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Effects of seed beetles on the performance of desert legumes depend on host species, plant stage, and beetle density

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ABSTRACT

Seeds of many arid habitat plants have a water-impermeable coat and can germinate only after being scarified. Bruchine seed beetles are important parasites of legume seeds in these environments, but their effect on germination can be unpredictable. Beetles deplete seed resources and can kill the embryo but also scarify seeds. We investigated the effects of a generalist parasite, *Stator limbatus*, on the germination and growth of two common legumes in the Sonoran desert, catclaw acacia (*Acacia greggii*) and blue paloverde (*Parkinsonia florida*). Feeding damage from a single larva greatly increased germination of paloverde but not acacia. This benefit was reduced if seeds were attacked by multiple larvae. Beetle-damaged seeds of both hosts germinated more quickly than did control seeds. Infestation by beetles reduced seedling size, though effects were greater for paloverde than for acacia. Our results demonstrate that the effect of *S. limbatus* can be highly host-specific. In addition, beetle infestation may enhance or reduce seedling recruitment, depending on the availability of other scarifying agents and the number of larvae per seed. Such contingencies make it difficult to predict the net effect of seed beetles on efforts to control invasive legume hosts or establish native hosts during aridland restoration.

1. Introduction

Seed germination is influenced by many abiotic and biotic factors, including rainfall, temperature, soil properties, leaf litter, burial depth, and vegetative cover (Bewley and Black, 1994). The timing of germination can have profound effects on seedling survival and later life-history traits in plants (Kalisz, 1986). Determining the factors that affect germination and early seedling growth is thus necessary to predict the establishment and regeneration of plants in native habitats and for choosing appropriate plants to restore degraded lands (Sy et al., 2001). Many desert legumes play an important role in restoration ecology because they can tolerate aridity, fix atmospheric nitrogen, and provide food, microhabitats, and shade for a diversity of organisms (Barnes, 2001; Camargo-Ricalde et al., 2004; Rohner and Ward, 1999).

In arid and semi-arid environments, germination and seedling growth are often limited by water availability (Bowers et al., 2004). Many arid habitat legumes produce seeds with an impermeable coat, so that seeds can germinate only after damage to the seed coat (scarification) that allows water imbibition. Seeds of many arid habitat plants have evolved in association with a variety of scarifying agents, including fire, floods, soil abrasion, and soil microorganisms (Bewley and Black, 1994). Germination and seedling recruitment may also be improved by ingestion and defecation of seeds by mammals and birds (Miller, 1994a; Rohner and Ward, 1999; Traveset et al., 2001). Finally, scarification can be mediated by insect damage, which often produces holes in the seed testa (Mucunguzi, 1995; Takakura, 2002).

Seed beetles (Coleoptera: Chrysomelidae: Bruchinae) are especially common predators and parasites of legume seeds in desert and tropical environments, and many are highly host-specific (Ramirez and Traveset, 2010). Females generally lay eggs on or inside fruits, or they glue their eggs directly to seeds that are exposed in dehiscent pods or are found on the soil surface (Southgate, 1979). Larvae typically bore into the seed and complete development within a single seed. In some cases, seed-beetle larvae may attack a majority of seeds in the local plant population, but infestation rates of some hosts can be chronically low (Miller, 1994a; Takakura, 2002).

The net effects of seed beetle infestation on the germination and recruitment of host legumes can be unpredictable (Southgate, 1979). In some cases, the insect clearly acts as a seed predator;

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larval feeding effectively kills the embryo or removes so much endosperm that the seed cannot germinate (Camargo-Ricalde et al., 2004; El Atta, 1993; Tomaz et al., 2007). Larval feeding may also create openings for pathogenic bacteria and fungi (Chang et al., 2011; Cipollini and Stiles, 1991; Mucunguzi, 1995). Even if germination occurs, prior infestation of seeds may distort the development of cotyledons or prevent the formation of true leaves (Hegazy and Eesa, 1991). Depletion of cotyledon reserves may slow plant growth and hence reduce the probability of establishment. Beetle damage may even negatively affect non-infested seeds developing in the same pods as infested ones, e.g., by mediating allocation of resource among seeds within the fruit (De Menezes et al., 2010).

Despite these detrimental effects of seed-beetle damage, some proportion of infested seeds will germinate successfully in most host populations (Halevy, 1974; Hoffman et al., 1989; Mack 1998; Miller, 1994b,c). The fate of a damaged seed often depends on its size relative to that of its parasite, as well as the number of larvae per seed (Fox et al., 2010). If beetle damage acts as a scarifying agent, it can have a large, positive effect on the frequency of germination (Arévalo et al., 2010; Mucunguzi, 1995; Nakai et al., 2011). In one study, beetle infestation was considered a prerequisite to successful germination (Takakura, 2002). Because their effects on host performance can be so variable, bruchine beetles have been implicated in aiding the spread of undesirable, invasive legumes (Arévalo et al., 2010) and, conversely, have been proposed as biological control agents for such invasive hosts (van Klinken and Flack, 2008).

This study examines the effects of seed beetle infestation and damage on two desert legumes: catclaw acacia, *Acacia greggii* Gray (Fabaceae: Mimosoideae), and blue paloverde, *Parkinsonia florida* (Bentham ex A. Gray) S. Watson (Fabaceae: Caesalpinioideae). Both species are common in the southwestern United States and northwestern Mexico. Germination of acacia and paloverde seeds is likely to be enhanced by scarification, which in Arizona may be accomplished by flash floods in gravelly soils, underground weathering, or ingestion by mammals (Longland et al., 2001; Or and Ward, 2003). Seeds of *A. greggii* and *P. florida* are also frequently attacked by a generalist seed beetle, *Stator limbatus* Horn (Fox et al., 1995, 1997; Siemens et al., 1992). This insect has a relatively wide geographic distribution and a broad host range (Stillwell et al., 2007, and references therein), but little is known about this beetle's impact on host performance. To determine the effect of *S. limbatus* on seedling recruitment in *A. greggii* and *P. florida*, we experimentally manipulated densities of *S. limbatus* in seeds and measured effects on both germination and seedling growth.

2. Materials and methods

2.1. Source populations of seeds and insects

Seeds were obtained from three sites in central Arizona: two populations of catclaw acacia and one population of blue paloverde. Seeds of the Oracle population of *A. greggii* were collected from mature pods on >20 trees along Hwy 77 and adjacent roads in Oracle, Pinal County (32.62° N 110.78° W). Seeds of the Phoenix population of *A. greggii* were similarly collected from >20 trees in the Cave Creek area north of Phoenix, Maricopa County (~32.4° N 112.0° W). Seeds of blue paloverde were also collected from >20 trees in Maricopa County (33.79° N 112.12° W). Based on a sample of >900 seeds from each population, average seed mass (±SD) was 195 ± 70 mg for Oracle acacia, 205 ± 72 mg for Phoenix acacia, and 199 ± 50 mg for blue paloverde.

Because the effects of insect damage on seed germination and plant growth may vary depending on the source population of the insect (Fox et al., 2010), we experimentally infested each host population with the same source population of *S. limbatus*. A laboratory population of *S. limbatus* was established >300 beetles that had been collected from >20 *A. greggii* trees at the Oracle site. Female *S. limbatus* oviposit directly onto host seeds inside fruits that have dehisced or have been damaged by other organisms, such as mice or other insects (including other seed beetles, Mitchell, 1977). Hatching larvae burrow into the seed beneath the oviposition site. Because larvae cannot move between seeds, we could control larval density by manipulating the initial number of eggs per seed.

2.2. Treatments

Seeds from each source population were divided among three treatments: control (no manipulation), beetle damage, or scarification. We scarified seeds by removing about 5% of the seed coat with sandpaper, which should promote imbibition of water without affecting the underlying cotyledons. This technique has been widely used for physical scarification of desert legume seeds (e.g., Patane and Gresta, 2006). To produce beetle-damaged seeds, mated pairs of beetles were individually confined with five pre-weighed seeds in a 30-mm Petri dish. Providing only five seeds per dish caused females to lay multiple eggs per seed. Females were allowed to lay eggs for ~72 h and discarded. Eggs were scraped from seeds to create densities ranging from one to five eggs per seed. Larvae within seeds were reared to adult emergence in a laboratory growth chamber at 30 °C. Because nearly all eggs hatch successfully (>95% of eggs hatch; Fox et al., 2007), the number of eggs on a seed is a good estimate of larval density. Seeds from the three treatments were randomly interspersed in the chamber to ensure that all seeds experienced similar environmental conditions.

After adult emergence from seeds had been completed, we removed beetle frass from seeds by tapping the seed and inserting a small brush into beetle exit holes. Seeds were then reweighed to estimate the amount of mass removed by beetle infestation. Because larval survival can be fairly low in seeds of blue paloverde (Fox et al., 1995; Fox and Mousseau, 1996), we carefully noted the total number of adults emerging from each seed. To remove biases caused by the presence of dead larvae inside seeds, we estimated beetle effects on plant performance using only those seeds from which all beetles emerged successfully as adults.

2.3. Germination and seedling growth

Control, infested, and mechanically scarified seeds were sown in pots in a University of Kentucky greenhouse furnished with supplemental lighting and maintained at 29 ± 1 °C (day) and 27 ± 1 °C (night) and a 15:9 L:D photocycle. Seeds were sown in 13 blocks over a year, with both species and treatments represented in each block. Each pot was filled with loosely packed ProMix® and received a single seed 2.5 cm below the soil surface. Pots were watered daily. Successful germination was noted when seedling tissue became visible above the soil surface. Seeds were classified as having failed to germinate if three months had elapsed with no visible tissue above the soil surface. This protocol would not detect instances in which seeds did germinate but seedlings died before emerging from the soil. However, later inspection of pots with no visible seedlings indicated that such 'cryptic' germination was rare and unlikely to influence any of the treatment effects. Fifteen days after germination, we measured the height of each seedling, carefully washed it to remove clinging soil, and dried it to constant weight at 60 °C. We used an electronic balance to measure above- and belowground dry mass of each seedling.

We planted 1913 seeds (560 Oracle acacia, 547 Phoenix acacia, and 806 paloverde), of which 837 (171, 322, and 344) plants germinated and were grown.

2.4. Analyses

Logistic regression was used to compare the frequency of germination among treatments. All other traits were subjected to analyses of variance to test for heterogeneity among classes (Proc MIXED, SAS Institute, Cary, North Carolina). Linear contrasts between particular treatment combinations were performed using the ESTIMATE statement. Initial seed mass and mass lost to beetle feeding were used as covariates in some analyses. We present *F* statistics for the overall ANOVA and ANCOVA results and a *t* statistic for linear contrasts. For all parametric analyses, "block" was included as a random effect. This effect was almost always significant, but was included only to control for among-block variation, and is not discussed further below. We present least-squares means to remove block effects on plant traits. These were estimated with the SAS PROC MIXED procedure. Estimates of least-squares means depend on the particular statistical model, and may differ slightly between analyses that included different treatment combinations.

Because we weighed and sowed only seeds from which all larvae emerged as adults, we obtained relatively low sample sizes of seeds infested with four or five larvae. Sample sizes were adequate for analyses of germination frequency (for which both germinated and non-germinated seeds are included). However, germination frequencies were low for these highly infested seeds (see Results), resulting in very low sample sizes for plant growth traits at these higher densities. We thus included all five larval densities in the analysis of germination frequency, but excluded larval densities greater than three beetles/seed in the analyses of germination time and seedling growth.

3. Results

3.1. Loss of mass in infested seeds

Infestation by a single larva reduced seed mass by about 6% in acacia seeds (both populations) and about 4% in paloverde seeds (Table 1). This difference between legume species was highly significant ($t_{372} = 11.12$, $P < 0.001$ for the contrast between paloverde and acacia), but the mass removed by a single larva did not differ between the two source populations of acacia ($t_{372} = 1.25$, $P = 0.21$). Increasing beetle density predictably increased the total mass lost for seeds of both acacia and paloverde (Table 1). However, the amount of seed mass lost per larva was not affected by larval density in Oracle acacia seeds ($F_{1,413} = 0.26$, $P = 0.6$) or in paloverde seeds ($F_{1,102} = 2.08$, $P = 0.15$). For acacia seeds from the Phoenix population, the amount of mass lost per larva decreased slightly but significantly with increasing larval density ($F_{1,420} = 4.0$, $P = 0.05$).

Table 1
Mean mass lost (±SE) by catclaw acacia and blue paloverde seeds after infestation by varying densities of the seed beetle *Stator limbatus*.

Host population	Larval density	No. of seeds	Total mass lost (mg)	Mass lost per larva (mg)	Proportion of initial mass lost
Catclaw acacia (Oracle)	1	156	11.0 ± 0.2	11.0 ± 0.2	0.062 ± 0.001
	2	109	22.1 ± 0.4	11.0 ± 0.2	0.115 ± 0.003
	3	72	32.7 ± 0.5	10.7 ± 0.2	0.165 ± 0.005
	4	52	43.8 ± 1.0	11.0 ± 0.2	0.203 ± 0.006
	5	28	55.5 ± 1.4	11.1 ± 0.3	0.229 ± 0.008
Catclaw acacia (Phoenix)	1	161	11.3 ± 0.2	11.3 ± 0.2	0.058 ± 0.001
	2	119	22.1 ± 0.3	11.0 ± 0.2	0.112 ± 0.002
	3	82	32.6 ± 0.4	10.9 ± 0.1	0.153 ± 0.004
	4	39	41.9 ± 0.8	10.5 ± 0.2	0.190 ± 0.005
	5	23	52.0 ± 1.5	10.4 ± 0.3	0.217 ± 0.011
Blue paloverde	1	58	7.6 ± 0.2	7.6 ± 0.2	0.043 ± 0.002
	2	23	16.0 ± 0.6	8.0 ± 0.3	0.080 ± 0.003
	3	18	23.3 ± 1.0	7.8 ± 0.3	0.120 ± 0.007

Mean initial seed mass (±SE) was 195 ± 2, 205 ± 2, 199 ± 1 mg for Oracle acacia, Phoenix acacia, and blue paloverde, respectively ($N > 900$ seeds per population).

3.2. Frequency and timing of germination

The proportion of control seeds that germinated varied among the three host populations (logistic regression; $\chi^2_2 = 117$, $P < 0.001$). Undamaged paloverde seeds germinated at a much lower frequency than seeds from either acacia population (Fig. 1A; $\chi^2_1 > 48$, $P < 0.001$ for both comparisons). In addition, control acacia seeds from the Phoenix population germinated at a higher frequency than those from the Oracle population ($\chi^2_1 = 15.6$, $P < 0.001$) (Fig. 1A). The frequency of germination of acacia seeds was not significantly affected by infestation by a single larva of *S. limbatus* ($\chi^2_1 < 0.46$, $P > 0.49$), but beetle infestation dramatically increased the proportion of paloverde seeds that germinated (Fig. 1A; $\chi^2_1 = 142$, $P < 0.001$).

Mechanical scarification significantly improved germination relative to control seeds (Fig. 1A; $\chi^2_1 > 5$, $P < 0.03$ for each of the three analyses), but it had an especially large effect on germination of paloverde, which rose from 5% to 95% (Fig. 1A). Scarified seeds were also more likely to germinate than singly infested seeds in all host populations (Fig. 1A; $\chi^2_1 > 4.2$, $P < 0.05$). Thus, rankings of germination frequency were scarified > control = infested for acacia, whereas they were scarified >> infested >> control for

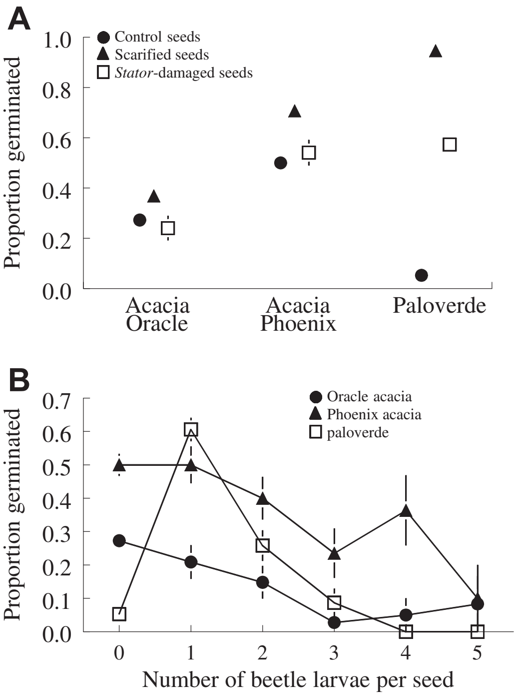

Fig. 1. A. Frequency of germination (mean ± SE) of control seeds, mechanically scarified seeds, and seeds previously infested by a single *Stator limbatus* larva. Seeds were from two populations of catclaw acacia and one population of blue paloverde. B. Germination frequencies of seeds previously infested with varying densities of *S. limbatus*. Some error bars are not visible because they are smaller than the points.

paloverde (Fig. 1A). It was also evident that Phoenix acacia seeds are consistently more likely to germinate than are Oracle acacia seeds subjected to the same treatment (Fig. 1A).

For all host populations, germination success generally decreased with an increasing number of larvae per seed (Fig. 1B; $\chi^2_1 > 7.2$, $P < 0.008$ for each host). However, the severity of this decline depended on host population, i.e., there was a significant host population × larval density interaction ($\chi^2_2 = 20.4$, $P < 0.001$). Despite gaining a very large benefit in germination frequency from infestation by a single larva, paloverde seeds then suffered the steepest decline in germination frequency with an increasing number of larvae per seed (Fig. 1B). Seeds from both species germinated at a very low frequency if they bore five larvae per seed (Fig. 1B).

Among control seeds, acacia took much longer to germinate than did paloverde ($t_{203} = 4.8$, $P < 0.001$; Fig. 2A). Although infestation by a single larva had no effect on germination frequency in acacia (Fig. 1A), it reduced the time required for germination by >65% in all three host populations (Fig. 2A; $t_{768} = 4.17$, $P < 0.001$ for the comparison of control versus one-larva seeds). Because mechanical scarification also greatly reduced germination time in all populations ($t_{768} = 4.72$, $P < 0.001$), there was no difference in the time required for germination between scarified and singly infested seeds (Fig. 2A; $t_{768} = 0.18$, $P = 0.86$). Seed mass had no effect on the time to germination of beetle damaged seeds for any host ($F < 1.0$, $P > 0.33$), and there was no relationship between the proportion of mass lost to feeding by a single larva and the time needed for germination ($F < 1.7$, $P > 0.20$ for each host population). Although infestation by at least one larvae substantially reduced the time until germination, the number of larvae per seed (from 1 to 3) had no effect on the time needed for germination (Fig. 2B; $F_{1,240} = 0.91$, $P = 0.34$).

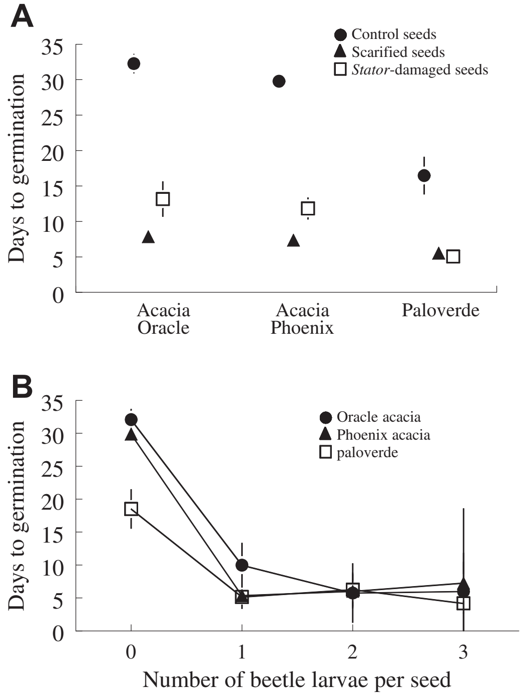

Fig. 2. A. Time required for germination (mean ± SE) of control seeds, mechanically scarified seeds, and seeds previously infested by a single *Stator limbatus* larva. B. Germination times of seeds previously infested with varying densities of *S. limbatus*. Some error bars are not visible because they are smaller than the points.

3.3. Seedling growth

Not surprisingly, larger seeds produced larger seedlings. In an analysis of covariance, there was a positive relationship between seed mass and the height and mass (above- and belowground) of control seedlings at 15 days after germination ($F > 5.4$, $P < 0.001$ for all traits). However, the effect of seed size differed between acacia and paloverde; the effect was large and highly significant for both acacia populations ($P < 0.001$ for all traits) and either non-significant (for height and belowground biomass) or only marginally significant ($P = 0.03$ for aboveground biomass) for paloverde seedlings.

The effect of infestation by one beetle larva depended on the type of host; for all traits, there was a significant interaction effect between host and the presence or absence of a single larva (Fig. 3). There were no differences in height among seedlings from control, scarified or singly infested seeds for either acacia population, ($F_{2,441} \leq 2.19$, $P > 0.33$ for each population), but the three treatment groups (control, scarified, and singly infested seeds) did vary in above- and belowground mass ($F_{2,439} > 6.1$, $P < 0.003$ for each trait); infestation by a single beetle larva reduced biomass compared to seeds not damaged by a beetle larvae ($t_{439} > 2.1$, $P < 0.04$). Paloverde seedlings germinating from singly infested seeds were significantly smaller for all three plant traits than seedlings germinating from seeds not infested by a beetle (Fig. 3; $P < 0.001$ for each trait), but there were no significant differences

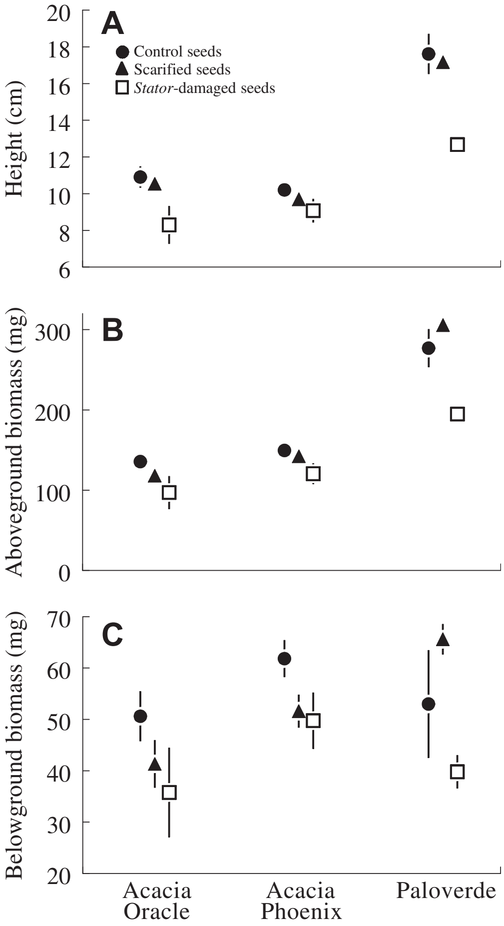

Fig. 3. A–C. Height and dry mass (mean ± SE) of seedlings derived from control seeds, mechanically scarified seeds, and seeds previously infested by a single *Stator limbatus* larva. Some error bars are not visible because they are smaller than the points.

between seedlings from control and scarified seeds ($P > 0.43$ for each trait). There was no significant difference between the two acacia populations for any of the plant traits (Fig. 3; $P > 0.11$ for all three traits). For all seedling traits in acacia, the effect of beetle damage also depended on initial seed mass, i.e., there was a significant interaction between the effects of seed mass and infestation by a single larva ($F > 12.0$, $P < 0.001$ for all traits). As expected, smaller seeds were much more negatively impacted by beetle damage. In contrast, the effect of beetle damage also did not depend on initial seed mass for paloverde seedlings ($F < 0.7$, $P > 0.4$).

Increasing the number of larvae per seed consistently reduced seedling height and biomass (Fig. 4; $F > 4.2$, $P < 0.04$ for all traits in an ANCOVA that included only beetle damaged seeds). There was no evidence that the effect of density differed among the three host populations, as there was no interaction between the effects of larval density and host for any seedling trait (Fig. 4; $F < 2.7$, $P > 0.07$ for each trait). As we observed for control seeds, seed mass was positively correlated to the size of beetle-damaged seedlings for acacia ($F > 11.0$, $P < 0.001$ for all three traits) but not for paloverde ($F < 1.0$, $P > 0.5$). However, there was no evidence that seed mass mediated the effects of beetle density on seedling size (there was no seed mass-x-beetle density interaction for any trait of either species; $F < 1.0$, $P > 0.47$). The proportion of seed mass lost to beetle consumption had a significant effect on all three measures of seedling growth ($F > 7.0$, $P < 0.007$ for all traits). Interestingly, though, the effect of beetle density remained significant after accounting for the amount of seed mass lost (using analysis of covariance) for above- and belowground biomass ($F > 4.4$, $P < 0.03$), but not height ($F_{1,91} = 1.33$, $P = 0.25$) of acacia, and for height and aboveground biomass ($F > 5.2$, $P < 0.03$), but not belowground biomass ($F_{1,130} = 1.54$, $P = 0.22$), of paloverde. This indicates that increasing beetle density reduced the size of seedlings beyond that accounted for by the increased amount of seed tissue lost to beetles in highly infested seeds.

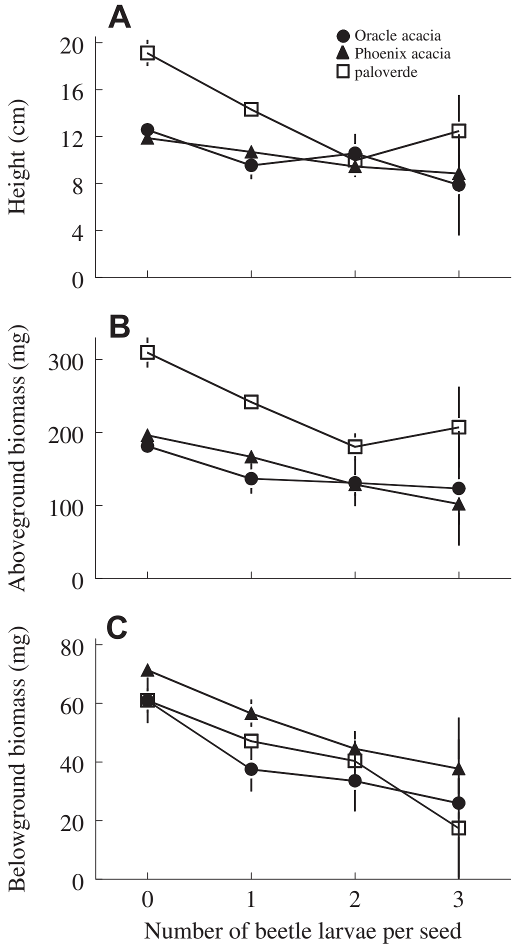

Fig. 4. A–C. Height and dry mass (mean ± SE) of seedlings derived from seeds previously infested by varying densities of *Stator limbatus*. Some error bars are not visible because they are smaller than the points.

4. Discussion

Taken together, our results highlight the complex and mixed effects of seed-beetle infestation on the performance of their native legume hosts. Because the body size of *S. limbatus* is small relative to the size of an acacia or paloverde seed, seeds supporting the complete development of one larva lost only a small fraction of their initial mass. Consequently, damage to embryonic tissue was less, and there was no decrease in the frequency of germination of singly infested seeds relative to control seeds for either species. On the contrary, feeding by a single larva greatly improved the germination success of blue paloverde, which relies on scarification much more than does catclaw acacia. However, the effect of beetle infestation was density-dependent; infestation by multiple larvae reduced the germination frequency of both species, but especially of paloverde seeds. Since acacia seeds, unlike paloverde seeds, did not benefit from infestation by a single larva, the effect of *S. limbatus* on the germination success of acacia would be neutral (in singly infested seeds) or detrimental (in multiply infested seeds) in relation to germination of control seeds.

Many legumes have seed coats that are largely impermeable to water, limiting their ability to imbibe water, an essential first event for germination (Takakura, 2002). Such species often require, or at least benefit from, scarification of their seed coat (Clemens et al., 1977). Our study demonstrates that beetle damage may be an important scarification mechanism in nature, but also that the effect of beetle damage on host germination must be considered in the context of other potential scarifying agents. For both species examined here, mechanical scarification improved germination relative to both control and beetle damaged seeds. This was especially true for paloverde, which is very dependent on scarification before it can germinate. For paloverde seeds, infestation by a single larva was beneficial relative to control seeds (beetle damage dramatically increased germination), but was costly relative to mechanically scarification (beetle damage dramatically reduced germination). Apparently, even one larva removes enough seed tissue to reduce the benefit of creating an opening in the seed coat. Optimal germination of both acacia and paloverde seeds would occur via physical disruption of the seed coat without insect colonization. Nevertheless, seed beetles could be an important scarification agent in natural populations of blue paloverde, since candidate abiotic processes, such as scraping of seeds during flash floods or weathering of seeds under the soil surface (Bewley and Black, 1994), may not be as frequent or effective as insect- or rodent-mediated scarification (cf., Mitchell, 1977; Takakura, 2002). More research is needed with respect to longer-term effects of either mechanical or beetle-mediated scarification. It is possible, for example, that either type of damage to the seed coat increases desiccation and reduces seed longevity.

In some desert species, seed dormancy is broken when germination inhibitors are leached from the seed coat after sufficient rainfall, or upon dilution of salt in the soil, ensuring that germination occurs when the soil moisture level can support seedling growth (Bewley and Black, 1994). Commonly, early germinating seeds (e.g., those germinating early in the rainy season) have a fitness advantage over later germinating seeds because they may experience a longer growing season (Gómez, 2004), although there are many exceptions (Kalisz, 1986, and references therein; Cheplick, 1996). Delayed germination may in fact be beneficial if it increases the total variance in germination times and makes it more likely that at least some individuals germinate under temporarily favorable conditions. For the desert species used in our study, infestation of seeds by beetles substantially shortened the time required for germination post-watering (by ~10 days for paloverde and >15 days for acacia). However, this benefit of beetle damage was generally less than the benefit of mechanical scarification, indicating again that the net effect of insect damage depends on the likelihood of mechanical scarification.

Despite the observed positive effects on germination frequency and timing, we must be cautious before concluding that low levels of beetle attack are generally beneficial. After germination, beetle damage to seeds has lingering, but species-specific, negative effects on seedlings. The negative effect of infestation by a single *S. limbatus* larva on 15-day-old seedlings was small for acacia seedlings but substantial for paloverde seedlings. It is not clear why this between-species difference occurred, since seeds of the two species are similar in size, and the amount of mass lost by a singly infested seeds was actually lower for paloverde than for acacia. The contrasting responses of acacia and paloverde may reflect fundamental differences in how seed reserves are used in each species. In some plants, part of the cotyledon may serve to buffer the negative impact of pre-dispersal seed predation, whereas any loss of cotyledon tissue in other plants greatly reduces seedling mass (Bonal et al., 2007; Mack, 1998). Control paloverde seeds produced larger seedlings than did control acacia seeds (Fig. 3), which may indicate that paloverde uses proportionally more reserves for seedling growth, and any loss of cotyledon tissue has negative consequences. For paloverde, the negative effects of beetle damage on seedling performance would partially offset the benefits of increased germination frequency and decreased time to germination, but this is not the case for acacia seeds.

In Arizona, it is typical for acacia and paloverde seeds to be attacked by multiple species of seed beetles in the Sonoran desert, and individual seeds often support multiple beetle larvae (C. Fox, pers. obs). For example, it is common for nearly all paloverde seeds to be attacked by either *S. limbatus* or another bruchine beetle (*Mimosestes amicus*), with more than half of all seeds producing at least one emerging adult beetle (Mitchell, 1977). Of seeds that are attacked by beetles, most receive multiple eggs. For example, an average blue paloverde seed is attacked by >3 *Mimosestes* eggs (Siemens et al., 1992), and most seeds attacked by *S. limbatus* receive more than one egg (Amarillo-Suarez, 2010; unpubl. data). We thus included variable beetle density in our study, and the results are generally clear and consistent for both species – the frequency of seed germination and seedling growth both decline quite substantially with increasing numbers of beetles, such that any benefit of beetle damage caused by scarification of the seed coat (e.g., in paloverde) is eliminated. In other seed/seed-parasite associations, the frequency and consequences of multiply infested seeds can be a complex function of the overall abundance of the seed crop as well as the average sizes of individual seeds (Bonal et al., 2007).

Our experiments probably underestimated the negative effects of beetle damage on seedling performance in nature. Experimental plants were provided ample water at regular intervals, and were reared in ProMix in the absence of competitors. Water availability is a major determinant of seedling survival and growth in arid habitat plant species, and even a modest decrease in initial plant size may lower survival in harsh habitats (Sher et al., 2004). In nature, beetle damage would also reduce seedling survival if it altered the seasonal timing of germination in a maladaptive way; e.g., if insect-caused scarification reduces the total amount of soil moisture needed for germination, seeds may germinate when average rainfall is suboptimal for seedling growth and persistence (Elberse and Bremen, 1990).

Our study also does not consider other ecological interactions that may have profound effects on germination and seedling growth in nature (Rodriguez-Pérez et al., 2011). For example, seeds cached in rodent dens provide the major source of recruitment for many desert shrubs (McAuliffe, 1990), but damaged seeds may more likely be eaten than cached, as has been demonstrated for squirrel predation on acorns (Steele et al., 1996). Alternatively, some rodents preferentially harvest undamaged seeds, such that low levels of insect damage may protect seeds from vertebrate predators (Miller, 1994b). Beetle damage may also reduce the beneficial effects of other scarifying agents. For example, coyotes, woodrats, deer and agricultural ruminants consume large numbers of desert seeds, many of which pass through the gut scarified and ready to germinate (Kneuper et al., 2003). However, insect-damaged seeds may be more likely to be destroyed by mammalian gut enzymes (Miller, 1993). We also do not know whether improved germination rates of singly infested seeds in this study would be somewhat lower in nature, where insect feeding may facilitate fungal attack (e.g., Chang et al., 2011).

5. Broader implications

Our study demonstrates that the overall effect of *S. limbatus* on the germination and growth of its legume host can range from highly negative to positive, depending on host species, plant stage, and larval density. At one extreme, attack by a single beetle improves the frequency and timing of germination of paloverde seeds, and thus can have very positive effects on plant fitness in the absence of other seed scarifying agents. At the other extreme, moderate beetle densities (within ranges commonly observed in nature) reduce germination frequency (probably by killing the embryo), and reduce growth of seedlings, substantially reducing plant fitness. It should not be surprising therefore that previous studies have reported highly beneficial, moderate, and highly detrimental effects of seed beetles on the fates of host seeds (Arévalo et al., 2010; De Menezes et al., 2010; Ernst et al., 1989; Or and Ward, 2003; Takakura, 2002; Tomaz et al., 2007). Even congeneric *Acacia* species in Africa savannas showed opposite responses to attack by a single beetle species (Mucunguzi, 1995).

Such complexities in the effects of beetle damage on plant performance make it difficult to quantify the role of a seed parasite in limiting or advancing the spread of invasive plants (Arévalo et al., 2010; Briano et al., 2002; van Klinken and Flack, 2008), or in decreasing population sizes of endangered plants (Hegazy and Eesa, 1991). Host-specific and density-dependent effects also make it difficult to predict how seed parasites will slow the re-establishment of native legumes in degraded or denuded habitats (Camargo-Ricalde et al., 2004; Khurana and Singh, 2001). To obtain an even more realistic assessment of the effect of a seed parasite on its host, one should also examine potential multi-species interactions involving seed beetles and their hosts (Brancalion et al., 2011; Mitchell, 1977; Miller, 1994a–c). For example, if seed beetle larvae are attacked by parasitoid wasps, they may accomplish scarification but not consume enough tissue to kill seeds (Nakai et al., 2011). Despite these caveats, experimental manipulations such as those in this study can begin to disentangle the complex interactions of seed-feeders and plants in natural communities.

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