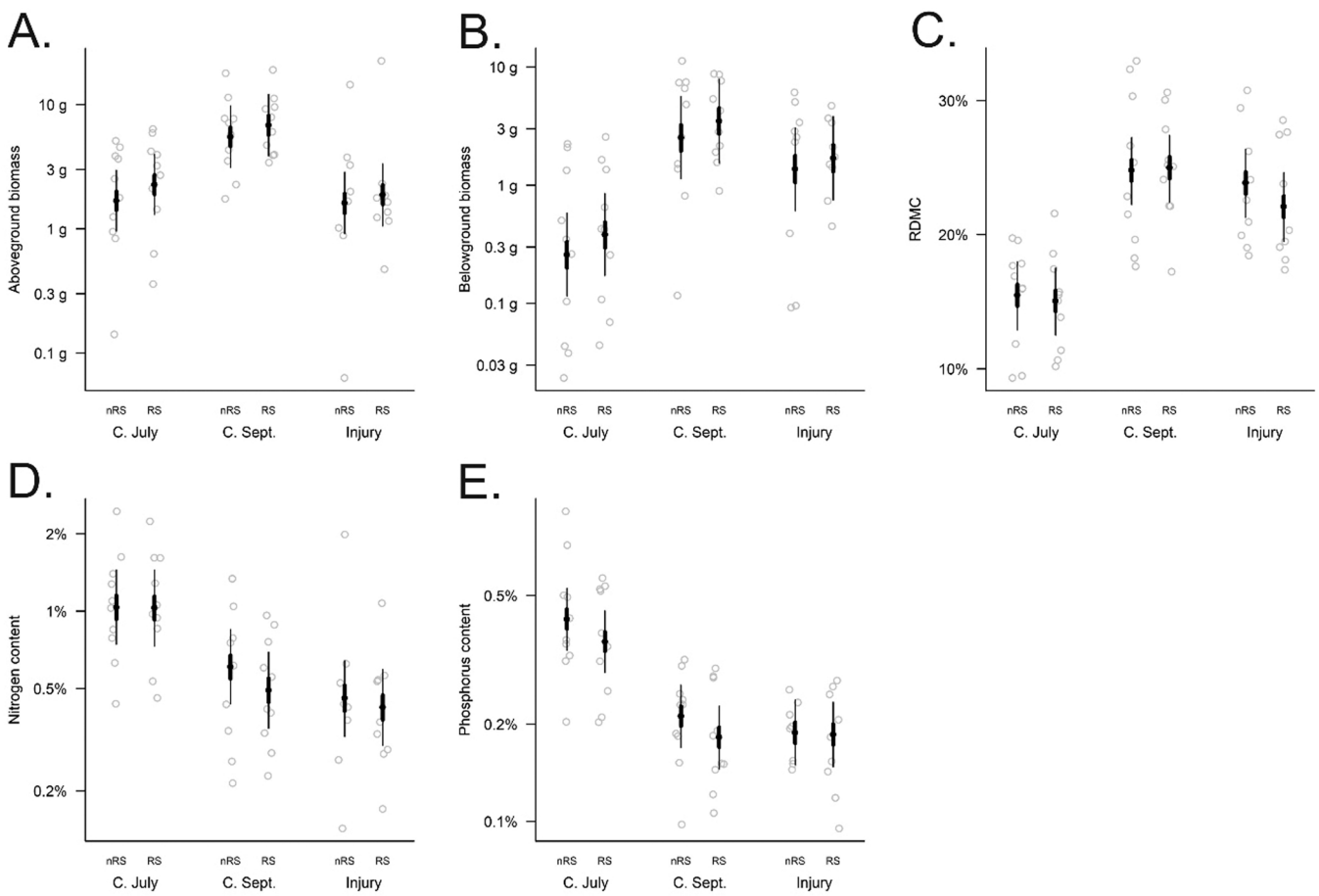


**Fig. 4.** Variability of biomass-related traits of the control and injured root-sprouter and non-root-sprouter plants. (A.) Aboveground biomass; (B.) Belowground biomass; (C.) RDMC – Dry matter content of root biomass; (D.) Nitrogen content – nitrogen content expressed as a percentage of belowground biomass weight; (E.) Phosphorus content – phosphorus content expressed as a percentage of belowground biomass weight. RS – root-sprouting species, nRS – non-root-sprouting species, C. July – control plants in July, C. Sept. – control plants in September, Injury – injured plants (injury applied in July, plants analysed in September). Thick lines denote 50% credible intervals, thin lines denote 95% intervals, and the midpoint is the posterior mean. Empty points are mean values per species. For the statistical results, see Tables 2 & 3.

et al., 2014; Herben and Klimešová, 2020). The formation of belowground organs of stem origin might last from several months to years, which might represent a disadvantage if disturbance occurs early in a plant's life (Klimešová and Klimeš, 2008). RS species are not developmentally limited in this way and can form root buds and regrow from them from the age of a few weeks (Martínková et al., 2016b, 2021). However, in our experiment, we found that RS and non-RS species injured at the age of four months did not differ in mortality or in their compensation of removed aboveground biomass. Therefore, according to our study, RS species are not better at coping with disturbance than non-RS herbs; however, nonclonal herbs were also included in our experimental RS group, which might have decreased the average ability of this group to respond to the disturbance. The reason for this observation might be the type of disturbance applied: namely, removal of all aboveground biomass 1 cm above the topsoil level at the age of four months. Some basal axillary buds were left present after the experimental injury in both groups, and basal axillary buds were as effective for regeneration in non-RS species as adventitious buds on roots in RS species at this plant age. Therefore, basal axillary buds help overcome the "developmental gap" when belowground organs of stem origin bearing buds are not formed yet or do not exist at all, such as in the case of nonclonal species.

RS species invested more in belowground biomass and had lower phosphorus (in June) and nitrogen (in September) contents in roots than non-RS species. Although the outcome of regeneration was the same for RS and non-RS plants at a young plant age, our experimental RS group reacted to injury with a lower RDMC. This suggests intensified growth after injury in this group (Freschet et al., 2010; Reich, 2014). These differences in belowground biomass investment and different reactions to injury probably point to different growth and acquisition strategies of both groups and thus possibly indicate that RS species are better prepared for severe aboveground biomass removal. However, the advantage of RS over non-RS species under disturbance further depends on the disturbance severity: namely, whether basal axillary buds are left intact or not. Our experiment was designed to mimic a severe disturbance that would not compromise the regeneration of non-RS plants. Therefore, we were not able to obtain results in favour of RS species, which we would probably obtain after removal of all axillary buds.

In addition to the presence of belowground buds, carbohydrate storage also plays a key role in biomass regeneration because of an apparent positive relationship among available carbohydrates, successful regeneration, and regenerated biomass (Iwasa and Kubo, 1997; Klimešová, 2018; Lubbe et al., 2021). While total carbohydrate content expresses the amount of potentially available energy for biochemical processes, the proportions of carbohydrate functional groups in tissues suggest the actual metabolic status of a plant and the species' ecophysiological strategy (Slewinsky and Braun, 2010). There are three main functional groups of carbohydrates: transport sugars (e.g., glucose and fructose) represent small simple soluble molecules that are easily transportable among organs to quickly provide energy. Starch is a larger

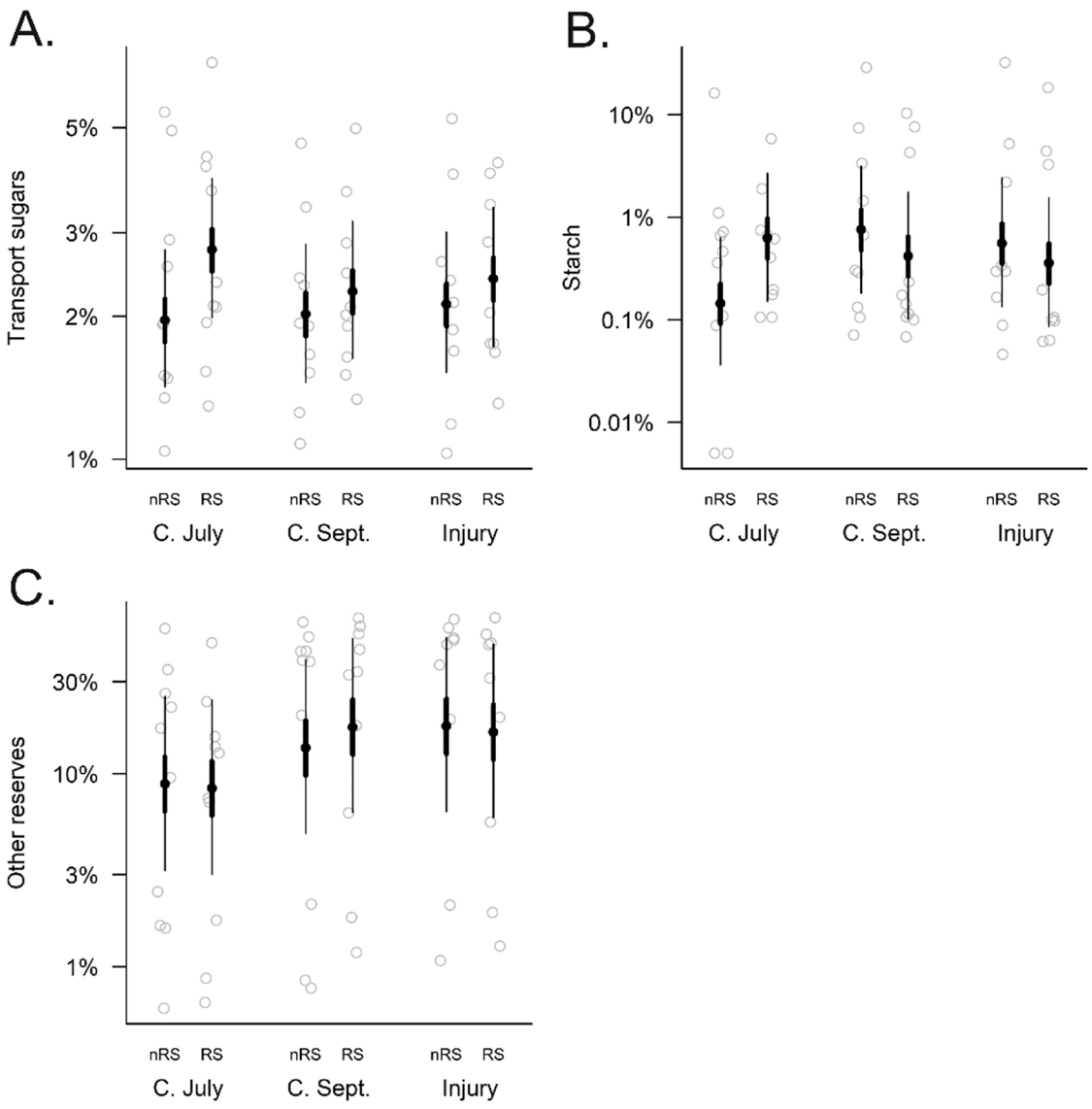


**Fig. 5.** Variability of root tissue carbohydrate concentrations of the control and injured root-sprouter and non-root-sprouter plants. (A.) Transport sugars; (B.) Starch; (C.) Other reserves – other carbohydrate concentrations. All contents are expressed as a percentage of belowground biomass weight. RS – root-sprouting species, nRS – non-root-sprouting species, C. July – control plants in July, C. Sept. – control plants in September, Injury – injured plants (injury applied in July, plants analysed in September). Thick lines denote 50% credible intervals, thin lines denote 95% intervals, and the midpoint is the posterior mean. Empty points are mean values per species. For the statistical results, see Tables 2 & 3.

and the most common insoluble storage molecule: it is not a standby carbohydrate and must be broken down back into monosaccharides to obtain energy, thus serving for "longer-term" storage. Finally, the group of other reserves represents soluble oligo- and polysaccharide molecules, such as fructans, galactose or raffinose. Although these molecules represent the major reserve carbohydrates in approximately 15% of flowering plant species (Hendry, 1993; Vijn and Smeekens, 1999; Van den Ende, 2013) (e.g., fructans in the *Asteraceae* family and raffinose in the *Plantaginaceae* family, Janeček et al., 2011), they are mostly responsible for osmotic regulation and act as signalling compounds under stress (Van den Ende, 2013; Moraes, 2016).

In our experiment, contrary to our expectations, injury did not affect total carbohydrate concentrations in either group, although a decrease in reserves due to aboveground biomass rebuilding could be expected (Iwasa and Kubo, 1997). This result probably suggests preferential rapid rebuilding of total reserves at the expense of photosynthetic biomass regrowth because even though injured plants had the same carbohydrate concentrations as control plants of the same age, they do not completely compensate for their aboveground biomass, even three months after injury. This "backup" storage might imply adaptation of plants to repeated disturbances (Janeček et al., 2015). Reduced use of stored carbohydrates might also be due to growth limitation by other factors, for example, by nutrients (Wise et al., 2008). Although RS species showed reduced root tissue concentrations of phosphorus and nitrogen during ontogeny, the level of added nutrients was maintained at a sufficiently high level in the experiment, and we do not expect that this could affect the regeneration of plants after injury.

Carbohydrates differ in their solubility, often in relation to phylogeny. In our study, we removed the effect of phylogeny by using very close relatives in pairs of RS and non-RS species; however, we still found different strategies of RS and non-RS in terms of carbohydrate functional group concentrations. The content of transport and other soluble sugars was higher in RS species, while starch was higher in non-RS species. Although other sugars, such as fructans, must also be metabolically broken down into monosaccharides similar to starch, the breakdown to monosaccharides is faster, and they are easier to use because they are

**Table 3**
Results of the main (A.) and additional analyses (B.) of the effects of root sprouting (RS) status on changes in trait values during growth and on the trait reaction to injury. The first column shows differences with age, with the positive values denoting a greater increase of a trait value from July to September in root-sprouting species than in non-root-sprouting species, whereas the negative values indicate the opposite (only non-injured control plants were tested; tested parameter of the model: β3_September; for details on the model, see Statistical methods). The second column shows differences in trait reaction to injury, with positive values denoting a higher increase in trait value in root sprouting species than in non-root-sprouting species due to injury and the negative values denoting the opposite (only injured plants were tested here; tested parameter of the model: β3_Injury – β3_September; for details on the model, see Statistical methods). Posterior means and 95% credible intervals are shown. Intervals not overlapping zero (in bold) indicate the difference in trait values between root-sprouting and non-root-sprouting species. R:S ratio – belowground to aboveground biomass ratio; N:P ratio – nitrogen to phosphorus ratio; aux/CK – auxin-to-cytokinin ratio; RDMC – dry matter content of belowground (root) biomass.

| Trait | Effect of being RS on growth | Effect of being RS on the reaction to injury |
|---|---|---|
| A. Main analyses | | |
| R:S ratio | 0.024 [-0.188, 0.235] | -0.072 [-0.298, 0.149] |
| N:P ratio | -0.353 [-0.765, 0.049] | -0.010 [-0.441, 0.428] |
| Carbohydrates | 0.137 [-0.220, 0.489] | -0.232 [-0.611, 0.144] |
| Aux/CK ratio | 0.025 [-0.518, 0.568] | -0.164 [-0.746, 0.419] |
| A. Additional analyses | | |
| Biomass | | |
| Aboveground biomass | -0.074 [-0.340, 0.199] | -0.051 [-0.343, 0.243] |
| Belowground biomass | -0.044 [-0.288, 0.190] | -0.064 [-0.318, 0.182] |
| RDMC | 0.094 [-0.172, 0.351] | **-0.292 [-0.577, -0.016]** |
| N content | -0.280 [-0.623, 0.064] | 0.175 [-0.195, 0.534] |
| P content | 0.016 [-0.350, 0.377] | 0.233 [-0.152, 0.619] |
| Carbohydrates | | |
| Transport sugars | **-0.485 [-0.825, -0.150]** | 0.028 [-0.329, 0.367] |
| Starch | **-0.962 [-1.305, -0.632]** | 0.071 [-0.285, 0.432] |
| Other reserves | 0.210 [-0.024, 0.452] | -0.222 [-0.470, 0.029] |
| Phytohormones | | |
| Auxins | 0.379 [-0.348, 1.086] | 0.121 [-0.624, 0.865] |
| Cytokinins | 0.219 [-0.315, 0.768] | 0.269 [-0.291, 0.828] |
| ABA-types | 0.170 [-0.469, 0.809] | -0.222 [-0.892, 0.445] |
| Gibberellins | 0.257 [-0.470, 0.997] | 0.248 [-0.523, 1.014] |
| Jasmonates | 0.510 [-0.132, 1.134] | -0.249 [-0.909, 0.423] |
| Phenolics | 0.003 [-0.626, 0.623] | -0.240 [-0.888, 0.413] |

soluble and commonly occur in the phloem and xylem (Isejima et al., 1991; Moraes et al., 2016). We can expect that plants with higher contents of standby transport molecules and other sugars at disposal have an advantage in situations when the rebuilding of photosynthetic biomass is probable, which might indicate greater adaptation of RS species for severe biomass removal (Moraes et al., 2016).

The release of buds from dormancy after an injury might be considered a similar process to the disruption or alleviation of apical dominance, and it seems that decreased sugar levels in the buds also play a role (Kebrom, 2017), but the process is not yet well known. Nevertheless, in general, during plant growth and development, dormant axillary buds are initiated, and branching occurs when the effect of auxin decreases and the effect of cytokinins produced mainly in roots prevails (Qiu et al., 2019). The decrease in auxin levels might be caused by the plant size; for example, meristems that produce auxin might be too far from buds responsible for branching, or the production of auxin may cease because growing meristems transform to flowering meristems (Noorden et al., 2006; Cheng and Zhao, 2007). The bud release from dormancy after an injury is similarly related to changes in phytohormones because biomass removal also causes a decrease in basipetal auxin flux from the apical parts and changes the auxin-to-cytokinin ratio (Kebrom, 2017). In our experiment, RS species preferentially regenerated from root buds even though basal axillary buds were left untouched. It seems that root buds either have an advantage in regeneration over axillary buds or are more easily released from dormancy. These behaviours could be related to lower sugar levels in root buds than in axillary buds: Kebrom (2017) suggests that low sugar levels might facilitate interactions with phytohormones and thus control the release of buds from dormancy. However, this supposition requires additional investigation: Bartušková et al. (2017) found the opposite effect and showed that axillary buds were more easily released from dormancy than root buds. Next, we found that the control noninjured RS species produced fewer auxins than the non-RS species but produced more cytokinins when injured; moreover, RS species had significantly lower aux/CK ratios. Lower aux/CK ratios in RS species than in non-RS species were also found by Martínková et al. (2022). In RS species, phytohormonal regulation, i.e., a low aux/CK ratio caused by an increase in cytokinin production, induces preferential regrowth from root buds after injury. Root buds seem to be sensitive to a low aux/CK ratio, and RS ability is connected to the ratio and cytokinin levels. However, the advantage of root buds over axillary buds in regeneration is not clear, although the cost of roots versus axillary buds as well as the number of unlimited root buds might provide an explanation (Vesk and Westoby, 2004; Ott et al., 2019).

Furthermore, we found that RS species produce a higher content of gibberellins in general than non-RS species, and the difference was more significant once injured, which is consistent with the study of Martínková et al. (2022). Gibberellins have been reported to stimulate regrowth after defoliation and are responsible for starch and fructans mobilization (Cai et al., 2016). Although regeneration after injury is based on a complex interplay of phytohormonal regulation and carbohydrate storage, a higher gibberellin content might provide an advantage because carbohydrate reserves are more promptly metabolized and energy for regrowth from buds is quickly available. This finding somewhat supports our hypothesis that RS species are more prepared for situations in which photosynthetic biomass is destroyed and needs to be quickly rebuilt.

## 5. Conclusions

We found differences in growth and acquisition strategies and carbohydrate concentrations between root-sprouting and non-root-sprouting herbs. These differences suggest that RS species are better prepared for severe biomass removal, although this advantage was not fully manifested by regenerated aboveground biomass in our experimental plants. Based on our findings, root spouting ability presumably represents a valuable strategy under disturbance, although it seems that only more severe disturbance that removes all axillary buds would unequivocally favour RS. Moreover, the barrier to the more frequent occurrence of root sprouting appears to be the low aux/CK ratio necessary for triggering RS ability, and such low levels likely do not occur in most plants in order to avoid the risk of body developmental deformities.

## Funding

The research was supported by the Czech Science Foundation GAČR [no. 19–13103S], the Ministry of Education, Youth and Sports of the Czech Republic from the European Regional Development Fund-Project "Centre for Experimental Plant Biology" [no. CZ.02.1.01/0.0/0.0/16_019/0000738], Premium Academiae awarded by the Czech Academy of Sciences, and a long-term research project of the Czech Academy of Sciences [no. RVO 67985939].

## CRediT authorship contribution statement

J. Martínková designed and conducted the experiment, co-formulated the concept, performed the biomass analysis, and wrote the manuscript; J. Klimešová formulated the original idea and cowrote the manuscript; Motyka V, P.I. Dobrev, R. Filepová, A. Gaudinová, and J. Lacek performed phytohormone analyses; A. Klimeš analysed the data; L. Adamec performed the nitrogen and phosphorus analyses; and IM

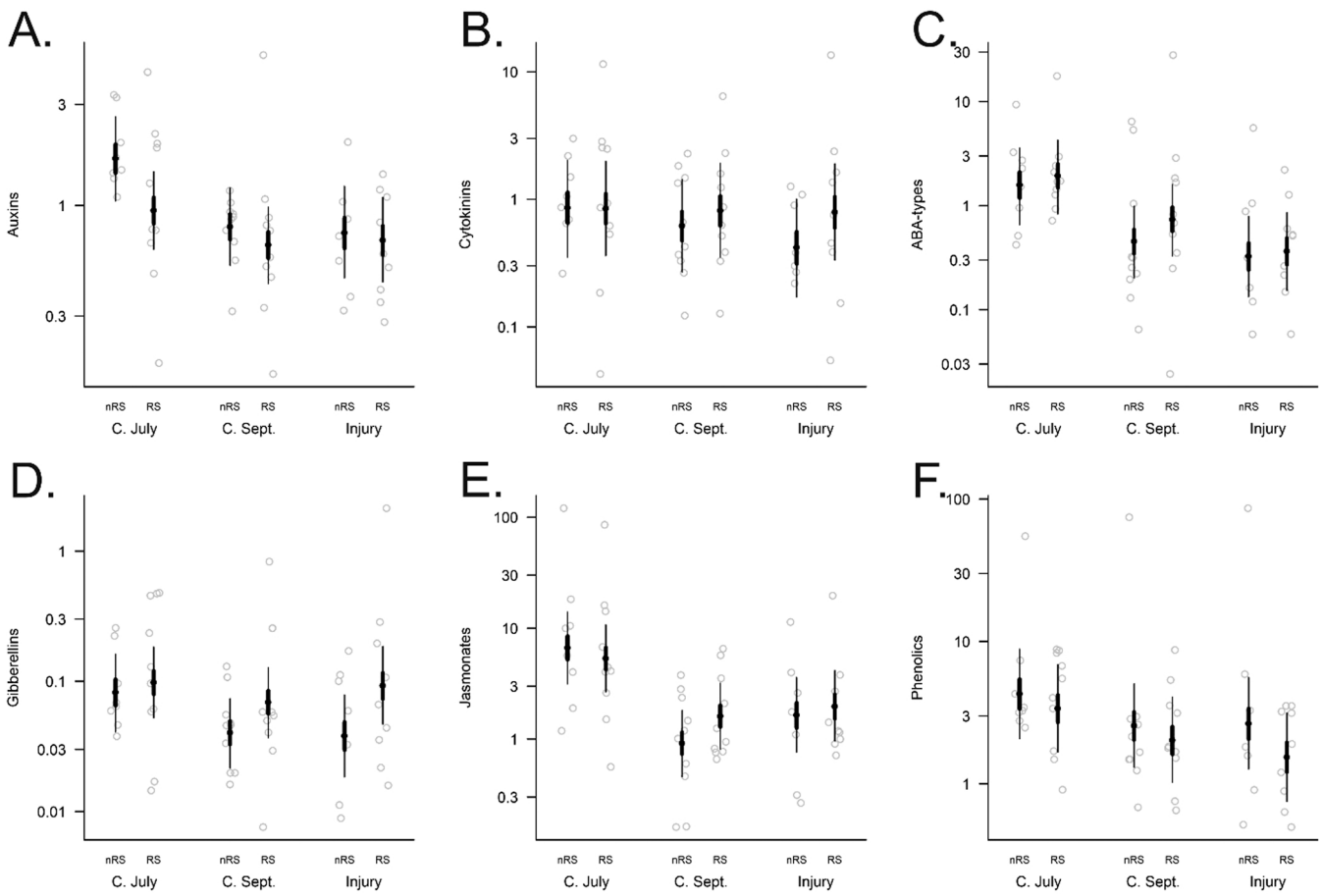


**Fig. 6.** Variability of phytohormone groups of the control and injured root-sprouter and non-root-sprouter plants. (A.) Auxins; (B.) Cytokinins; (C.) ABA types; (D.) Gibberellins; (E.) Jasmonates; (F.) Phenolics. RS – root-sprouting species, nRS – non-root-sprouting species, C. July – control plants in July, C. Sept. – control plants in September, Injury – injured plants (injury applied in July, plants analysed in September). Thick lines denote 50% credible intervals, thin lines denote 95% intervals, and the midpoint is the posterior mean. Empty points are mean values per species. For the statistical results, see Table 2 & Table 3. For phytohormone group descriptions, see Methods and Table S2. All values are in picomoles per g of dry belowground biomass [pmol/g].

performed the carbohydrate analysis.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgement

The authors thank to Šárka Haumerová, Lenka Leštinová and Michael Bartoš for technical assistance with the experiment and to Alena Bartušková for drawing Fig. 1.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2022.105147.

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