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Research Article

Title: Intraspecific leaf trait variation across and within wine grape varieties

Running Title: Leaf trait variation in wine grapes

Authors Information: Samantha C. Macklin [1], Rachel O. Mariani [1], Emily N. Young [1], Rosalyn Kish [1], Kimberley A. Cathline [2], Gavin Robertson [2], and Adam R. Martin [1*]

[1] Department of Physical and Environmental Sciences, University of Toronto Scarborough, Canada

[2] Horticultural & Environmental Sciences Innovation Centre, Niagara College, Canada

*Corresponding author: adam.martin@utoronto.ca

Abstract

Variability in traits forming the Leaf Economics Spectrum (LES) among and within crop species play a key role in governing agroecosystem processes. However, studies evaluating the extent, causes, and consequences of within-species variation in LES traits for some of the world's most common crops remain limited. We quantified variation in nine leaf traits measured across 90 vines of five wine grape (*Vitis vinifera*) varieties at two ontogenetic stages. Grape traits covary along an intraspecific LES, in patterns similar to those documented in wild plants. Across varieties, high rates of photosynthesis (*A*), and leaf nitrogen (N) concentrations, are coupled with low leaf mass per area (LMA), while the opposite suite of traits defines the "resource conserving end" of this intraspecific LES in grape. Variety identity predicted of leaf physiological (*A*) and morphological traits (i.e., leaf area and leaf mass), while leaf chemical traits and LMA were best explained by ontogenetic stage. All varieties expressed greater resource conserving trait syndromes (i.e., higher LMA, lower N, lower A_{mass}) later in the growing season. Traits related to leaf hydraulics, including instantaneous water-use efficiency (WUE), were unrelated to LES and other resource capture traits, and were better explained by spatial location. Our results highlight

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the relative contributions of genetic vs. phenotypic factors in structuring this variation and point to a key role of domestication in governing trait relationships in the world's crops.

Keywords: Agroecology; functional trait; intraspecific trait variation; Leaf Economics Spectrum; plant trait spectra; *Vitis vinifera*.

Introduction

The Leaf Economics Spectrum (LES) represents a suite of six leaf functional traits—maximum photosynthetic assimilation (*A*) and dark respiration rates (*R*), leaf nitrogen (N) and phosphorus (P) concentrations, leaf mass per area (LMA), and leaf lifespan (LL)—that covary with one another across [1,2] and within [3,4] plant species. The LES trait syndromes expressed by species or individual plants in turn underpin plant resource-use or ecological strategies, which range from resource acquiring strategies on one end of the LES, vs. resource conserving strategies on the other [1,2,5]. In general, resource acquiring species and plants express high rates of *A* and *R*, high leaf N, which are coupled with low LMA, and short LL; the opposite suite of traits reflects the resource conserving end of the LES [2].

The LES trait syndromes of plants scale-up to influence different aspects of whole-plant physiology, form, and function [5,6]. For instance, species expressing resource conserving LES traits or trait syndromes, are commonly associated with shade tolerant life-history strategies, while resource acquiring species often represent early successional pioneer vegetation [7-9]. At the same time, LES traits also represent the mechanism by which plant diversity influences rates of ecosystem functioning. For example, certain LES traits, including leaf N, are widely reported to predict rates of leaf-litter decomposition and soil N availability [10], while other LES traits, including *A* and LMA, are central in vegetation dynamics models [11].

To date, much of the research on the ecological and evolutionary determinants of LES trait variation and relationships in plants, has focused on LES trait expression in wild plants growing in unmanaged ecosystems [2,12]. However, more recently, studies have begun to quantify the extent, causes, and consequences of inter- and intraspecific LES trait variation in crops or their progenitors growing in managed systems. This includes studies on soy [13], coffee [14,15], wheat [16], maize [17], cocoa [18], rice [19], and sunflower [20], cultivated across field and lab-based conditions. These studies have largely focused on: 1) quantifying how plants of the

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same crop species or variety differ from one another across the LES [e.g., 15]; 2) elucidating the role environmental conditions, genetics, and/ or domestication history plays in structuring LES trait variation in crops [e.g., 18]; and finally, 3) assessing relationships between LES trait variation in crops and agroecosystem functions, including yield [13,21], tissue decomposition [22], soil microbial diversity [23], and plant-soil interactions such as N_2 fixation [24].

While results differ across studies and systems, some generalities have emerged from this line of research. First, most studies on crop LES trait variation have indicated that artificial selection has shifted some crops towards expressing among the most extreme resource acquiring LES trait values observed among plants globally [17,25]. Second, multiple studies have reported that individual plants of the same crop species or genotype differ along an "infraspecific LES" (i.e., a LES that exists below the species level), which is largely driven by environmental conditions. Specifically, within a given crop, the resource conserving end of an infraspecific LES is dictated by plants growing in unfavourable conditions (e.g., hot, dry, nutrient limited, and/or under soil compaction), while favourable growing conditions confer expression of resource acquiring LES trait syndromes [13,15].

Finally, research has consistently shown that the shape of infraspecific LESs (i.e., the slope of a bivariate statistical model that describes trait relationships) are both unique to a given crop, and often (but not always) differ from LES trait relationships observed among plants globally [13,15,19]. For example, compared to wild plants, coffee expresses lower *A* at a given leaf N concentration, which likely reflects the role selection for non-photosynthetic N-based compounds (i.e., caffeine) plays in governing coffee LES trait relationships [14,15]. Alternatively, versus wild plants, rice expresses higher rates of *A* for a given leaf N concentration, which likely reflects a history of artificial selection for improved N-use efficiency and growth [19]. Still, other crops including soy, express relationships between *A* and leaf N that are statistically indistinguishable from those in wild plants [13]. While certain generalities have emerged from this literature, multiple studies indicate that infraspecific LES relationships are unique to individual crops. However, to date there still remain relatively few studies testing for the presence of LES trait relationships in crops, and evaluating whether these crop-specific LES trait relationships differ from a "universal LES" hypothesized to describe plant trait variation globally.

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In this study, we evaluate LES trait relationships in wine grapes (*Vitis vinifera* L.): one of the world's most commercially important crops that, along with table grapes, is currently estimated to cover ~6.95 million ha of agricultural land globally. Leaf physiological, chemical, and morphological trait variation has long been the focus of many studies in the areas of crop biology and viticulture [26]. However, to our knowledge there have been no studies explicitly evaluating if wine grapes vary along an infraspecific LES, or if the shape of an infraspecific wine grape LES differs from that observed among plants globally. Here, we fill this gap by quantifying nine LES and related leaf traits in five widely cultivated wine grape varieties ('Chardonnay', 'Pinot Gris', 'Cabernet Sauvignon', 'Merlot', and 'Syrah') at two growth stages (post-flowering and veraison). We use this data to 1) quantify differences in LES traits across wine grape varieties and throughout the growing season; 2) determine if an infraspecific LES in wine grapes exists; and 3) test if wine grapes differ from wild plants in their LES trait relationships.

Materials and Methods

Study site and design

Our study was situated at the Niagara College Teaching Vineyard (previously known as "Coyote's Run" winery), situated in Niagara-on-the-Lake, Ontario, Canada (43.1697 °N, 79.1193 °W). This vineyard is situated within the Lakeshore Plains Region in the Niagara Region, which is characterised by gentle slopes, lake-effect moderated temperatures, and high incident-sunlight during the growing season. More specifically, our study site was a ~24 ha vineyard operated by Niagara College, Canada, as a teaching and operational vineyard. The vineyard is situated on top of sandy loam/red shale soils, which are well drained. Five of Ontario's most common grape varieties were selected for this study, including 'Chardonnay', 'Cabernet Sauvignon', 'Merlot', 'Pinot Gris', and 'Syrah'.

For each of these varieties, we sampled leaf traits on a total of nine plants, which were evenly distributed across three distinct sampling rows spaced ~10 m apart. Within each row, we selected three individual vines for assessment of leaf traits. Sampling rows and individual vines were marked with flagging tape to allow for sampling at two different sample times, including immediately following flowering (i.e., June 15-20, 2021), and during veraison/fruit ripening (i.e, August 10-15). All vines chosen for our study were between 1-3 cm in basal diameter. On each

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vine, we selected one individual leaf for detailed assessment of leaf traits. Leaves were all situated at ~1.5 m aboveground, which corresponded to the top of each vine canopy. Leaves chosen for sampling were all recently developed, fully expanded, fully sun-exposed, and free of pigmented blemishes or other signs of damage [27]. In sum, our trait dataset included measurements on five varieties, with each variety being represented by nine vines, with each vine spaced across three planting rows (alternatively, 45 planting rows in total). Each vine was sampled at two points in the growing season (June and August) for a total sample size of n=90 leaves.

Functional trait measurements

For each leaf we measured a total of nine physiological, morphological, and chemical traits. In the field, we used an LI-6800 portable gas exchange analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA) to evaluate leaf physiological traits, including maximum photosynthetic capacity on a per leaf area basis (A_{max}, μmol CO_2 m^{-2} s^{-1}), evapotranspiration rates (E, mmol H_2O m^{-2} s^{-1}), and stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}). All physiological measurements were taken before 13:00 to avoid stomatal closure, and under the following conditions: CO_2 concentrations of 400 ppm, relative humidity at 53-74%, leaf vapour pressure deficits of 1.2-1.7 KPa, and leaf temperatures between 24.3-31.6 °C. Physiological measurement data were also used to calculate instantaneous water-use efficiency (WUE, mmol CO_2 mol H_2O) as A_{max}/ E.

Once physiological measurements were completed, leaves were collected and transported to the University of Toronto Scarborough, Canada, for analyses of morphological and chemical traits. Here, leaves were first weighed for fresh leaf mass (g), and we then used an LI-3100C leaf area meter (LI-COR Biosciences, Lincoln, Nebraska, USA) to measure leaf area (cm^2). After this, all leaves were dried at 60°C to constant mass and re-weighed for dry mass (g). This data was used to calculate LMA (g m^{-2}) as dry mass/fresh area, and LMA data was in turn used to derive mass-based maximum photosynthetic rates (A_{mass}, $m$mol CO_2 g^{-1} s^{-1}) as A_{max}/ LMA. Finally, leaves were ground into a homogeneous fine tissue using a MM400 Retsch ball mill (Retsch Ltd., Hann, Germany), and ~0.1 mg of leaf tissue weighed, placed into foil capsules, and analysed for leaf N and C concentrations (both on a % dry mass) using a LECO CN 628 elemental analyzer (LECO Instruments, Ontario, Canada).

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Data analysis – causes of intraspecific trait variation in grape

We first evaluated statistical distributions for all traits using the '*fitdist*' function in the '*fitdistrplus*' R package [28], to identify which traits were normally or log-normally distributed, as inferred by the highest log-likelihood values. Based on these results, we calculated descriptive statistics for each trait across our entire dataset (n=90 observations for each trait), which included means and standard deviations (SD) for normally distributed traits, and medians and median absolute deviations (MAD) for log-normally distributed traits; coefficients of variation (CV) were also calculated for all traits.

An Analysis of Variance (ANOVA) procedure, coupled with Tukey's Honestly Significant Difference (HSD) post-hoc tests, was then used to evaluate if traits varied as a function of sampling time, variety identity, and planting row, as well as all two- and three-way interactions. This procedure was then paired with a variance partitioning analysis employed in previous analyses of intraspecific trait variation [15,29], to identify the factors that explained the highest proportion of variability in grape traits. This entailed first fitting a linear mixed effects model with nested random effects using the '*lme*' function in the '*nlme*' R package [30]. In this model, nested random effects were parameterized as planting rows nested within varieties which were nested within sampling time; a random intercept was included as the only fixed effects [29]. We then used the '*varcomp*' function in the '*ape*' R package [31] to partition the variance of a given trait across the nested random effects, while also quantifying the proportion of trait variability unexplained by our nested factors considered here.

Data analysis – bivariate and multivariate trait correlations

We used Pearson correlation tests to evaluate all pairwise trait relationships across our entire dataset (n=90 observations total for each test). We then further examined multivariate trait relationships in our dataset using principal components analysis (PCA) implemented with the '*rda*' in the '*vegan*' R package [32]. In this PCA, all trait data was scaled to unit variance, and A_{max} was excluded due to its strong correlation with A_{mass} (r=0.801, p<0.001). We then used the '*dimdesc*' function in the '*FactoMineR*' R package [33] to evaluate the statistical relationships between individual traits and the first two principal component axes. Our multivariate analysis also included a permutational multivariate analysis of variance (PerMANOVA) which was

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designed to test if multivariate trait syndromes varied significantly as a function of planting row, variety, and sampling time, as well as all two- and three-way interactions among these factors. Our PerMANOVA was performed using the '*adonis*' function in the '*vegan*' R package [32] and was based on n=999 permutations.

Data analysis –an intraspecific LES in grape

Our final analysis evaluated relationships among three leaf traits that are central in the LES hypothesis including LMA, A_{mass}, and leaf N [2]. We used standardized major axis (SMA) regression analysis to quantify these pairwise relationships in grape, and compare their shape (i.e., SMA slopes) and strength (i.e., SMA r^2 values) to those same trait relationships observed among plants globally. This analysis entailed first fitting an SMA regression to our grape trait dataset (n=90 leaves total) using the '*sma*' function in the '*smatr*' R package [34], and then performing this same analysis on plant species in the GLOPNET dataset of Wright et al. [2]. These GLOPNET analyses were based on n=764 species with paired LMA-A_{mass} data, n=1,958 species with paired LMA-leaf N data, and n=706 plant species with paired A_{mass}-leaf N data. We tested for statistically significant differences in the slopes of these LES trait relationships in grape vs. wild plants in GLOPNET, using the '*slope.test*' function in the '*smatr*' R package [34].

Results

Trait variation across grapevines

All traits ranged widely across the varieties and sampling times evaluated here, with all traits except LMA, leaf C, and leaf N expressing CVs ≥ 20 (Table 1). Physiological traits were particularly variable, such that A_{max} ranged from 2.3-20.1 μmol CO_2 m^{-2} s^{-1} (CV=26.7) and A_{mass} from 0.043-0.338 μmol CO_2 g^{-1} s^{-1} (CV=30.2). Similarly, WUE ranged widely from 1.0-19.1 mmol CO_2 mol H_2O^{-1} (CV=63.9) and g_s from 0.012-0.83 mol H_2O m^{-2} s^{-1} (CV=97.3). Though for these groups of traits, the factors best explaining this variability differed. Variation in both A_{mass} and A_{max} was best explained by grape variety identity (explained variance=27.3 and 37.8%, respectively), while variation in WUE and g_s was best explained by spatial location/ row identity (explained variance=9.2 and 12.9%, respectively). Variation in traits related to leaf size, including leaf dry mass (range=0.399-2.04 g) and leaf area (range=52.6-241.7 cm^2), was also best explained by variety identity (explained variance=29.4 and 35.5%, respectively). Leaf

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chemical traits, including leaf C and N concentrations, were the least variable (CV=2.0 and 17.0, respectively), with values ranging from 41.4-45.6 % C and 2.2-4.3% N. Leaf chemical traits, along with LMA, were best explained by sampling month, therefore reflecting trait variation that occurred as plants develop from June to August (Table 1, Figure 1).

With the exceptions of WUE and g_s, variance partitioning and ANOVA indicated that variety identity, sampling time, as well as a variety-by-sampling time interaction term, were the most important factors determining leaf trait variation in our dataset. Across all traits except WUE and g_s, the combination of variety identity and sampling time explained between 27.3-81.5% of trait variation (Table 1). Moreover, except in the case of A_{max}, traits varied significantly as a function of variety and sampling time (Figure 1, Table S1). Across varieties, 'Pinot Gris' most consistently expressed a suite of traits that was the clearest 'resource acquiring' trait syndrome. In our dataset, 'Pinot Gris' expressed among the highest values of A_{max} (15.1 and 13.7 μmol CO_2 m^{-2} s^{-1} in June and August, respectively), A_{mass} (0.256 and 0.186 μmol CO_2 g^{-1} s^{-1} in June and August, respectively), and leaf N (3.4 and 2.5% in June and August, respectively), and the lowest LMA values (59.9 and 73.4 g m^{-2} in June and August, respectively; Figure 1).

One of the most consistent patterns observed in our analysis is that across all varieties and traits, grape generally expresses more 'resource conservative" trait syndromes in their leaves through the growing season. This entailed all varieties expressing statistically significant (Tukey's HSD $p<0.05$) increases in LMA between June and August sampling periods, four varieties expressing statistically significant (Tukey's HSD $p<0.05$) declines in leaf N, and three varieties expressing statistically significant (Tukey's HSD $p<0.05$) declines in A_{mass} over the same time (Figure 1). Also consistent with plants moving towards more resource conserving trait syndromes through the growing season, leaves were smaller in area in August vs. June within all varieties, though these differences were not statistically significant (Figure 1). Four of five varieties also expressed statistically significant declines (Tukey's HSD $p>0.05$) in leaf C concentrations between the two sampling periods.

Relationships among LES and other leaf traits in wine grape

Trait relationships in grape were largely consistent with patterns observed of the LES, including positive relationships among A_{mass} and leaf N (Pearson $p<0.001$, $r=0.448$), both of which traded-off with LMA (Pearson $r=-0.424$ and $r=-0.727$, respectively, $p<0.001$ in both

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cases; Figure 2, Table S2). Leaf C concentrations also expressed significant relationships with certain LES traits, notably a positive correlation with leaf N (Pearson r=0.63, p<0.001) and a negative relationship with LMA (Pearson r=-0.359, p<0.001; Figure 2, Table S2). While traits associated with plant water relations were correlated with one another (Pearson r=-0.67, p<0.001), WUE and g_s were unrelated to any other traits measured here associated with C assimilation, leaf chemistry, or leaf size (Figure 2, Table S2).

Multivariate analysis revealed that the first two principal components explained 36.2% and 21.6% of the trait variation in grape traits (Figure 3). Consistent with results from bivariate analyses, the first PCA axis was significantly associated with LES traits including A_{mass} (r=0.559, p<0.001), and leaf N (r=0.932, p<0.001), which trade-off against LMA (r=-0.782, p<0.001; Figure 3, Table S2). Other traits including leaf C (r=0.721, p<0.001) and leaf area (r=0.375, p<0.001) also loaded onto the first principal component axis, thereby contributing to the suite of traits that reflect resource acquisition (Figure 3, Table S2). The second principal component was primarily defined by WUE (r=0.806, p<0.001) which traded-off against g_s (r=-0.851, p<0.001; Figure 3, Table S2).

Our PerMANOVA analysis was consistent with univariate analysis of traits and causes of trait variation, with both sampling time and variety being statistically significant predictors of multivariate trait syndromes. These two factors explained a total of 50.3% of the variation in traits, with variety identity explaining 27.4% and sampling time explaining an additional 22.9% of variation (PerMANOVA p<0.001 in both cases; Table S3). While variety differences were more difficult to distinguish visually in our PCA, trait observations measured at different sampling times were clearly differentiated across the first PCA axis. Specifically, leaves from all varieties sampled in June were strongly associated with the resource acquiring end of the first PCA axis, which reflected larger leaves with higher A_{mass}, leaf N, leaf C, and lower LMA. The opposite suite of traits characterized leaves from all varieties sampled in August (Figure 3).

A Leaf Economics Spectrum in wine grape varieties

Relationships among three core LES traits evaluated here including A_{mass}, leaf N, and LMA, closely matched patterns of LES trait variation observed among plants globally. This included positive SMA relationships among A_{mass} and leaf N (SMA model r^2=0.189, p<0.001), and negative relationships between LMA and A_{mass} (SMA model r^2=0.191, p<0.001), and LMA

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and leaf N (SMA model r^2=0.507, p<0.001; Figure 4, Table S4). Positive scaling relationships between A_{mass} and leaf N in grape (SMA model slope=0.12) were statistically indistinguishable from the A_{mass}-leaf N observed in the GLOPNET dataset of plants globally (SMA model slope=0.11, slope test r=0.03, p=0.77; Figure 4, Table S4). Our analysis did detect statistically significant differences in LES trait scaling relationships between LMA and leaf N in grape which were steeper (SMA model slope=-0.04) vs. the GLOPNET dataset (SMA model slope=-0.008; slope test r=0.96, p<0.001; Figure 4, Table S4). Similarly, relationships between A_{mass} and LMA in grape (SMA model slope=-225.2) differed statistically from that observed in the GLOPNET dataset (SMA model slope=-868.1; slope test r=-868.1, p<0.001; Figure 4, Table S4).

Discussion

Our study reveals that the five wine grape varieties evaluated here differ significantly in their leaf physiological, chemical, and morphological traits. Specifically, our analysis revealed that variety differences in A_{mass}, leaf C, leaf N, leaf size (both mass and area), and to a lesser extent LMA, reflect differences in ecological strategies among varieties (Figure 3). Across our entire dataset, we detected some evidence that white varieties ('Chardonnay' and 'Pinot Gris') express more resource acquiring trait syndromes vs. red varieties ('Cabernet Sauvignon', 'Syrah', and 'Merlot') including higher A_{mass} and lower LMA (Figure 1). Yet although variety identity explains up to 37.8% of leaf trait values (Table 1), seasonal change in trait syndromes was also a pronounced determinant of trait syndromes in wine grape.

As vines of all varieties enter veraison, leaves shift from resource acquiring to resource conserving leaf trait syndromes. With few exceptions, all varieties expressed declines in A_{max}, A_{mass}, leaf N, and leaf area, alongside increases in LMA, between the two sampling times, and leaves were strongly and statistically differentiated in multivariate trait space according to sampling time (Figure 3). Taken together, variety differences, seasonal change in traits, and their interaction, are the most important factors structuring intraspecific leaf trait variation in grape. Alternatively, finer-scale spatial variation in traits within a given variety at a given sampling time—accounted for here as sampling row identity—explained little variation in leaf traits, particularly those associated with the C economy of leaves.

Systematic varietal differences in longer-term leaf hydraulic traits including water potential at turgor loss point have been well documented, and indeed represent a primary basis of

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variety selection under climate change [e.g., 35]. However, in our study, unlike traits reflecting hydraulic safety margins or resource capture traits, we found g_s and WUE did not strongly vary across varieties or sampling times. Instead, these traits were better explained by planting rows variation, though systematic differences across rows were not statistically significant. This finding is consistent with previous work on crop traits reporting that at the farm scale, leaf hydraulic traits reflecting short-term water fluxes such as WUE are often explained by localized environmental conditions [13].

We detected evidence that grape vines fall along an infraspecific LES consistent with that observed in wild plants [1,2]. This includes statistically significant positive covariation between A_{mass} and leaf N, both of which trade-off with LMA (Figure 4). This finding contributes to existing literature on intraspecific LES trait variation, showing that plants of multiple domesticated plant species differentiate along intraspecific Leaf Economics Spectra [13,15,19]. This also aligns with our recent work showing that within 'Chardonnay' alone, vines differ in their resource capture trait in response to soil compaction [36]. Here we find that in wine grape varieties, relationships between leaf N and A_{mass} are statistically indistinguishable from the same relationship observed in plants globally (Figure 4). And while other LES trait relationships in grape evaluated here, including those between LMA, $A_{mass,}$ and leaf N, did differ statistically from those quantified in the GLOPNET dataset, grape LES traits and their relationships were broadly aligned in both datasets (Figure 4).

This is unlike certain crops including rice [19] and coffee [15], which owing to their domestication syndromes that favour intensive resource-use efficiencies or secondary compounds, express LES trait relationships that differ statistically from those observed in wild plants. Instead, wine grapes appear to align more closely with crops such as soy whose LES relationships match closely with wild plants [13]. When taken with the broader literature on crop traits, the following hypothesis is emerging: LES trait relationships in crops are altered from those in wild plants, when the domestication syndrome entails targeted alteration to the N economy of leaves and plants. In our study, the sample sizes for individual varieties (n=18) are likely too small to evaluate if varieties differ in their LES trait relationships [37]. However, one emerging theme in studies on intraspecific leaf trait relationships, is whether plants are constrained along a single LES that is unique to a given species or genotype. To date, this has been only weakly addressed, with different datasets from coffee indicating that a single

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intraspecific LES describes plant trait syndrome differences across growing conditions [15] and ontogeny [14]. Therefore, expanding our study design here to include a greater number of wine grape varieties, and expanding sampling designs, would inform this question.

In defining the traits that form the LES in plants, we also detected evidence that in wine grapes, leaf C concentrations correlate positively with traits associated with resource acquisition including leaf N and (in multivariate space) A_{mass}, while trading off against LMA (Figures 2 and 3). In other crops, leaf C has been found to reflect leaf construction costs and therefore positively correlate with LMA and leaf dry matter content, such that higher leaf C values generally reflect a resource conserving trait strategy [15,21]. However, here and in our previous research on 'Chardonnay' traits [36], higher leaf C values are associated with a resource acquiring trait syndrome. In addition to strong inter-varietal variation in leaf C, we also detected consistent and pronounced seasonal declines in leaf C in all varieties except 'Merlot' (Figure 1). These trait relationships may reflect a component of the domestication syndrome that is unique to wine grapes, namely strong artificial selection for starches and sugars throughout the growing season which then deplete during veraison [26].

Our work here contributes to the growing literature indicating that plants of the same species differ from one another in their leaf traits, with plants differentiating along infraspecific LESs that show important similarities and differences from the LES observed in plants globally. Here we find that variety and seasonal differences in leaf traits are most important in structuring trait-based ecological strategies of wine grapes, with fine scale spatial variation conditions being a smaller component. Therefore, for studies on the comparative ecology of plants, our data indicates significant genetic variation across wine grape varieties, and temporal variation in relation to reproduction.

Supplementary Materials

This manuscript also contains Supplementary Information as follows:

Table S1. Results of Pearson correlation tests evaluating bivariate relationships across nine leaf traits measured across five wine grape varieties in Southern Ontario, Canada, at two sampling times.

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Table S2. Contributions of leaf traits towards two primary axes in a principal component analysis (PCA) across wine grape leaves, measured on five varieties at two different times during the growing season.

Table S3. Results of a permutational multivariate analysis of variance (PerMANOVA) evaluating variation in seven leaf traits measured in *n*=90 leaves from five different grape varieties at two points in the growing season.

Table S4. Standardized major axis (SMA) regression models evaluating bivariate correlations in three traits forming the Leaf Economics Spectrum (LES).

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Author Contributions

Conceptualization, Samantha Macklin, Rachel Mariani , Emily Young and Adam Martin; Data curation, Samantha Macklin, Rachel Mariani , Emily Young and Rosalyn Kish; Formal analysis, Samantha Macklin and Adam Martin; Funding acquisition, Kimberley Cathline, Gavin Robertson and Adam Martin; Investigation, Samantha Macklin, Rachel Mariani , Emily Young and Rosalyn Kish; Methodology, Rachel Mariani and Adam Martin; Project administration, Kimberley Cathline, Gavin Robertson and Adam Martin; Resources, Kimberley Cathline, Gavin Robertson and Adam Martin; Supervision, Adam Martin; Visualization, Samantha Macklin and Adam Martin; Writing – original draft, Samantha Macklin and Adam Martin; Writing – review & editing, Rachel Mariani , Emily Young, Rosalyn Kish and Kimberley Cathline.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data are not yet provided but will be archived in the TRY Functional Trait Database upon publication of the manuscript.

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Tables and Figures

Table 1. Descriptive statistics for nine leaf functional traits measured across five grape varieties at two different sampling times. Trait distributions were determined based on the highest log-likelihood scores (highlighted in bold). For normally distributed traits, means and standard deviations (SD) are presented, while for log-normally distributed traits, median and median absolute deviations (MAD) are presented. Sample sizes in all cases are n=90 leaves, and trait acronyms are as follows: A_{max}: light saturated maximum photosynthetic rate on a per unit leaf area basis; A_{mass}: light saturated maximum photosynthetic rate on a per unit leaf mass basis; g_s: stomatal conductance; WUE: instantaneous water use efficiency; LMA: leaf mass per unit area.

		Distribution Fitting		Descriptive Statistics				Variance Partitioning			
Trait group	**Trait**	**Normal**	**Log-normal**	**Mean/ Median**	**SD/ MAD**	**Range**	**CV**	**Time**	**Variety**	**Row**	**Unexplained**
Physiological	A_{max} (μmol CO_2 m^{-2} s^{-1})	**-240.4**	-261.8	13.2	3.52	3.0-20.1	26.7	0.000	**0.273**	0.041	0.686
	A_{mass} (μmol CO_2 g^{-1} s^{-1})	**129.6**	115.5	0.19	0.06	0.04-0.34	30.1	0.108	**0.378**	0.036	0.478
	g_s (mol H_2O m^{-2} s^{-1})	-0.9	**44.2**	0.188	0.13	0.012-0.83	97.3	0.075	0.064	**0.129**	0.732
	WUE (mmol CO_2 mol H_2O^{-1})	-318.0	**-222.5**	4.3	2.2	1.02-19.1	63.9	0.000	0.000	**0.109**	0.891
Morphological	Leaf dry mass (g)	-32.8	**-25.1**	0.88	0.36	0.4-2.0	37.9	0.000	**0.294**	0.000	0.706
	Leaf area (cm^2)	-464.7	**-462.1**	125.6	37.5	52.6-241.7	32.5	0.150	**0.355**	0.000	0.495
	LMA (g m^{-2})	-357.9	**-357.2**	68.8	14.6	44.9-102.8	18.4	**0.748**	0.021	0.000	0.231
Chemical	Carbon (% mass)	**-116.8**	-117.1	43.6	0.9	41.4-45.6	2.0	**0.476**	0.205	0.045	0.275
	Nitrogen (% mass)	-64.6	**-61.6**	2.9	0.5	2.2-4.3	17.0	**0.779**	0.037	0.048	0.136

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Table 2. Results of Analysis of Variance (ANOVA) testing variation in nine leaf traits measured across two times in the growing season, five varieties, and individual planting rows. Values shown here are *F*-statistics and associated *p*-values (in parentheses), where sample size for all ANOVAs was n=90 leaves distributed equally across two sampling times (n=45 leaves per sampling time total), five varieties (n=18 leaves per variety total), and three rows for each sampling time-by-variety combination. Statistically significant effects (where p<0.05) are highlighted in bold, and trait acronyms are as follows: A_{max}: light saturated maximum photosynthetic rate on a per unit leaf area basis; A_{mass}: light saturated maximum photosynthetic rate on a per unit leaf mass basis; g_s: stomatal conductance; WUE: instantaneous water use efficiency; LMA: leaf mass per unit area. Results here and associated post-hoc tests are presented visually in Figure 1.

Trait group	Trait	Month	Variety	Row	Month* Variety	Month* Row	Variety* Row	Month* Variety* Row
Physiological	A_{max}	3.18 (0.08)	1.92 (0.119)	0.64 (0.529)	**7.99 (<0.001)**	0.08 (0.92)	1.04 (0.417)	1.73 (0.11)
	A_{mass}	**18.49 (<0.001)**	**6.46 (<0.001)**	1.17 (0.317)	**10.22 (<0.001)**	0.07 (0.933)	0.94 (0.495)	1.82 (0.091)
	log-g_s	**6.95 (0.011)**	1.96 (0.112)	2.1 (0.132)	**2.66 (0.041)**	1.61 (0.208)	1.52 (0.17)	1.38 (0.225)
	log-WUE	0.05 (0.832)	1.795 (0.146)	0.773 (0.466)	0.368 (0.83)	0.92 (0.403)	2.78 (0.011)	0.45 (0.889)
Morphological	log-Dry mass	0.65 (0.425)	**8.17 (<0.001)**	1.01 (0.369)	0.54 (0.705)	0.02 (0.984)	0.64 (0.744)	0.43 (0.897)
	log-Area	**18.72 (<0.001)**	**12.39 (<0.001)**	1.17 (0.318)	0.82 (0.52)	0.12 (0.885)	0.61 (0.77)	0.44 (0.895)
	log-LMA	**146.87 (<0.001)**	2.26 (0.074)	0.26 (0.775)	1.38 (0.253)	0.12 (0.885)	1.23 (0.297)	1.11 (0.372)
Chemical	Leaf C	**86.13 (<0.001)**	**9.26 (<0.001)**	0.07 (0.937)	**7.15 (<0.001)**	0.66 (0.521)	2.02 (0.059)	1.53 (0.168)
	log-Leaf N	**261.85 (<0.001)**	**4.2 (0.005)**	0.82 (0.45)	**4.79 (0.002)**	1.5 (0.232)	**2.76 (0.012)**	1.83 (0.089)

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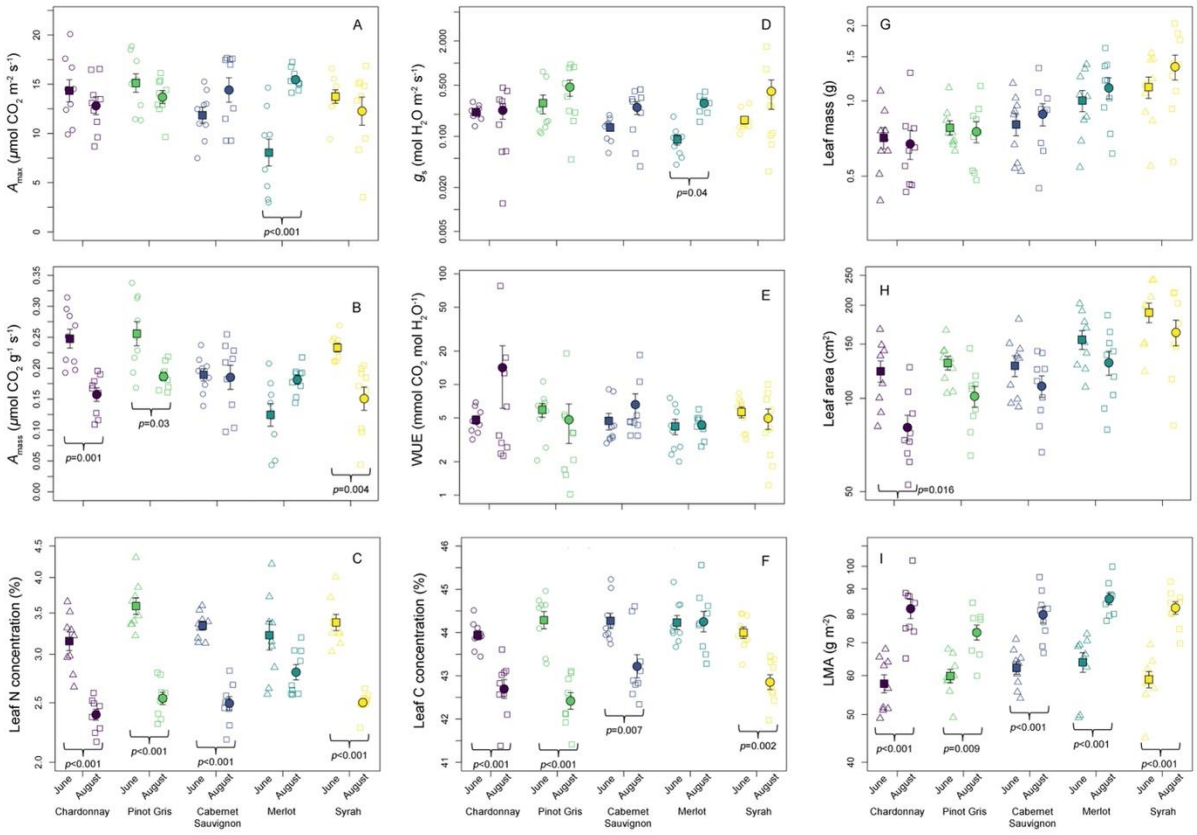

Figure 1. Functional trait variation across five wine grape varieties at two times during a growing season. Colours correspond to different wine grape varieties, with square symbols represent trait values measured early in the growing season (June) and circles represent trait values measured late in the growing season during veraison (August). Trait data are presented on log-scales where appropriate as informed by summary statistics presented in Table 1, and results from analyses of variance (ANOVA) testing for differences in traits across varieties, sampling times, planting rows, and all interactions, are presented in Table 2. Also presented are certain results from Tukey's Honestly Significant Difference (HSD) post-hoc tests. For clarity, only instances where traits varied significantly within varieties across sampling times (Tukey's HSD $p<0.05$) are shown below a given contrast. Trait acronyms are as follows: A_{max}: light saturated maximum photosynthetic rate on a per unit leaf area basis; A_{mass}: light saturated maximum photosynthetic rate on a per unit leaf mass basis; g_s: stomatal conductance; WUE: instantaneous water use efficiency; LMA: leaf mass per unit area.

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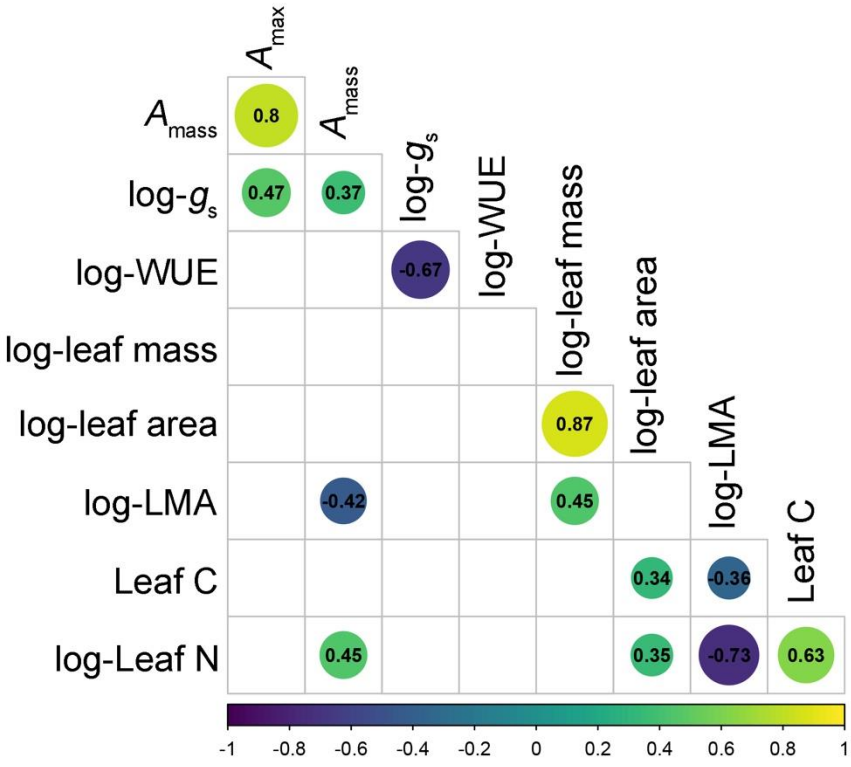

Figure 2. Pearson correlation tests analyzing relationships between nine leaf traits measured across five wine grape varieties in Southern Ontario, Canada. Shades of circles correspond to Pearson correlation coefficients for each test which are presented numerically within the circles. Sample sizes for each correlation test were n=90 leaves, and only statistically significant trait correlations represented by circles/correlation coefficients (where $p \leq 0.05$) are presented here. A full trait correlation matrix is presented in full in Table S1. Trait acronyms are as follows: A_{max}: light saturated maximum photosynthetic rate on a per unit leaf area basis; A_{mass}: light saturated maximum photosynthetic rate on a per unit leaf mass basis; g_s: stomatal conductance; WUE: instantaneous water use efficiency; LMA: leaf mass per unit area.

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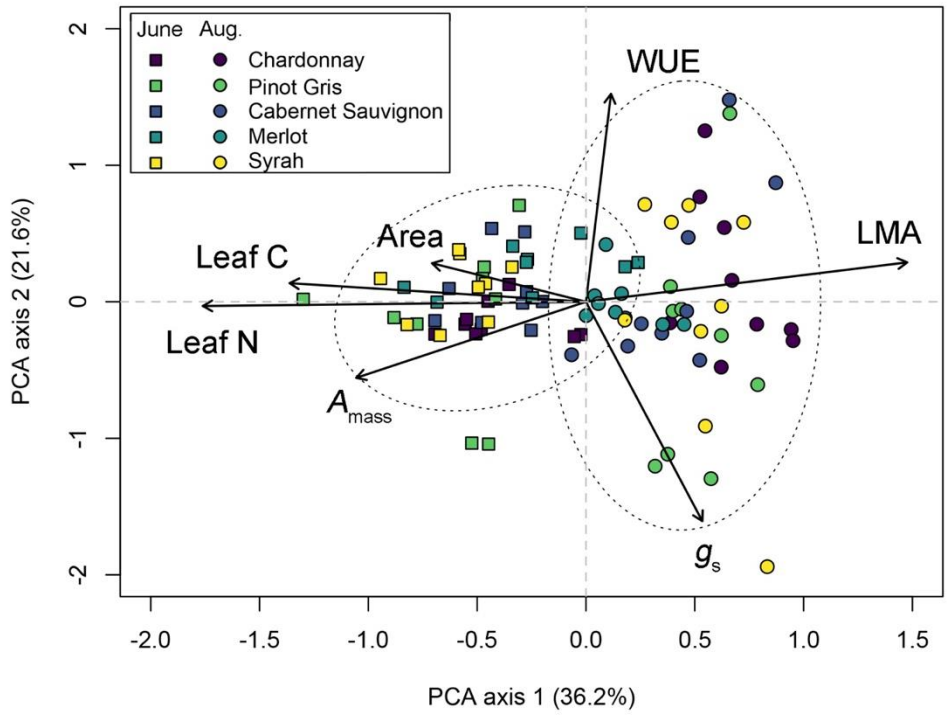

Figure 3. Principle components analysis (PCA) evaluating multivariate trait relationships across five wine grape varieties across two time points in the growing season. Only seven of nine traits quantified in this study were included here, due to strong collinearity in certain traits (see Figure 2). Colours correspond to different varieties, while symbols represent different sampling times. To aid in visualization, also presented here are 95% confidence ellipses surrounding the two different sampling times which explained 22.9% of the variation in the seven traits analyzed here. Associated permutational analysis of variance (PerMANOVA) and relationships between individual traits and PCA axes are presented in Tables S2 and S3. Trait acronyms are as follows: A_{max}: light saturated maximum photosynthetic rate on a per unit leaf area basis; A_{mass}: light saturated maximum photosynthetic rate on a per unit leaf mass basis; g_s: stomatal conductance; WUE: instantaneous water use efficiency; LMA: leaf mass per unit area.

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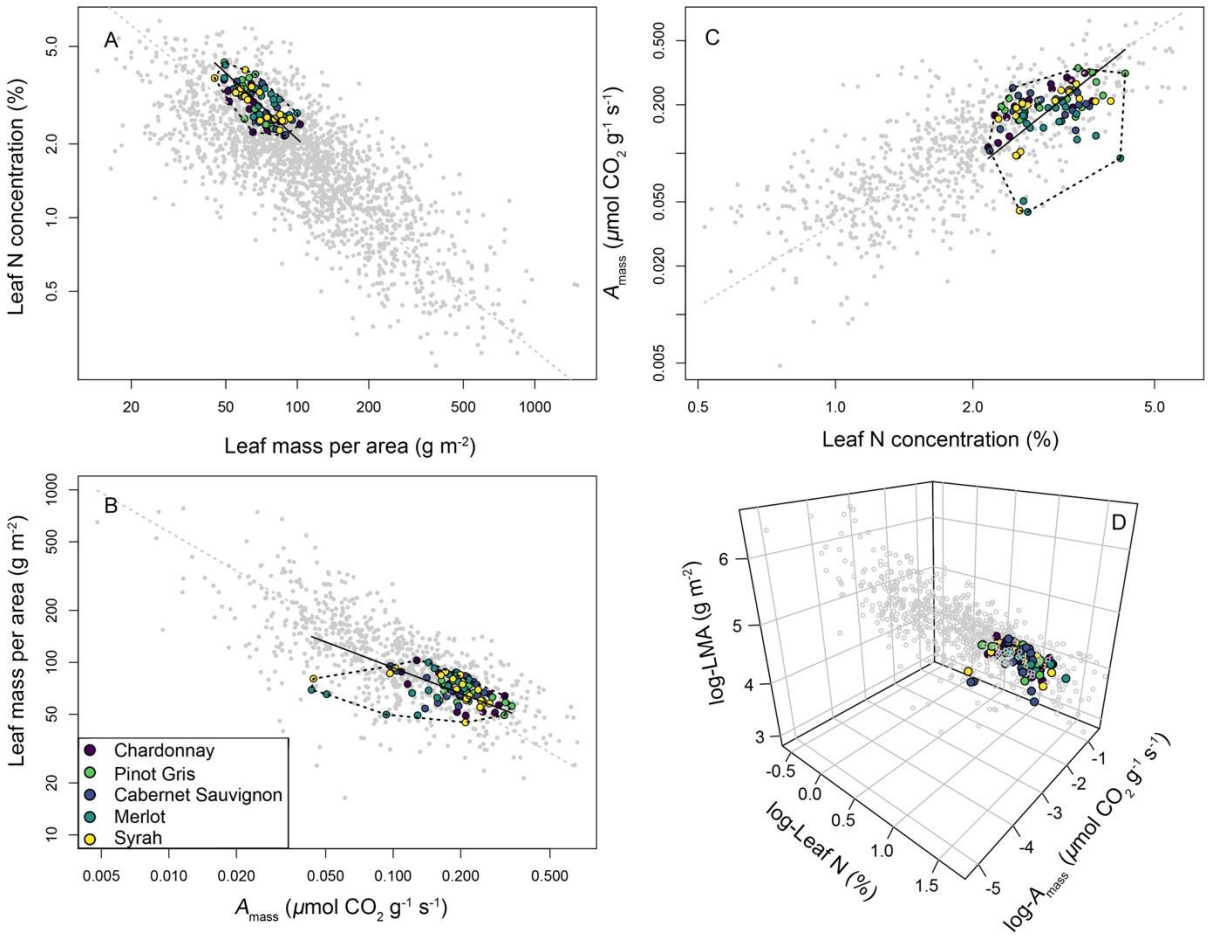

Figure 4. Leaf Economics Spectrum trait relationships in wine grapes. Coloured points correspond to different grape varieties, which are not differentiated based on sampling time here to aid in visualization. Black solid trend lines correspond to the standardized major axis (SMA) regression model of a given bivariate trait relationship across wine grapes (where SMA model $p<0.05$ and $r^2 \geq 0.189$ in all cases) and dashed black trend lines in panels A-C represent convex hull models that encapsulate the two-dimensional trait space occupied by wine grape leaves. Also shown in all panels are the data and SMA models for the same LES trait relationships observed among wild plants in the GLOPNET dataset (grey dashed trend lines and points). SMA models were fit to the 'Wine grape variety' dataset, and wild plants derived from the GLOPNET dataset (Wright et al. 2004). Full model diagnostics for each SMA model in panels A-C are presented in full in Table S4. Trait acronyms are as follows: A_{mass}: light saturated maximum photosynthetic rate on a per unit leaf mass basis; LMA: leaf mass per unit area.