

Root sprouting in *Knautia arvensis* (Dipsacaceae): effects of polyploidy, soil origin and nutrient availability

Jana Martínková · Jitka Klimešová · Jiří Doležal · Filip Kolář

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Abstract Plants able to resprout from roots have a potential bud bank that gets initiated after injury to overcome meristem limitation after loss of all stem parts and to facilitate regeneration. *Knautia arvensis* is reportedly able to sprout from its roots on arable land, but information is missing regarding such ability in serpentine populations or how it might differ between diploids and tetraploids. We hypothesized that (1) ‘ruderal’ non-serpentine populations better tolerate severe disturbance than relic, serpentine ones; (2) tetraploid populations resprout more readily than diploids due to enhanced growth of higher ploidy levels; and (3) plants of different ploidy levels from serpentine soils are, for evolutionary reasons, more similar in their response to disturbances than plants from non-serpentine soils. To test these hypotheses, we conducted a pot experiment. Its results do not

support our hypothesis that the ability to sprout from roots is a factor driving the spread of new weedy taxa into central Europe or the hypothesis that it is related to polyploidization in the genus *Knautia*. Both tetraploids and plants from non-serpentine populations regenerated less vigorously than diploids and plants from serpentine populations. However, the genetically closer populations of serpentine origin were more similar in their response to experimental manipulations than their genetically distinct non-serpentine counterparts. The success of non-serpentine taxa in disturbed habitats of central Europe might be related to traits other than the ability to resprout.

Keywords Bud bank · Ploidy · Disturbance · Regeneration · Serpentine · Sprouting

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J. Martínková (✉) · J. Klimešová · J. Doležal
Institute of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, 379 82 Třeboň, Czech Republic
e-mail: jana.martinkova@ibot.cas.cz

F. Kolář
Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 128 01 Prague, Czech Republic

F. Kolář
Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, 252 43 Průhonice, Czech Republic

Introduction

Disturbances shape plant evolution by selecting genotypes that better cope with them, be it through tolerance, resistance or avoidance mechanisms. Strong selective pressures exerted by severe disturbances lead to the evolution of certain traits which can be regarded as typical of heavily disturbed habitats. Good examples are serotiny or lignotubers in flammable ecosystems (Bellingham and Sparrow 2000; Bell 2001). Another trait that has been studied intensively in recent years is the ability to form adventitious buds on roots (Klimešová and Martínková 2004; Malíková

et al. 2010). Plants carrying this trait have a potential bud bank that gets initiated after injury to overcome meristem limitation after severe damage and to facilitate regeneration (Klimešová et al. 2014). Root sprouting is concentrated in some taxonomic groups and missing in others (Rauh 1937), which makes it difficult to assess the evolutionary significance of this trait. Plant species able to resprout from roots have inherited this trait from an ancestor or possess it as an adaptation to past or current conditions. Moreover, the anatomy, morphology and ecology of adventitious sprouting differ depending on the taxonomic group (Rauh 1937; Klimešová and Martínková 2004; Klimešová 2007), so it has probably arisen from independent evolutionary events. Differences in the role of root sprouting also exist among related species, and these differences are particularly related to the plant life cycle (e.g. in members of *Euphorbia* L. and *Linaria* Mill., see Rauh 1937 for descriptions of the genera).

On the intraspecific level, populations are known to differ in their root sprouting ability. This ability can manifest either as survival or biomass compensation, as demonstrated both in the field (Malíková et al. 2010) and under standard experimental conditions (Latzel et al. 2010; 2011). There are also indices that polyploids resprout from roots more readily than diploids (e.g. *Rumex acetosella* L., den Nijs 1983, or *Biscutella laevigata* L., Herben unpubl. data). It is, however, difficult to speculate about the adaptive value of the root sprouting in a species without knowledge of the phylogenetic background of the group under study with regard to its cytotype structure.

Groups of closely related species with known evolutionary history are ideal for studying the functioning of plant traits in an evolutionary context. One such model group is the diploid-tetraploid complex of *Knautia arvensis* (L.) Coulter in Central Europe (Kolář et al. 2009, 2012). The complex has two so-called soil lines, both of which comprise diploids and tetraploids. While serpentine tetraploids are recent derivatives of serpentine diploids, non-serpentine diploids are genetically very distinct and presumably separated from their non-serpentine tetraploid counterparts a long time ago (Kolář et al. 2012). While serpentine populations are a relic lineage surviving for a long time on isolated serpentine outcrops, non-serpentine populations are relatively recent newcomers whose spread has been facilitated by deforestation and farming (Štěpánek

1989; Kaplan 1998; Kolář et al. 2012). Non-serpentine populations nowadays inhabit semi-natural grasslands, where they are subjected to much more intensive agricultural management including ploughing. Their serpentine counterparts occupy stable communities such as open forests and rocky grasslands (e.g. Štěpánek 1997) and exhibit tolerance to extreme chemical properties of serpentine soil (Kolář et al. 2014). *K. arvensis* has been reported to possess the ability to resprout from roots after injury on arable land (Wehsarg 1954), but no information exists on how this ability varies in different soil lines or ploidy levels.

We hypothesize that (1) newcomers to Central Europe, i.e. non-serpentine populations, better tolerate severe disturbances than relic serpentine populations; (2) tetraploid populations resprout more readily due to enhanced growth of higher ploidy levels; and (3) different ploidy levels from serpentine soils are more similar in their response to disturbances than different ploidy levels from non-serpentine populations because serpentine diploids and tetraploids are more closely related than non-serpentine cytotypes.

To test these hypotheses, we conducted a pot experiment on plants of two ploidy levels from nine populations belonging to two ‘soil lines’. These plants were so severely damaged that they could resprout only from roots. Moreover, we cultivated the plants under two nutrient levels because productivity of the environment may substantially affect the response of plants to severe injury (Banta et al. 2010, but see Klimešová et al. 2014).

Materials and methods

Study species

Knautia arvensis (L.) Coulter (Dipsacaceae) is a polymorphic species complex comprising co-occurring diploid and tetraploid cytotypes (Štěpánek 1997; Kolář et al. 2009). Its individuals are perennial hemicryptophytic herbs with a taproot and sympodial stem bases (Štěpánek 1997). They may form underground stems originating in axillary buds and are also able to form root buds, which predominantly serve regeneration after severe disturbances (Klimešová and de Bello 2009). The species is also a recognized weed of arable land (e.g. Wehsarg 1954).

Knautia arvensis is widely distributed throughout Europe. It inhabits dry and mesophilous, often human-influenced grasslands, shrub lands, forest and field margins, road embankments, etc. It is nonetheless also known from several natural habitats with low competition such as open relict forests on serpentine outcrops and subalpine grasslands (Štěpánek 1997; Kaplan 1998; Vange et al. 2004). Its semi-natural and semi-ruderal habitats contrast in nutrient availability as well as the probability and severity of disturbance events.

Experimental design

Seeds of nine diploid and tetraploid populations of *K. arvensis* growing on serpentine and non-serpentine soil were collected during 2008 in the Czech Republic on localities where populations of *K. arvensis* had already been studied by Kolář (2009, 2012). Four populations were diploid, five populations were tetraploid, five populations originated from serpentine soil, and four populations were of non-serpentine origin. For a list of localities and detailed descriptions of populations see Table 3 in Appendix. For the number of population crossed between soil origin and ploidy level see Tables 4 and 5 in Appendix. Seeds were stored in paper bags at room temperature. In late spring of 2010, seeds were germinated in a growth chamber (light 16 h/25 °C, dark 8 h/10 °C) after two months of moist stratification at 5 °C.

Two-day old seedlings were transplanted into 2 l pots filled with sand. The pots were weekly fertilized with a full-strength basic Rorison solution in the high-nutrient treatment and with a 12.5 % dilution of the same solution in the low-nutrient treatment (Hunt et al. 1993). As seeds from all populations germinated over 2 weeks and because germination was very low, 2-day old seedlings were transplanted evenly into pots belonging to each treatment to minimize the effect of different seedling age. Due to uneven germination, the final number of replicates differed among the treatments. The precise numbers of replicates is presented in Tables 4 and 5 in Appendix.

In September 2010, after 12 weeks of growing inside an open unheated glasshouse, plants of each population under both nutrient treatments were randomly assigned to two groups. The first group was the control without injury, and the second group was artificially injured; their entire aboveground biomass with all axillary buds was removed, so the plants could

regenerate only from roots. The biomass removed from the injured plants was sampled, dried and weighed.

Injury survival (yes/no) was recorded at the end of the 2010 growing season. Consequently, all plants were left to overwinter in 2010–2011 in an open-side unheated glasshouse while being continuously supplied with the Rorison solution.

In June of 2011, individuals that overwintered (scored as yes/no) and the number of flower heads per plant were counted; plants were harvested afterwards. Aboveground and belowground biomass of all plants was separated, roots were washed on a sieve, and their biomass was dried and weighed; for a description of all characteristics see Table 1. Compensation of aboveground biomass and compensation of the number of flower heads were calculated; for descriptions of compensation calculations see Table 1.

Statistical analyses

The effects of soil origin, ploidy level, disturbance (injury) and nutrient availability on (A) injury event characteristics—removed biomass and injury survival; (B) growth after injury characteristics—overwintering 2010/2011, aboveground biomass, belowground biomass and the number of flower heads per plant; and (C) compensation characteristics (for a description of calculations see Table 1)—compensation of aboveground biomass and compensation of the number of flower heads were analysed using a generalized mixed-effect model with assumed binomial, Gamma or Poisson distribution of dependent variables. Plant population identity was a random factor whereas soil origin, ploidy level, disturbance and nutrient level were cross-fixed effects. The inclusion of the random effect of plant population identity created a split-plot structure reflecting the dependence of plant individuals coming from particular populations. All the tests were based on the restricted maximum likelihood (REML) approach. The statistical significance of the main effects and interactions were assessed by computing Bayesian highest probability (HPD) intervals using Markov chain Monte Carlo simulations (1000 permutation in each test), as this is favoured over normal confidence limits for GLMMs. The test statistic is approximated by a χ^2 distribution. The analyses were done using the ‘lme4’ (Bates and Maechler 2010), ‘Stat5303libs’ and

Table 1 List of characteristics obtained from the pot experiment with *Knautia arvensis*

Variable	Description
(A) Injury event	
Removed biomass	Amount of removed (destroyed) biomass at the time of injury, dried (g)
Injury survival	If individual survived the injury, recorded at the end of growing season 2010, the same year as injury was applied, yes/no characteristic
(B) Growth after injury	
Overwintering 2010/2011	If individual survived winter 2010/2011, yes/no characteristic
Aboveground biomass	Aboveground biomass at the end of the experiment, dried (g)
Belowground biomass	Belowground biomass at the end of the experiment, dried (g)
Number of flower heads per plant	Overall number of flower heads per plant in 2011, including buds and dry inflorescences
(C) Compensation	
Compensation of aboveground biomass	Ratio of aboveground biomass of the plant survived injury and average aboveground biomass of control plant (both measured at the end of the experiment)
Compensation of the no. of flower heads	Ratio of the number of flower heads per plant survived injury and average number of flower heads of control plant (both measured at the end of the experiment)

A Characteristics related to injury event, *B* characteristics related to growth after injury and *C* calculated compensation characteristics with their descriptions

‘cfdade’ packages in R Development Core Team (2010).

The regression between removed biomass at the time of injury and final aboveground biomass for different soil origins, ploidy levels and nutrient levels as well as the difference between obtained regression lines were evaluated by general linear models (GLM). The test statistic was approximated by F statistics, and both biomass characteristics were log transformed in all cases. The relationship between removed biomass and injury survival under different nutrient levels was tested by generalized linear models (GLZM, Wald statistics) with a binomial distribution followed by a post hoc Tukey HSD test. All GLM and GLZM analyses were done using STATISTICA 8. (StatSoft, Inc. 2007).

Results

Injury survival and the amount of removed biomass

Soil origin, ploidy level and nutrient availability did not have any significant effect on injury survival (Table 2a). Injury survival did not differ either between diploids and tetraploids or between plants of serpentine and non-serpentine origin, and not even between plants cultivated under high and low nutrient availability.

The injury survival was significantly influenced by the amount of removed biomass, which served as a proxy of plant size at the time of disturbance (Fig. 1, GLZM, Gamma distribution: Injury survival Wald stat. = 6.75, d.f. = 1, $p < 0.01$). Plants that survived the injury had significantly higher biomass at the time of injury than plants that did not (Fig. 1). However, the post hoc Tukey HSD test showed this to be true only for plants under high nutrient availability (Fig. 1). Plants grown under the low-nutrient regime which survived the injury did not differ in removed biomass from those which did not survive (Fig. 1).

The amount of removed biomass at the time of injury differed significantly between the high- and low- nutrient treatment (Table 2a; Fig. 2a). Plants cultivated under the high-nutrient regime had a higher amount of removed biomass. The amount of removed biomass was significantly influenced also by the interaction between soil origin and ploidy level (Table 2a; Fig. 2a). Serpentine tetraploids exhibited higher amounts of removed biomass than serpentine diploids, but non-serpentine tetraploids had lower amounts of removed biomass than non-serpentine diploids (Table 2a; Fig. 2a).

Regrowth after injury

Overwintering from autumn 2010 to spring 2011 was significantly influenced only by disturbance

Table 2 Effects of soil origin (serpentine vs non-serpentine), ploidy level (diploid vs tetraploid), disturbance (injured vs control) and nutrient availability (high vs low) on the performance of *Knautia arvensis*

GLMM results are presented with χ^2 statistic values, and corresponding Type I error estimates (p). D.f. = 1 in all cases. Effects of individual predictors (*rows*) and their interactions were examined for each dependent variable (*columns*). Statistical significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. A Characteristics related to injury event B characteristics related to growth after injury C calculated compensation characteristics (for description of calculations see Table 1). For A and C characteristics, statistical analysis only on injured individuals was relevant *n.s.* non significant

(A) Injury event				
	Removed biomass (g) $\chi^2 + p$		Injury survival $\chi^2 + p$	
Soil origin (SO)	0.03n.s.		0.06n.s.	
Ploidy (PL)	0.07n.s.		0.40n.s.	
Nutrients (NU)	45.26***		0.27n.s.	
SO:PL	3.97*		0.66n.s.	
SO:NU	0.53n.s.		0.21n.s.	
PL:NU	0.00n.s.		0.02n.s.	
(B) Growth after injury				
	Overwintering 2010/2011 $\chi^2 + p$	Aboveground biomass (g) $\chi^2 + p$	Belowground biomass (g) $\chi^2 + p$	Number of flowerheads per plant $\chi^2 + p$
Soil origin (SO)	0.24n.s.	0.42n.s.	0.18n.s.	23.99***
Ploidy (PL)	0.74n.s.	10.96***	3.67*	5.08*
Disturbance (DI)	76.57***	37.02***	38.43***	157.14***
Nutrients (NU)	1.88n.s.	74.14***	71.67***	87.42***
SO:PL	2.12n.s.	11.53**	3.76*	0.13n.s.
SO:DI	0.52n.s.	0.01n.s.	1.13n.s.	25.40***
PL:DI	0.82n.s.	0.86n.s.	1.70n.s.	2.11n.s.
SO:NU	1.00n.s.	0.76n.s.	0.19n.s.	9.10**
PL:NU	0.01n.s.	5.83*	2.27n.s.	3.10n.s.
DI:NU	1.66n.s.	12.68***	15.26***	79.99***
(C) Compensation				
	Compensation of aboveground biomass $\chi^2 + p$		Compensation of the number flower heads $\chi^2 + p$	
Soil origin (SO)	0.39n.s.		0.04n.s.	
Ploidy (PL)	2.56n.s.		5.83*	
Nutrients (NU)	0.41n.s.		0.52n.s.	
SO:PL	0.59n.s.		0.90n.s.	
SO:NU	0.61n.s.		0.71n.s.	
PL:NU	2.93n.s.		0.47n.s.	

(Table 2b). Injured plants survived winter less frequently than uninjured ones.

Aboveground biomass as well as belowground biomass was significantly influenced by ploidy level, disturbance and nutrients (Table 2b). Soil origin did not have a significant effect on the amount of aboveground and belowground biomass. The aboveground and belowground biomass was also significantly affected by the interaction between soil origin and ploidy level, by the interaction between disturbance and nutrients and in the case of aboveground biomass also by the interaction between ploidy

and nutrients (Table 2b). Injured individuals and plants cultivated under the low-nutrient level formed lower amount of aboveground and belowground biomass compared to uninjured individuals and individuals under high nutrient availability (Fig. 2b), except injured non-serpentine tetraploids, which formed a higher amount of aboveground biomass under the low-nutrient treatment than under the high nutrient availability (Fig. 2b). Serpentine tetraploids had a higher biomass than non-serpentine tetraploids, and non-serpentine diploids had a higher biomass than serpentine diploids (Fig. 2b), except injured diploids

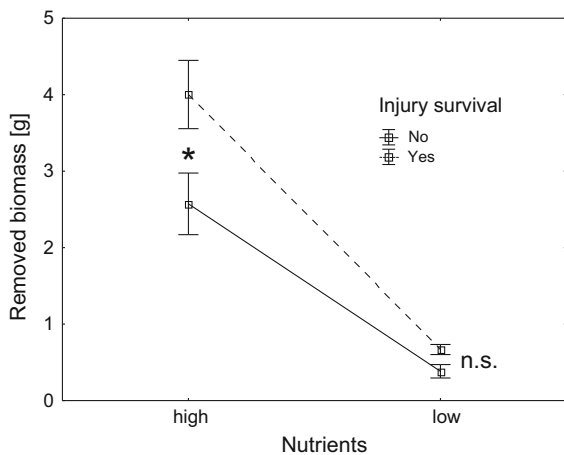


Fig. 1 Relationship between removed biomass (amount of biomass destroyed by injury as a proxy of plant size at the time of injury) and injury survival (yes/no) under two different nutrition levels (high/low). Means and standard errors are shown, * $p < 0.05$; n.s. non significant

cultivated under the high-nutrient treatment where the biomass of non-serpentines was not higher than that of serpentines.

The number of flower heads was influenced by all factors tested, i.e. by soil origin, ploidy level and disturbance, and also by nutrient level (Table 2). The interaction of soil origin and disturbance, soil origin and nutrients and also nutrients and disturbance were found to significantly influence the number of flower heads per plant (Table 2). In uninjured individuals, plants under high nutrient availability and plants of non-serpentine origin had a higher number of flower heads compared to plants cultivated under low nutrient availability and serpentine ones (Fig. 2c). In injured plants, the relationship was not so clear, since the number of flower heads per plant was very low in all cases and most of the plants had no or only one flower head (Fig. 2c).

Compensation

Compensation of aboveground biomass was not affected by any of the factors tested. Soil origin, ploidy level and nutrient did not have any effect on the ability to rebuild the plant body in terms of aboveground biomass after injury (Table 2; Fig. 2d). Only ploidy significantly influenced the compensation of

the number of flower heads per plant. Diploids compensated better for the loss of flower heads than tetraploids (Table 2; Fig. 2e).

Removed vs regenerated biomass

The amount of aboveground biomass at the end of the experiment was found to be positively correlated with biomass formed at the time of injury, i.e. with removed biomass. However, no difference in regression lines among diploids and tetraploids (GLM, d.f. = 1, $F = 0.00$, n.s.), plants of serpentine and non-serpentine origin (GLM, d.f. = 1, $F = 2.53$, n.s.) and plants cultivated under low and high nutrient availability (GLM, d.f. = 1, $F = 0.54$, n.s.) was found.

Discussion

Our study does not support hypothesis No.1 outlined in the introduction that root sprouting ability is a factor driving the spread of new weedy taxa into central Europe. Neither does it support the proposition (hypothesis No. 2) that the ability to sprout from roots is related to polyploidization within the genus *Knautia*. Tetraploids and plants from non-serpentine populations regenerated less vigorously after severe injury, which mimicked agricultural management, than diploids and plants from serpentine populations. On the other hand, their response to the experimental manipulations corresponded well with the genetic proximity of the populations under study (hypothesis No. 3). Specifically, the genetically closer populations of serpentine origin (Kolář et al. 2012) were more similar in their response to experimental manipulations than their genetically distinct non-serpentine counterparts.

Ploidy levels

The virtual ubiquity of polyploidy among the angiosperms (Soltis et al. 2009) indicates that polyploids are evolutionarily advantaged over their diploid ancestors. Indeed, various genetic consequences of polyploidy resulting in higher overall genome flexibility have been suggested as key factors promoting the success of polyploids (Parisod et al. 2010; te Beest et al. 2012). Polyploidization can alter plant morphology, phenology, physiology and ecology within

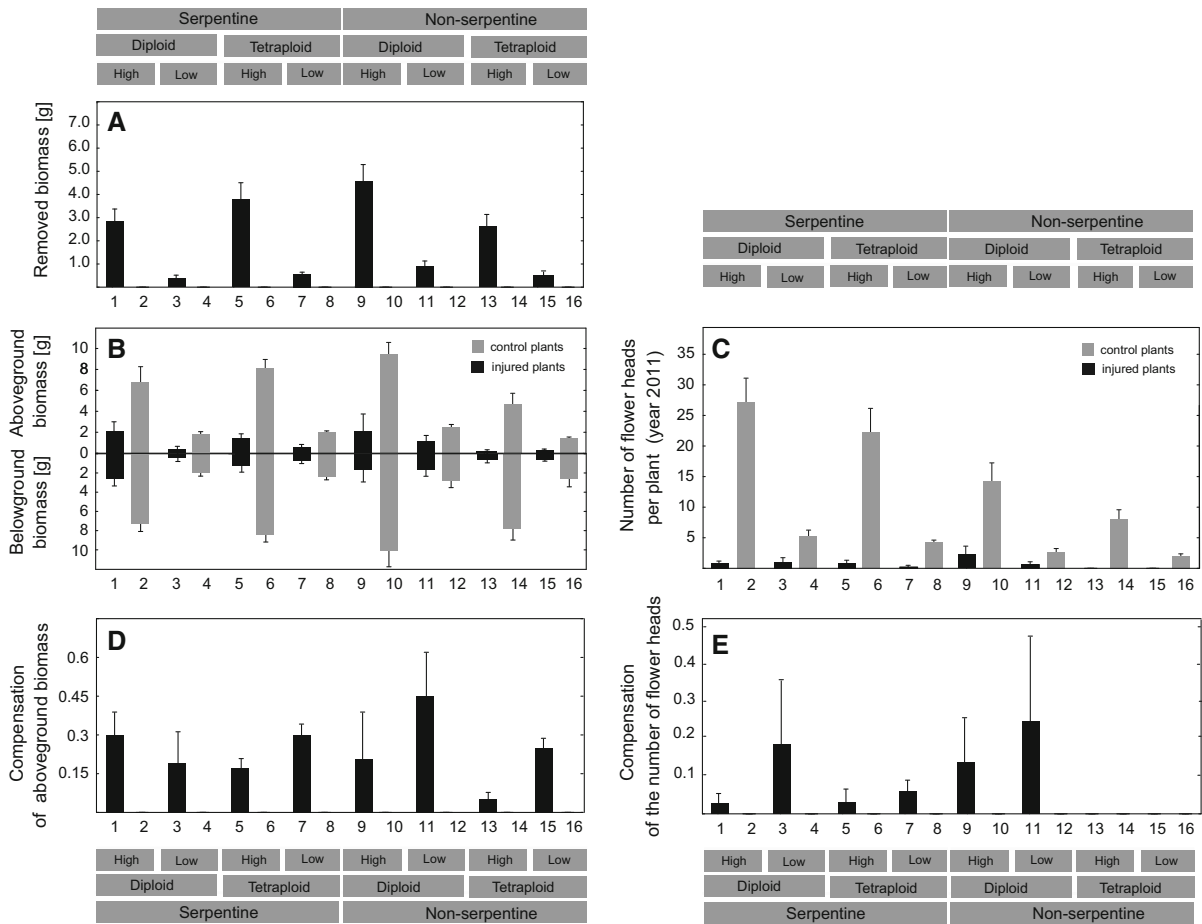


Fig. 2 Characteristics plotted individually for all 16 treatments of three factors: soil origin—serpentine, non-serpentine; ploidy level—diploid, tetraploid, nutrient availability—high, low. Treatments were set hierarchically in the figure. *Black bars* are for injured plants, *grey* for uninjured controls. **a** Removed biomass at the time of injury, i.e. amount of biomass destroyed by injury, **b** above and belowground biomass at the end of the experiment, **c** number of flower heads per plant during the

second year of the experiment, **d** compensation of aboveground biomass; i.e. ratio of aboveground biomass of the regenerated plant to the mean aboveground biomass of control plants, **e** compensation of the number of flower heads, i.e. ratio of the number of flower heads per regenerated plant to the mean number of flower heads of control plants. All values are means with standard errors

only one or a few generations (Levin 1983). Still, evidence that general ecological traits are behind the success of polyploids is lacking. Our study failed to confirm the root sprouting ability as a key trait of *K. arvensis* polyploids. It also did not confirm the usually observed tendency towards better vegetative growth linked to clonality in polyploids, which in some cases contrasts with better generative reproduction in diploids (Černá and Müenzbergová 2013; Henery et al. 2010; Hroudová and Zákavský 1993).

As pointed out by Boalt et al. (2010), when examining tolerance to damage in populations of

different ploidy, it is important to take into account other possibly linked factors such as phenology. As shifts in phenology promote sympatric speciation (Schluter 2001), phenological differences in different ploidy levels are to be expected. Phenological differences are important for herbivores because herbivory is characterized by strongly seasonal patterns. Shifts in phenology are to be expected in the *K. arvensis* complex, which is subject to frequent anthropogenic disturbances. Arable practices provide weeds with opportunities to adjust their phenology and thus avoid damage in their most susceptible stage.

Effect of nutrients

Nutrients were the most decisive factor for the plant response to severe injury because nutrients affected the plants' size. Interestingly, plants grown under low nutrient levels that were able to survive had lower aboveground biomass than plants grown under high nutrient levels that failed to regenerate. This might have been caused by relatively higher investments of such plants into root biomass and carbon stored belowground, which promote biomass regeneration. Results of various experiments differ because experimental conditions probably not always match the nutrient conditions to which species are adapted (compare Martínková et al. 2008; Latzel and Klimešová 2010; Latzel et al. 2010, 2014). Similarly, the nutrient-rich conditions in our experiment probably had a different meaning for serpentine populations adapted to generally nutrient-poor soils (Brady et al. 2005) than for non-serpentine populations often growing on managed meadows or arable land.

Compensatory capacity

Injured plants were much smaller at the end of the experiment than control plants. This contradicts the results of previous experiments conducted on short-lived weedy species, which usually effectively compensated for biomass and seed loss (Lennartsson et al. 1998; Huhta et al. 2000; Martínková et al. 2008; Latzel et al. 2009). The different response of *K. arvensis* might stem from its different life strategy, as it is a polycarpic perennial species (Štěpánek 1997). Non-serpentine diploids grown under low nutrient levels achieved nearly 50 % compensation, which was the best result. Perennials can therefore profit from having the ability to resprout after severe damage only in cases of large-scale disturbances reducing the vigour of all plants, not when only individual plants are injured.

Our results indicate that the ability to sprout from roots after severe injury is important for actual populations in which all plants suffer severe damage at the same time. If uninjured plants are present, their ability to regrow after injury and subsequently reproduce generatively cannot keep pace with the vigour of uninjured plants. Such full-area injury events are frequent on arable land and in other habitats strongly

impacted by man. Under such conditions, root sprouting can be useful not only for already established populations, but also for plants colonizing places devoid of a seed bank.

From the evolutionary point of view, the heritable ability to sprout from roots can help survive rare bottlenecks even though its importance seems to be non-essential for actual populations. The advantage of having this trait increases as *K. arvensis* moves to more anthropogenic habitats, which are becoming more widespread. Occasional disturbances such as grazing or droughts can nevertheless influence even populations in environmentally stable habitats, for example, serpentine outcrops. Indeed, serpentine plants exhibited good regeneration abilities, in some cases even better than semi-ruderal non-serpentine populations. Diploid and tetraploid, serpentine and non-serpentine, and especially relict and anthropogenic populations of *K. arvensis* have set off on different evolutionary paths. The longer they are evolutionarily separated, the more important will their ability to sprout from roots become.

Conclusion

To sum up, the success of non-serpentine taxa in disturbed habitats in central Europe is likely related to traits other than the ability to resprout from roots after injury. These traits possibly differ in plants of distinct evolutionary origin such as non-serpentine diploids vs tetraploids. Our data suggest that the success of *K. arvensis* in unstable; human-influenced environments might be partly responsible for the fact that non-serpentine diploids are able to grow better in both nutrient-rich and nutrient-poor conditions, and possibly to the early flowering of non-serpentine tetraploids (besides other characteristics not addressed in our experiments).

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Appendix

See Tables 3, 4, and 5.

Table 3 Details on populations of *Knautia arvensis* represented in the experiment

Soil type	Ploidy level	Site	Geographic co-ordinates	Site description	Population code	Population code ^b	Genetic group ^a
Serpentine	2×	Borovsko (E. Bohemia, CZ)	49°40'57.7"N, 15°07'49.7"E	Open pine forest	D	S1/263	Relict 2× + 4×
	2×	Staré Ransko (E. Bohemia, CZ)	49°39'04.9"N, 15°48'57.3"E	Coniferous forest margins	B	S2/71	Relict 2× + 4×
	4×	Pluhův Bor (W. Bohemia, CZ)	50°03'01.3"N, 12°46'24.3"E	Open pine forest	G	S3/259	Relict 2× + 4×
	4×	Křížky (W. Bohemia, CZ)	50°03'54.2"N, 12°45'03.6"E	Rock outcrops, semidry grassland	F	S4/260	Relict 2× + 4×
	4×	Dominova Skalka (W. Bohemia, CZ)	50°04'16.7"N, 12°47'8.9"E	Rock outcrops, semidry grassland	H	-/261	Relict 2× + 4×
Non-serpentine	2×	Tvarožná Lhota (S. Moravia, CZ)	48°51'43.6"N, 17°23'23.3"E	Mesophilous meadow	C	NS1/21	Non-relict 2×
	2×	Lajdovci (W. Slovakia, SK)	48°28'29.8"N, 17°38'59.2"E	Dry meadow	E	NS2/63	Non-relict 2×
	4×	Aš (W. Bohemia, CZ)	50°13'12.9"N, 12°13'19.2"E	Abandoned meadow	A	NS3/-	Relict 2× + 4×
	4×	Chanovice (SW. Bohemia, CZ)	49°24'39.0"N, 13°43'55.5"E	Dry meadow	I	NS4/-	Relict 2× + 4×

^a Major genetic grouping according to AFLP genotyping of European *Knautia* (Kolář et al. 2012)

^b Population codes before the slash correspond to Doubková et al. (2011) and (2012), codes after the slash to Kolář et al. (2009)

Table 4 Details on the number of replicates for individual treatments, ploidy levels, soil types and nutrient levels in the pot experiment with *Knautia arvensis*

Total no. of plants	Treatment	No. of plants	Ploidy level	No. of plants	Soil	No. of plants	Nutrients	No. of plants
208	Control	108	4n	76	Serpentine	27	High	12
						Low	15	
					Non-serpentine	49	High	25
			Low	24				
			2n	32	Serpentine	12	High	6
					Low	6		
	Injured	100	4n	74	Serpentine	28	High	13
						Low	15	
					Non-serpentine	46	High	23
			Low	23				
			2n	26	Serpentine	9	High	5
					Low	4		
Non-serpentine	17	High	9					
		Low	8					

Table 5 Number of populations represented in the experiment for each soil origin and ploidy level

Soil origin	Population ploidy level		Total
	Diploid	Tetraploid	
Serpentine	2	3	5
Non-serpentine	2	2	4
Total	4	5	9

References

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