

Łukasz Dylewski, Dawid Adamczyk, Roma Żytkowiak,
Andrzej M. Jagodziński*

Seed mass and seed resources – testing the defense trade-off hypothesis in woody plants

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Abstract: Physical and chemical defenses of seeds are essential for plant survival and reproduction. By protecting their seeds from herbivores and other threats, plants can ensure that their offspring have a better chance of surviving and growing into adult plants. Plant seed-size variation can affect their ability to allocate defense resources and nutrients. Smaller seeds tend to contain fewer resources and are likely to be more constrained in resource allocation to defense or to nutrient supply for the germinating seedling than larger seeds. In this study, we hypothesized that (1) the trade-off between physical and chemical defenses and reserve materials (in this study TNC content) in seeds is related to the allocation of those resources to seeds, 2) the direction of allocation of these resources is mediated by seed size. Based on seeds of 77 woody plant species collected in the Kórnik Arboretum located in western Poland, we measured several physical and chemical seed traits: seed mass, total phenols, tannin content, crude fiber, lignin content, and total non-structural carbohydrates. We revealed that chemical defense (phenol content) and total non-structural carbohydrates increased with seed mass, whereas physical defense (fiber content) had the opposite effect. We found that the trade-off between physical and chemical defense in plants was often mediated by seed size.

Keywords: seed size, trade-off hypothesis, plant traits, physical and chemical defense, woody plants

Addresses: Ł. Dylewski, Poznań University of Life Sciences, Department of Zoology, Wojska Polskiego 71C, 60-625 Poznań, Poland;  <https://orcid.org/0000-0002-1370-7625>, e-mail: lukasz.dylewski@up.poznan.pl
D. Adamczyk, R. Żytkowiak, AM Jagodziński, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland; DA  <https://orcid.org/0000-0001-7193-3332>;
RŻ  <https://orcid.org/0000-0003-1024-8694>; AMJ  <https://orcid.org/0000-0001-6899-0985>
* corresponding author

Introduction

The success of organisms depends on how their functional traits interact with the environment and other organisms (Grime, 1977; Harper, 1977). A plant functional trait can be defined as any attribute that will influence establishment and survival and plays a crucial role in determining fitness (Grime,

1977; Kunstler et al., 2016). Most theoretical frameworks in ecology postulate that predicting the success of organisms within their environments requires understanding how species traits interact with key environmental processes (Grime, 1977; Chesson, 2000). Plant functional traits are important elements in community assembly theory to explain plant success in the context of plant–plant and plant–animal

interactions. Recent studies demonstrate that by focusing on traits linked to fundamental plant life-history trade-offs, ecologists can begin to predict plant community structure at global scales (Dylewski et al., 2020).

Seed functional traits, are crucial for understanding a plant's success in the context of various ecological factors, encompassing interactions with biotic elements like seed predation and dispersal, as well as responses to abiotic factors such as drought and water limitation (Tweddle et al., 2003; Kijowska-Oberc et al., 2022, 2023). Seed size is recognized as a fundamental trait for understanding the functioning of plant communities (Moles & Westoby, 2006; Adler et al., 2014) and is linked with seedling establishment (Leishman & Westoby, 1994) as well as plant animal interactions (Moles et al., 2003; Maron et al., 2012; Dylewski et al., 2020).

Plants have evolved various defense mechanisms, both physical and chemical, which impede access to nutrients in the affected organs or are more toxic to protect against damage by herbivores as well as attack by pathogens (Hanley et al., 2007). The defensive trade-off hypothesis in plants proposes that plants allocate resources towards either growth or defense (Fine et al., 2006). This means that when a plant is facing a high level of herbivory or other environmental stress, it may allocate more resources towards defense mechanisms such as chemical compounds or physical structures that deter herbivores or protect against damage (Hanley et al., 2007; Fürstenberg-Hägg et al., 2013). This can come at the expense of growth, as the plant must divert resources that could be used for growth toward defense instead. There is evidence to support the defensive trade-off hypothesis in plants (Twigg & Socha, 1996; Hanley & Lamont, 2002), but it is also thought that plants may be able to allocate resources towards both growth and defense simultaneously to some extent, depending on the specific circumstances and the availability of resources (Koricheva et al., 2004; Moles et al., 2013). Additionally, the strength of the trade-off may vary among different plant species and in different environments (Agrawal & Fishbein, 2006).

Trade-offs with plant seed size result from energy constraints on reproductive efficiency that generate alternative life history strategies in which plant species produce either a few large or many small seeds. Large-seeded species devote resources to fewer, better-supplied seeds that can settle in various conditions. Small-seeded species maximize the number of seeds, thus increasing the chances that some seeds will reach favorable micro-sites (Turnbull et al., 1999; Moles & Westoby, 2004; Muller-Landau, 2010). Several seed traits can influence foraging decisions; seed size affords an overriding trait allowing foragers to assess overall energetic benefits vs. handling costs of

seeds (Wang et al., 2013; Lichti et al., 2017), with optimal foraging theory predicting that larger seed sizes will be favored for higher energetic returns up to the point where larger size inhibits handling time (Pyke et al., 1977; Dylewski et al., 2020). Such size dependence links seed predation to plant seed-size fecundity trade-offs (Shmida & Ellner, 1984; Tilman & Pacala, 1993) and long-term life-history trade-offs via trait syndromes (Adler et al., 2014). Some authors have argued that seeds can form coordinated defense compounds (Agrawal & Fishbein, 2006). A recent study found a marginally significant correlation between a species overall level of chemical and physical defenses that do not support arguments for compromise or coordinated defense compounds (Moles et al., 2013).

A seed's mass may determine its ability to allocate resources to defense, since seeds vary in mass by several orders of magnitude (Moles et al., 2015). For example, Wang et al., (2018) tested 163 plant species from the Xishuangbanna tropical forest and revealed that the trade-off between physical (fiber content) and chemical (total phenolics) defenses in seeds was mediated by total resource allocation, specifically showing stronger negative relationships in smaller seeds compared to larger ones. These findings emphasize the importance of considering overall resource acquisition when assessing defense trade-offs and suggest using easily measurable proxies to quantify such variations.

Consequently, smaller seeds may show: 1) a trade-off between chemical and physical defense, and 2) a trade-off between defense (both physical and chemical) and nutrient contents. In contrast, large seeds tend to have abundant resources that far exceed their requirements, i.e., many large-seeded plant species can endure a significant loss of their cotyledon biomass and continue to germinate. This excess of resources may show that large seeds may not be compromised between chemical and physical defenses as well as allocation to reserve materials. We hypothesized that (1) the trade-off between physical and chemical defenses and reserve materials (in this study TNC content) in seeds is related to the allocation of those resources to seeds, 2) the direction of allocation of these resources is mediated by seed-size. According to Wang et al. (2018), we expect that trade-off between physical and chemical defenses weakens with increasing seed weight. In this study we used crude fiber content ("proxy trait" of seed coat, i.e., Hudson, 1984; Mueller & Van der Valk, 2002; Wang et al., 2018) as an indicator of physical defense, phenols and tannins as indicators of chemical defense (regulate crucial physiological functions in plants to provide resistance against various biotic and abiotic stress conditions i.e., Hättenschwiler & Vitousek, 2000; Wang & Chen, 2011; Shimada et al., 2015). The nutritional value of seeds was indicated

by total non-structural carbohydrates. If there is a trade-off between physical and chemical defenses and nutritional content (total non-structural carbohydrates), we anticipate that plants will develop large seeds to allocate resources to both chemical defense and nutrition, thereby enhancing dispersal rates and post-dispersal survival in the soil. Conversely, according to Wang et al. (2018), we expect that small-seeded species exhibit a trade-off favoring physical defense, rather than chemical defense.

Materials and Methods

Study area

The seed materials were collected from tree and shrub species growing in the Kórnik Arboretum located in western Poland (52.2448°N, 17.0969°E, 75 m a.s.l.). The Kórnik Arboretum is the biggest and oldest arboretum in Poland, covering ca. 3500 taxa of woody plant species (Paż-Dyderska et al., 2020).

Study species

We included 77 woody plant species (trees and shrubs) belonging to 46 genera and 26 families. Selected species naturally occurring in the temperate biome (except *Melia azedarach*, occurring in the tropical biome but it is naturalized in temperate biome), (Tab. S1). Twenty-nine species are native in Europe, 23 have been introduced status in Europe, and 25 species are not native and have been not naturalized in Europe, but cultivated in botanical collections and gardens (POWO, 2023).

Chemical analysis

We collected seeds from September to November in 2020. We collected seeds or fruits directly from branches or fallen seeds from the ground. In the case of gymnosperm species, we collected cones which were not yet open and seeds were extracted in the laboratory. For the species producing fleshy fruits, we removed all the fleshy parts wrapping the seeds; for the wind-dispersed seeds, we removed the wings. We estimated dry mass of all collected seeds (oven-dried at 40 °C for at least 72 h) using 30–50 seeds. We weighed seeds using BP 210 S scales (Sartorius, Göttingen, Germany) with accuracy of 0.001 g for large-seeded species and AS 60/220.R2 (Radwag, Poland) with accuracy of 0.00001 g for small-seeded species. For chemical analysis we used 200g of dried and crushed seeds with seed coats. We determined total non-structural carbohydrates (hereafter TNC; i.e., soluble carbohydrates and starch) as described by Hansen and Møller (1975), and Haissig

and Dickson (1979). We assayed soluble carbohydrates in methanol–chloroform–water extracts ($\lambda = 625$ nm), and TNC results were expressed as % d.m. We determined the fiber content using the methodology of Brinkmann et al. (2002). We suspended powdered dry mass (200 mg) in 20 mL of washing buffer (100mM K_2HPO_4/KH_2PO_4 , pH=7.8, 0.5% Triton X-100), slowly stirred for 30 min at room temperature and centrifuged (20 min, 5500 g, swing rotor). Next, we resuspended the pellet in washing buffer and washed as above. Subsequently, we washed the pellet four times (30 min) in 100% MeOH. The resulting pellet consisted mainly of structural biomass and was dried (12h at 80 °C), weighed, and used for spectrophotometric lignin analysis by the thioglycolic acid (TGA) method (Bruce & West, 1989). Next, to determine lignin content we weighed a 1–2 mg sample of structural biomass (three replicates per individual sample) into Eppendorf tubes and mixed with 1.5 mL of 2M HCl and 0.3 mL thioglycolic acid. We incubated samples at 95 °C for 4h with repeated mixing. The samples were rapidly cooled on ice and centrifuged for 10 min at 15000 g (desk top centrifuge). The supernatant was discarded. We washed pellets three-times with distilled water (1000 μ L). Thereafter, we incubated pellets with 1000 μ L 0.5M NaOH for 18h on a shaker at room temperature. The suspension was centrifuged for 10 min at 15 000 g. We transferred the supernatant into a 2 mL Eppendorf tube. We resuspended the pellet in 0.5 mL 0.5M NaOH, vigorously mixed, and centrifuged. The resulting supernatant was combined with the first alkaline supernatant and mixed with 0.3 mL concentrated HCl. We incubated samples for 4h at 4 °C to precipitate the lignothioglycolate derivatives. We centrifuged samples, discarded the supernatant, and the pellet was solubilized in 1000 μ L 0.5M NaOH. We measured the absorbance of the resulting solution at 280 nm. We generated calibration curves by subjecting increasing amounts of 0.5–2.5 mg of commercial lignin (alkaline spruce lignin, Aldrich, Milwaukee, WI, USA) to the same procedure. We used a total of 0.1 g of tissue powder to determine the concentration of total phenols. Samples were boiled for 15 min in 95% ethanol and 10 min in 80% ethanol. Folin–Ciocalteu Phenol Reagent (Sigma F-9252) was used and the concentration of total phenols was determined spectrophotometrically by measuring absorbance at 660 nm as described by Johnson and Schaal (1957) and modified by Singleton and Rossi (1965). The concentration of total phenolic compounds (TPhC) is expressed as μ mol of chlorogenic acid per g⁻¹ dry mass. Chlorogenic acid was the standard used in these assays, and the results were expressed in terms of μ M of chlorogenic acid g⁻¹ dry mass (d.m.). Condensed (catechol) tannins, after extraction with absolute methanol, were determined

colorimetrically ($\lambda = 500$ nm) using a color reaction with vanillin in an acid medium (Price et al., 1978). We converted these results to μM catechin $\text{g}^{-1}\text{d.m.}$. Absorbance (tannins, phenols, and carbohydrates) was determined with a spectrophotometer (UV-1700 Visible Spectrophotometer; PharmaSpec, Shimadzu, Japan).

Statistical analyses

Before conducting the analyses, we applied a natural logarithm transformation to the seed mass, phenolic content, tannin content, lignin content, TNC and fiber content to achieve distributions that did not differ from normality. Next, we used z-scores standardization (centered around the mean and divided by the standard deviation) of each variable to reduce skewness in the residuals and stabilize the estimation procedure for each model. We used linear regression. First, we determined the relationships between seed mass and other seed traits (phenolic, tannin, lignin, TNC and fiber content). Second, we determined trade-offs between chemical (phenolic and tannin content), physical (fiber content) and nutrient (TNC). Third, we determined if these trade-offs were mediated by seed masses. To do that we calculated the simple correlation between total phenolic and fiber content, tannin and fiber content, TCN and phenolic content, TNC and tannin content, and TNC and fiber content for subsets of the full dataset defined by seed mass windows according to Wang et al. (2018). To determine whether seed mass affects the trade-off between seed defensive traits, we divided our dataset into six equally sized subsets based on seed mass quantiles as described by Wang et al. (2018). Then we built a linear model with trait correlations for each subset predicted as a function of the corresponding seed mass window. Analyses were carried out in R 4.0.2 (R Core Team, 2020). The simple regression models were carried out using the stats package in R. The data visualizations were performed using the ggplot2 package (Wickham, 2016).

Results

The seed masses used in our study ranged from 0.00048 g to 12.94 g (mean \pm SD: 1.14 \pm 2.96). The phenolic content ranged from 14.09 μM $\text{g}^{-1}\text{d.m.}$ to 822.93 μM $\text{g}^{-1}\text{d.m.}$ (126.40 \pm 151.18), tannin levels ranged from 0.04 μM $\text{g}^{-1}\text{d.m.}$ to 811.92 μM $\text{g}^{-1}\text{d.m.}$ (76.67 \pm 138.87). The fiber ranged from 0.24% to 0.97% (0.67 \pm 0.18) and lignin content ranged from 3.78% to 10.96% (7.04 \pm 1.64). The non-structural carbohydrates ranged from 0.94% to 53.76% (9.37 \pm 12.60).

Our results indicated that seed mass was significantly positively correlated with the tannin content ($\beta \pm \text{SE}$ 0.285 \pm 0.11, $R^2=0.07$, $F_{1,75}=6.62$, $p=0.012$, Fig. S1), phenol content (0.372 \pm 0.11, $R^2=0.13$, $F_{1,75}=12.08$, $p<0.001$, Fig. S2) and total non-structural carbohydrates (0.611 \pm 0.09, $R^2=0.36$, $F_{1,73}=42.18$, $p<0.001$, Fig. S3), but was negatively correlated with the fiber content (-0.279 ± 0.12 , $R^2=0.07$, $F_{1,75}=6.33$, $p=0.014$, Fig. S4). We did not find any relationship between seed mass and lignin content (-0.023 ± 0.12 , $R^2=0.00$, $F_{1,75}=0.40$, $p=0.838$, Fig. S5).

Our results showed a significant negative relationship between phenolic content and fiber content in seeds (-0.283 ± 0.11 , $R^2=0.07$, $F_{1,75}=6.54$, $p=0.013$,

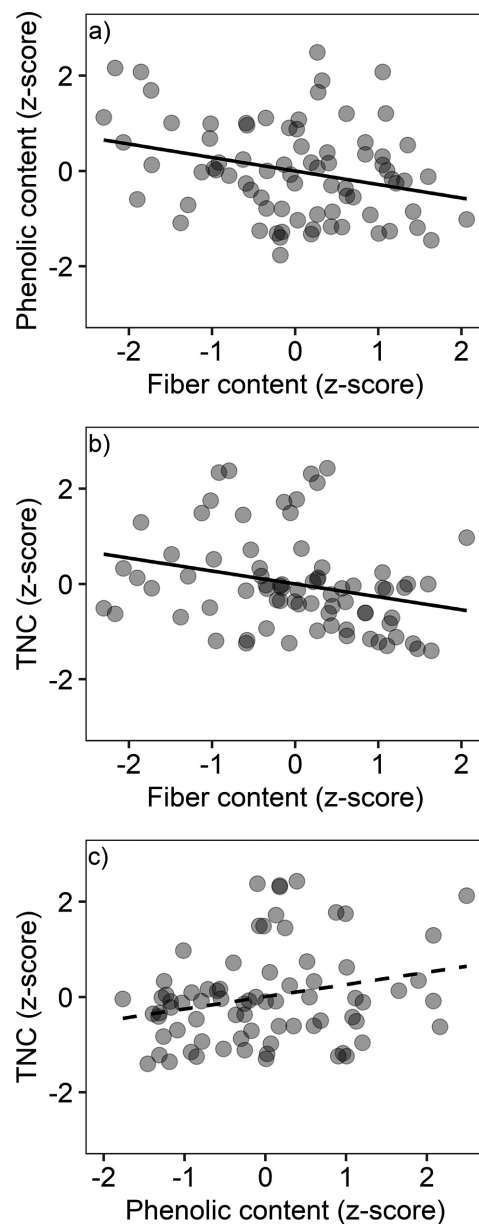


Fig. 1. The relationship between a) total phenolic content and fiber content, b) total non-structural carbohydrates and fiber content, and c) total non-structural carbohydrates and total phenolic content

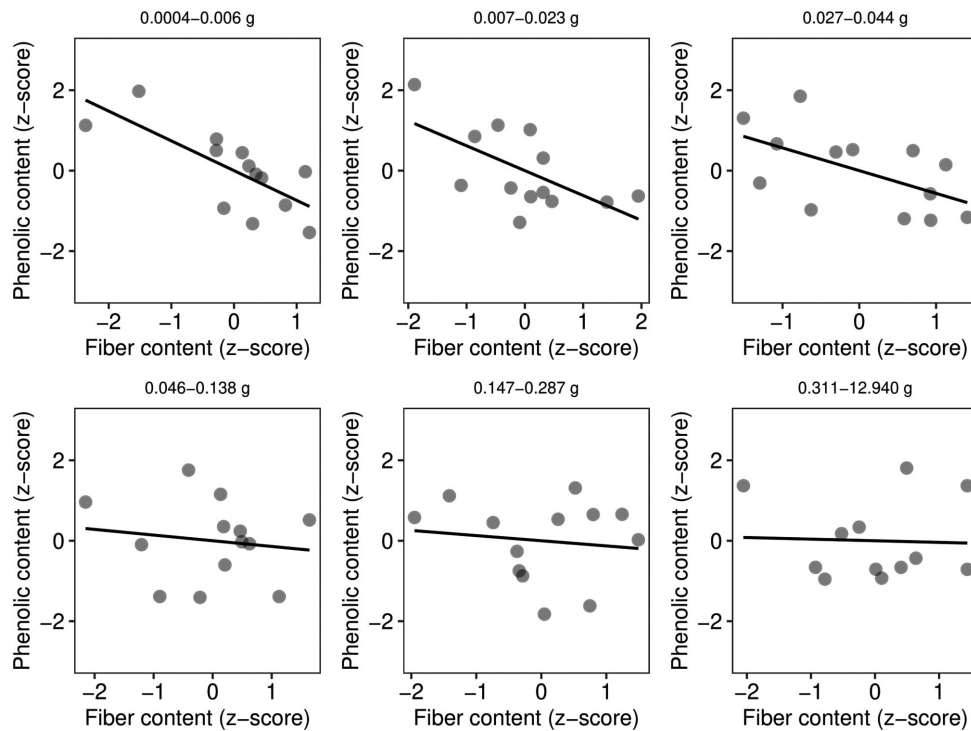


Fig. 2. The relationship between total phenolic and fiber contents across species for six seed mass windows

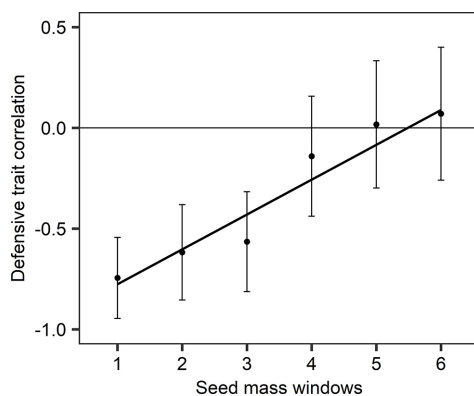


Fig. 3. The relationship between total phenolic and fiber contents across species for six seed mass windows (1: 0.0004–0.006 g; 2: 0.007–0.023 g; 3: 0.027–0.044 g; 4: 0.046–0.138 g; 5: 0.147–0.287 g; 6: 0.311–12.940 g)

Fig. 1a). However, this relationship was more strongly negative for small seeded species than for large seeded species, where the line flattens out with increasing seed mass windows (compared slope across seed mass windows: 0.153 ± 0.02 , $df=4$, $p=0.003$, $R^2=0.88$, Fig. 3). In the case of tannin content, the relationship with the fiber content was negative but non-significant (-0.194 ± 0.11 , $R^2=0.02$, $F_{1,75}=2.94$, $p=0.090$, Fig. S6). This relationship also was more strongly negative for small seeded species and became less negative for large seeded species, however, the compared slope was non-significant across seed mass windows (0.049 ± 0.04 , $df=4$, $p=0.340$, $R^2=0.03$, Fig. S7).

We found that non-structural carbohydrates were significant negatively correlated with the fiber content (-0.271 ± 0.11 , $R^2=0.06$, $F_{1,73}=5.61$, $p=0.021$, Fig. 1b) and positively correlated with the phenol content (0.257 ± 0.11 , $R^2=0.05$, $F_{1,73}=5.08$, $p=0.027$, Fig. 1c). We did not find a significant relationship between non-structural carbohydrates and tannins (0.202 ± 0.11 , $R^2=0.03$, $F_{1,73}=3.21$, $p=0.077$). Moreover, we did not find that seed size mediated a trade-off between TNC and chemical (both phenolic and tannins contents) or between TNC and physical defense ($p>0.05$, Tab. S2).

Discussion

Seeds typically possess stored nutrients, serving as a temporary source of sustenance for the establishment of seedlings (Zangerl & Bazzaz, 1992). However, several biotic and abiotic pressures may have had a selective effect on the evolutionary trajectory for allocation of energy to chemical or physical defense strategies to protect the seed embryo (Dalling et al., 2011). Across a variety of woody plant species used in the analyses, we showed several correlations between seed masses and seed resources. We found that contents of tannins, phenolics and total non-structural carbohydrates increased with the seed mass, but fiber content decreased with the seed mass. Moreover, our results supported that the trade-off between chemical and physical defenses was

mediated by seed mass and nutrients (non-structural carbohydrates). Furthermore, our results indicated a stronger trade-off between physical and chemical defenses for small-seeded species than for large-seeded species.

In this study the relationships between seed mass, physical, chemical and nutrient contents were significant. The role of chemical and physical defenses in seeds may affect interactions with animals, due to increasing attractiveness for seed dispersers (Janzen, 1969), but also avoiding seed predators (Janzen, 1971), or may affect the ability of microbial pathogens to attack seeds in the soil (Dalling et al., 2011). Even though both small- and large-seeded species are consumed by different guilds of seed predators (e.g., invertebrates and small mammals) other factors, like physiological adaptation to survive (dormancy and desiccation), may affect the allocation of energy in small and large seeds. Our results indicated that large seeds have a high level of energy content (starch and glucose) and chemical component, but lower content of fiber. Large-seeded species are associated with higher recruitment survival rates (Metz et al., 2010; Lönngberg & Eriksson, 2013) and provide an advantage for seedling establishment in dry conditions. Moreover, seedlings from large-seeded species had higher emergence and survival percentages than seedlings from small-seeded species in dry soil conditions in glasshouse experiments (Leishman & Westoby, 1994). Our results indicated that small-seeded species were characterized by higher fiber, which corresponds with a previous study (Wu et al., 2019). Small-seeded species may invest much more energy in physical defense (i.e., seed coat mass ratio) than large-seeded species due to the adaptation for defense against parasitic microorganisms (Dalling et al., 2011), seed predation by insects (Louda, 1982), but also survival of passage through the digestive system in the case of endozoochory dispersion (Chen & Moles, 2015).

Seed traits are linked to their predation and dispersion (Janzen, 1968). Due to predation pressure, seeds invest energy to various components of physical and chemical defenses against seed-eating animals (Janzen, 1971). In this study the relationships between seed mass, physical, chemical, and nutrient contents were weak but significant. Our results indicated negative correlations between physical and chemical components. We found that a nutritional trait (in our study non-structural carbohydrates) was associated with the physical and chemical defensive traits. Non-structural carbohydrates were significantly positively correlated with the phenol content, but not with the tannins. An opposite significant relationship was found between non-structural carbohydrates and fiber content. Many field-based research studies indicated various effects of seed traits

on removal rates, where seed quality interacted with other environmental factors (e.g., precipitation, shrub level, moonlight) to influence seed selection by rodents (Perea et al., 2011; Chiufo et al., 2018; Moore et al., 2022). Testing 40 tree species, Blate et al. (1998) found that seed predation decreased with seed size and hardness of seed coat. Rodents first select seeds with higher caloric values, which is correlated with seed size (Westoby et al., 1992), however, chemical components in seeds can modulate this relationship. Gong et al. (2015) found that seeds with high levels of phenolic content were better defended against seed predators, whereas other traits like seed size and coat hardness did not affect seed predation. Total phenolic contents are an important component of defense among plant species, and high content of phenolics might protect seeds from predators. Flores-Peredo and Cime (2016) revealed that rodents preferred small seeds of *Pinus* species with high levels of lipids and low tannin contents. However, some empirical evidence suggests that high levels of chemical defense like tannins and nutrition like starch increased seed removal and consumption by rodents, whereas high seed coat thickness and low tannin levels decreased seed consumption (Zhang et al., 2016). Other studies showed that seed size played the main role in seed caching behavior by small mammals, and nutrients and chemical components had less impact (Wang & Chen, 2009) or no effect (Kuprewicz & Garcia-Robledo, 2019). Our results suggest that large-seeded species which invested in both chemical and nutritional components of seeds may affect seed caching behavior by small mammals, increasing seed survival after dispersion.

Trade-offs in seeds traits may have a predominant role in shaping the relationship between plants and seed predation (Zhang et al., 2016). Our results partially support the trade-off defense hypothesis of Wang et al. (2018) in seeds of temperate woody plant species, where we found that phenolic content, but not tannins, significantly decreased with the fiber content in seeds of woody plant species. In the case of tannin content the slope was weakly negative. We also found that this trade-off was mediated by seed size, where in small-seeded species the trend was more negative than for large-seeded species. The non-significant relationship for tannins may be the result of the fact that tannins are one of the compounds belonging to phenols, and there can be no or very low tannin production in seeds in our plant species (for example a tannin level of $0.04 \mu\text{M g}^{-1}\text{d.m}$ compared with $32.64 \mu\text{M g}^{-1}\text{d.m}$ total phenolic in *Picea abies* 'virgata'). The total phenolic content varies greatly among species and represents a wide range of components, therefore it is a good indicator of carbon-based defense response chemicals in plants (Xiao et al., 2006; Steele et al., 2001; Smallwood et

al., 2001; Wang et al., 2018). Allocation of resources in plants is related to the physiological and ecological costs that a plant incurs to protect against herbivores. Most plant defense hypotheses predict a trade-off between resource investments in different types of defenses against herbivores (Koricheva et al., 2004). However, evidence of the occurrence of trade-offs between chemical and physical defenses are strongly debated. Trade-offs between chemical and physical defenses in plant leaves and seeds were tested in several studies and supported by some (Twigg & Socha, 1996; Hanley & Lamont, 2002; Zhang et al., 2016; Wang et al., 2018) or rejected by others (Read et al., 2009; Koricheva et al., 2004; Moles et al., 2013; Tiansawat et al., 2014). The global comparative research focused on large numbers of species provide little evidence to support the trade-off defensive hypothesis (Koricheva et al., 2004; Moles et al., 2013). The reason for lack of support for the trade-off defensive hypothesis is that due to selective pressures, plants deploy a different combination of defenses to compete with coexisting species. Moreover, previous studies that concentrated on seeds argued that combinations of traits like nutrients or seed size mediates the trade-off between physical and chemical defenses (Zhang et al., 2016; Wang et al., 2018). Based on 23 tree species Zhang et al. (2016) showed that seed coat thickness (physical defense) was negatively correlated with the tannin content, but the relationship was non-linear. Our results correspond with previous research conducted on seeds of 163 tropical plant species (Wang et al., 2018), where it was found that the trade-off between physical and chemical defenses was stronger for small-seeded species than among large-seeded species.

In conclusion, we found several significant relationships between seed mass and other seed traits (phenol content, fiber content and TNC). We confirmed that the trade-off between chemical and physical defenses is mediated by seed size, where in small-seeded species the trend was more negative than for large-seeded species. Moreover, we showed that TNC increased with chemical defense but decreased with the fiber content, however, these trade-offs were not mediated by seed mass. Our study found that plant species that produce small seeds tend to contain fewer resources and are likely to be more limited in their allocation to defense than plants that produce larger seeds.

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Literature

- Adler PB, Salguero-Gomez R, Compagnoni A, Hsu JS, Ray-Mukherjee J, Mbeau-Ache C & Franco M (2014) Functional traits explain variation in plant life history strategies. *Proceedings of the National Academy of Sciences* 111: 740–745. doi:10.1073/pnas.1315179111.
- Agrawal AA & Fishbein M (2006) Plant defense syndromes. *Ecology* 87: 132–149. doi:10.1890/0012-9658(2006)87[132:PDS]2.0.CO;2.
- Blate GM, Peart DR & Leighton M (1998) Post-dispersal predation on isolated seeds: a comparative study of 40 tree species in a Southeast Asian rainforest. *Oikos* 82: 522–538. doi:10.2307/3546373.
- Brinkmann K, Blaschke L & Polle A (2002) Comparison of different methods for lignin determination as a basis for calibration of near-infrared reflectance spectroscopy and implications of lignoproteins. *Journal of Chemical Ecology* 28: 2483–2501. doi:10.1023/A:1021484002582.
- Bruce RJ & West CA (1989) Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. *Plant Physiology* 91: 889–897. doi:10.1104/pp.91.3.889.
- Chen SC & Moles AT (2015) A mammoth mouthful? A test of the idea that larger animals ingest larger seeds. *Global Ecology and Biogeography* 24: 1269–1280. doi:10.1111/geb.12346.
- Chesson P (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics* 31: 343–366. doi:10.1146/annurev.ecolsys.31.1.343.
- Chiuffo MC, Moyano J, Rodriguez-Cabal MA & Nuñez MA (2018) Seed predation of non-native species along a precipitation gradient. *Plant Ecology* 219: 1307–1314. doi:10.1007/s11258-018-0880-9.
- Dalling JW, Davis AS, Schutte BJ & Arnold AE (2011) Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. *Journal of Ecology* 99: 89–95. doi:10.1111/j.1365-2745.2010.01739.x.
- Dylewski Ł, Ortega YK, Bogdziewicz M & Pearson DE (2020) Seed size predicts global effects of small mammal seed predation on plant recruitment. *Ecology Letters* 23: 1024–1033. doi:10.1111/ele.13499.
- Fine PV, Miller ZJ, Mesones I, Irazuzta S, Appel HM, Stevens MHH, Sääksjärvi, Schultz JC & Coley PD (2006) The growth–defense trade-off and habitat specialization by plants

- in Amazonian forests. *Ecology* 87: 150–162. doi:10.1890/0012-9658(2006)87[150:TG-TAHS]2.0.CO;2.
- Flores-Peredo R & Cimé BBS (2016) Pine seed predation by mice: an experimental assessment of preference. *Animal Biodiversity and Conservation* 39: 173–184. doi:10.32800/abc.2016.39.0173.
- Fürstenberg-Hägg J, Zagrobelny M & Bak S (2013) Plant defense against insect herbivores. *International Journal of Molecular Sciences* 14: 10242–10297. doi:10.3390/ijms140510242.
- Gong H, Tang C & Wang B (2015) Post dispersal seed predation and its relations with seed traits: a thirty species comparative study. *Plant Species Biology* 30: 193–201. doi:10.1111/1442-1984.12051.
- Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169–1194. doi:10.1086/283244.
- Haissig BE & Dickson RE (1979) Starch measurement in plant tissue using enzymatic hydrolysis. *Physiologia Plantarum* 47: 151–157. doi:10.1111/j.1399-3054.1979.tb03207.x.
- Hanley ME & Lamont BB (2002) Relationships between physical and chemical attributes of congeneric seedlings: how important is seedling defence? *Functional Ecology* 16: 216–222. doi:10.1046/j.1365-2435.2002.00612.x.
- Hanley ME, Lamont BB, Fairbanks MM & Rafferty CM (2007) Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics* 8: 157–178. doi:10.1016/j.ppees.2007.01.001.
- Hansen J & Møller I (1975) Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. *Analytical Biochemistry* 68: 87–94. doi:10.1016/0003-2697(75)90682-X.
- Harper JL (1977) *Population biology of plants*. Academic Press, London, UK.
- Hättenschwiler S & Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution* 15: 238–243. doi:10.1016/S0169-5347(00)01861-9.
- Hudson BJB (1984) Evening primrose (*Oenothera* spp.) oil and seed. *Journal of the American Oil Chemists' Society* 61: 540–543. doi:10.1007/BF02677026.
- Janzen DH (1969) Seed-eaters versus seed size, number, toxicity and dispersal. *Evolution* 23: 1–27. doi:10.2307/2406478.
- Janzen DH (1971) Seed predation by animals. *Annual Review of Ecology, Evolution, and Systematics* 2: 465–492. doi:10.1146/annurev.es.02.110171.002341.
- Johnson G & Schaal LA (1957) Accumulation of phenolic substances and ascorbic acid in potato tuber tissue upon injury and their possible role in disease resistance. *American Journal of Potato Research* 34: 200–209. doi:10.1007/BF02854948.
- Kijowska-Oberc J, Wawrzyniak MK, Staszak AM & Ratajczak E (2022) Exogenous seed treatment with proline and its consequences to Norway spruce (*Picea abies* (L.) H. Karst) seedling establishment. *Dendrobiology* 87: 149–162. doi:10.12657/denbio.087.011.
- Kijowska-Oberc J, Dylewski Ł & Ratajczak E (2023) Proline concentrations in seedlings of woody plants change with drought stress duration and are mediated by seed characteristics: a meta-analysis. *Scientific Reports* 13: 15157. doi:10.1038/s41598-023-40694-5.
- Koricheva J, Nykänen H & Gianoli E (2004) Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? *American Naturalist* 163: 64–75. doi:10.1086/382601.
- Kunstler G, Falster D, Coomes DA, Hui Fm Kooyman RM, Laughlin DC, Poorter L, Vanderrwel M, Vieilledent G, Wright SJ, Aiba M, Baraloto C, Caspersen J, Cornelissen JH, Gourlet-Fleury S, Hanewinkel M, Herault B, Kattge J, Kurokawa H, Onoda Y, Peñuelas J, Poorter H, Uriarte M, Richardson S, Ruiz-Benito P, Sun IE, Ståhl G, Swenson NG, Thompson J, Westerlund B, Wirth C, Zavala MA, Zeng H, Zimmerman JK, Zimmermann NE & Westoby M (2016) Plant functional traits have globally consistent effects on competition. *Nature* 529: 204–207. doi:10.1038/nature16476.
- Kuprewicz EK & García Robledo C (2019) Deciphering seed dispersal decisions: Size, not tannin content, drives seed fate and survival in a tropical forest. *Ecosphere* 10: e02551. doi:10.1002/ecs2.2551.
- Leishman MR & Westoby M (1994) The role of seed size in seedling establishment in dry soil conditions – experimental evidence from semi-arid species. *Journal of Ecology* 82: 249–258. doi:10.2307/2261293.
- Lichti NI, Steele MA & Swihart RK (2017) Seed fate and decision-making processes in scatter-hoarding rodents. *Biological Reviews* 92: 474–504. doi:10.1111/brv.12240.
- Lönnberg K & Eriksson O (2013) Rules of the seed size game: contests between large-seeded and small-seeded species. *Oikos* 122: 1080–1084. doi:10.1111/j.1600-0706.2012.00249.x.
- Louda SM (1982) Distribution ecology: variation in plant recruitment over a gradient in relation to insect seed predation. *Ecological Monographs* 52: 25–41. doi:10.2307/2937343.
- Maron JL, Pearson DE, Potter T & Ortega YK (2012) Seed size and provenance mediate the joint effects of disturbance and seed predation on communi-

- ty assembly. *Journal of Ecology* 100: 1492–1500. doi:10.1111/j.1365-2745.2012.02027.x.
- Metz MR, Sousa WP & Valencia R (2010) Widespread density-dependent seedling mortality promotes species coexistence in a highly diverse Amazonian rain forest. *Ecology* 91: 3675–3685. doi:10.1890/08-2323.1.
- Moles AT, Peco B, Wallis IR, Foley WJ, Poore AGB, Seabloom EW, Vesk PA, Bisigato AJ, Cella-Pizarro L, Clark CJ, Cohen PS, Cornwell WK, Edwards W, Ejrnæs R, Gonzales-Ojeda T, Graae BJ, Hay G, Lumbwe FC, Magaña-Rodríguez B, Moore BD, Peri PL, Poulsen JR, Stegen JC, Veldtman R, von Zeipel H, Andrew NR, Boulter SL, Borer ET, Cornelissen JHC, Farji-Brener AG, DeGabriel JL, Jurado E, Kyhn LA, Low B, Mulder CPH, Rardon-Smith K, Rodríguez-Velázquez J, De Fortier A, Zheng Z, Blendinger PG, Enquist BJ, Facelli JM, Knight T, Majer JD, Martínez-Ramos M, McQuillan P & Hui FKC (2013) Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? *New Phytology* 198: 252–263. doi:10.1111/nph.12116.
- Moles AT & Westoby M (2004) Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology* 92: 372–383. doi:10.1111/j.0022-0477.2004.00884.x.
- Moles AT & Westoby M (2006) Seed size and plant strategy across the whole life cycle. *Oikos* 113: 91–105. doi:10.1111/j.0030-1299.2006.14194.x.
- Moles AT, Warton DI & Westoby M (2003) Do small seeded species have higher survival through seed predation than large seeded species? *Ecology* 84: 3148–3161. doi:10.1890/02-0662.
- Moore NB, Stephens RB & Rowe RJ (2022) Nutritional and environmental factors influence small mammal seed selection in a northern temperate forest. *Ecosphere* 13: e4036. doi:10.1002/ecs2.4036.
- Mueller MH & van der Valk AG (2002) The potential role of ducks in wetland seed dispersal. *Wetlands* 22: 170–178. doi:10.1672/0277-5212(2002)022[0170:T-PRODI]2.0.CO;2.
- Muller-Landau HC (2010) The tolerance–fecundity trade-off and the maintenance of diversity in seed size. *Proceedings of the National Academy of Sciences* 107: 4242–4247. doi:10.1073/pnas.0911637107.
- Paź-Dyderska S, Dyderski MK, Nowak K & Jagodziński AM (2020) On the sunny side of the crown—quantification of intra-canopy SLA variation among 179 taxa. *Forest Ecology and Management* 472: 118254. doi:10.1016/j.foreco.2020.118254.
- Perea R, Gonzalez R, San Miguel A & Gil L (2011) Moonlight and Shelter Cause Differential Seed Selection and Removal by Rodents. *Animal Behaviour* 82: 717–723. doi:10.1016/j.anbehav.2011.07.001.
- POWO (2023) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. <http://www.plantsoftheworldonline.org/>
- Price ML, Van Scoyoc S & Butler LG (1978) A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry* 26: 1214–1218. doi:10.1021/jf60219a031.
- Pyke GH, Pulliam HR & Charnov EL (1977) Optimal foraging: a selective review of theory and tests. *The Quarterly Review of Biology* 52: 137–154. doi:10.1086/409852.
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Read J, Sanson GD, Caldwell E, Clissold FJ, Chatain A, Peeters P, Lamont BB, De Garine-Wichatitsky M, Jaffré T & Kerr S (2009) Correlations between leaf toughness and phenolics among species in contrasting environments of Australia and New Caledonia. *Annals of Botany* 103: 757–767. doi:10.1093/aob/mcn246.
- Shimada T, Takahashi A, Shibata M & Yagihashi T (2015) Effects of within-plant variability in seed weight and tannin content on foraging behaviour of seed consumers. *Functional Ecology* 29: 1513–1521. doi:10.1111/1365-2435.12464.
- Shmida A & Ellner S (1984) Coexistence of plant species with similar niches. *Vegetatio* 58: 29–55. doi:10.1007/BF00044894.
- Singleton VL & Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144–158. doi:10.5344/ajev.1965.16.3.144.
- Smallwood PD, Steele MA & Faeth SH (2001) The ultimate basis of the caching preferences of rodents, and the oak-dispersal syndrome: tannins, insects, and seed germination. *American Zoologist* 41: 840–851. doi:10.1093/icb/41.4.840.
- Steele MA, Smallwood PD, Spunar A & Nelsen E (2001) The proximate basis of the oak dispersal syndrome: detection of seed dormancy by rodents. *American Zoologist* 41: 852–864. doi:10.1093/icb/41.4.852.
- Tiansawat P, Davis AS, Berhow MA, Zalamea PC & Dalling JW (2014) Investment in seed physical defence is associated with species' light requirement for regeneration and seed persistence: evidence from *Macaranga* species in Borneo. *PLoS One* 9: e99691. doi:10.1371/journal.pone.0099691.

- Tilman D & Pacala S (1993) The maintenance of species richness in plant communities. Species diversity in ecological communities (ed. by RE Ricklefs & D Schluter) University of Chicago Press, Chicago, Illinois, USA, pp. 13–15.
- Turnbull LA, Rees M & Crawley MJ (1999) Seed mass and the competition/colonization trade-off: a sowing experiment. *Journal of Ecology* 92: 899–912. doi:10.1046/j.1365-2745.1999.00405.x.
- Tweddle JC, Dickie JB, Baskin CC & Baskin JM (2003) Ecological aspects of seed desiccation sensitivity. *Journal of Ecology* 91: 294–304. doi:10.1046/j.1365-2745.2003.00760.x.
- Twigg LE & Socha LV (1996) Physical versus chemical defence mechanisms in toxic *Gastrolobium*. *Oecologia* 108: 21–28. doi:10.1007/BF00333210.
- Wang B & Ives AR (2017) Tree-to-tree variation in seed size and its consequences for seed dispersal versus predation by rodents. *Oecologia* 183: 751–762. doi:10.1007/s00442-016-3793-0.
- Wang B & Chen J (2009) Seed size, more than nutrient or tannin content, affects seed caching behavior of a common genus of Old World rodents. *Ecology* 90: 3023–3032. doi:10.1890/08-2188.1.
- Wang B, Phillips JS & Tomlinson KW (2018) Tradeoff between physical and chemical defense in plant seeds is mediated by seed mass. *Oikos* 127: 440–447. doi:10.1111/oik.04867.
- Wang B, Ye CX, Cannon CH & Chen J (2013) Dissecting the decision making process of scatter hoarding rodents. *Oikos* 122: 1027–1034. doi:10.1111/j.1600-0706.2012.20823.x.
- Westoby M, Jurado E & Leishman M (1992) Comparative evolutionary ecology of seed size. *Trends in Ecology & Evolution* 7: 368–372. doi:10.1016/0169-5347(92)90006-W.
- Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*, Springer.
- Wu LM, Chen SC & Wang B (2019) An allometry between seed kernel and seed coat shows greater investment in physical defense in small seeds. *American Journal of Botany* 106: 371–376. doi:10.1002/ajb2.1252.
- Xiao Z, Wang Y, Harris M & Zhang Z (2006) Spatial and temporal variation of seed predation and removal of sympatric large-seeded species in relation to innate seed traits in a subtropical forest, Southwest China. *Forest Ecology and Management* 222: 46–54. doi:10.1016/j.foreco.2005.10.020.
- Zangerl AR & Bazzaz FA (1992) Theory and pattern in plant resistance to herbivores and pathogens: Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics (ed. by RS Fritz & EL Simms) Chicago: Univ. Chicago Press, pp. 363–91.
- Zhang Z, Wang Z, Chang G, Yi X, Lu J, Xiao Z, Zhang H, Cao L, Wang F, Li H & Yan C (2016) Tradeoff between seed defensive traits and impacts on interaction patterns between seeds and rodents in forest ecosystems. *Plant Ecology* 217: 253–265. doi:10.1007/s11258-016-0566-0.