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Extending Plant Defense Theory to Seeds

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Keywords

dormancy, chemical defense, physical defense, endophytes, seed predation, seed-microbial interactions

Abstract

Plant defense theory explores how plants invest in defenses against natural enemies but has focused primarily on the traits expressed by juvenile and mature plants. Here we describe the diverse ways in which seeds are chemically and physically defended. We suggest that through associations with other traits, seeds are likely to exhibit defense syndromes that reflect constraints or trade-offs imposed by selection to attract dispersers, enable effective dispersal, ensure appropriate timing of seed germination, and enhance seedling performance. We draw attention to seed and reproductive traits that are analogous to defense traits in mature plants and describe how the effectiveness of defenses is likely to differ at pre- and postdispersal stages. We also highlight recent insights into the mutualistic and antagonistic interactions between seeds and microbial communities, including fungi and endohyphal bacteria, that can influence seed survival in the soil and subsequent seedling vigor.

1. PLANT DEFENSE THEORY

Plant defense theory provides a powerful framework for understanding the ecological and evolutionary dynamics of interactions between plants and their antagonists, the distribution of plant traits across communities, and, consequently, the distributions of plant species. Detailed explorations of plant defenses, and the ways plants' natural enemies can overcome them, also can provide insights into how closely related species coexist (Kursar et al. 2009) and the success of invasive species (Callaway & Ridenour 2004) and may account for species radiations in diverse genera (Coley et al. 2018). Predictions of plant defense theory have highlighted how resource availability mediates investment in defenses, the determinants of the production of different suites of protective chemicals, conditions favoring constitutive versus induced defenses, and which tissues should be most strongly defended (Stamp 2003). Comparative studies have further revealed how individual defense traits can be combined, highlighting the effectiveness and orthogonality of resultant defense syndromes (Coley et al. 2018). Plant defense theory, however, centers mostly on the life-history and defense traits expressed by juvenile and mature plants and focuses primarily on the most conspicuous of plant organs: leaves.

As plant defense theory matures, opportunities have arisen to apply this framework to other plant organs. Root herbivory can have as large an effect on plant fitness as folivory (Maron 1998), and therefore analyses have explored whether similar structural and chemical defense traits are expressed above- and belowground and whether expression across roots and shoots is coordinated (Rasmann & Agrawal 2008). Although seed defenses are well studied, few attempts have been made to explore defense allocation in seeds in the context of existing defense theory, despite analogies between whole-plant defensive traits and those of seeds (**Table 1**). Because seeds are exposed to natural enemies while developing on the maternal plant, when dispersed to the soil surface, and when incorporated into the soil, they are likely to encounter a greater diversity of natural enemies than shoots and roots. Broader comparative analyses of seed defenses and seed defense syndromes are also needed to reveal potential trade-offs or constraints with seed and fruit traits that attract dispersers, enable effective dispersal, ensure the appropriate timing of seed germination, and impact seedling performance (**Figure 1**).
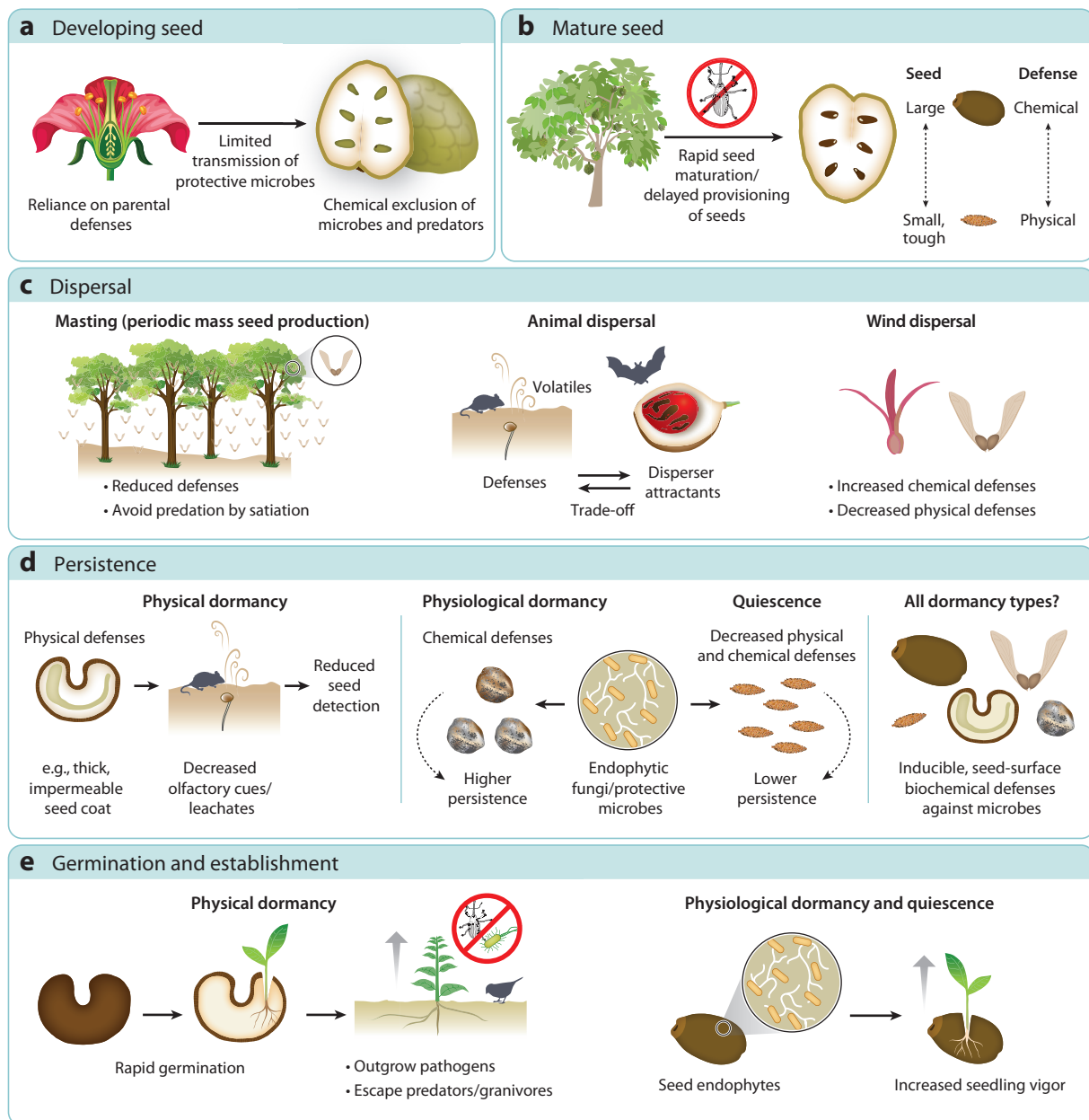
In this review we explore the limitations and opportunities arising from applying plant defense theory to seeds and also highlight broader insights that seeds can provide into how plant defenses at the seed stage can mediate plant community composition (Larios et al. 2017). We start by identifying the suite of seed defenses expressed during seed development, seed dispersal, and subsequent

Table 1 Comparison of leaf or whole-plant defense resistance and avoidance traits with seed or reproductive defense traits

Juvenile or adult plant traits	Analogous seed or reproductive traits
Leaf toughness	Hard or fibrous seed-protecting structures, low seed moisture content, seed shape
Spines, trichomes, latex	Seed/fruit wall structures, resin ducts, mucilage
Root exudates	Seed exudates
Delayed greening	Delayed ripening
Synchronous leaf flush	Synchronous flowering, masting, seed dormancy
Rapid leaf expansion	Rapid seed maturation and rapid seedling emergence
Induced defenses	Seed-surface biochemical defenses
Indirect defenses (e.g., domatia, extrafloral nectaries, foliar endophytes)	Indirect defenses (e.g., arils and chemical ant attractants, seed endophytes)
Secondary metabolites (e.g., phenolics and alkaloids)	Many shared secondary metabolites (e.g., phenolics and alkaloids)

persistence in soil seed banks. We then place seed defense traits into a broader framework by exploring linkages among defense, dispersal, and dormancy traits. We evaluate the degree of coordination of defenses between seeds and other plant organs and assess the contribution of different defense traits to seed persistence in the soil. Finally, we highlight the complexity and specificity of seed-microbial interactions affecting both seed survival and postgermination processes. We focus primarily on angiosperms with dispersal by means other than water and emphasize the

Dormancy: state in which a viable and mature seed fails to germinate under favorable conditions due to endogenous characteristics

a Developing seed

Reliance on parental defenses → Limited transmission of protective microbes → Chemical exclusion of microbes and predators

b Mature seed

Rapid seed maturation/ delayed provisioning of seeds

Seed	Defense
Large	Chemical
Small, tough	Physical

c Dispersal

Masting (periodic mass seed production)
• Reduced defenses
• Avoid predation by satiation

Animal dispersal
Volatiles
Defenses ⇄ Disperser attractants
Trade-off

Wind dispersal
• Increased chemical defenses
• Decreased physical defenses

d Persistence

Physical dormancy
Physical defenses → Reduced seed detection
e.g., thick, impermeable seed coat
Decreased olfactory cues/ leachates

Physiological dormancy
Chemical defenses
Higher persistence ← Endophytic fungi/protective microbes

Quiescence
Decreased physical and chemical defenses
Lower persistence

All dormancy types?
Inducible, seed-surface biochemical defenses against microbes

e Germination and establishment

Physical dormancy
Rapid germination →
• Outgrow pathogens
• Escape predators/granivores

Physiological dormancy and quiescence
Seed endophytes → Increased seedling vigor

(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Defense traits during the life of seeds with a focus on dicot angiosperms. (*a*) During maturation, seeds rely mostly on chemical defenses; maternal transmission of protective microbes is limited. (*b*) Before dispersal, seeds can avoid predation by rapid seed development or by delayed resource provisioning of seeds. A physical scaling relationship confers a toughness advantage to small seeds, implying that large seeds should be more dependent on chemical defenses. (*c*) Masting can reduce defenses when predators are satiated (*left*). In animal-dispersed species, seed defenses may evolve into or trade off with dispersal attractants (*middle*); wind-dispersed species may reduce investment in heavy physical defenses and increase metabolizable chemical defenses (*right*). (*d*) Persistence traits differ for seeds that have physical dormancy versus physiological dormancy and quiescence. To persist, physically dormant seeds evade detection by reducing leachate and olfactory cues (*left*), while all seed types may use inducible biochemical defenses to counteract pathogen attack (*right*). (*e*) During germination, physically dormant seeds may germinate fast to outgrow pathogens and escape granivores (*left*), while seed endophytes may increase seedling vigor (*right*). Flower illustration in panel *a* adapted from **https://www.vecteezy.com/vector-art/302683-science-of-common-flower-parts**.

diversity of defense traits exhibited by tropical trees. We also take an ecological perspective by considering diaspores (i.e., dispersal units that leave the maternal plant) as seeds, even though the key enclosing and protective structures of diaspores often contain fruit-derived maternal tissues.

2. INVESTMENT IN SEED DEFENSES

Seeds are critical components of fitness for most plants and thus can be among the most highly defended plant organs (Zangerl & Bazzaz 1992). Furthermore, seeds contain rich nutritional resources often present in concentrated form to reduce seed size and weight (Janzen 1971). Seeds can be physically defended with hard or fibrous layers that exclude generalist seed predators, or they can feature chemical defenses with activity against both predators and pathogens. Especially robust physical protection is found in the stony endocarps of plants such as tropical palms and legumes. However, the hardest true seeds belong to vegetable ivory palms (*Phytelephas* spp.), which have an endosperm composed of the polysaccharide mannan that develops over 3 years within a thick, spiny, and fibrous exocarp (Bergtoft Pedersen 1993) and in a weight loading machine require a load of 1,260 kg to crack (Kiltie 1982). Chemical defenses of seeds can be as concentrated as those in leaves and roots. For example, wild parsnip (*Pastinaca sativa*) seeds contain up to 2% by mass of furanocoumarins, with a significant biosynthetic cost that affects total seed production (Zangerl & Berenbaum 1997).

2.1. Effectiveness of Defenses During the Life of a Seed

Understanding selection on seed defenses requires breaking down the stages in the life of seeds from development and maturation on the maternal plant, to initial dispersal to the soil surface and subsequent incorporation in the soil seed bank. Although some seed traits govern the attractiveness of seeds throughout their lives (e.g., seed nutritional quality), the distinct suites of natural enemies seeds encounter at each stage may select for different defense traits (**Figure 1**; **Table 1**).

Diaspore: plant dispersal unit that leaves the maternal plant consisting of a seed plus additional tissues that assist dispersal

2.1.1. Predispersal stage.
The predispersal stage for seeds is analogous to the immature leaf stage: it describes the period when seed filling occurs and physical protective structures develop. Because physical defenses are ineffective until they can be fully developed, defensive traits might be expected to be more similar to those of the soft tissues of shoots and leaves. In addition to investing in chemical defenses to reduce seed palatability and microbial infection (Tewksbury et al. 2008), defense adaptations can include (*a*) rapid seed development, analogous to rapid leaf expansion (Aide & Londoño 1989), which shortens the window of opportunity for larval development (Sallabanks & Courtney 1992) and increases the probability of dispersal in herbaceous plants when

foraging herbivores consume both leaves and seeds (Janzen 1984); (*b*) elongated seed shape, reducing space for insect development (Szentesi & Jermy 1995); (*c*) delayed ripening of fruit and development of seeds, analogous to delayed greening in leaves (Kursar & Coley 1992), where seeds are fully provisioned only at the moment that fruit or infructescences mature and become attractive to dispersers (e.g., *Cecropia* spp.; J.W. Dalling, personal observation); and (*d*) masting, i.e., supra-annual variation in seed crop size at the individual level, or synchronized across populations or communities, to satiate pre- and postdispersal seed predators, analogous to synchronous leaf flush (Lieberman & Lieberman 1984).

2.1.2. Postdispersal stage.
Most seed mortality occurs immediately postdispersal and appears to be independent of seed size (Moles et al. 2003a). Defenses at this stage should be directed primarily to reducing rates of seed detection, for example, by blocking olfactory cues (Paulsen et al. 2013), reducing seed attractiveness to vertebrate and invertebrate seed predators by physical or chemical defenses, impeding the ability of granivores to handle seeds [e.g., through mucus production (Engelbrecht & García-Fayos 2012)], or, conversely, satiating seed predators by masting (Kelly 1994). Seed losses also can be reduced by directed dispersal to safe microsites with reduced predation risk (Wenny 2001), for example, by attracting secondary dispersers via arils and elaiosomes (Beattie & Culver 1982). However, disperser rewards may attract seed predators such as granivorous ants (Edwards et al. 2006) and birds (Janzen 1971).

Seed losses on the soil surface also may be reduced by rapid seed germination, because the transformation of seed reserves into less palatable tissue can make seedlings less attractive than seeds (Hadj-Chikh et al. 1996). In addition to germination soon after dispersal, high germination velocity (i.e., a rapid transition from the vulnerable stage of initial radicle emergence to seedling establishment) can allow some plants to evade pathogen infection (Beckstead et al. 2007). Some species also can tolerate high levels of seed damage and successfully establish as seedlings. Seeds of the neotropical trees *Gustavia superba* (Lecythidaceae) and *Prioria copaifera* (Fabaceae) often are infested by multiple insect larvae or have more than half of their seed reserves consumed by rodents before successfully germinating, and they are capable of surviving multiple episodes of browsing damage to their emerging seedlings by reallocating remaining seed reserves (Dalling & Harms 1999). Additionally, insect, bird, and mammalian predators of acorns often avoid consuming the tannin-rich embryo end of the seed, allowing germination (Steele et al. 1993).

2.1.3. Seed bank persistence.
Seeds that persist for prolonged periods before germination, and consequently become incorporated in the soil seed bank, may have a reduced risk of predation (Hulme 1998). However, once in soil they are increasingly susceptible to microbial infection from pathogens and decay of seed-protecting structures by saprophytes (Wagner & Mitschunas 2008). Seed bank–forming species might therefore be expected to undergo selection for particularly effective antimicrobial defenses relative to species with a more transient seed stage. Effective defenses for persistent seeds may include indirect defenses via microbial mutualistic partnerships that exclude potential pathogens or promote germination (Sarmiento et al. 2017) or the induction of biochemical defenses consisting of latent enzymes that are activated in the presence of pathogens (Fuerst et al. 2017).

2.2. Relationship Between Seed Defense Investment and Broader Trait Syndromes

Defense of foliar tissue and reproductive output are linked directly because leaf damage can strongly impact seed production (Rockwood 1973). More generally, plant defenses are tied to

overall resource acquisition and allocation, as heavily defended leaves and shoots often are associated with low-resource environments where the cost of tissue replacement is high (Coley et al. 1985). Given the high investment of mineral nutrients in seeds, we therefore expect seeds to be more defended when produced in nutrient-poor environments or where light limitation precludes investment belowground in resource acquisition to provision seeds. Conversely, if resource limitation strongly limits total reproductive output, then resources that could be invested in seed defense might better be used to increase seed production. Indeed, mast seeding appears to be associated with nutrient scarcity (Fernández-Martínez et al. 2019).

To date, only limited evidence links characteristics of individual seeds to broader resource-conservative trait syndromes. In a comparison of tropical forests on three continents, Kraft et al. (2015) found that leaf toughness, a key component of resistance to herbivores (Coley 1983), was correlated positively with seed size, whereas seed size was not related to leaf tannin and phenol concentrations. Seed size–plant defense relationships may arise because seed mass is positively related to the ability of seedlings to tolerate a broad range of factors, including competition, herbivory, and resource limitation (Muller-Landau 2010). Although Kraft et al. (2015) did not explore seed traits other than volume and mass, other community-wide studies have linked seed mass to investment in seed defense. In a study of 70 plant species with seed masses ranging from 0.01 to 300 g at Barro Colorado Island, Panama, the proportion of total seed mass invested in protective tissues was not correlated with seed mass (Fricke & Wright 2016). However, an important relationship emerged from the study: Absolute toughness (the energy required to break a seed) increased with seed mass, but the slope of this relationship was <1, indicating that the specific toughness of seeds (i.e., the energy required to break a seed per gram of seed mass) declined with increasing seed mass. In fact, the smallest seeds required three orders of magnitude more force per gram to break than the largest ones (Fricke & Wright 2016). Thus, smaller seeds obtain a greater benefit per unit energy invested in physical defenses than do larger seeds.

In a comprehensive analysis of phenolic and polyphenol constituents in seeds of 196 tree and liana species from Barro Colorado Island, Gripenberg et al. (2017) found that these chemical constituents were widespread (occurring in >80% of species). However, the highest concentrations were found in species with large seeds, low investment in seed physical defenses, and conservative plant traits (high wood density and leaf mass per unit area and low radial growth rates; Gripenberg et al. 2017). Collectively, Kraft et al. (2015) and Gripenberg et al. (2017) suggest that increased investment in correlated plant-defense traits in trees associated with shade-tolerant life histories and low-resource environments also is manifested in the defenses of seeds.

2.3. Coordination of Chemical Defenses in Shoots, Roots, and Seeds

Plants that invest heavily in chemical defenses in their leaves may allocate the same compounds in seeds, reflecting coordination of multiple defense components across different tissue types (van Dam 2009). With the exception of cyanogenic glycosides, the major classes of chemical defenses recognized to have activity against insect or vertebrate herbivores (glucosinolates, alkaloids, terpenoids, saponins, phenolics, phytoalexins, polyphenol oxidases, proteinase inhibitors, and nonprotein amino acids) also have been reported to have antifungal or antibacterial activity (Mayer 2004). Consistent with a broad spectrum of activity of their chemical constituents, roots and shoots, which often have little similarity in their herbivore communities, often have a high degree of overlap in their defensive chemistry, with little consistent evidence for bias in allocation above- and belowground (Rasmann & Agrawal 2008). Coordination across tissue types may also extend to inducible defenses. For example, leaf damage to evening primrose plants

(*Oenothera biennis*) by Japanese beetles induces phenolic production in reproductive tissues, greatly reducing seed predation by native lepidoptera (McArt et al. 2013).

While defense coordination of root and shoot tissue is well established, insights into selection on seed defenses are hampered by a paucity of comparative studies of seed and leaf chemistry. A comparison of ten *Macaranga* species from Borneo differing in seed mass and shade tolerance found 23 phenolic compounds in seeds via high-performance liquid chromatography, while 70 phenols were recovered from leaves (Tiansawat 2013). Overall, leaves had higher phenolic concentrations and, on average, three times higher phenolic diversity than seeds. Only one phenol was unique to seed tissue. Other studies suggest greater specialization of seed defensive chemistry. An analysis of the amide class of alkaloids prevalent in many species of *Piper* showed that *P. reticulatum* seeds had higher diversity and concentrations of amides than leaves, suggesting independent selection on seed defenses (Whitehead et al. 2013). Similarly, in *Geranium*, seeds and roots accumulated proanthocyanidins whereas ellagitannins dominated photosynthetic organs (Tuominen et al. 2013). In a broader survey of phenolic compounds, Gripenberg et al. (2017) observed that multiple flavonol derivatives (myricetin, kaempferol, and quercetin) were detected rarely in seeds even though they are common components of foliar polyphenols. Future comparative analyses of leaf and seed tissues that address a broad spectrum of potential defense chemicals may provide a useful approach to screen for candidate chemicals that selectively protect seeds against granivores and seed pathogens and/or facilitate microbial mutualisms (Martins et al. 2017).

3. LINKAGES AMONG DEFENSE, DORMANCY, AND DISPERSAL TRAITS IN PLANTS

Daniel Janzen coined the term prodigal parent theory to describe a once-prevalent idea that seed production is so prodigious that variation in seed mortality, and therefore seed defenses, is unimportant (Janzen 1969). While this idea has been rejected, a broader criticism invoked in the paper is valid: Janzen (1969, p. 1) states that "nearly all studies in wild plant biology have regarded the traits of seeds and seed crops as the direct result of physiological processes that are in turn correlated with the immediate environment." In the emerging framework of seed defense syndromes, plant traits influencing dormancy, and to some extent dispersal, can be viewed as shaped by the need to defend seeds.

3.1. Dispersal-Defense Relationships

Dispersal effectiveness may directly affect selection on seed defenses because seeds that are diffusely distributed across the landscape, or that are removed to safe sites, are less conspicuous or more energetically costly for seed predators to locate and may therefore need less investment in defense in order to evade predation. Such escape may be seed- and crop-size dependent. In a review of seed predation, Janzen (1971) argued that grass seeds generally have low toxicity, reflecting low seed production by individual plants, dispersal of individual achenes that are difficult to harvest, and high rates of vegetative spread in perennial grasses. Furthermore, chemical toxicity may be difficult to evolve in grasses because the seeds consumed by a single predator are likely to come from multiple parent plants, with little opportunity for predators to learn to discriminate among them. It is notable therefore that toxicity in grasses is primarily caused by infection with endophytic fungi in the Clavicipitaceae, such as *Epichloë/Neotyphodium* (Schardl et al. 2004).

Linkages between defense and dispersal are also apparent when traits that reduce losses to seed predators negatively impact either the probability or distance of dispersal. Here, the distinction between wind- and biotically dispersed seeds may be especially important. For wind-dispersed seeds

where the diaspore is a samara (e.g., ash and maple species and species in numerous families of tropical trees and lianas), rate of descent, and therefore dispersal distance, is highly correlated with the square root of wing-loading—the ratio of diaspore mass to wing surface area (Green 1980). Selection on dispersal effectiveness therefore constrains investment in heavy seed-protecting structures and favors investment in (*a*) more energy-dense lipid reserves over carbohydrates (Lokesha et al. 1992) and/or (*b*) chemical defenses such as alkaloids or lectins that can also be used for energy storage after germination (Kato et al. 2012, Liener 1979). However, more nutritious lipid- and protein-rich seeds are also often preferred by seed predators (Reichman 1977).

For biotically dispersed seeds, conflicts also may arise between attracting dispersers and repelling predators. Attractive fruit that are removed rapidly by dispersers have lower concentrations of compounds such as tannins and phenols in their pulp than unattractive fruit that persist in a ripe but undispersed state for longer (Cazetta et al. 2008, Whitehead et al. 2016). In contrast, fruit infection by certain fungi may enhance the attractiveness of fruit (Peris et al. 2017). Toxic compounds used to deter seed predators through learned behavior could be sequestered within seeds to avoid contact with dispersers, while reducing seed losses to pathogens may require allocating defensive chemicals on exterior seed surfaces. For plant species that are secondarily dispersed and cached by ants or scatter-hoarding rodents, external cues that initially stimulate dispersal should be lost rapidly once seeds are buried to reduce the risk of subsequent recovery and predation. The degradation of remaining sugars or fruit pulp on surfaces by saprotrophic fungi in soil or seed caches may play a beneficial but understudied role.

Mast seeding may provide additional selective pressures on seed defenses. If masting is an effective reproductive strategy because predators are locally satiated by seed production, rather than excluded from consuming them, then selection might be expected to favor reallocating resources from defense to seed production. Masting may in general be more effective in temperate forests where high densities of conspecific trees may permit local predator satiation through population-level rather than individual-based seed production; however, there is no evidence of a latitudinal gradient in postdispersal seed defenses (Chen & Moles 2018).

The relationship between dispersal traits and defense against seed predation has been studied in many systems, but escape from seed pathogens may be equally important and has received less attention (Sarmiento et al. 2017). Density-dependent processes that are thought to play a key role in regulating population sizes, and therefore maintaining diversity, are increasingly recognized to be mediated by soil-microbial interactions (Bever et al. 2015). Soilborne pathogens can be a major source of seed mortality, particularly in high-density seed patches and for species that form soil seed banks (Dalling et al. 1998). Such effects occur not only in tropical and temperate forests and grasslands (e.g., Kotanen 2007, Mordecai 2012) but in arid lands, where desert annuals (Li et al. 2019) and woody perennials (Kissell 2020) can suffer major seed losses due to infections by soilborne fungi, often in a site-, soil-, and season-specific manner (Hamzazai et al. 2020). Several of these studies have shown that fungal strains pathogenic or otherwise detrimental to some plant species may have little negative effect on others (Li et al. 2019, Sarmiento et al. 2017). Primary dispersal via gut passage also may decrease the fungal load on seeds and contribute to higher recruitment success compared to uningested seeds. Fricke et al. (2013) found that chili pepper seeds (*Capsicum chacoense*) that had passed through a gut both were less attractive to seed predators and had a lower fungal infection score than unprocessed seeds from the same fruit. Coupled with movement away from maternal plants, frugivorous seed dispersers thus may doubly benefit the plants with which they establish mutualisms in tropical forests and other biomes.

Similarly, secondary dispersal of seeds may either increase or decrease seed losses due to pathogens. Damage to seeds' protective structures during gut passage or by insect handling may provide opportunities for fungi, nematodes, and bacteria (and the viruses they may transmit) to

enter seeds once in the soil (Davis et al. 2008). Alternatively, scat and dung may provide seeds with a pathogen-suppressive external microbiome (Nelson 2018). Secondary dispersers also may process seeds in ways that reduce losses to pathogens. Ants can secrete chemicals from metapleural glands that inhibit mycelial growth of soil fungi (Beattie et al. 1986), resulting in reduced richness of phytopathogenic fungi in seed-harvesting ant nests (Tarsa et al. 2018). Ants have long been proposed to chemically protect seeds from pathogens inside seed caches (Levey & Byrne 1993), but direct evidence that ant-associated antimicrobials are effective in prolonging seed survival is lacking.

3.2. Dormancy-Defense Relationships

Although ecologists often describe ungerminated seeds in the soil as dormant, the term should be used to refer specifically to intrinsic characteristics of seeds that prevent germination under a range of normal physical environmental conditions (Vleeshouwers et al. 1995). Seeds can survive for prolonged periods in soil either while maintaining dormancy or in a quiescent state, where seeds lack any intrinsic barrier to germination but fail to receive a suitable environmental cue. Although a wide range of dormancy classes exist for seeds, most species that exhibit seed dormancy have either physical dormancy, where seeds are enclosed inside a water-impermeable layer in the seed or fruit coat, or physiological dormancy, where seeds are permeable but can only germinate following specific chemical or environmental cues (Baskin & Baskin 2004).

Dormancy classes generally have been considered to be functionally equivalent, in that each imparts a delay in germination to avoid adverse environmental conditions for emergence and seedling establishment (Thompson 2000). Defense traits and dormancy classes, however, are linked and therefore are related to seed persistence (Zalamea et al. 2018). Notably, a single trait that distinguishes physically dormant seeds from those in other dormancy classes, namely seed permeability, may play a role in determining attractiveness or accessibility of seed contents to both granivores (Paulsen et al. 2013) and pathogens (Zalamea et al. 2014). The linkage between defense traits and dormancy classes was identified by Dalling et al. (2011), who proposed the existence of distinct seed-defense syndromes analogous to the defense syndromes at the center of whole-plant defense theory. Within this framework, species with physical dormancy use physical defenses to exclude predators and germinate rapidly to evade pathogens. Species with physiological dormancy are defended with varying investments in chemical and physical defenses. Quiescent seeds, which have comparatively limited chemical and physical defenses, may instead depend on beneficial seed-infecting microbes for protection.

A subsequent study of pioneer tree species from lowland tropical forest in Panama found support for the three dormancy-defense syndromes (Zalamea et al. 2018). For 16 species with physical dormancy, physiological dormancy, or quiescent seeds, an array of physical and chemical defenses were measured and related to dormancy and seed persistence through discriminant analysis and structural equation modeling. This revealed that seed permeability distinguished physically dormant species, which were associated positively with physical defenses and negatively with chemical defenses, from physiologically dormant and quiescent species. Quiescent species that had relatively transient soil seed banks had lower chemical and physical defenses than species with seed dormancy, consistent with subsequent studies in desert and grassland systems (see Hamzazai et al. 2020, Kissell 2020). In tropical forests, persistence lasting several decades (Dalling & Brown 2009) could be achieved with either physical or chemical defenses (Zalamea et al. 2018). Additional analyses of defense traits, and their efficacy against particular suites of natural enemies, are needed to determine whether variation in the prevalence of dormancy types across biomes is related to seed-defense requirements.

Quiescent: the state in which a nondormant seed does not germinate due to the absence of adequate environmental factors needed for germination

Physical dormancy: dormancy class in which seeds are enclosed inside a water-impermeable layer

Physiological dormancy: dormancy class in which seeds are permeable but can only germinate following specific chemical or environmental cues

Persistence: period of time after dispersal in which seeds can remain viable in the soil

Dormancy-defense syndromes also may explain why dormancy is maintained in ecosystems where delayed germination appears to be maladaptive. In forests where canopy gaps created by treefalls occur once a century, persistence in the seed bank increases recruitment success (Dalling & John 2008). In these settings, dormancy should reduce recruitment success if it prevents germination of seeds dispersed directly to gaps. Widespread pioneer species in Panama with both physical (e.g., *Luehea seemannii*) and physiological dormancy (e.g., *Zanthoxylum ekmanii*) have reduced germination rates in greenhouse conditions simulating large tree-fall gaps for the first two years after dispersal, but the conditions required to break dormancy relax as seeds age in the soil (P.-C. Zalamea, unpublished data). Incorporating avoidance of natural enemies, in addition to unfavorable abiotic conditions, may also provide new perspectives on selection on delayed germination in deserts and other ecosystems (Li et al. 2019). Before we examine the relationship between defense and seed persistence in the soil (Section 5), we first review evidence for trade-offs in defense investment.

4. TRADE-OFFS IN CHEMICAL AND PHYSICAL DEFENSES IN SEEDS

The potential fitness costs of allocating resources to defense implies that developing one set of effective defenses precludes simultaneously developing others. Thus, plants have been predicted to show trade-offs between investing primarily in chemical versus physical defenses or between direct chemical and physical defenses versus indirect defenses mediated by biotic interactions (Janzen 1966, Steward & Keeler 1988). However, evidence for the trade-off model has been weak when applied to foliar or whole-plant defenses: Some clades show the presence or absence of specific defenses consistent with the trade-off model (Twigg & Socha 1996), but a meta-analysis of plant defense traits showed only a marginally significant relationship between chemical and physical defenses for 261 species (Moles et al. 2013).

Defense-trait relationships might be stronger for seeds than for foliar and whole-plant traits. Although physical defenses of leaves are highly effective deterrents of herbivory (Coley 1983), their effectiveness is compromised by accommodating functional demands on leaves to optimize gas exchange and light transmission through the epidermis. In contrast, the protective structures of seeds are free to enclose the embryo and endosperm, creating an impermeable barrier in seeds with physical dormancy. Impermeable seeds have the added advantage of reducing seed detection opportunities by natural enemies that can result from the escape of leachates, mucilage, and/or volatiles (Paulsen et al. 2013).

In the broadest analysis of seed chemical and physical defenses to date, Wang et al. (2018) compared investment in 163 plant species sampled from the seed bank of the Xishuangbanna Tropical Botanical Garden in China. In this phylogenetically diverse collection, total seed phenolics (0.01–20.5% seed mass) and seed fiber content (a good proxy of physical defense; 4.5–81.5% of seed mass) varied widely. The smallest seeds (<0.001 g) showed a strong negative correlation between chemical and physical defense. However, the slope of this relationship became flatter with increasing seed mass and disappeared for seeds >0.02 g. The authors attributed the loss of this relationship in larger seeds to selection for investment in both defense classes for seeds that are more resource rich. However, these findings are also consistent with the allometric scaling relationship of seed mass and specific seed toughness that implies reduced effectiveness of physical defenses with increasing seed size (Fricke & Wright 2016). A similar relationship at least partially underpins the distribution of chemical and physical defenses in seeds in a lowland tropical forest (Gripenberg et al. 2017), where polyphenols are present in higher concentrations in larger seeds.

5. CONTRIBUTION OF DEFENSE TRAITS TO SEED PERSISTENCE IN THE SOIL

Under natural conditions, seeds of most species persist for some period in the soil seed bank prior to germination. Many authors have classified species as transient or nonpersistent in the soil (variously defined as <1 year to <4 years of survival) or as persistent, if they are capable of longer survival times. Persistence time is potentially a more easily measured trait than predation rate because seeds can be artificially buried and recovered to measure survival. However, in a detailed review, Long et al. (2015) noted that persistence is a function of a complex set of interactions including a seed's resistance to physiological aging, predation, pathogen infection, and microbial decay and its exposure to climate, soil, and site conditions. Furthermore, persistence also is affected by losses due to fatal germination, where seeds germinate but seedlings die before reaching the soil surface (Davis & Renner 2007).

Evidence for a prevailing role of chemical versus physical defenses for seed persistence is mixed. In a study of 80 species, mostly from the native British flora, Hendry et al. (1994) found that species that formed persistent seed banks (retaining viability for >4 years) had significantly higher *ortho*-dihydroxyphenol concentrations in their seeds than species with nonpersistent seed banks. *Ortho*-dihydroxyphenols were found in all species and contain many of the widespread forms of phenols, phenolic acids, and tannins. Associations of persistence with chemical defenses also were seen at the congeneric level in this study. For example, three species of *Galium* with nonpersistent seed banks had low phenolic concentrations. The phenolic concentrations of a fourth species that forms persistent seed banks, *G. saxatile*, were an order of magnitude higher (Hendry et al. 1994).

In contrast, agricultural weeds in the midwestern United States (Davis et al. 2016) and France (Gardarin et al. 2010), *Macaranga* trees in Borneo (Tiansawat et al. 2014), and perennial Fynbos plants from South Africa (Holmes & Newton 2004) all indicate that physical defenses are more strongly associated with persistence. These contradictory results may show that persistence can be achieved with high investment in either physical or chemical defenses (Zalamea et al. 2018). More generally, seed trait–seed persistence relationships may be context dependent, reflecting the exposure component of the persistence framework described by Long et al. (2015). For example, physical defenses may be favored in communities with small-seeded species where seed toughness traits are intrinsically more effective (Fricke & Wright 2016) or in deserts where hard seeds are effective defenses against granivores (Paulsen et al. 2013). Alternatively, chemical defenses may be favored in some fire-prone ecosystems where smoke is a germination cue that cannot penetrate impermeable seeds with strong physical defenses (Long et al. 2015).

Variation in exposure to different sources of seed mortality in the soil may also explain contrasting relationships found between seed shape and seed persistence. In some ecosystems persistence is associated with small seed mass and compact, rounded seed shape (Long et al. 2015). This relationship may reflect lower rates of predation for small or more easily buried seeds. The absence of this relationship in drier ecosystems may reflect reduced size-dependent predation given the greater difficulty for mammalian seed predators to detect dry seeds than hydrated seeds (Moles et al. 2003b and references therein). Alternatively, weak relationships between seed-defense traits and seed persistence (or seed predation rate) may reflect conflicting evolutionary and ecological processes. Moles et al. (2003b) noted that species with high rates of seed predation over evolutionary time are expected to have increased defense allocation, which may result in a negative relationship between defense and persistence, while in ecological time, defense and persistence are predicted to be positively related. Predicting seed persistence from seed traits is therefore complex. Future analyses will need to integrate measurements across many more seed traits to determine

covariance relationships between individual defense characteristics, physical habitat conditions, and biotic causes of seed losses.

6. SEED-MICROBIAL INTERACTIONS: AN UNEXPLORED AXIS OF SEED DEFENSE

Seeds engage in a range of interactions with microbes, particularly fungi, that impact their persistence, defense against pathogens, release from dormancy, and subsequent seedling vigor (Khalaf & Raizada 2018, Nelson 2018, Ridout et al. 2019, Sarmiento et al. 2017). The microbiomes of individual seeds remain only infrequently explored, but a clear pattern has emerged from culture-based studies showing that the richness of bacteria and fungi isolated from seeds often is lower than that of leaves and roots from the same plants (Ganley & Newcombe 2006, Newcombe et al. 2018). Some fungal species may occur as endophytes in both seeds and leaf or root tissue, even if not vertically transmitted (e.g., Shaffer et al. 2016, U'Ren et al. 2009). Next-generation sequencing of seeds provides additional support, with most fresh seeds harboring very few bacterial and fungal operational taxonomic units (a proxy for species) (Ridout et al. 2019). These observations extend to seeds even after incubation in soils, which consistently result in isolation of not more than 1–2 fungal strains per seed in culture (Kissell 2020, Li et al. 2019, Sarmiento et al. 2017) or 1–3 operational taxonomic units from next-generation sequencing (P.-C. Zalamea, C. Sarmiento, A.S. Davis, A.E. Arnold & J.W. Dalling, unpublished data).

Observations to date of limited microbial diversity in intact, surface-sterilized seeds of dicot angiosperms have led to the suggestion that seeds represent a bottleneck in plant microbiomes, where a combination of strong chemical defense allocation to developing seeds and exclusionary interactions among potential seed-infecting microbes limits both fungal and bacterial diversity to one or a few primary symbionts (Newcombe et al. 2018, Raghavendra et al. 2013). Low microbial richness implies that the effects of seed-infecting microbes on seed traits and seedling performance should be highly tractable to investigation when compared to other plant-microbial interactions. This may reveal that endophytic seed microbes, in common with endophytes of other tissues such as leaves, play a key role in modifying plant disease severity (Arnold et al. 2003, Busby et al. 2016).

Another implication of the primary symbiont hypothesis (Newcombe et al. 2018) is that the identity of initial microbial colonists of seeds, either before or after dispersal, will strongly impact seed losses to pathogens. Primary colonization of seeds by pathogenic fungi may account for the initially high rates of seed mortality sometimes observed in seed-burial experiments (Dalling et al. 1997, Davis et al. 2016). In contrast, initial colonization by nonpathogenic fungi may defend seeds against subsequent pathogen infection due to exclusionary interactions, accounting for a long tail of seed survivorship and possibly forming a critical seed-defense mechanism, particularly for quiescent species (Dalling et al. 2011). Culture-based studies show that viable seeds commonly acquire asymptomatic fungal and bacterial infections in the seed bank (Gallery et al. 2007a, Sarmiento et al. 2017) and that across species, overall infection rate is uncorrelated with seed survival—a potential consequence of the capacity of seeds to acquire both beneficial and detrimental microbial associates at the population or replicate level (Gallery et al. 2010).

In a large-scale experiment, Sarmiento et al. (2017) used a common-garden seed-burial experiment to show that fungal taxa associated with seed mortality in one host species (i.e., putative pathogens) are associated with asymptomatic infections in others. This functional specificity in seed-fungal interactions provides an additional layer of context dependency to observations that seed fungi successfully infect only a subset of potential host plant species (Wassermann et al. 2019). Functional specificity also was observed in the outcome of seed-inoculation experiments, where fungal isolates killed some hosts but not others (Sarmiento et al. 2017), and can be

modulated further by the presence of additional symbionts such as endohyphal bacteria (Shaffer et al. 2018). Additional seed inoculation and burial experiments are needed to determine whether seed endophytes are as effective in preventing pathogen infection as foliar endophytes. However, seed-associated fungi from desert and tropical plants often can have beneficial impacts on seedling growth and can demonstrate robust in vitro antagonism of plant pathogenic fungi in preliminary trials (A.E. Arnold, unpublished data).

While seed-infecting fungi can show strong host and functional specificity that restrict their host range as pathogens, it remains unclear whether their infection potential extends beyond seeds to other plant tissues. Fungi that infect seeds in the soil are closely related to foliar endophytes across four ascomycete genera (U'Ren et al. 2009), suggesting that seed infections have the potential to be retained by germinating seedlings. In lowland forest in Panama, Gallery et al. (2007b) compared mortality rates of seeds of the dioecious pioneer tree *Cecropia insignis* that were buried beneath isolated female trees, beneath isolated male trees (that therefore lack a *Cecropia* seed bank), and at sites distant from *Cecropia* adults. Seed survival rates were lower below female trees than sites without *Cecropia*, consistent with density- and/or distance-dependent mortality from seed-infecting pathogens, but survival rates beneath male and female trees did not differ. This suggests that a pathogen inoculum source for seeds may arrive from *Cecropia* leaf litter or roots. Potential dualities in fungal life histories, where fungi are potentially pathogens in seeds but endophytes in other tissues of the same host species, remain to be explored.

In addition to exclusionary interactions, seeds also may counter pathogen attack via biochemical defenses. In predicting how seeds are defended, Dalling et al. (2011, p. 90) argued that seeds, unlike shoots, are "unable to tailor defense responses to individual threats." Anderson et al. (2010) and Fuerst et al. (2014) have added a new dimension to seed defenses, however. They show that even seeds with physiological dormancy can be quite responsive when challenged with a pathogen, activating and releasing polyphenol oxidase (PPO) enzymes and potentially also peroxidase, chitinase, and oxalate oxidase enzymes that can generate toxicity, create lignin-like physical barriers to infection, and participate in the production of reactive oxygen species (Fuerst et al. 2014, 2017). Furthermore, these biochemical defenses, characterized by high-molecular-weight protein- and enzyme-based mechanisms, appear to be associated with dead external tissues (e.g., hulls of wheat and wild oats) and in inactive forms are tolerant to temperature changes and resistant to leaching. More broadly, pericarp tissues and other dead, enclosing structures of fruits and seeds appear to contain many biologically active proteins that influence seed survival and germination (Raviv et al. 2017). The generality and efficacy of these inducible defenses, their contribution to seed longevity, and the specificity of their induction by microorganisms remain important and unresolved questions (Fuerst et al. 2014, Pollard 2018).

7. FUTURE DIRECTIONS

More than 25 years ago, Zangerl & Bazzaz (1992) noted that remarkably few comparisons exist of the chemical defenses of seeds and shoots. While still limited, we have highlighted how such comparisons can lead to new insights into the evolution of seed defenses and their relationship to resource availability. New surveys of seed toughness are also beginning to reveal how investments in seed physical and chemical defenses are potentially mediated by seed size and are related to dormancy type. As plant-microbial interactions belowground are increasingly recognized as critical determinants of plant performance and abundance (Bever et al. 2015), attention also needs to be paid to microbial selection on plant defenses at the seed and seedling stage. The degree to which the development of these defenses carries over to influence well-studied foliar defenses and the composition of their herbivore communities remains unstudied. Integrating seeds into

a whole-plant defense framework will also require further efforts to standardize measurements of a full range of seed functional traits (Saatkamp et al. 2019). Likewise, seed ecologists need to keep in mind the multifunctionality of seed traits with which defensive functions can be gained or lost. For example, seed phenols not only play a key role in plant defense but also may limit the permeability of seeds with physical dormancy or protect seeds against UV damage (Waterman & Mole 1994), mucilage may function to reduce seed removal rates or to maintain seed hydration (LoPresti et al. 2019, Yang et al. 2012), and fleshy fruits may have initially evolved to reduce seed predation rather than to attract dispersers (Mack 2000).

An improved understanding of how seeds are defended also could be used to extend seed survival or storage for agriculture or restoration, or, conversely, to hasten losses of weed seeds. Some seed defenses, including those associated with physical protection or that affect seed palatability, have been bred out of crop plants to facilitate harvesting and processing (Bergvinson & García-Lara 2004). Intrinsic resistance traits can be bred back into cultivated plants, but new discoveries regarding how seeds interact with their microbiomes offer new management opportunities. For example, novel management approaches to control weed seed populations might include applications of soil organic matter or green manure to accelerate seed decay by microbes (Davis et al. 2006, Mohler et al. 2012) or enhancing seed damage from either seed predators or mechanical seed treatments to increase losses to seed pathogens (Davis et al. 2008). In contrast, isolation of key taxa or microbial consortia with antibiotic activity present on seed surfaces may provide effective seed pretreatments to protect crop plants against pathogens.

SUMMARY POINTS

1. Most chemical and physical plant defenses are likely effective against a range of natural enemies. Nonetheless, we encourage additional comparisons across plant organs to determine the prevalence and identity of tissue-specific defenses of seeds against granivores and pathogens.

2. Limited evidence to date suggests that increased investment in foliar and whole-plant defense traits observed in plants from resource-poor environments is also reflected in increased defense of seeds.

3. Declining seed-specific toughness (energy required to break a seed per unit of seed mass) with increasing seed size indicates that small seeds obtain a greater benefit per unit energy invested in physical defenses than do large seeds.

4. Consistent with declining effectiveness of physical defenses with increasing seed mass, a trade-off between chemical and physical seed defenses is most apparent for the smallest size classes of seeds.

5. Seed defense allocation is linked to seed dormancy class. Quiescent seeds with transient seed banks invest less in defense. Seeds with physical dormancy invest in more physical than chemical defenses. Seeds with physiological dormancy vary in physical and chemical defense investment.

6. No consistent relationship has been found between the duration of seed persistence in soil and investment in chemical versus physical defense; the effectiveness of these defenses may depend on ecosystem context.

7. Seed-microbial interactions remain poorly explored. Dicot angiosperm seeds appear to harbor very low fungal and bacterial diversity. However, the symbionts they do acquire may have profound effects on seed survival, seedling vigor, and potentially species coexistence.

8. Even the dormant seeds of some species may have inducible responses to microbial antagonists that consist of protein- and enzyme-based defenses present on the enclosing structures of fruits and seeds.

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