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Cytotypes of *Centaurea stoebe* found to differ in root growth using growth pouches

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Summary

Centaurea stoebe is native to Europe and Western Asia and was introduced into North America in the late 19th century, where it has become highly invasive. In its native range, *C. stoebe* occurs in two cytotypes, namely diploids (2n = 18) and tetraploids (2n = 36), but only the tetraploid form has been identified in the invaded range. We used special growth pouches to determine whether diploid and tetraploid cytotypes from the native range differed in root growth and architecture. We grew seeds from five populations of each cytotype in growth pouches during a period of sixteen days and measured root growth traits both by hand and using a root-scanning software package (WinRHIZO). Tetraploid cytotypes had significantly larger total root length, taproot length, surface area, root volume, above- and below-ground biomass and root to shoot ratios than diploid cytotypes. We suggest that increased early root growth of tetraploid cytotypes as compared with diploids may be one factor that pre-adapted them towards the colonisation of warmer and drier climates in Europe and North America, where tetraploids are currently expanding and invasive respectively.

Keywords: cytotype, root growth, root morphology, biomass, WinRHIZO, biological invasion, spotted knapweed.

Introduction

Centaurea stoebe L. is native to Europe and Western Asia and was introduced to North America *c*. 120 years ago (Roché *et al*., 1986; Treier *et al*., 2009). In its native range, *C. stoebe* occurs in two different cytotypes, namely diploids (2n = 18) and tetraploids (2n = 36), but only the tetraploid form is invasive in North America (Treier *et al*., 2009). The invasion success of *C. stoebe* has been partly explained by the pre-adaptation of tetraploid cytotypes in Europe to drier environments and then further adaptation to drier and warmer conditions in the introduced range (Broennimann *et al*., 2007; Treier *et al*., 2009). Furthermore, the diploids and tetraploids of this species have distinctly different life cycles; the tetraploids have a polycarpic life cycle forming rosettes and bolting in the first year, while diploids have a monocarpic biennial life cycle forming rosettes in the first year and bolting in the second year (Müller, 1989; Ochsmann, 2000). Most studies of the *C. stoebe* cytotypes have focused on aboveground traits for plant performance (Treier *et al*., 2009; Henery *et al*., 2010; Mráz *et al*., 2010) rather than differences in root growth. Larger root growth may confer a competitive

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advantage, particularly in the early stages of invasion, a stage often characterised by establishment failure.

Growth pouches offer an alternative to glasshouse pot experiments as a way to precisely measure and visualise root growth and architecture in the absence of soil. Growth pouches consist of a flat paper wick enclosed in a thin transparent plastic envelope where root growth can be visualised two-dimensionally. This system allows root growth and root architecture to be measured over a period of *c*. 1–3 weeks, depending on the species. No root washing is required, and therefore, there is no loss of fine roots. Furthermore, root-scanning software can be easily used to quantify root area, root volume and other growth characteristics that cannot be measured by hand. Growth pouches and scanning software have been commonly used in studies of agronomic crops such as common bean (Liao *et al*., 2001), but so far, they have not been used in invasion ecology. Root growth parameters between plants grown in growth pouches and plants grown in soil filled pots have been shown to be significantly correlated under glasshouse conditions (Mia *et al*., 1996), indicating that the growth pouch technique is a reliable tool for screening root characteristics.

Here, we use growth pouches to determine how root growth differs between diploid and tetraploid cytotypes of *C. stoebe* from the native range over short timescales. We hypothesise that tetraploid cytotypes will have longer taproots, greater root area, length and volume and greater root to shoot ratios than diploid cytotypes, because of the known trends associated with polyploidy, including increased growth and wider ecological tolerance (Levin, 2002).

Materials and methods

Seed material

Seeds from at least 20 mother plants were collected from five populations of each cytotype (10 populations total, Table 1) across the European range (Austria, Germany, Hungary, Romania and Switzerland) in 2009 and 2010. Within each country, climatically similar diploid and tetraploid population pairs were chosen using an outlier mean index analysis (Treier *et al*., 2009). For each population, we randomly selected 10 mother plants and pooled five seeds per mother plant into a composite seed sample for each population. Composite samples were then germinated in Petri dishes (three Petri dishes per population) with moistened filter paper for 3 days in optimal growing conditions (23°C/16°C, day/night, 50% relative humidity) prior to the start of the experiment.

Experimental design

To visualise the root development, we used seed germination pouches (18 × 16.5 cm, CYG Germination Pouches; Mega International, St. Paul, MN, USA). One seedling was transferred to each growth pouch once the two cotyledons had emerged (3 days after germination), with six replicate pouches per population (60 pouches total). For each of the 10 populations, we chose seedlings that were of similar size by measuring cotyledon length. Each pouch was covered with black poster board and was secured using four paper clips. Pouches were placed randomly in plastic boxes (15 pouches per box) with foam inserts, so that pouches remained upright (Fig. 1).

The pouches were placed in a growth chamber under optimal growing conditions consisting of 23°C/16°C (day/night), 16 h of light and natural humidity. Twenty millilitres of half strength Hoagland's solution (Hoagland's No. 2 basal Salt Mixture H2395; Sigma) was added to each pouch at the start of the experiment. After 4 days, the pouches were checked daily for drying and were topped up with the nutrient solution and double distilled water (2–3 mL) when necessary. In total, for the 16 day experiment, each pouch received 56 mL of the nutrient solution.

Table 1 Population information for the five populations used from each cytotype

Populations	Country	Ploidy*	Origin	Latitude	Longitude	Altitude (m)	Habitat type
SAD	Austria	2x	EU	48.60	15.95	279	Semi-natural grassland
H1	Hungary	2x	EU	46.72	17.77	140	Semi-natural grassland
Alecu2x	Romania	2x	EU	47.11	27.28	110	Natural grassland
DE7	Germany	2x	EU	51.85	12.77	72	Ruderal roadside
CH1basel	Switzerland	2x	EU	47.55	7.64	298	Ruderal railway
SAC	Austria	4x	EU	48.43	15.65	248	Natural dry meadow
Robert4x	Hungary	4x	EU	46.01	18.40	188	Loess grassland with some disturbance
Alecu4x	Romania	4x	EU	46.16	27.37	164	Semi-natural grassland
DE3	Germany	4x	EU	49.42	11.09	329	Ruderal railway
CH1	Switzerland	4x	EU	47.28	8.15	519	Natural dry meadow

*2x and 4x refer to ploidy level.

Fig. 1 Experimental set-up of germination pouches in a growth chamber.

Measurements and analysis

On day four, eight and twelve following transfer to the growth pouches, the length of the taproot and the number of lateral roots were measured. After 16 days, the roots were removed from the pouches for root architecture analysis. The roots were scanned with a black paper background using a Canon scanner (CanoScan Lide 70) with a resolution of 600 pixels. Root analysis was performed using WinRHIZO$_{TM}$ Basic V 2009 c (Regent Instruments, Canada). The parameters evaluated were total root length, root area, mean root diameter, surface area and root volume. After root analysis, the above- and belowground biomass was separated and dried for 48 h at 60°C before being weighed.

Statistical analysis

Statistical analyses were performed using JMP (Version 8.0.2; SAS Institute, Cary, NC, USA). A standard expected mean squares ANOVA was used for all models. ANOVAs including the main effect of cytotype were performed for above- and belowground biomass and all WinRHIZO measurements to determine whether this factor differed systematically. Running the models with and without population as a random effect provided qualitatively similar outcomes and thus was excluded from further analyses. The mean length of lateral roots, the root volume and belowground biomass was log transformed to achieve normality of the residuals.

Results

Root measurements

There was a significant effect of cytotype ($F_{1,49}$ = 10.82, P = 0.0019) for taproot length, where tetraploids had a mean taproot length of 10.40 ± 0.41 cm (mean ± SE) compared with 6.99 ± 0.52 cm for diploids. Both cytotypes produced few lateral roots during the 16-day experiment (4.16 ± 0.43), and there was no significant difference in lateral root number ($F_{1,48}$ = 0.058, P = 0.81) between cytotypes.

Root-scanning measurements

There was a significant effect of cytotype for root surface area ($F_{1,49}$ = 10.50, P < 0.001) (Fig. 2A), root volume ($F_{1,49}$ = 12.58, P < 0.001) (Fig. 2B) and root length ($F_{1,49}$ = 10.50, P = 0.0021) (Fig. 2C), where tetraploids consistently had greater performance than diploids. Root surface area, root volume and root length were also positively correlated with belowground biomass (r^2 = 0.62 $F_{1,49}$ = 83.02, P < 0.001; r^2 = 0.54 $F_{1,49}$ = 59.79, P < 0.001; r^2 = 0.57 $F_{1,49}$ = 66.52, P < 0.001 respectively).

There was a significant effect of cytotype for both above- ($F_{1,49}$ = 9.26, P = 0.0037) and belowground ($F_{1,49}$ = 10.85, P = 0.0018) biomass, where tetraploid plants produced significantly greater above- and belowground biomass than diploids (Fig. 3A,B). Moreover, tetraploids had greater root to shoot ratios than diploid plants ($F_{1,49}$ = 7.54, P = 0.0084, Fig. 3C).

Discussion

In the European range, tetraploids occupy a drier climatic niche than diploids (Broennimann *et al*., 2007; Treier *et al*., 2009) and have been hypothesised to put more energy towards roots (i.e. longer taproots), to better exploit available resources and thus survive in drier habitats (Henery *et al*., 2010). Our results support this hypothesis, as tetraploids had 33% longer taproots than diploids after only 16 days of growth. This response may permit tetraploids to survive drier conditions by allowing plants to access water from deeper in the soil profile than would be tapped during periods of adequate water supply. Plants have been shown to have longer taproots when exposed to dry conditions (Pace *et al*., 1999) and deeper rooted seedlings have been shown to have a greater probability of surviving summer drought than those that are shallow-rooted (Padilla & Pugnaire, 2007).

Tetraploids also had significantly greater total root length and root surface area than diploids, indicating that tetraploids may be much better adapted to low nutrient and moisture conditions. Total root length and root surface have been shown to be relevant traits in models showing plant access to water and minerals in soil (Tinker & Nye, 2000), as increasing root length and area are adjustments of the plants

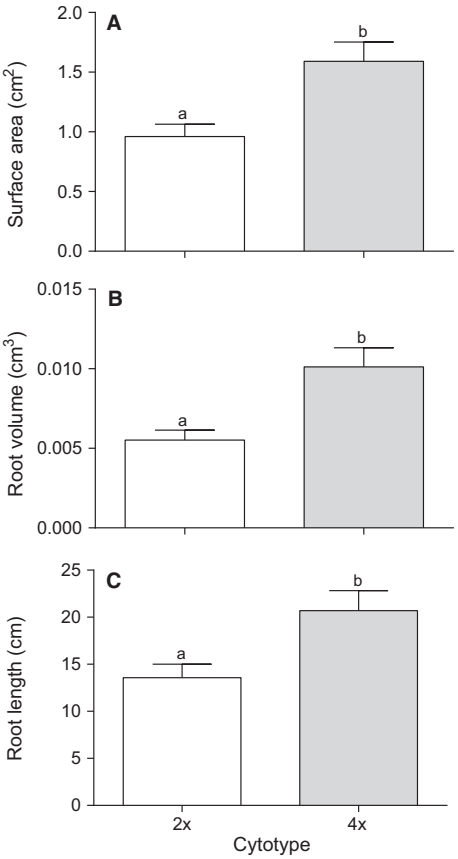

Fig. 2 Means and standard errors (vertical bars) of diploid and tetraploid cytotypes for (A) surface area, (B) root volume and (C) total root length measured using WinRHIZO (cf. text for details). Different letters indicate statistical differences at the level of $P \leq 0.05$ and 2x and 4x refer to ploidy level.

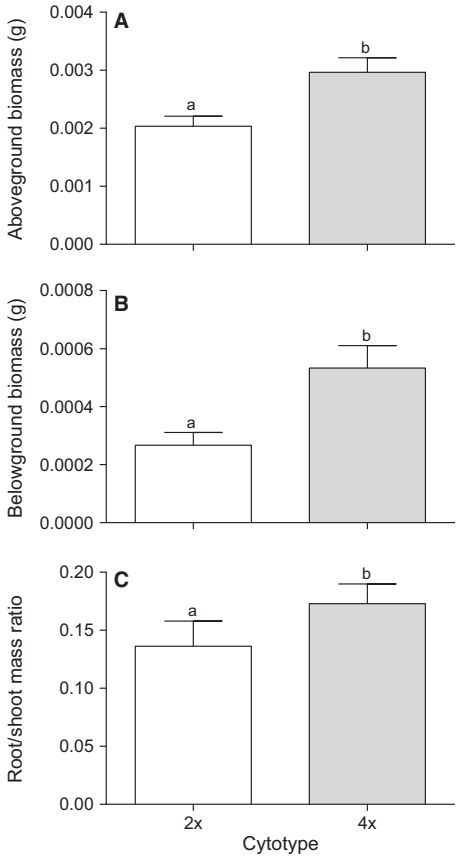

Fig. 3 Means and standard errors (vertical bars) of diploid and tetraploid cytotypes for (A) aboveground biomass, (B) belowground biomass and (C) root/shoot mass ratio. Different letters indicate statistical differences at the level of $P \leq 0.05$ and 2x and 4x refer to ploidy level.

absorptive surfaces to find scarce water resources (Markhart, 1985). Greater root to shoot ratios have also been shown to serve as an adaptation to water-stress (Chapin *et al.*, 1993), and for many plant systems, this response has been shown to have a strong genetic component (e.g. Dhanda *et al.*, 2004). Our result of greater root to shoot ratios among tetraploids is consistent with a previous glasshouse experiment that found that tetraploids of *C. stoebe* allocated a greater proportion of their biomass to belowground roots compared with shoots (A.R. Collins unpubl. obs.).

We showed that the two cytotypes of *C. stoebe* differ significantly in root growth, even over very short timescales. We suggest that this greater investment of tetraploid cytotypes towards longer taproots and larger root systems may be one factor that pre-adapted them to occupy drier climates and ultimately to invade even drier climates in their new range in North America. Still, invasive plants have to succeed in a much more complex soil environment than a growth pouch, and more experiments will be necessary to further our understanding of how root growth will influence the invasion process.

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