Rapid evolution or preadaptation in invasive Jacobaea vulgaris

Doorduin, L.J.

Chapter

2

Enemies lost: Changes in anatomy and physiology of the invasive plant Jacobaea vulgaris (Asteraceae)

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Introduction

One of the benefits for invading plant species in an invaded area is a reduced impact of specialist herbivores compared to the native area (Keane and Crawley 2002). Specialized herbivores are often held responsible for a high plant biomass loss. The absence of the selection pressure of specialist herbivores in the invasive area may lead to evolutionary changes in morphological, physiological and growth patterns of invasive plants (Feng et al. 2009).

The Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey and Notzold 1995), states that the release from specialist herbivores allows for an evolutionary change of invasive plants in energy allocation from defence to growth. Defence is often divided in two types of defences, quantitative defences (digestibility reducers) which are costly to produce and qualitative defences (toxins) which are cheaper to produce. Quantitative defences act against specialist as well as generalist herbivores. Qualitative defences act against generalist herbivores but specialist herbivores are often adapted to these defences and even can use these compounds as a cue to locate their host plant, and act as an oviposition and feeding stimulant (Bernays et al. 2003, Macel and Vrieling 2003). Qualitative defences have lower allocation costs and defend the plant against generalists, but simultaneously make the plant more vulnerable to adapted specialist herbivores. This dilemma is referred to as the specialist- generalist dilemma (van der Meijden 1996). In the invasive area, where specialists are absent, it is the best strategy for a plant to increase its cheap qualitative defence against generalist herbivores without having the side effect of attracting specialist herbivores and decreasing their quantitative defences. As a result of the changed allocation patterns to the different defences a net gain is achieved, by exchanging costly quantitative defences for cheap qualitative defences that can be allocated to growth (Doorduin and Vrieling 2011). This evolutionary shift of quantitative defence to qualitative defence in the invasive area is called the Shifting Defence Hypothesis (SDH) (Muller-Scharer et al. 2004, Joshi and Vrieling 2005). In a common environment without herbivores several invasive plant species indeed showed a more vigorous growth (Blair and Wolfe 2004, Lewis et al. 2006, Ridenour et al. 2008) and a higher level of qualitative defences (Joshi and Vrieling 2005, Lewis et al. 2006, Cano et al. 2009) compared to individuals in the native area.

Feng et al (2009) explained the vigorous growth of invaded plants as a consequence of an enhanced investment in photosynthesis at the cost of a reduced cell wall content both in mass and nitrogen allocation (Feng et al. 2009). Nitrogen (N) is one of the most important limiting resources for plant growth (Niinemets 2007) and most leaf N is allocated to chloroplasts, needed for photosynthesis (Evans 1989, Pons and Anten 2004). However, the primary plant cell wall consists for 5-10% of proteins (Loomis 1997) and therefore cell walls can be considered as an important N sink as well. An increased N allocation to cell walls is related to better defence against herbivores (Showalter 1993). In the absence of herbivores allocation of N may therefore shift from cell walls to photosynthesis. Feng et al. (2009) indeed found that *Ageratina adenophora* shrubs from the invasive area allocated 40-50% less proteins to cell walls and 13% more nitrogen to photosynthesis compared to native plants (Feng et al. 2009). The nitrogen Use Efficiency (PNUE) was also 20% higher, indicating more efficient use of nitrogen in photosynthesis. This N reallocation was coupled with a decrease in leaf mass per area (LMA) indicating poorer structural defences. In the native area leaf toughness can be beneficial because it possibly reduces palatability of *A. adenophora* to specialist herbivores whereas in the invasive area these specialists are not present. This selection for increased photosynthesis, albeit at the expense of defence, allows for a higher reproductive output in invasive populations. This allocation change is beneficial in the light of competition and dispersal and may have contributed to the invasive character of *A. adenophora*. Besides defence, another strategy to cope with herbivory by specialists is tolerance. This is the innate capacity of plants to reduce fitness loss in spite of tissue losses (van der Meijden et al. 1988). Fitness loss is often reduced by the ability of plants to regrow fast after defoliation (Rosenthal and Kotanen 1994).

We did a study on *Jacobaea vulgaris* to investigate if the invasive individuals have an increased photosynthetic capacity and a decreased allocation to quantitative structural defences as shown in *A. adenophora*. Furthermore we investigated whether in the invasive area, in the absence of specialists, regrowth capacity is lower in invasive *J. vulgaris* individuals compared to native individuals.

Jacobaea vulgaris (Asteraceae, syn. *Senecio jacobaea*) or common ragwort is a plant native to Europe and invasive in parts of Australia, New Zealand and North America. It contains pyrrolizidine alkaloids (PAs) which are toxic and can be lethal to cattle (Johnson 1978, Stegelmeier et al. 1999). In the invasive areas *J. vulgaris* is considered a weedy species because of its wide spread and distribution. Furthermore it received a pest status because infestations have resulted in significant livestock losses due to alkaloid poisoning and decreased pasture yields (Jakobs et al. 2004). Because of its weedy character, research is being devoted to discover how *J. vulgaris* has evolved into a pest species in the invasive areas (Willis et al. 2000, Joshi and Vrieling 2005, Stastny et al. 2005). A major difference in the invasive communities compared to the native community is the absence of specialist herbivores on *J. vulgaris*. The Cinnabar moth *Tyria jacobaeae* and the flea beetle *Longitarsus jacobaeae* are both specialists and absent in the invaded area, though they have been introduced between 30-40 years ago in some areas of North America, Canada and Australia to act as biological control (James et al. 1992, McEvoy et al. 1993, Ireson et al. 2000). In a common garden experiment Joshi and Vrieling showed that invasive individuals of *J. vulgaris* had more vegetative growth and had reached a 37% higher inflorescences dry weight compared to native individuals (Joshi and Vrieling 2005). Moreover, as predicted by the shifting defence hypothesis, plants from invasive *J. vulgaris* populations produced on average 90% more PAs (a qualitative defence) than plants from native areas (Joshi and Vrieling 2005) which was shown to result in a better defence against generalist herbivores. The leaf mass area (LMA) which is the inverted value of SLA, is often used as indicator of structural biomass (Reich et al. 1991) and is considered as an estimator of quantitative defences. Although we expect that native plants have thicker leaves and therefore a higher LMA, in the study of Joshi and Vrieling native and invasive individuals of *J. vulgaris* did not differ. The same holds true for leaf nitrogen content that was similar for invasive and native populations. Interestingly, regrowth ability of invasive individuals was decreased compared to that of native individuals by 12% (Joshi and Vrieling 2005).

The aim of this study is to investigate whether invasive *J. vulgaris* individuals increased their growth due to an increase of photosynthesis per leaf area (P_{max}), more efficient use of nitrogen in fotosynthesis (PNUE) and a decreased allocation to quantitative structural defences. Besides LMA we also investigated leaf structure, the amount of cell wall material and leaf toughness as estimation for structural biomass. As an indicator of regrowth capacity we measured the root to shoot ratio because fast regrowth is positively correlated with the root/shoot ratio (van der Meijden et al. 1988, van der Meijden et al. 1988, Iwasa and Kubo 1997).

Methods

Study species

J. vulgaris is a self-incompatible monocarpic perennial plant (Harper and Wood 1957, Kirk et al. 2005). It is a serious pest in Australia, New Zealand, the United States and Canada. *J. vulgaris* was first spotted outside its native distribution in the 1850s on the east coast of Canada (Bain 1991), around 1874 in New Zealand (Thomson 1922) and Australia (McLaren 1997) and in 1901 on the west coast of the USA (Gilkey 1957).

Plant material and growth conditions

Seeds were collected from 19 native populations in Europe and from 20 invasive populations in New Zealand, Australia and the USA (Table 1). Seeds were germinated in a petri dish with moistened filter paper and per population 5 seedlings each of a different maternal line were potted in 0.5 L pots with 5% potting soil, 95% sand mixture and 0.75g osmocote® (N:P:K:MgO 15:9:11:2.5). Plants were grown in a climate room for 17 weeks at 20°C, 70% humidity (day and night), 16 hours daylight with a light intensity of 113 umol PAR $m^{-2}s^{-1}$. They were watered when needed. After 10 weeks, 50 mL Pokon solution NPK 7-5-6 (8 mL/L) and Fe-EDTA of 3.2 g/L was given to the plants twice a week. Per population two plants were randomly picked to use in the analysis of photosynthesis and cell wall measurements (after 12 weeks), microscopy (after 14 weeks) and for toughness measurements (after 16 weeks). After 17 weeks all plants were harvested. For details of measurements see Table 1.

Physiology

Photosynthesis and chlorophyll content

Twelve weeks after planting the light saturated rate of photosynthesis per unit leaf area (Pmax), respiration (R), stomatal conductance ($g_{st)}$ and intercellular CO_2 concentration (C_i) were measured on the middlemost leaf of a plant using a LICOR 6400 at atmospheric CO_2 (ca. 380 µmol/mol in the leaf chamber), growth temperature and 1250 (µmol/s) PAR. After two minutes of incubation in the light, each leaf was measured and three minutes after switching the light off, dark respiration was measured. Gas exchange rates were corrected for dark respiration under the leaf chamber gasket according to Pons and Welchen (2002) (Pons and Welschen 2002). Pmax was also calculated for total shoot. First Pmax was calculated for dry shoot by Pmax (µmol m^{-2} s^{-1}) * % DM of the shoot (1). Then the surface of the dry shoot was calculated by dry mass shoot (g)/ leaf mass area (g m^2) (2). Finally, total µmol photosynthesis in the shoot was calculated by (1) * (2). The measured leaf sections (2x3 cm) were cut out and two 1 cm diameter leaf punches were removed (Fig. 1). After weighing, leaf sections, punches and leaf remainder were frozen in liquid nitrogen. The remainder of the dissected leaf part was used for analysis of C and N concentration in dry matter using an elemental analyser (Carlo Erba, Milan, Italy)**.** Photosynthetic Nitrogen Use Efficiency (PNUE) was calculated by dividing Pmax (µmol CO2 m^{-2} s^{-1}) by N per leaf area, N_{LA} (mmol/m^2). Chlorophyll content was determined on the two punches using DMF (N,N-Dimethylformamide) according to Porra *et al.* (1989) (Porra et al. 1989). Total chlorophyll content in the dry shoot (mg) was calculated as: chlorophyll mg/g fresh * % DM in shoot.* shoot dry mass (g).

Leaf anatomy

Microscopy

Fourteen weeks after planting, coupes were cut from the middlemost leaf at the tip of the leaf (Fig. 1) using a hand microtome. Coupes were then stained using Propidium Iodide for 15 minutes. Propidium iodide stains DNA as well as cell wall material. Images were then scanned using a Zeiss Observer with a LSM 5 exciter scanhead confocal microscope at 545 nm. A full cross section of the leaf was obtained by tile scanning the specimen with a 40 x 1.2 NA Plan APO water immersion objective. This gave an image size of 321.43 µm x 482.14 µm with a resolution of 80 nm. per pixel. Measurements were made using ImageJ® 1.42q. Each measurement was made 5 times on different parts of the cross-section as outlined in Figure 2.

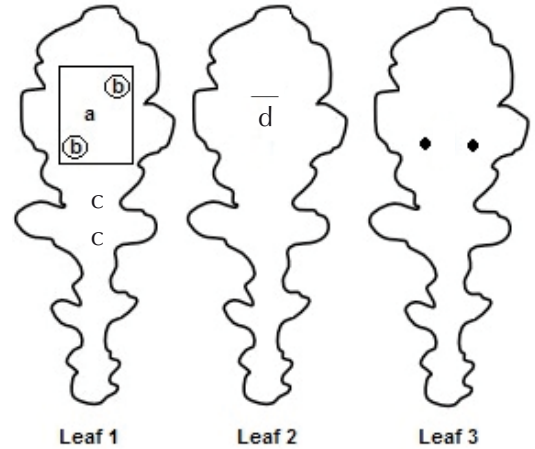

Fig.1 Parts of the leaf measured. **Leaf 1;** a: area measured for photosynthesis and for nitrogen analysis. b: Punches for chlorophyll determination. c: Punches for cell wall material analysis. **Leaf 2**; d: coupe location microscopy. **Leaf 3**; e: Toughness measurements.

a) Cuticle thickness
b) Epidermis cell length & width
c) Epidermis – epidermis cell wall thickness
d) Epidermis- palisade parenchyma cell wall thickness
e) Palisade parenchyma cell wall thickness
f) Palisade parenchyma cell length & width
g) Sponge parenchyma cell length & width
h) Sponge parenchyma cell wall thickness
i) Epidermis cell wall thickness
j) Epidermis cell length & width
k) Cuticle thickness
l) Palisade parenchyma layer thickness
m) Sponge parenchyma layer thickness
n) Leaf thickness

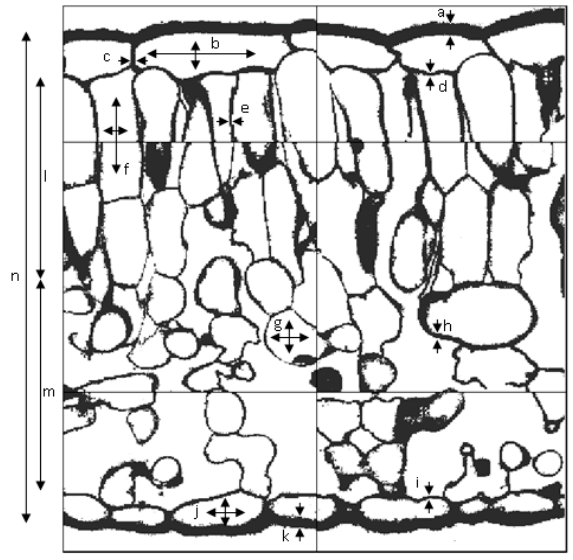

Fig 2. Measurements made on leaf cross section. Arrows indicate measurements made. Length is defined as top to bottom and width as side to side. This sample cross section is a cropped version of a coupe from Landsborough/ Haast New Zealand.

Toughness

After sixteen weeks of growth the then middlemost leaf was removed and used in toughness measurements (Fig. 1). Toughness was measured by using a punch and die method on an Instron 4000 after Onoda *et al.* (2008) (Onoda et al. 2008). A flat ended sharp-edged cylindrical steel punch and a steel die with a sharp-edged hole were used. The punch and die were installed into the machine. When the punch started to compress the leaf, a sharp increase in force was observed. Maximum force (N) was recorded just before the leaf fractured. Work (µ Joule) was recorded during the whole process and the total work to penetrate the leaf was calculated. Each leaf was measured twice and for analysis the average of two measurements was taken. Maximum force and work to puncture the leaf were calculated from a force-displacement curve (Aranwela et al. 1999).

Cell walls

Two one cm diameter punches were extracted from the same leaf used for the photosynthesis analysis (material after twelve weeks of growth). Cell wall material was extracted using the protein extraction protocol of Takashima *et al* (2004) (Takashima et al. 2004). Water soluble material and SDS soluble material was removed. The remaining cell wall material was oven dried at 60°C for 18 hours and weighed. Cell wall material extraction was replicated thrice on the same leaf. For statistical analysis, the average of three measurements was taken.

Growth

After 17 weeks all plants were harvested. Fresh mass of roots and shoots were determined. After oven drying at 60°C for a minimum of 48 hours, dry mass of shoots and roots was determined. From leaves that were used in physiological and morphological measurements, fresh and dry mass was determined and added to the total plant mass.

LMA

After 17 weeks, on the same day of total harvest, the fifth leaf was used to determine the area by using a portable area meter. After oven drying at 60°C for 48 hours the dry mass of the leaf was determined and used to define the leaf mass area (dry mass leaf (g)/ surface of leaf (m^2)).

Data analysis

As the main interest of this study was to find differences in invasive versus native areas, statistical analysis was performed by a nested ANOVA. When covariates were taken into account a nested ANCOVA was used. Individual plants were nested within population of origin which was nested within either the invasive or native area. Averages of invasive or native areas are based on estimated marginal means from the analysis. Normality of the residuals was checked with a Kolmogorov-Smirnov test and equality of the variances with a Levene's test. When variances were found to be significantly different, a Mann-Whitney U test was performed. The significance of the correlations between variables was tested using a Pearson correlation. All analyses were carried out using SPSS 18.0 (SPSS: An IBM Company).

Table 1. Origin of populations used in this study and number of plants measured. Growth (harvest): measurement of fresh mass, dry mass and LMA. (17 weeks) Microsc.:measurement of cell wall parameters (14 weeks). Photosyn.: photosynthetic measurements, Nitrogen and Carbon content (12 weeks). Chl.: measurement of chlorophyll content (12 weeks). Tough.:measurement of leaf toughness (16 weeks). CW: measurement of cell wall weight (12 weeks).

				Number of plants used in measurements					
Range	***Country***	***Location***	***Coordinates***	***Growth***	***Microsc.***	***Photosyn.***	***Chl.***	***Tough.***	***CW***
Invasive	*Australia*	Barramunga	*Lat38.33 Lon143.41*	4	2	2	2	2	1
		Beech forest	*Lat38.38 Lon143.33*	4	2	2	2	3	1
		Dairy Plains	*Lat41.34 Lon146.31*	4	1	2	1	1	1
		Franklin	*Lat43.05 Lon147.00*	5	2	2	2	2	1
		Mayberry	*Lat41.33 Lon146.18*	4	2	2	2	2	1
		Turton's Creek	*Lat38.33 Lon146.15*	5	2	3	2	1	1
	New Zealand	Craigieburn/Grey valley	*Lat39.25 Lon174.13*	4	2	1		2	1
		Landsborough/Haast	*Lat43.53 Lon169.02*	5	2	1	1	2	
		Maruia	*Lat42.11 Lon172.13*	2	2	2	2	2	1
		Opunake/Taranaki	*Lat39.25 Lon174.13*	5	2	2	2	2	1
		Soutland/New Zealand	*Lat45.28 Lon167.55*	1	1	1	1	1	1
		Whatipu	*Lat37.01 Lon174.31*	4	2	2	2	2	1
	USA	Basket Slough, Oregon	*Lat44.58 Lon123.19*	5	2	2	2	2	1
		C. spur/Montana	*Lat47.48 Lon111.35*	5	2	2	2	2	1
		Del Norte Clifornia	*Lat41.42 Lon123.57*	4	2	2	2	2	1
		Kootenai National Park, Montana	*Lat48.17 Lon114.53*	4	2	2	2	2	1
		No Bear/Oregon	*Lat43.00 Lon120.00*	5	2	2	2	2	1
		S. Cooper/Oregon	*Lat45.40 Lon122.50*	4	2	3	3	2	1
		Salem, Oregon	*Lat44.56 Lon123.02*	4	2	3	3	2	1
		Surprise Hill/Montana	*Lat48.15 Lon115.00*	4	1	2	1	1	1
Native	*Belgium*	Brussels	*Lat50.51 Lon04.25*	5	2	2	1	2	1
		Spa	*Lat50.29 Lon05.50*	4	2	2	2	2	1
	Denmark	Sundstrup	*Lat56.37 Lon18.30*	5	2	3	3	2	1
	England	Deal	*Lat51.13 Lon01.24*	4	2	1	2	2	1
	Finland	Kirkkonummi	*Lat26.15 Lon24.53*	5	1	2	2	1	1
	France	Mt. St. Michel	*Lat48.37 Lon01.32*	4	2	2	2	2	1
	Germany	Holzlarchen	*Lat47.53 Lon11.43*	4	2	1		2	1
		near Lubeck	*Lat54.05 Lon10.42*	3	2	2	2	2	1
	Hungary	Csokvaomany	*Lat48.10 Lon20.22*	4	2	1	1	2	1
	Ireland	near Caherdaniel	*Lat53.07 Lon8.02*	4	2	2	2	2	1
	Netherlands	Veluwe	*Lat52.19 Lon06.00*	5	2	2	2	2	1
		Wageningen	*Lat52.01 Lon05.34*	4	2	2	2	2	1
	Norway	Sor Trondelag/Malvik	*Lat60.33 Lon7.53*	5	2	2	2	2	1
	Poland	Near Warsaw	*Lat51.52 Lon19.25*	5	2	2	2	2	1
	Scotland	Dundee	*Lat56.29 Lon03.02*	2	2	1	1	2	1
	Spain	Porto de San Glorio	*Lat40.01 Lon3.37*	5	2	2	2	2	1
	Sweden	Lund	*Lat55.43 Lon13.13*	5	2	2	1	2	1
	Swiss	l'Himelette	*Lat47.07 Lon07.00*	5	2	2	2	2	1
		Rothenthurm	*Lat47.06 Lon08.040*	1	1	1	1	1	1

Results

Physiology

Maximum photosynthesis per leaf area (P_{max}) was 11.4 % lower for invasive *J. vulgaris* plants compared to native ones (Table 2). However Pmax of native and invasive individuals did not differ, if Pmax was calculated for the whole shoot (Table 2). Furthermore no significant difference was found in respiration, stomatal conductance and CO2 concentration in the intercellular spaces (Ci).

Leaf nitrogen content per area (N_{LA}) did not differ between native and invasive individuals of *J. vulgaris*. However the amount of nitrogen calculated as mmol/g dry weight (N_W) was higher in native individuals whereas invasive individuals contained significantly more carbon (Table 2). As a consequence the N:C ratio was significantly higher in native individuals. When the total amounts of N and C were calculated for the total shoot, invasive individuals contained significantly more carbon and also more nitrogen. The latter because they had larger shoots (Table 2). The PNUE, the Photosynthetic Nitrogen Use Efficiency, was not significantly different between *J. vulgaris* plants of both areas.

J. vulgaris from the invasive area contained on average 13.3 % less chlorophyll (mg g freshmass^{-1}) compared to individuals from the native area (Table 2). Nevertheless, the total amount of chlorophyll (mg) in the total shoot was higher in invasive individuals due to the their larger shoots (Table 2).

Both nitrogen (N_{LA}) and chlorophyll were correlated with Pmax. If nitrogen and chlorophyll were used as a covariate Pmax did not significantly differ between native and invasive individuals ($F_{1,34}$= 1,203, NS, for both covariates: $p<0.05$)

Leaf anatomy

Microscopy

Of the nineteen measurements of *J. vulgaris* leaf cross sections (see Figure 2) made on 73 individuals, only the lower epidermis cell wall was significantly thicker in invasive individuals compared to native individuals (Table 2).

Toughness

By using a punch and die method, maximum force and work required to puncture a leaf was measured. For invasive plants significantly more work was needed to puncture a leaf compared to native plants (Table 2). The LMA (leaf dry mass (g)/ leaf area (cm^2)) as indicator of the amount of leaf dry matter per area did not differ. However, the thickness of the lower epidermis was positively correlated with work to puncture a leaf (Pearson correlation r= 0,415, p=0.001) (Fig. 3).

Cell walls

The amount of cell wall material was not significantly different between *J. vulgaris* plants from the invasive and native area (Table 2). Leaf nitrogen (N_{LA}) content and leaf cell wall material were not significantly correlated, for both native and invasive *J. vulgaris* plants.

Table 2: Results of nested ANOVA's for physiological, morphological and mass measurements on native and invasive *J. vulgaris* plants grown under standardized conditions in a climate room. Mass refers to dry mass unless otherwise indicated. Df indicates the degrees of freedom of the ANOVA among the native and invasive area. F indicates F ratio, Z indicates the Z value of a Mann-Whitney(MW) test when the requirements of an ANOVA were not met. p indicates the significance level of the ANOVA. ns= not significant. P_{max} = light saturated rate of photosynthesis per unit leaf area, NLA= leaf nitrogen content, PNUE= photosynthetic nitrogen use efficiency %DM= percentage dry mass.

Physiology and chemistry

ANOVA	Native	Invasive	Df	F/Z	p
Pmax (µmol m^{-2} s^{-1})	15,8	14	1/36	7,674	<0.01
Pmax dry shoot (µmol g)	0,196	0,24	1/37	3,094	ns.
Respiration (µmol m^{-2} s^{-1})	1,18	1,35	1/ 37	0,559	ns.
Intercelulair CO2 (µmol mol $^{-1}$)	248	270	1/37	0,274	ns.
Stomatal conductance (mol m^{-2} s^{-1})	0,261	0,281	1/37	0,937	ns.
NLA (mmol m $^{-2}$)	0,102	0,091	1/36	0,197	n.s.
Leaf Nitrogen (mmol g^{-1}) (Nw)	3,05	2,56	1/36	13	**<0.01**
Leaf Nitrogen dry shoot (mmol)	17,29	19,87	1/37	4,939	**<0.05**
Leaf Carbon (mmol g^{-1}) (C)	33,6	34,2	1/36	5,605	**<0.05**
Leaf Carbon dry shoot (mmol)	207	269	1/37	12,293	**<0.01**
N/C Ratio	0,092	0,075	1/36	21,511	**<0.001**
PNUE (µmol CO2 mol N $^{-1}$ s^{-1})	154,9	153,8	1/36	0,866	ns.
Chlorophyll a+b (mg g freshmass^{-1})	1,65	1,43	1/33	10,665	**<0.01**
Chlorophyll a+b dry shoot (mg)	1,04	1,27	1/35	4,679	**<0.05**
Chlorophyll a/chlorphyl b	3,07	3,13	1/33	3,622	ns.
Leaf anatomy					
Microscopy (all measurements in µm)					
Upper cuticle thickness	3,4	3,42	1/37	0,607	ns.
Upper epidermis cell length	25,2	25,7	1/37	0,24	ns.
Upper epidermis cell width	54,2	49,5	1/37	1,144	ns.
Epidermis-epidermis cell wall thickness	1,44	1,43		-0,001	ns.^{MW}
Epidermis-palisade parenchyma cell wall thickness	1,13	1,11	1/37	0,086	ns.
Palisade parenchyma cell length	71,3	70,1	1/37	0,113	ns.
Palisade parenchyma cell width	32,2	30,3	1/37	2,204	ns.
Palisade parenchyma cell wall thickness	0,87	0,93		-1,072	ns.^{MW}
Sponge parenchyma cell length	27,4	27,6	1/37	0,002	ns.
Sponge parenchyma cell width	37,7	38,4	1/37	0,017	ns.
Sponge parenchyma cell wall thickness	0,89	0,89	1/37	0,005	ns.
Lower epidermis cell length	16,7	17,2	1/37	0,029	ns.
Lower epidermis cell width	30,8	32,3	1/37	0,386	ns.
Lower epidermis cell wall thickness	1,03	1,12	1/37	4,607	**<0.05**
Lower cuticle thickness	2,18	2,26		-0,451	ns.^{MW}
Leaf thickness	277	268	1/37	0,682	ns.
Palisade parenchyma layer thickness (Pal)	117	112	1/37	0,898	ns.
Sponge parenchyma layer thickness (Spo)	122	121	1/37	0,06	ns.
Pal/Spo	1,00	0,95	1/37	0,357	ns.
Toughness					
Maximum Force (N)	0,90	0,90	1/37	0,001	ns.
Work (µjoule)	297	330	1/37	4,163	**<0.05**
Cell walls					
Cell wall material (g • m^{-2})	39,2	37,2	1/37	0,348	n.s.
Cell wall material (g • g^{-1})	0,29	0,28	1/37	0,536	n.s.
Growth					
Plant (g)	11,7	12,2	1/37	0,698	ns.
Plant %DM (g • g^{-1})	13,20%	12,70%	1/37	2,216	ns.
Shoot (g)	5,00	5,68	1/37	5,164	**<0.05**
Shoot %DM (g •g^{-1})	11,0%	11,3%	1/37	0,533	ns.
Root (g)	5,61	5,23	1/37	0,177	ns.
Root % DM (g •g^{-1})	17,20%	15,50%	1/37	7,092	**<0.01**
Ratio Root/Shoot	0,92	0,75	1/37	5,001	**<0.05**
LMA of the 5th leaf (g·m^{-2})	57,5	55,8	1/37	0,024	n.s.

Growth

Invasive *J. vulgaris* plants had a 12 % higher shoot dry mass than native plants (Table 2). No difference was found in root dry mass. As a result,invasive individuals had an 18.1 % lower root to shoot ratio compared to native individuals (Table 2). With an increase in plant mass, plants showed an increase in root to shoot ratio (r= 0,472 p=0.01) (Fig. 4). Shoot mass was negatively correlated with N_w (Pearson correlation r= -0,471 p< 0.01) (Fig. 5).

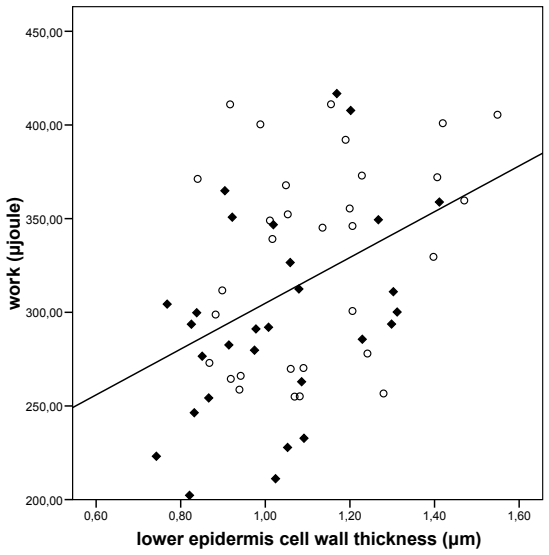

Fig. 3 Thickness of the lower epidermis cell wall measured in µm plotted against the work to puncture a leaf measured in µm. Native individuals are indicated with closed diamonds and invasive individuals are indicated with open circles.

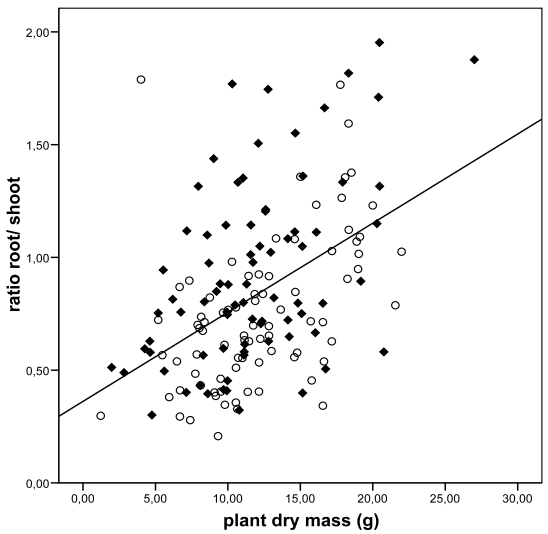

Fig. 4 Plant dry mass (g) plotted against the root to shoot ratio. Native individuals are indicated with closed diamonds and invasive individuals are indicated with open circles.

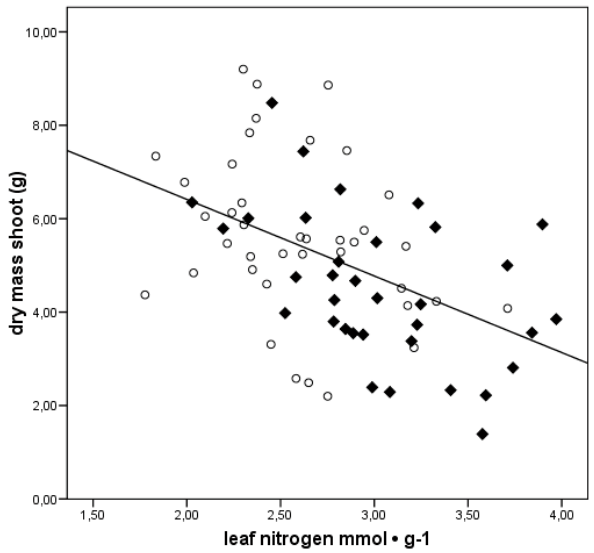

Fig. 5 Leaf nitrogen in mmol per gram dry mass plotted against dry mass of the shoot. Native individuals are indicated with closed diamonds and invasive individuals are indicated with open circles.

Discussion

In the study of Feng et al. (2009) invasive individuals of *A. adenophora* showed a change in nitrogen allocation from structural defences in cell walls into photosynthesis (Feng et al. 2009). According to this study and also to the EICA hypothesis we expected to find an increased maximum photosynthesis and higher photosynthetic efficiency and a decrease in leaf mass area (LMA), cell walls and regrowth capacity in invasive plants compared to native plants. In contrast we found that the maximum photosynthetic rate (Pmax) and chorophyll content per leaf area were significantly lower for invasive *J. vulgaris* plants. As expected, Pmax was influenced by the amount of chlorophyll and nitrogen (N_{LA}) (Jia and Gray 2004). PNUE did not differ between plants from the native and invasive area. Also, Pmax per total shoot did not differ between native and invasive individuals. Chlorophyll content for the total shoot is significantly higher in invasive individuals. Like chlorophyll, gram nitrogen in the total shoot was also significantly higher in invasive individuals. So, invasive plants accumulated more chlorophyll and leaf nitrogen although Pmax for the total shoot did not differ from native individuals. These data show that initially plant growth of invasive individuals was higher than that of native individuals. During the experiment nutrients in the pots became more limiting for the invasive plants due to a higher growth rate and as a result N_{LA} declined although the total amount of nitrogen in the shoots of invasive plants was still significantly higher due to the larger shoot (Fig. 5).

The shoot to root ratio was also influenced by leaf nitrogen. However, an ANCOVA with N_W as a covariate still showed a significantly higher shoot to root for invasive individuals. Although invasive plants

had a significantly higher shoot mass than native *J. vulgaris* plants, plant mass did not differ between native and invasive individuals. Invasive individuals had a slightly lower root mass albeit not significantly different. If nutrients become limiting plants alter there root to shoot ratio in the direction of larger roots. Invasive plants grew faster and therefore experienced nutrient limitations earlier than native plants. Larger plants started to adjust there root to shoot ratio to decreasing nutrient availability (Fig. 4) but still invasive plants maintained a lower root to shoot ratio than native plants. The data show that PNUE and Pmax of total shoot do not differ between native and invasive individuals as it differed in *A. adenophora* (Feng et al. 2009) but that a higher growth rate is obtained by a lower root to shoot ratio of invasive individuals. Regular complete defoliation by the cinnabar moth might select for higher root to shoot ratio in native individuals to be able to quickly regrow after defoliation (Van der Meijden et al. 1988).

Native individuals of *J. vulgaris* allocated more biomass to the roots compared to invasive plants, resulting in a 23 percent higher root to shoot ratio for native individuals compared to invasive individuals.

Only the lower epidermis cell wall showed a significant difference, with thicker cell walls for invasive individuals. Furthermore measurements on maximum work to penetrate a leaf showed also a higher value for invasive individuals of *J. vulgaris*. Although we did not find a significant correlation between leaf thickness and maximum work, we did find a significant positive correlation between thickness of the lower epidermis alone and maximum work (Fig. 3).

The lower epidermis may play a role to retain leaves moisture in dry climates. However following the climate classification of Köppen-Geiger individuals from the native and invasive areas belong in general to the same classification, namely a temperate climate without a dry season and with a warm summer (Peel et al. 2007).

LMA, which is an indication for the amount of leaf dry matter per area, did not differ between native and invasive plants. This is in line with the finding that the amount of cell wall material in a leaf did not differ between native and invasive plants of *J. vulgaris*.

In this study we did not find evidence for higher photosynthetic rates, leaf anatomy and allocation to cell walls. However an alternative strategy of native individuals to cope with herbivory is a high regrowth capacity. The amount of roots, which were found to be positively correlated with fast regrowth capacity (van der Meijden et al. 1988) was indeed higher for native individuals. Joshi and Vrieling (2005) already showed that plants from invasive areas had a 12% reduction in regrowth capacity (Joshi and Vrieling 2005). This is in line with a high herbivore pressure of specialists in the native area compared to the absence of specialist herbivores in the invasive area.

As *J. vulgaris* is not predated by specialised herbivores in its invasive range, regrowth capacity is not necessary to maintain thus mass can be re-allocated from roots to shoots that would otherwise stand a high risk of being eaten. Bigger shoots are known to be favourable in an environment with high levels of competition (Burns 2006).

These findings differ from the study of Feng et al 2009, where invasive success of *Ageratina adenophora* was ascribed to the allocation of leaf nitrogen (g/m2) from defence (cell walls) into photosynthesis (Feng et al. 2009). Although in both cases a release from herbivory seems to be an important factor for invasive success, reaction to this ecological change differs between both plant species.

In conclusion, in the native range of *J. vulgaris* there is heavy herbivory by specialist herbivores and plants that are introduced in a new area are released from this burden. Specialist herbivores in the native area have broken through the main lines of defence, so plants have to find other ways to cope with the damage done. For *J. vulgaris* this is not achieved by reducing palatability through costly investment in structural defence, but to invest in re-growth capacity which is detrimental to the fast growth rate. In the invasive range it is no longer necessary to maintain this potential and mass can be allocated into fast growth of shoots.

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