ABSTRACT

HABINCK, EMILY MARIE. Correlated Ecophysiological and Growth-Related Leaf Traits among *Arabidopsis thaliana* Ecotypes. (Under the direction of William A. Hoffmann).

Across plant taxa there are large-scale correlations among ecophysiological leaf traits: Specific leaf area (SLA), maximum photosynthetic rate (A_{max}), leaf nitrogen content (N_L) and leaf lifespan (L_L). Suite syndromes of leaf traits are associated among r and K-selected species and *Arabidopsis thaliana* ecotypes exhibit growth syndromes similar to the r and K- selection theory and range from a wide variety of latitudes of origin. The purpose of this study was to determine if the ecophysiological leaf traits: SLA, A_{max}, N_L, L_L and other growth-related traits were correlated across *A. thaliana* ecotypes from a wide range of latitudes of origin. Fifteen *A. thaliana* ecotypes were grown in an experimental growth chamber to determine if these ecophysiological leaf traits and other growth-related traits such as leaf initiation rate and bolting time were correlated across the ecotypes. Results indicate that SLA, A_{max} and N_L were correlated across the ecotypes as consistent with global trends while L_L was not significantly correlated. The life history trait, bolting time, was correlated with leaf traits. There was a wide range of leaf age and change in longevity among the leaves, making leaf-lifespan difficult to determine. The results from this study indicate that *A. thaliana* will be a good model in order to understand the developmental and genetic links with these correlations.

Correlated Ecophysiological and Growth-Related Leaf Traits among *Arabidopsis thaliana* Ecotypes

by
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DEDICATION

For my mother, Veloy L. Habinck; my father Thomas J. Habinck; and stepmother Sandra Y. Habinck.

BIOGRAPHY

Emily Marie Habinck was born in South Dakota and was raised in Iowa. She graduated from T. Roosevelt High School in Des Moines, Iowa in 1995. She attended college in Charlotte, North Carolina and graduated with a B.S. in Biology and a B.A. in Earth Science from the University of North Carolina at Charlotte. As an undergraduate she completed an honors project on the effect of cadmium on mitochondrial function of oysters in the lab of Inna Sokolova in the Biology Department at UNC-Charlotte. In the Department of Geography and Earth Sciences she studied fall leaf color change with Martha C. Eppes. In the fall of 2004, she entered the Plant Ecophysiology lab of William A. Hoffmann in the Botany (now Plant Biology) department at North Carolina State University to pursue her interests in plant biology. Throughout her career she has taught a range of students from pre-K to adults, including a position as a 9^{th} Grade Earth Science Teacher. She is now an Interpretive Ranger for Maricopa County Parks and Recreation and resides in Phoenix, Arizona.

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LIST OF ABBREVIATIONS

RGR-relative growth rate

A_{max}-maximum photosynthetic rate

L_L-leaf lifespan

N_L-leaf nitrogen content

ETR-electron transport rate

PFD-photon flux density

INTRODUCTION

There are different suites of traits associated with the two extremes of life history variation according to the r/K selection theory. Fast growth rate, short life-spans and high reproductive effort is indicative of the r-type, while the converse is indicative of the K-type (Mac Arthur and Wilson, 1967). Within these two extremes of life history variation are correlations in leaf traits among plant species, and specific suites of leaf traits commonly co-occur with other associated growth-related traits. Weedy, or fast growing (r-selected), species typically have high relative growth rates (RGR), which are associated with high maximum photosynthetic capacity (A_{max}), short leaf lifespan (L_L), high leaf nitrogen content (N_L) and high specific leaf area (SLA, which is leaf area per mass); while the converse trend occurs in longer-lived K-selected species (Grime, 1977; Kikuzawa, 1995). Therefore, this axis of leaf traits not only reflects interspecific correlations among these growth-related ecophysiological leaf traits (Reich et al., 1997; Ackerly and Reich, 1999; Reich et al., 2003; Wright et al., 2004), but also corresponds to the continuum from r to K life-history strategies. In turn, we can expect plants to exhibit syndromes involving this suite of functional traits due to evolutionary constraints and developmental links (Ackerly et al., 2000; Chapin II et al., 1993).

A recent review has called for an understanding of both the genetic and developmental factors underlying these leaf and other growth-related traits (Ackerly et

al., 2000). To begin the process of understanding the mechanisms behind these correlations, we look to the model organism in plant genetics, physiology and ecology, *Arabidopsis thaliana* (Mitchell-Olds, 2001; Pigliucci, 2002; Shimizu and Purugganan, 2005; Tonsor et al., 2005). *A. thaliana* is a good model for understanding life history trade-offs because it has ecotypes which are genetically different (Hardtke et al., 1996), that are adapted to particular environments (Koorneef et al, 2004) and that range naturally in a wide range of latitudes (Hoffmann, 2002) and climatic niches (Hoffmann, 2005). In addition, studies have shown that *A. thaliana* ecotypes exhibit a range of r-selection and k-selection behaviors, in which larger *A. thaliana* ecotypes have less fecundity and grow slower than other ecotypes (Taylor et al., 1990) and there are trade-offs among age and size at first reproduction (Mitchell-Olds, 1996).

The ecophysiological leaf traits, SLA, A_{max}, L_L and N_L are correlated across species (Wright et al., 2005), and the strategy of rapid acquisition of resources is a major evolutionary specialization for a subset of species across different environmental conditions (Diaz et al., 2004). One complex environmental gradient, altitude, has been shown to be correlated with this suite of ecophysiological leaf traits (Barusch, 1979; Diemer, 1998; Woodward, 1983). Altitude is associated with temperature, rainfall and soil conditions, all of which have been associated with these ecophysiological leaf traits (e.g. Reich and Oleksyn, 2004; Kiado, 2006; Schultze et al., 2006; Woodward, 1979; Berry and Bjorklund, 1980; Loveys et al., 2002). *A. thaliana* ecotypes have been shown

to differ in leaf and growth-related traits. They differ in L_L (Laquez et al., 2006), SLA (Pigliucci and Koldodynska, 2002), RGR (Li et al., 1998) and whole-plant amino acid content (Pinghun et al., 2006); however it is not known whether are correlated across *A. thaliana* ecotypes.

The purpose of this study was to:

(1) determine if SLA, L_L, A_{max} and N_L and growth-related plant and leaf traits are correlated across *A. thaliana* ecotypes. (2) determine if those leaf traits are correlated with latitude (which is similar to altitude in change of environmental factors) of seed origin.

I expected that leaf traits would be correlated across *A. thaliana* ecotypes according to the global spectrum (Reich et al., 1977). I also predicted that bolting time would be associated with these leaf traits. *A. thaliana* ecotypes that bolt earlier have higher A_{max} and produced more leaves and die more quickly than those that bolt later (Levey and Wingler, 2005). Bolting time in *A. thaliana* is correlated with other plant traits, particularly total leaf number as indicated by plant size (Clauss and Aarssen, 1994) and is an adaptive trait to the environment (Karlsson et al., 1993). Bolting time also varies with latitude (Li et al., 1998); and ecotypes from different latitudes have different tolerances to stressors or selective forces due to phenotypic differences (Abarca et al., 2001), therefore I also expected trait correlations with latitude of seed origin.

MATERIALS AND METHODS

Sixteen individuals of 15 stock accessions of *A. thaliana*, Br-0 (CS22628), Bay-0 (CS22633), Col-0 (CS22626), Col-4 (CS933), Cvi-0 (CS22614), Cvi-1 (CS902), Kondara (CS22651), Ler-0 (CS20), Ler-2 (CS8581), LL-0 (CS1338), Mso (CS22655), Mok-3 (CS22643), Sha (CS22652), Sorbo (CS22653), Var2-1 (CS22580), were chosen and sown based on different latitudes of seed origin (Table 1), their wide range in SLA (appendix i) and availability of genetic information for future study (Alonso-Blanco et al., 1998; Loudet et al., 2002).

Seeds were cold stratified in 1% v/v agar solution for four days at 4ºC. They were grown in an experimental growth chamber at 22ºC under mean photon flux densities (pfd) of 200 μmol m^{-2} s^{-1}, supplied by a combination of incandescent and fluorescent light. Plants were potted in Premier Pro-Mix PGX (Canadian sphagnum peat moss 65-75% by volume, vermiculite, dolomitic and calcitic limestone, wetting agent) fertilized once at potting with 106 ppm nitrogen, 10.41 ppm phosphorous and 111.03 ppm potassium solution. They were watered daily from underneath the pots.

Bolting day was recorded as the date of first bolt appearance. Leaves on each rosette were counted every three days to determine the number of leaves emerged per day for leaf initiation rate. The first leaf primordia initiated was considered to be leaf number 1, for experimental purposes, and subsequent leaves were numbered sequentially as 2, 3,

4 etc. so that the oldest leaf on the plant was experimental leaf number 1. Leaf death rate was determined by calculating the number of leaves that died per day on each plant. My observation indicated that leaf lifespan changed with leaf ontogeny such that leaves produced later in development persisted longer than leaves produced earlier. I quantified this ontogenetic shift by calculating the slope of the relationship between leaf lifespan and date of leaf emergence.

Photosynthetic yield as proxy by electron transport rate (ETR) was determined by using a portable chlorophyll florometer PAM-2100 (Heinz Walz, Germany) at a pfd of 400 μmol m^{-2} s^{-1}, at which photosynthetic rates were near maximum (unpublished data), (Maxwell and Johnson, 2000). Plants were allowed to acclimate to this pfd for 30 minutes before measurements were taken. Because photosynthetic yield varies spatially on the leaf (unpublished data), yield measurements were taken only on the tip of each leaf. ETR was calculated as $ETR=\Phi_{PSII} * PFDa * 0.5$, where Φ_{PSII} is quantum yield of photosystem II, and PFDa is absorbed photon flux density (PFD, Maxwell and Johnson, 2000). PFDa was assumed to be 0.85 PFDi, where PFDi is incident PFD.

Photosynthetic yield measurements were taken on leaf numbers 7, 10, 13, 16, 19 and 22, and were repeated regularly during plant development on expanded leaves to determine maximum leaf values per each plant.

N_L was determined by collecting leaf numbers 8, 11 and 14 on different experimental days, when each of these approached maturity (before yellowing, after full

leaf expansion). These three leaves were pooled, finely ground and the N concentrations determined by the by the North Carolina State University Soils Laboratory.

L_L was recorded for leaf numbers 9, 12, 15 and 18 and determined by date of leaf senescence minus leaf emergence. A leaf was considered to be dead when less than 5% of its leaf area was green. Because leaf lifespan increased with increasing date of leaf emergence, only the earliest emergent leaf collected for L_L, leaf 9, was used to characterize L_L. For example, leaf 9 on a given experimental individual may have had an L_L of 12 days, whereas the L_L of leaf 18 on that same plant may have been 30 days. To quantify the rate at which L_L increased with leaf position, I calculated the slope of the relationship between experimental day (x-axis) and L_L (y-axis) for each experimental individual.

Linear regression analyses for all correlations were performed to test for correlation among parameters using SAS 9.1. Each of the 16 individuals for each of the 15 ecotypes studied here was treated as an independent sample.

RESULTS

There were significant positive correlations between ETR/leaf mass and N_L ($p=0.04$, $R^2=0.30$), SLA and N_L ($p=0.0009$, $R^2=0.58$), and SLA and ETR/leaf mass

(p=0.0004, R^2=0.64) across *A. thaliana* ecotypes (Figure 1). There were no significant correlations between L_L and ETR/leaf mass (p=0.54), N_L (p=0.99) or SLA (p=0.70). Bolting day was significantly and negatively correlated with SLA (p=0.03, R^2=0.36) and N_L (p=0.02, R^2=0.36), but only marginally correlated with ETR/leaf mass (p=0.057, R^2=0.25, Figure 2).

N_L and SLA were negatively correlated with latitude of seed origin (p=0.0007, R^2=0.67 and p=0.05, R^2=0.28 respectively), (Figure 3) while leaf initiation rate and leaf number at bolt were positively correlated with latitude (p=0.04, R^2=0.30 and p=0.01, R^2=0.44 respectively, Figure 4). Other traits were not correlated with latitude (Table 2). Leaf initiation rate was negatively correlated with N_L (p=0.002, R^2=0.55, Figure 5), and leaf death rate was also negatively correlated with leaf longevity rate (p=0.003, R^2=0.51) and N_L (p=0.01, R^2=0.39, Figure 6).

Leaf number at bolting was correlated with leaf initiation rate (p=0.008, R^2=0.46), plant age at bolting (p=0.002, R^2=0.58), leaf longevity factor (p=0.009, R^2=0.45), SLA (p=0.003, R^2=0.53) and NL (p=0.0003, R^2=0.67, Figure 6).

DISCUSSION

The positive correlations among SLA, ETR/leaf mass and N_L (Figure 1) were consistent with previous findings among plant species independently of phylogenetic

constraints (Reich et al., 1997; Ackerly and Reich, 1999), suggesting that *A. thaliana* ecotypes span a range of strategies of resource uptake and utilization. These rates tend to be associated with a r-selection strategy, characterized by rapid growth and short generation times. These tend to be negatively correlated with time of bolting, indicating that ecotypes with large values of these traits would therefore be expected to have a short generation time. Growth rate, as inferred by leaf initiation rate was negatively correlated with N_L (Figure 5), so that the earlier bolting time does not seem to result from more rapid growth. Instead, among ecotypes with high ETR, SLA and N_L, bolting is earlier because it occurs when fewer leaves are present.

These different ecotypes do not represent different ecological strategies within a site, but instead are associated with latitude. Leaf initiation rate and leaf number at bolting were positively correlated with latitude (Figure 4) suggesting that low leaf number and slow initiation rate are adaptive to cold, high latitudes, while these leaves in colder climates also have lower N_L and SLA (Figure 3).

In contrast to the correlation among SLA, ETR, and N_L, there were no significant correlations between these traits and L_L, which is not consistent with previous findings across plant species. These results suggest that *A. thaliana*, a monocarpic plant, may be under different genetic or developmental controls than those across plant species, perhaps because leaf senescence is related to flowering time. Age at bolting ranged from

approximately 1 to 2.7 times leaf lifespan, so that plant lifespan is quite short relative to leaf lifespan.

The constraint that such a short plant lifespan places on leaf lifespan is evident from the observation that, for some ecotypes (e.g. Cvi-1), the marked leaves survived until whole plant senescence (data not shown), placing an upper limit on possible leaf lifespan. For other ecotypes (e.g. Col-0), the leaves died prior to full senescence (data not shown) which is consistent with previously reported data for one ecotype (Hensel et al., 1993).

Leaf lifespan was also difficult to characterize for each ecotype, as leaves extended in longevity across ecotypes with increasing leaf rosette number, up to three fold difference in young and old leaves, and therefore, a single leaf lifespan of leaf number 9 as used in this study could not characterize the value of leaf lifespan for each experimental individual. However, leaf death rate was negatively correlated with the rate at which leaf lifespan increased with date of leaf production. Therefore ecotypes with leaves that stayed on the plant also died more slowly. In addition, it has been shown that early and late-senescing leaves in *A. thaliana* are under different genetic controls (Nam, 1997), while most of the leaves collected in this study were from leaves 9-11, some ecotypes had as few as 12 leaves at bolting while more than 50 leaves before bolting, suggesting that leaves were collected under different developmental programs. To my knowledge, there is no previous report of increased leaf longevity with increasing age,

however these results are consistent with previous findings that there are no correlations with leaf lifespan and latitude of seed origin (Levey and Wingler, 2005). In addition, the negative correlation of leaf nitrogen and longevity indicates that longer lived leaves have lower overall leaf nitrogen content which is consistent with models of leaf senescence (Wingler et al., 2006).

The correlations of SLA, ETR/leaf mass and N_L, with bolting day and leaf number at bolt suggest there are constraints among leaf traits in flowering as consistent with previous studies among plant age and size at first reproduction. In general, the results from this study indicate that *A. thaliana* will be a useful model to study the developmental links and genetic factors including quantitative trait loci mapping among ETR/leaf mass, SLA and N_L.

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Table 1. P-values and R^2 values for correlations of functional traits with latitude across *A.thaliana* ecotypes.

	R^2	p-value
Leaf initiation rate (leaves/day)	.30	0.04
Leaf number at bolt	.44	0.01
SLA	.284	.05
Leaf % N	.63	0.0007
Leaf number at bolt/leaf lifespan	.43	0.01
Bolting day	.24	0.08
ETR/leaf mass	.13	.22
Leaf longevity	0.10	.28
Leaf death rate	.004	.83

Table 2. *Arabidopsis thaliana* ecotypes used in this study and country and latitude of origin

Ecotype	Country of Origin	LATITUDE (N)
Br-0	Czechoslovakia	49
Bay-0	Germany	49
Col-0	USA	38
Kondara	Tajikistan	37-39
Sorbo	Tajikistan	37-39
LL-0	Spain	42
Ler-0	Germany	48-52
Mso-0	Russia	55.5
Sha	Tajikistan	34.5
Ler-2	Germany	48-52
Col-4	USA	N/A
Var2-1	Sweden	59
Nok-3	Netherlands	52
Cvi-1	Cape Verdi Islands	15-17
Cvi-0	Cape Verdi Islands	15-17

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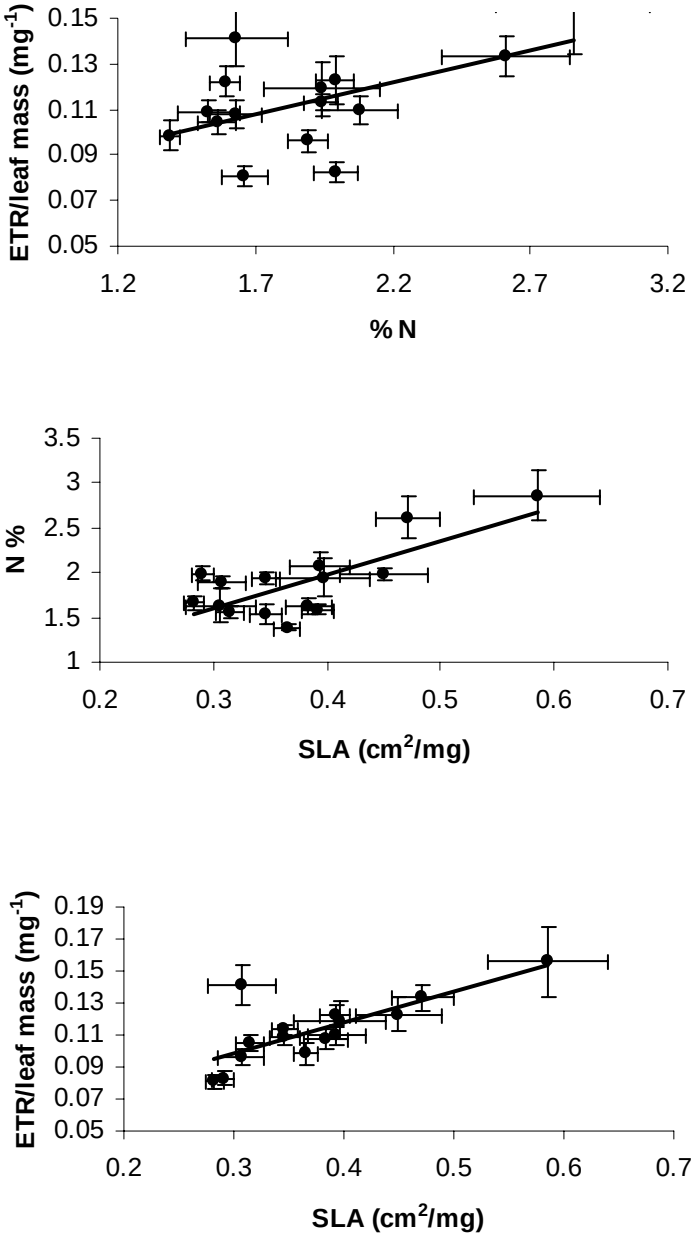


Figure 1. Positive correlations of photosynthetic electron transport rate, ETR/leaf mass (mg-1) and leaf nitrogen content, L_N (R^2=0.30, p=0.04), specific leaf area, SLA and L_N (R^2=0.58, p= 0.0009) and SLA and ETR/leaf mass (mg-1) (R^2=.64, p=0.0004) across *A. thaliana* ecotypes.

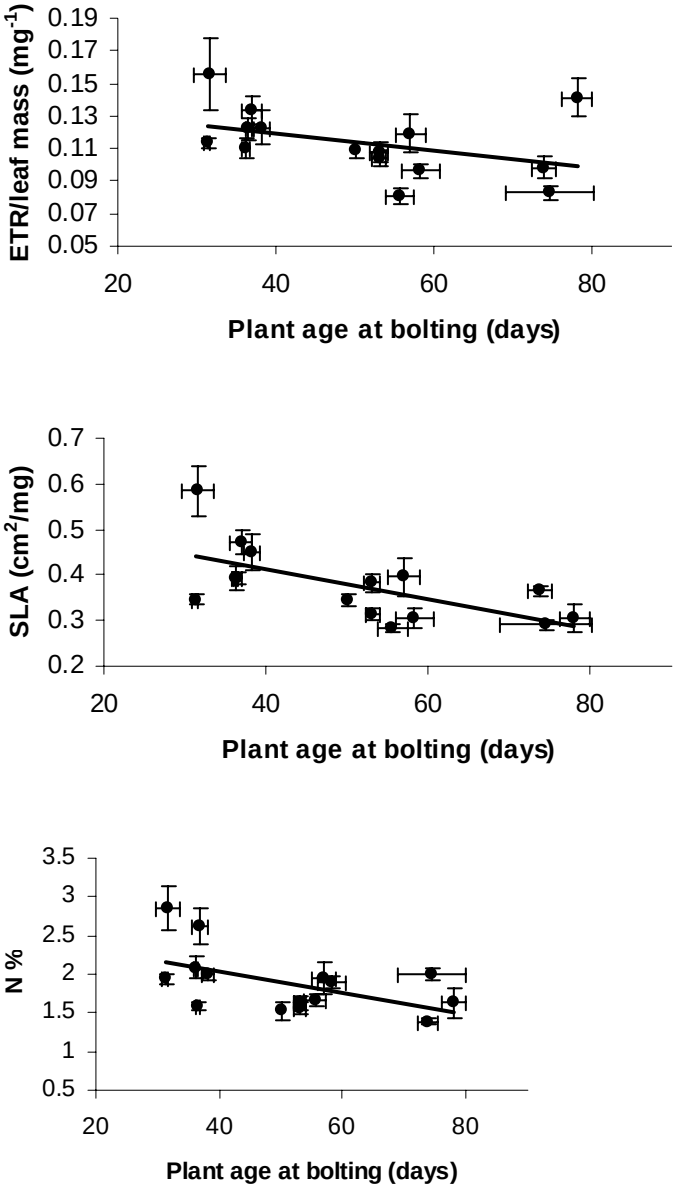


Figure 2. Marginally significant correlation with plant age at bolting and photosynthetic electron transport rate, ETR/leaf mass (mg^{-1}) ($R^2$0.25, p=0.06); but significant negative correlations with plant age at bolting and specific leaf area, SLA and leaf nitrogen content, L_N (R^2 = 0.36, p=0.03; and R^2=0.36, p=0.02 respectively).

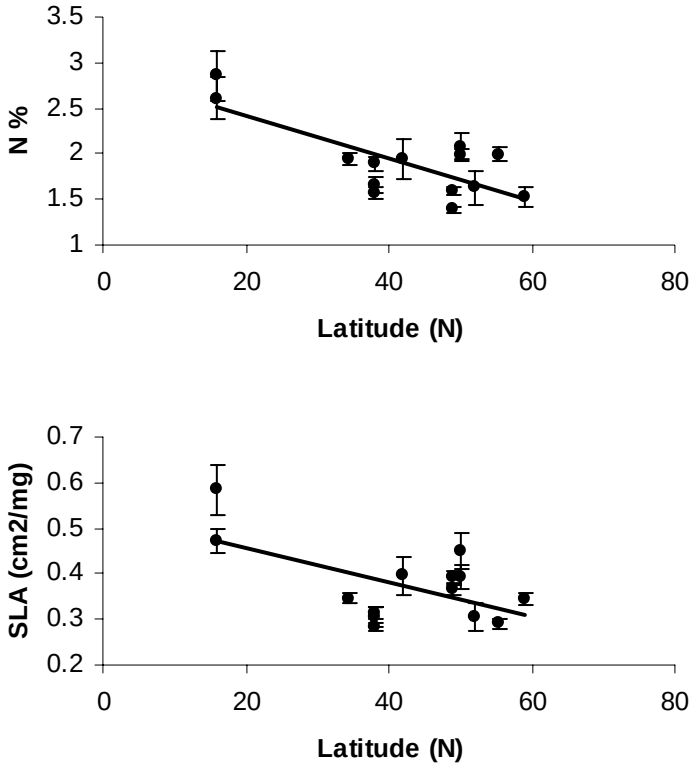


Figure 3. Leaf nitrogen content, N_L and specific leaf area, SLA are negatively correlated with latitude of seed origin (R^2=0.67 p=0.0007 ; and R^2=0.28, p=0.05 respectively).

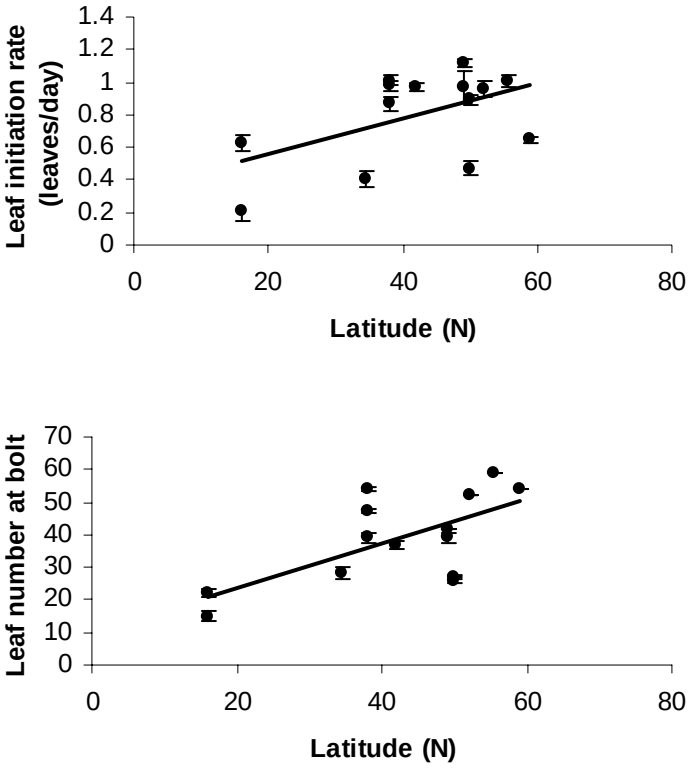


Figure 4. Leaf initiation rate and leaf number at bolting is positively correlated with latitude (R^2=0.30, p=0.04 and R^2=0.434 p=0.01 respectively).

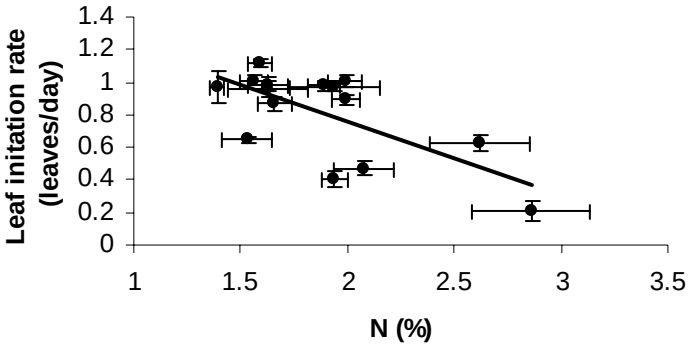


Figure 5. Leaf initiation rate is negatively correlated with leaf nitrogen content L_N (R^2=0.55, p= 0.002) across *A. thaliana* ecotypes.

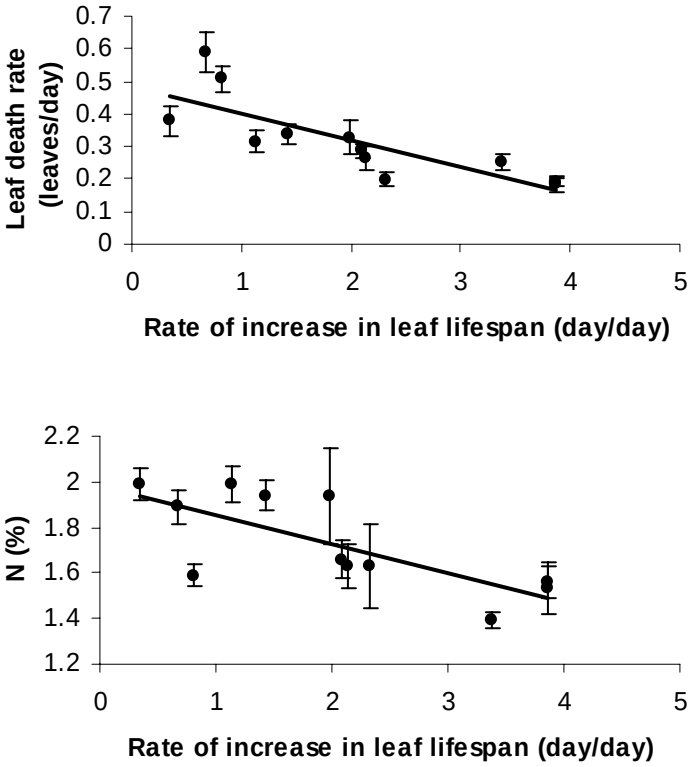


Figure 6. Relationships between the rate at which leaf lifespan increased over time, negatively correlated with leaf death rate (R^2=0.51, p=0.003) and leaf nitrogen content, L_N (R^2=0.40, p=0.02).

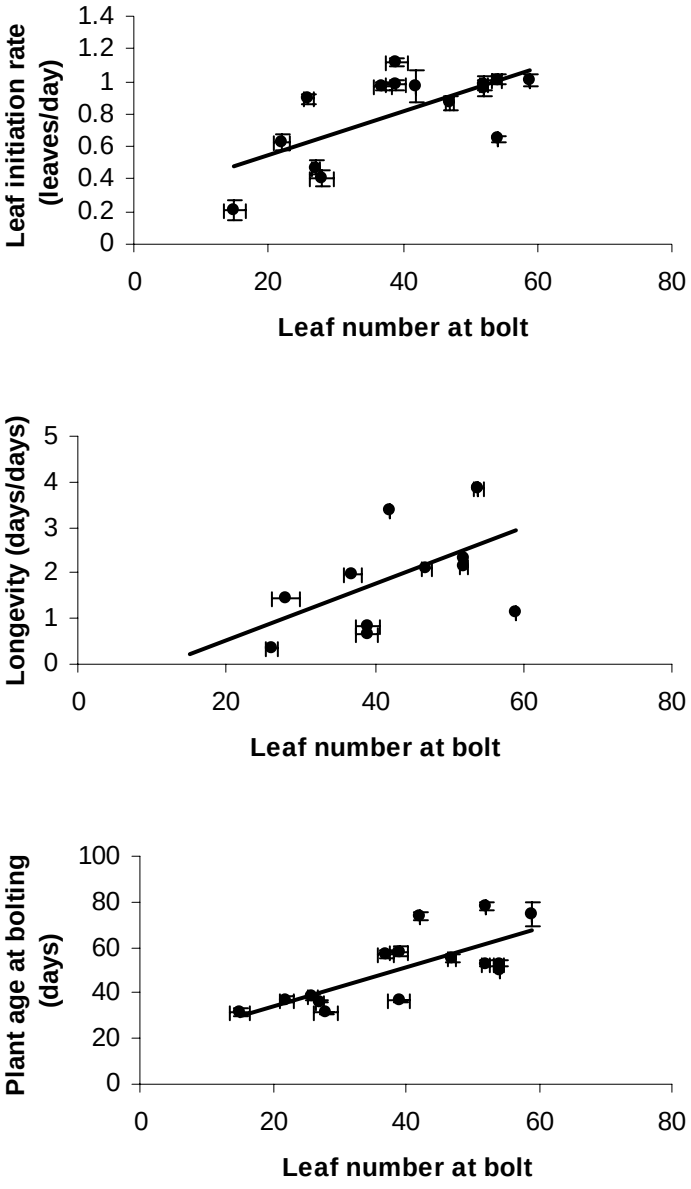


Figure 7. Leaf number at bolting positively correlated with leaf initiation rate (R^2=0.46, p=0.008), plant age at bolting (R^2=0.58, p=0.002), leaf longevity factor (R^2=.45, p=0.009).

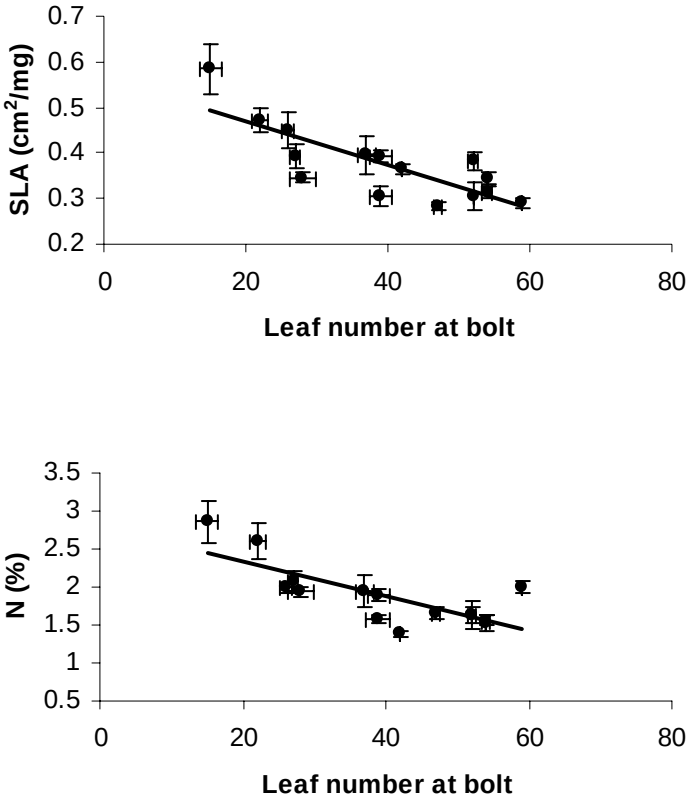


Figure 8. Leaf number at bolting negatively correlated with specific leaf area, SLA (R^2=0.53, p=0.003) and leaf nitrogen content, L_N (R^2=0.67, p=0.0003).

APPENDIX

APPENDIX 1

Appendix 1. Mean values of specific leaf area, SLA; leaf nitrogen content, L_N; photosynthetic electron transport rate per leaf mass, ETR; leaf number at bolting, leaf death rate, longevity, leaf lifespan, L_L, and leaf initiation rate for *A. thaliana* ecotypes used in this study.

Ecotype	SLA (cm2/mg)	L_N (%)	ETR/leaf mass (mg^{-1})	Leaf number at bolt	Leaf death rate (leaves/day)	Longevity (day/day)	L_L	Leaf initiation rate (leaves/day)
Br-0	0.365	1.39	0.098	42	0.0253	3.37	27.0	0.97
Bay-0	0.392	1.59	0.122	39	0.508	0.81	28.8	1.12
Col-0	0.314	1.56	0.104	54	0.189	3.87	37.2	1.01
Kondara	0.283	1.66	0.081	47	0.288	2.09	29.5	0.97
Sorbo	0.307	1.89	0.096	39	0.589	0.67	26.3	0.97
LL-0	0.397	1.94	0.119	37	0.327	1.98	33.0	0.97
Ler-0	0.050	1.99	0.122	26	0.379	0.35	22.7	0.89
Mso-0	0.290	1.99	0.082	59	0.316	1.13	33.1	1.00
Sha	0.346	1.94	0.113	27	0.338	1.43	29.8	0.41
Ler-2	0.393	2.08	0.110	27	0.385	N/A	31.8	0.47
Col-4	0.383	1.63	0.107	52	0.263	2.14	42.9	0.99
Var2-1	0.346	1.53	0.109	54	0.186	3.86	43.1	0.65
Nok-3	0.307	1.63	0.141	52	0.199	2.33	30.9	0.96
Cvi-1	0.472	2.61	0.133	22	0.347	N/A	37.5	0.63
Cvi-0	0.585	2.85	0.156	15	0.255	N/A	32.9	0.21