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VIEWPOINT: PART OF A SPECIAL ISSUE ON SEEDLING HERBIVORY

Seedling–herbivore interactions: insights into plant defence and regeneration patterns

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- *Background* Herbivores have the power to shape plant evolutionary trajectories, influence the structure and function of vegetation, devastate entire crops, or halt the spread of invasive weeds, and as a consequence, research into plant–herbivore interactions is pivotal to our understanding of plant ecology and evolution. However, the causes and consequences of seedling herbivory have received remarkably little attention, despite the fact that plants tend to be most susceptible to herbivory during establishment, and this damage can alter community composition and structure.
- *Scope* In this Viewpoint article we review why herbivory during early plant ontogeny is important and in so doing introduce an *Annals of Botany* Special Issue that draws together the latest work on the topic. In a synthesis of the existing literature and a collection of new studies, we examine several linked issues. These include the development and expression of seedling defences and patterns of selection by herbivores, and how seedling selection affects plant establishment and community structure. We then examine how disruption of the seedling–herbivore interaction might affect normal patterns of plant community establishment and discuss how an understanding of patterns of seedling herbivory can aid our attempts to restore semi-natural vegetation. We finish by outlining a number of areas where more research is required. These include a need for a deeper consideration of how endogenous and exogenous factors determine investment in seedling defence, particularly for the very youngest plants, and a better understanding of the phylogenetic and biogeographical patterns of seedling defence. There is also much still be to be done on the mechanisms of seedling selection by herbivores, particularly with respect to the possible involvement of volatile cues. These inter-related issues together inform our understanding of how seedling herbivory affects plant regeneration at a time when anthropogenic change is likely to disrupt this long-established, but all-too-often ignored interaction.

Key words: Seedling herbivory, plant–herbivore interactions, plant defence, regeneration patterns, establishment, community structure.

INTRODUCTION

That herbivory has a pivotal role in plant ecology and evolution is unlikely to be challenged by anyone reading this Special Issue. A substantial body of literature documents the impact herbivores have on plant phenotypes, fitness, populations and communities. Most readers would also probably accept that plant regeneration biology is pivotal to our understanding of plant evolution and ecology. Thus, it is surprising that relatively little research has focused on the effects and consequences of herbivory on seedlings, the plant regeneration stage. Some readers might well be aware of the dramatic impact herbivores have on seedlings – entire cohorts can be destroyed by just a few hours of feeding by invertebrate or vertebrate herbivores – and that among studies that have identified sources of mortality in natural populations, herbivory is the greatest cause of seedling mortality (Moles and Westoby, 2004). Nonetheless, it has been suggested that large-scale losses early on simply reduce subsequent self-thinning such that there are few significant population-level implications for recruitment into the established plant community (Crawley, 1983). We now know that the interplay of herbivore selectivity and variation in seedling characteristics can alter the direction of plant community development. Moreover, research on defence expression in seedlings is providing new insights into plant defence theory. For example, in this issue, novel data show that allocation patterns often deviate from simplistic predictions assuming defence constraints early in ontogeny, and that seedlings can be extremely toxic, tolerate high levels of defoliation, and employ volatile chemical cues to signal their unpalatability to putative herbivores. In addition to revealing much about plant community assembly, we argue that a deeper understanding of plant–herbivore interactions during early plant ontogeny can contribute to plant population ecology, evolution, chemical ecology, invasion biology, food security and many other major themes in contemporary plant biology.

Research has moved apace over recent years, but the discipline remains fragmented largely along the lines of work focused on organisms (the ecophysiological expression of early plant defence traits) and communities (the effects of herbivore exclusion on plant community establishment). Our aim in this Special Issue is to bring together a body of new research to highlight and synthesize the wider biological importance of plant–herbivore interactions during early plant ontogeny. In so doing we ask four main questions.

1. How do seedlings defend against herbivores?
2. What is the role of seedling herbivory in community dynamics and regeneration?

(3) Can information about seedling defence inform us about patterns of herbivore selection on seedlings and subsequent patterns of establishment and community structure?
(4) How does disruption of the seedling–herbivore interaction affect patterns of plant community establishment within a conservation context?

In answering the first question, we combine both new (Barton, 2013; Goodger *et al.*, 2013; Hanley *et al.*, 2013; Kitajima *et al.*, 2013; Queenborough *et al.*, 2013; Quintero and Bowers, 2013; Villamil *et al.*, 2013) and old studies to show that many different kinds of defence traits vary markedly across early ontogeny. The second and third questions are tackled via the recent literature on herbivore-induced shifts in the establishing plant community, and ideas and evidence from related disciplines such as plant defence theory show how and why selective herbivory during plant establishment can exert such marked effects. It is this poorly explored interaction between early ontogenetic trait expression and shifts in plant community composition that we most wish to highlight for future research. A host of new studies in this Special Issue (Barlow *et al.*, 2013; Lieurance and Cipollini, 2013; Orians *et al.*, 2013; Shaw *et al.*, 2013; Wang *et al.*, 2013) allow us to answer the fourth question. In so doing, we show why a better understanding of the link between the ecophysiology of early plant defence and the effects of herbivory on community structure and function is desirable from an applied as well as the theoretical perspective.

Our contributors present work from as far afield as the south Australian mallee, British grasslands, the Amazonian rainforest, and Hawai'i. They consider a range of plant species from tropical trees, to upland temperate shrubs and invasive herbs. This biogeographical scope reflects the fact that plant–herbivore interactions during early establishment are globally important and may do much to explain the evolution of plant ecophysiological traits as well as the structure and function of vegetation worldwide.

SEEDLING DEFENCE AGAINST HERBIVORES

Perhaps the most notable aspect of plant defences is that they vary tremendously within and among species. Identifying important sources of variation in defence has been one of the primary goals of plant evolutionary ecology research. Although patterns are sometimes complex, there is clear evidence that plant defence traits vary genetically within and among populations and species, and in response to biotic and abiotic factors as a result of phenotypic plasticity (Denno and McClure, 1983; Karban and Baldwin, 1997; Endara and Coley, 2011). In addition, it has become clear over the past decade that plant development and ontogeny are important sources of variation, with marked changes in defence expression from the seedling to juvenile to mature and senescent plant stages being documented in hundreds of plant species across all kinds of defences, including chemical and structural resistance, and tolerance traits (Nykänen and Koricheva, 2004; Boege and Marquis, 2005; Barton and Koricheva, 2010). In addition to the characterization of ontogenetic patterns in defence trait expression, this research has provided new insights into long-standing debates on plant defence theory. For example, while a growth–defence trade-off is the backbone of contemporary plant defence theory (Herms and Mattson, 1992; Stamp, 2003), these trade-offs are not universal, and there are many examples of plants showing no or even positive relationships between growth and defence (Bergelson and Purrington, 1996; Purrington, 2000; Koricheva, 2002; Strauss *et al.*, 2002). Ontogenetic studies have shed light on this apparent inconsistency by revealing that defence may incur costs early in ontogeny, but then decrease as plants grow and have greater access to resources (Orians *et al.*, 2010).

Prior to the realization that defence changes dramatically across plant ontogeny, most research quantifying defence traits has focused on juvenile/saplings and mature plants. Very few studies explicitly measured defence in seedlings or early juvenile stages, an important oversight considering that herbivory is the primary source of seedling mortality (Moles and Westoby, 2004), and that seedlings may thereby represent the life-stage at which herbivore selection pressure is strongest (Swihart and Bryant, 2001; Fenner and Thompson, 2005). Consequently, we still have a relatively poor understanding about how seedlings defend themselves against herbivores, and this is particularly true for seedlings in the strict sense, including only plants that are still dependent on stored reserves (usually the cotyledon) for early nutrition (Hanley *et al.*, 2004). Thus, here and throughout this Special Issue, we broadly accept studies on true seedlings as well as young juvenile plants (i.e. no longer dependent on stored reserves/maternal provisioning), and we collectively refer to these young plants as 'seedlings' and 'juvenile', and use 'regeneration' and 'establishment' to refer to the active recruitment of these plants into their communities.

This Special Issue provides novel data on seedling defences and how they differ from that of older ontogenetic stages. Furthermore, by examining previously under-explored aspects of seedling defence, such as the development of extrafloral nectaries for indirect defence (Villamil *et al.*, 2013) and mechanisms of tolerance (Barton, 2013), we gain a better understanding of the anatomical and physiological constraints associated with early ontogenetic defence.

Seedling chemical resistance

In general, and assuming that defence costs are most severe in the seedling stage due to their limited photosynthetic area and root biomass, it is predicted that seedlings have low investment in defences (Boege and Marquis, 2005). Thus, an increase from the seedling to the juvenile stage is expected, followed by continued increase, plateau or decrease depending on the plant's allocation priorities as it reaches reproductive maturity (Boege and Marquis, 2005). While some evidence supports these general patterns (Gregianini *et al.*, 2004; Donaldson *et al.*, 2006, Goodger *et al.*, 2006,), it is apparent that ontogenetic patterns may vary among classes of plant secondary compounds (Elger *et al.*, 2009; Barton and Koricheva, 2010). In one of the first studies to simultaneously examine ontogenetic patterns in multiple classes of plant secondary compounds, Goodger *et al.* (2013) demonstrate that phenolics and terpenoids show contrasting ontogenetic patterns in *Eucalyptus froggattii*. While phenolic levels are highest in seedlings compared to juveniles and mature trees, terpenoids show the opposite pattern. Terpenoids in *E. froggattii* require specialized secretory ducts to avoid autotoxicity, and so low levels in seedlings may reflect either anatomical constraints or costs associated with developing these secretory ducts early in ontogeny.

In some cases, seedlings may produce very high levels of secondary compounds as defence (Sinclair *et al.*, 1988), presumably in response to strong selection pressure by herbivores on seedlings. The early expression of chemical resistance is thought to be particularly important in woody plants in boreal forests because of the importance of ground-dwelling mammalian herbivores in those systems (Swihart and Bryant, 2001). In contrast, relatively little is known about herbs with highly toxic seedlings. Quintero and Bowers (2013) reveal that *Penstemon virgatus* seedlings are extremely well defended, with iridoid glycosides comprising up to 20 % dry weight in leaf tissues. In contrast to the model predicting an increase in defence from the seedling to juvenile stage (Boege and Marquis, 2005), iridoid glycoside levels remain constant through the seedling–juvenile transition, and seedlings are more inducible than juvenile plants, increasing iridoid glycosides by 8 %. This study demonstrates very high chemical resistance (both constitutive and induced) in herbaceous seedlings.

Despite growing evidence of seedling chemical resistance, few studies have attempted to link seedling secondary chemistry to herbivore selection patterns, a necessary step to relate ontogenetic patterns in plant defence to patterns of herbivore-mediated seedling mortality. In one of the first such studies, Hanley *et al.* (2013) used macerated leaf tissue from seven age classes of *Plantago lanceolata* in feeding trials with a generalist herbivore, the snail *Helix aspersa*. They find that snails strongly prefer younger ontogenetic stages, and that this selection is based on olfactory cues of the leaf tissue. Chemical analyses reveal that green leaf volatiles increase significantly in older plants and that the composition shifts across ontogeny, providing an olfactory cue for patterns of selection by snails.

In addition to whole-plant developmental/ontogenetic patterns, it is well established that leaves undergo strong shifts in defence during development (Kursar and Coley, 2003; Koricheva and Barton, 2012). In general, young leaves are attractive to herbivores because they lack structural carbohydrates that contribute to leaf toughness (Coley, 1983). In response, delayed greening has evolved as a defence strategy in young leaves (Kursar and Coley, 1992*b*, 2003). During delayed greening, chlorophyll synthesis or chloroplast development is deferred until leaves mature in order to make young leaves less nutritious to herbivores (Kursar and Coley, 1992*a*; Whatley, 1992). At the same time, young leaves often have high anthocyanin levels, presumably functioning in pathogen defence, photoprotection, or crypsis against herbivores that cannot see red light (Dominy *et al.*, 2002; Hughes *et al.*, 2007). Although delayed greening has been documented extensively in mature trees, Queenborough *et al.* (2013) provide the first evidence that delayed greening is common in seedlings. In forests in Ecuador and Panama, they observe that 53 % and 82 %, respectively, of seedlings demonstrated delayed greening. These rates are even higher than delayed greening in saplings and mature trees, suggesting that delayed greening may be even more important in young plants than in older ontogenetic stages.

Seedling structural resistance

In contrast to chemical resistance, much less is known about ontogenetic patterns in plant structural defence traits such as spines, thorns, prickles, trichomes and toughness, particularly during the seedling stage (Hanley *et al.*, 2007). Nonetheless, there is evidence that pubescence (Del Val and Dirzo, 2003; Traw and Feeny, 2008) and leaf toughness (Kearsley and Whitham, 1989; Loney *et al.*, 2006) can increase during plant ontogeny, and there are also examples of ontogenetic declines in prickles (Givnish *et al.*, 1994), spines (Gowda and Palo, 2003) and leaf toughness (Boege, 2005). In the most comprehensive study of seedling structural defences to date, Kitajima *et al.* (2013) demonstrate for 104 Neotropical plant species that some measures of toughness positively contribute to leaf longevity in both sun and shade habitats. Toughness also changes during seedling development, showing a significant increase when measured as leaf and stem density, suggesting that young seedlings are weakly defended by structural traits.

Seedling indirect defence

In addition to expression of resistance traits that directly deter herbivores, many plants produce food bodies and extrafloral nectar to attract the natural enemies of their herbivores as a form of indirect defence (Heil, 2008; Koptur *et al.*, 2013; Marazzi *et al.*, 2013). While there is some evidence that extrafloral nectar can be abundant in some juvenile plants (Doak *et al.*, 2007; Wooley *et al.*, 2007), it is more common that food rewards increase across ontogeny (Veena *et al.*, 1989; Kwok and Laird, 2012; Pringle *et al.*, 2012). Thus, although it appears that seedlings commonly express low levels of food rewards, it has not been clear whether this results from allocation priorities and growth/defence trade-offs or from anatomical and physiological constraints. Villamil *et al.* (2013) shed light on this through their anatomical examination of the extrafloral nectaries in three ontogenetic stages of *Turnera velutina*. They document that while extrafloral nectary abundance does increase across ontogeny, more importantly, the nectaries do not become functional until they develop a transcuticular pore that forms a channel through which nectar can be released from the gland; this occurs only in a late juvenile stage. Moreover, reproductively mature plants double the secretory cells in the extrafloral nectaries, leading to significantly higher nectar secretion. Thus, although nectaries may be present in seedlings, they are not functional and so cannot be considered part of the seedling defence syndrome. This study highlights the value in applying anatomical/physiological approaches to ecological studies.

Seedling tolerance

In general, it is predicted that seedlings are constrained in their ability to tolerate (maintain fitness despite) damage (Strauss and Agrawal, 1999; Haukioja and Koricheva, 2000; Kelly and Hanley, 2005). Yet, tolerance has no overall ontogenetic pattern when synthesized across studies (Barton and Koricheva, 2010), with several examples of higher damage tolerance in younger ontogenetic stages (Weltzin *et al.*, 1998; Thomson *et al.*, 2003; Barton, 2008). If seedlings can tolerate herbivory better than expected, it is likely that they depend on different mechanisms than older plants, which have greater stored reserves and a higher capacity to access underground resources and light for photosynthesis.

Barton (2013) provides new insight into ontogenetic patterns in the mechanisms of damage tolerance using two species of

Plantago exposed to 50 % defoliation treatments at the seedling, juvenile and mature plant stages. While damage tolerance is very high in *P. lanceolata*, it is much lower in *P. major*, and neither species shows an ontogenetic pattern in tolerance; however, mechanisms associated with tolerance do change across ontogeny in both species. In *P. lanceolata*, tolerance is associated with early flowering time in juvenile plants and with pre-damage shoot biomass in mature plants. Although it is less clear how *P. lanceolata* seedlings tolerate the 50 % defoliation treatment, it is likely that their high rates of photosynthesis and plasticity in root/shoot allocation are important factors. In *P. major*, all plant stages show low tolerance, but seedlings that have invested in more root biomass prior to damage have higher tolerance, and the induction of photosynthetic parameters is associated with tolerance in juvenile plants. This study highlights how physiological and morphological data can shed light on the constraints and mechanisms of seedling defence.

HERBIVORE IMPACTS ON THE ESTABLISHING COMMUNITY

> 'Seedlings are also destroyed in vast numbers by various enemies; for instance on a piece of ground three feet long and two wide, dug and cleared, and where there could be no choking from other plants, I marked all the seedlings of our native weeds as they came up, and out of the 357 no less than 295 were destroyed, chiefly by slugs and insects.'

Darwin's oft quoted passage from *Origin of Species* is more interesting for what he doesn't say, than what he does (Darwin, 1859). It is clear that he recognized that the numerical impact of slugs and 'insects' on seedling cohorts can be devastating; a fact that would be readily corroborated by any gardener or farmer. But Darwin tells us nothing about the 62 surviving seedlings and their transition into the mature phase. What species were they? What germination, morphological or growth characteristics did they have? Did they survive beyond this experiment and reach reproductive maturity? What would have happened to the 357 seedlings if Darwin had excluded invertebrate herbivores during this most vulnerable stage? Unfortunately, rather than stimulate interest into the fate of seedlings and their interaction with herbivores, Darwin's observation remained unchallenged and unrefined for well over a century.

Interest was reawakened following the outbreak of myxomatosis and the dramatic post-World War II decline of rabbit populations in the UK. Changes in rabbit grazing pressure had enormous effects on grassland vegetation, particularly in the East Anglian Breckland region where the first seminal work (Watt, 1962) and subsequent studies (Bishop and Davy, 1984; Marrs *et al.*, 1986) highlighted how rabbits greatly affected seedling recruitment. The impacts of post-fire seedling herbivory by Californian chaparral rabbits were also widely reported (Mills, 1983, 1986; Swank and Oechel, 1991). Although informative, these studies were done largely from an autecological or population-level perspective, seldom extending beyond a discussion of herbivore-limitation for particular plant species to the recruitment patterns structuring communities. Indeed, the conventional wisdom suggested that seedling herbivory simply offset later losses to self-thinning and other forms of mortality (Crawley, 1983), so perhaps there was no need to consider seedling herbivory as any kind of selective filter in patterns of plant community assembly.

No systematic studies of the community-level effects of herbivory on seedling regeneration (via herbivore-exclusion experiments) were published until the late 1980s onwards, when research showed how terrestrial molluscs could alter the development, structure and composition of experimental plant communities (Edwards and Gilman, 1987; Hulme, 1994; Hanley *et al.*, 1995, 1996*a*, *b*). In all cases, excluding mollusc herbivores led to shifts in the success and establishment of different plant species. Not only did these authors highlight the wider importance of seedling herbivory, they also joined a number of contemporary studies to overturn the established view that invertebrate herbivores have little influence over vegetation dynamics (Hairston *et al.*, 1960). Subsequently, a number of experiments, undertaken in a variety of habitats, have shown how selective herbivory during plant establishment impacts upon community assembly. These studies include work conducted in tropical (Lindquist and Carroll, 2004; Asquith and Mejia-Chang, 2005) and temperate forests (Beckage and Clark, 2005), mangrove forests (Farnsworth and Ellison, 1997), oceanic islands (Green *et al.*, 1997), Mediterranean shrubland (Izhaki and Neeman, 1996), North American prairie (Burt-Smith *et al.*, 2003) and riparian vegetation (Hensgen *et al.*, 2011). The most consistent and perhaps most important observation to emerge from these experiments is that recruitment to the established community is differentially affected by the presence or absence of herbivores during an often narrow establishment window. That herbivores target some seedlings while avoiding/ignoring others indicates an important role of seedling palatability and defence and illustrates why a better integration of seedling trait analysis with regeneration dynamics will provide insights into this keystone interaction. In particular, investigating seedling defence among multiple species simultaneously will provide insights into herbivore selection patterns and consequential regeneration dynamics, providing links between plant defence theory, community structure and plant diversity.

We know of only two studies that have examined seedling defence in a community context. In the first, Kelly and Hanley (2005) compared herbivory and plant growth rate for five sympatric congeneric pairs of British herbs and grasses. They found that seedlings of the congener more prone to mollusc attack were consistently the faster growing. Assuming that relative acceptability is a good surrogate for allocation to constitutive seedling defence, the negative relationship between growth and acceptability strongly supports a growth–defence trade-off very early in plant ontogeny (Herms and Mattson, 1992). However, whether such interspecific variation in seedling defence and growth translate into community-level patterns of species abundances were not determined.

A subsequent study conducted on red (*Trifolium pratense*) and white (*T. repens*) clover suggested that the relationship between seedling defence and growth can determine plant community composition (Hanley and Sykes, 2009). Red and white clover seedlings vary considerably in their susceptibility to herbivore attack and in their growth rate: *T. pratense* is faster growing but more acceptable to snails (*Helix aspersa*); *T. repens* is relatively slower growing, but has lower acceptability. By varying the numbers of snails placed on pots when seedlings were only 14 days old, Hanley and Sykes (2009) showed that variation in the intensity of herbivory, coupled with species-specific variation in seedling growth and defence allocation could dictate which species came to dominate the mature community. In the absence of herbivory, the faster growing *T. pratense* came to dominate the established community, presumably by virtue of the fact that its greater competitive ability at the seedling stage facilitated niche pre-emption. Moderate snail herbivory resulted in species coexistence, while more intense seedling herbivory removed the majority of *T. pratense* seedlings early enough to allow domination by the grazing-resistant *T. repens*.

By altering the number of snails placed on each assemblage, Hanley and Sykes (2009) imposed variation in the intensity of seedling herbivory. Such variation is likely to be commonplace in natural systems, but the impact of long-term fluctuations in herbivore populations on plant regeneration remains speculative. It is not unreasonable to suppose, however, that the interplay of spatio-temporal variation in herbivore activity with species-specific variation growth–defence trade-offs during recruitment could facilitate species coexistence (Hanley and Sykes, 2014). A series of locally 'high-' 'intermediate-', and 'low-herbivory' years would almost certainly allow plants with different regeneration strategies to establish and so coexist in mature vegetation.

Any disruption of this interaction via the invasion of new plant or herbivore species, or unnatural shifts in the populations of native herbivores through factors such as changing climatic conditions, could have serious repercussions for plant community structure and function. We consider some of these issues in the next section.

SEEDLING HERBIVORY AND CONSERVATION

The seedling stage clearly plays a critical role in community structure and dynamics. Therefore, it is no surprise that successful conservation of rare or threatened native plants and the eradication and control of invasive plants depend on seedling interactions. For example, in Hawai'i, invasive slugs threaten 59 rare plant species, reducing seedling survival more in endangered seedlings compared to non-endangered native and invasive seedlings (Joe and Daehler, 2008). In many communities, herbivores threaten the seedlings of rare species, and successful restoration requires specific information about seedling susceptibility to herbivores. In this Special Issue, two articles present novel data on the role of seedling herbivory for the restoration of threatened upland hay meadows in the UK (Barlow *et al.*, 2013) and for the success of outplanted willow species in Scottish montane willow scrub (Shaw *et al.*, 2013).

Barlow *et al.* (2013) present acceptability data for 23 meadow species to herbivory by the generalist slug, *Deroceras reticulatum*. They report that species vary markedly in their acceptability to slugs, and that preferred plant species have much lower survival than species that slugs avoid. This information can inform land managers about which species are likely to have higher success in restored meadows due to their low acceptability to slugs and resulting high seedling recruitment. For example, *Geranium sylvaticum*, *Rumex acetosa*, *Leontodon hispidus* and *Anthoxanthum odoratum* are all avoided by slugs, and these are target species for restoration as a result of the likelihood of high seedling survival.

In Scottish montane willow scrub, mammalian herbivores are known to be an important impediment to successful restoration and conservation of rare plants (Shaw *et al.*, 2010). While considerable effort has been done to eradicate large mammals through fencing, less attention has been paid to the effects of small mammals to plant survival and growth. Shaw *et al.* (2013) investigate the effect of small mammals (bank voles) on the survival and growth of three outplanted willow species. While they find that vole damage rarely causes mortality in saplings, the ability of willows to re-grow and compensate for vole damage depends on microsite characteristics. Damaged willow saplings compensate better when planted into disturbed sites where above- and below-ground competition is reduced. This study highlights how plant defence interacts with other plant interactions (namely competition) and how plant conservation can benefit from careful manipulation of multiple factors, such as the simultaneous removal of seedling herbivores and competitors, in order to achieve greater restoration success.

Seedling herbivory can also play a role in the invasion biology of plants. For example, invasive plant species are more tolerant of seedling herbivory than native species (Rogers and Siemann, 2002; Gleason and Ares, 2004; Zas *et al.*, 2011), indicating that seedling herbivory may play a crucial role in the replacement of native plants by alien ones during plant invasions. Lieurance and Cipollini (2013) demonstrate that while tolerance of 50 % herbivory is generally quite high in the invasive shrub, *Lonicera maackii*, resource availability strongly influences the size, growth rate and chemistry of damaged and undamaged plants. This study emphasizes the need to include multiple factors in studies on seedling defence and herbivory in order to more fully understand the complexity of conditions in natural communities.

To further understand traits associated with the successful establishment and spread of invasive species, many studies compare populations from the invasive vs. native range (Liu and Stiling, 2006). These studies have revealed that herbivory and defence can often differ among native and invasive populations, although few of these studies have focused on seedlings. Wang *et al.* (2013) present novel data on the production of extrafloral nectar (constitutive and induced) in native vs. invasive populations of *Triadica sebifera* in order to assess whether invasive populations have lost these defences due to a relaxation of selection pressure in the introduced range, as predicted by the enemy release hypothesis (Keane and Crawley, 2002). In contrast to enemy release predictions, seedlings from the invasive populations have more leaves with extrafloral nectaries than seedlings from the native range. Evidence for the induction of extrafloral nectar is more complex, with differences between the magnitude of induction by specialists vs. generalists in the native and introduced populations. This study provides compelling evidence that indirect defences are important for seedlings within the context of plant invasions.

Seedling defence may also play a crucial role in the persistence of native plants when threatened with herbivory by non-native animals. When non-native herbivores reduce plant fitness via seedling damage, there is the potential for novel evolutionary trajectories in plants. Orians *et al.* (2013) present the results of a selection experiment that examines how North American hybrid willows (*Salix sericea* × *S. eriocephala*) respond to herbivory by the exotic slug, *Arion subfuscus*, which causes extensive mortality in young willows in North America. They find that, contrary to expectations, surviving plants do not have higher foliar concentrations of defence traits, but they do have higher foliar nutrients and greater above-ground biomass, indicating that vigorously growing plants are inherently more resistant to slugs. Interestingly, selected plants are more susceptible to three other phytophages, an indigenous pathogen (*Melampsora epitea*), a native herbivorous beetle (*Chrysomela knabi*) and an exotic willow leaf beetle (*Plagiodera versicolora*). Other exotic herbivore species may have similar direct and indirect effects on native plant populations.

CONCLUSIONS AND FUTURE DIRECTIONS

Seedling herbivory and defence play a fundamental role in the structure and composition of plant communities. However, relatively few studies focus on this early life history stage, despite growing evidence that defence and herbivory change dramatically across plant ontogeny. This Special Issue provides new insights into the evolutionary ecology of plants by demonstrating how seedling defence differs from that of older ontogenetic stages, through the beginnings of a link between seedling defence and community dynamics and by elucidating how specific knowledge about seedling defence and herbivory can inform restoration and conservation efforts.

Nonetheless, there remain important unanswered questions, and we suggest future research should address the following. (1) How do very young seedlings defend against herbivores? 'Seedling' in the broad sense refers to all young/small plants, but technically, the seedling stage lasts only until seed reserves are exhausted (Hanley *et al.*, 2004). Almost nothing is known about defence in this youngest, 'true seedling' stage. (2) What endogenous (i.e. allocation costs, anatomical and physiological constraints) and exogenous (i.e. ecological costs associated with competition, timing of mycorrhizal infection, pathogens, etc.) factors determine investment in seedling defence? (3) What are the general patterns of seedling defence across species and within a phylogenetic context? (4) What are the biogeographical patterns of seedling defence, and how does this relate to intensity of herbivory? (5) How do herbivores select seedlings? In other words, what are the volatile, visual or tactile cues associated with herbivore selection patterns? (6) How do patterns of seedling herbivory translate into plant regeneration patterns and community composition? (7) How does seedling herbivory influence crop species? Can selection for seedling defence improve crop performance and yield?

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