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RESEARCH ARTICLE

Top-down cascading effects of seed-feeding beetles and their parasitoids on plants and leaf herbivores

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Abstract

1. When feeding on a plant, herbivorous insects alter the quality of the plant as a food source. This affects other organisms interacting with the same plant. These so-called 'plant-mediated interactions' can be altered by parasitoids that attack the herbivores. So far, this research area has mainly focussed on interactions at the leaf level, and very little is known about plant-mediated interactions via seeds. It is still poorly understood if seeds that survive insect damage have fewer resources to allocate to plant growth and defence against leaf herbivores, and whether parasitoids that kill seed-feeding insects mitigate such negative effects.
2. Using seeds of wild lima bean plants *Phaseolus lunatus*, we studied the effect of the intensity of infestation by seed beetles *Zabrotes subfasciatus* and their parasitoids *Stenocorse bruchivora* on the following parameters under lab conditions: seed mass and germination, plant growth and defensive compounds (cyanogenic glycosides and flavonoids) and performance of a leaf herbivore species *Spodoptera latifascia*. In addition, we performed a field experiment using seeds with or without insect damage to investigate the consequences on plant performance and fitness in the wild.
3. Seed beetle infestation had an overall negative impact on seed germination. Lab experiments revealed that damaged seeds produced plants with slower growth and reduced concentration of defensive compounds, which increased the performance of the leaf herbivores. Effects of seed-feeding on seed germination and plant growth were attenuated by parasitism, resulting in a net increase of the number of viable offspring. In the field, we observed that seed damage impaired germination, delayed flowering time and increased leaf herbivory.
4. Our results show that plant-mediated interactions between insect herbivores are not limited to leaf herbivores, but extend to seed herbivores. In our study system, parasitoids had no apparent effect on these interactions, despite their strong beneficial effects on germination and plant performance. These findings confirm the long-lasting consequences of indirect plant-mediated interactions in

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a community-wide ecological context. Furthermore, they contribute to a better understanding of the important but understudied effects of parasitoids on plant fitness.

KEYWORDS
defence, germination, performance, *Phaseolus lunatus*, plant fitness, plant–mediated interactions, pre-dispersal seed damage, tritrophic interactions

1 | INTRODUCTION

Interactions among plants and their herbivores occur within complex communities where organisms from different trophic levels affect each other directly and indirectly (Abdala-Roberts et al., 2019; Kaplan & Denno, 2007; Price et al., 1980). During the last decades, studies have specifically examined how plants can mediate interactions among herbivores that attack the plant at different developmental stages (Ohgushi, 2008; Utsumi et al., 2013), and how this results in cascading effects on different insect guilds and trophic levels (Hernández-Cumplido et al., 2016; McArt et al., 2013). The most recent studies have taken a top-down approach and examine how parasitoids of leaf-feeders can alter plant responses to herbivory and how this affects other insects in the community (Bustos-Segura et al., 2020; Cuny et al., 2018; Cusumano et al., 2018). Seed-feeding insects have not yet been considered in this context, possibly because it is often assumed that when a seed is attacked it is no longer viable. However, seeds are not always fully consumed by insects, and, in certain cases, damage by seed predators may even facilitate germination (Fox et al., 2012; Takakura, 2002; Vallejo-Marin et al., 2006). The ability to successfully germinate depends on the degree of damage to the embryo. Even if seeds germinate, the development of the seedling may be negatively impacted by insufficient remaining resources for growth and defence. How these top-down effects of seed insects affect plant performance and cascade to subsequent leaf herbivores, has not yet been investigated.

Also unexplored are the top-down effects of the third trophic level on the interactions between seed predators and their host plants. Larvae of seed feeders are frequently parasitized by parasitic wasps (Gómez & Zamora, 1994; Hernández-Cumplido et al., 2016; Moreira et al., 2015). These parasitoids halt host development and ultimately kill the larvae. Therefore, parasitism is expected to reduce seed damage, thereby, possibly increasing seed germination success and seedling performance (Nakai et al., 2011). For instance, parasitoids of *Bruchidius* sp. increase the seed germination success rate of *Acacia drepanolobium* (Martins, 2013), but it is unknown if parasitoids affect the performance of the germinated plants. Whether this is the case is particularly relevant in the context of the ongoing debate concerning the benefits that plants derive from recruiting parasitoids as part of the plant's indirect defence strategies (Cuny et al., 2021; Pearse et al., 2020; Turlings & Erb, 2018).

Here, we hypothesise that: (a) seeds that survive damage by seed-feeding insects have fewer resources to allocate to plant growth and defence, which makes plants more susceptible to leaf herbivores, and (b) parasitoids of seed-feeding larvae reduce seed damage, thereby mitigating the negative effects on plant resistance and plant performance parameters. To test these hypotheses, we evaluated the impact of seed beetle larvae on the germination success rate of *Phaseolus lunatus* (wild lima bean) seeds and on the subsequent seedling growth, the level of leaf defensive chemical compounds and interactions with a leaf-feeding insect. Moreover, we studied how parasitoids of seed beetle larvae may affect these measures of plant performance and resistance.

2 | MATERIALS AND METHODS

2.1 | Biological system

Phaseolus lunatus (wild lima bean) is an annual legume species that occurs naturally along the Pacific coast of Mexico (Heil, 2004; Hernández-Cumplido et al., 2016). When seeds are undamaged, they can become dormant, induced by environmental conditions such as high temperature and low humidity, creating a seed bank (Degreef et al., 2002). Seeds of *P. lunatus* are attacked by several bruchid (Bruchinae) species, and among these, *Zabrotes subfasciatus*, is one of the main granivorous pests species of beans worldwide (Benrey et al., 1998; Leroi et al., 1990; Shlichta et al., 2014; Figure 1a). Adult females enter dry pods before seed dispersion and affix their eggs on the seed coat. Upon hatching, larvae bore into the seed where they complete their development (Benrey et al., 1998). Before pupation, each beetle larva creates its own cavity close to the seed coat. Therefore, newly developed adults emerge from seeds by chewing their own exit hole from their individual cavity. Seed beetle larvae feed mainly on the cotyledons inside the seed, which generally does not have lethal consequences for the seed. Thus, seeds can germinate if the embryonic meristem is almost intact (personal observation). Beetle larvae are attacked by several hymenopteran ectoparasitoids that lay their eggs on their larval hosts developing inside the seed, halting their development (Campan & Benrey, 2004). Upon completion of their development, adult parasitoids exit the seed by chewing their own exit holes, which are smaller than the exit holes created by adult beetles (Figure 1b). The effect of seed damage

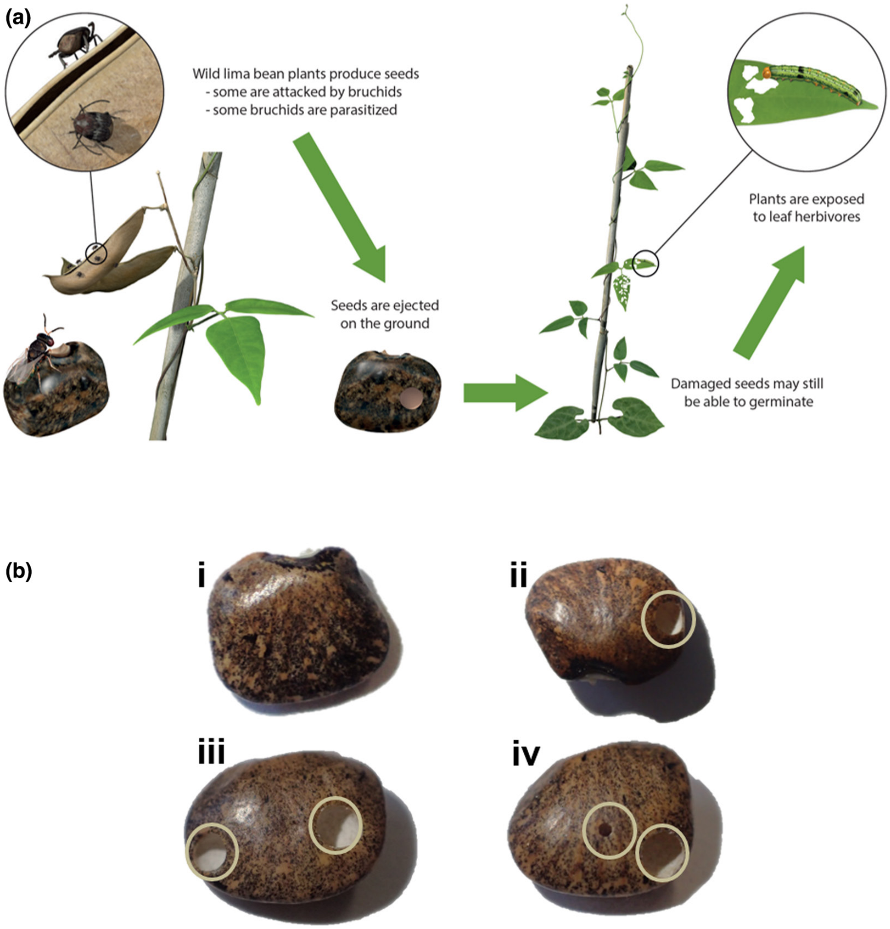

FIGURE 1 (a) Schematic diagram of the experimental system. (b) Example of seeds used in the laboratory (i, ii, iii, iv) and in the field (i, ii) experiments. (i) Undamaged seed, (ii) seed with one beetle exit hole, (iii) seed with two beetle exit holes and (iv) seed with one beetle exit hole and one parasitoid exit hole. Exit holes are circled

on the interaction between the newly germinated plant and a leaf herbivore was tested using *Spodoptera latifascia* (velvet armyworm), a generalist leaf herbivore species naturally present in Mexico that readily infests wild *P. lunatus* (Cuny et al., 2018). *Spodoptera latifascia* caterpillars were collected in the field in Mexico (Table S1) in October 2014 and reared on artificial diet ('beet armyworm diet', BioServ) at the University of Neuchâtel.

2.2 | Seed selection, morphological and chemical characterization

The level of infestation by seed insects was not manipulated. Instead, we collected seeds already damaged with different degrees of infestation by seed beetles and parasitoids. To confirm that the observed differences between seed-infestation categories are real rather than a result of patterns of seed infestation by insects being driven by specific seed traits, seeds were morphologically and chemically characterized. Seeds were collected directly from mature pods from wild lima bean plants. Once in the lab, seeds were categorized by their degree of damage indicated by the number of exit holes of seed beetles and their parasitoids. We used seeds from one population (AZUL) to perform a preliminary analysis to determine if undamaged seeds and seeds with insect damage differed in size and chemical traits. Seeds with either no insect hole, at least one beetle hole or at least one parasitoid hole were individually measured (length, from the two most distant points, with an electronic digital Vernier calliper, Vogel, Germany) and weighed (analytical balance, Mettler AE163, Switzerland). We also measured the concentration of their main defensive chemical compounds, two cyanogenic glycosides (CNGs), linamarin and lotaustralin, (Shlichta et al., 2014, 2018) and the total amount of flavonoids (Moreira et al., 2015). We used the same protocol as it is described below for leaf defensive chemical compounds (Shlichta et al., 2014).

2.3 | Effects of seed damage by healthy and parasitized beetle larvae on seed mass, germination and seedling growth

For this first lab experiment, we used *P. lunatus* seeds collected from four different populations during the field season of 2013 (YEL, AZUL, ITC, MAR, Table S1; Hernández-Cumplido et al., 2016; Shlichta et al., 2014). After the emergence of beetles and parasitoids, seeds were stored in a cold incubator (4°C) until further use. Beetles and parasitoids that emerged from these seeds were visually identified but were not quantified. These granivorous insects were all bruchid beetles, mainly *Zabrotes subfasciatus*, and the parasitic wasps were mainly *Stenocorse bruchivora*, an idiobiont parasitoid (i.e. that halts the development of its host after parasitism), which is a specialist on *Z. subfasciatus* [pers. obs., Campan & Benrey, 2004; Hernández-Cumplido et al., 2016; Moreira et al., 2015)]. Seeds were assigned to different seed-infestation categories according to the initial number of beetles in the seed and to their parasitism status: (a) undamaged seeds, (b) seeds infested by one, (c) two or (d) three unparasitized beetles and (e) seeds infested by one, (f) two or (g) three parasitized beetles (if at least one beetle was parasitized, the seed was included in the parasitized category). These seed-infestation categories were used to determine the effect of seed beetle and parasitoid infestation on seed mass, germination success, time to germination and plant growth. In November 2016, a total of 218 seeds from the four populations with different infestation categories were taken out of the cold incubator. Seeds were weighed to the nearest 0.01 mg using an analytical balance (Mettler AE163, Switzerland; number of replicates for control seeds: $n = 43$; seeds damaged by one, two or three beetles: $n = 43, 33, 30$, respectively; seeds damaged by one, two or three beetles with at least one parasitized: $n = 21, 19, 29$, respectively). Then, these seeds were individually sown in pots (11 cm in height and 4 cm in diameter) with soil (profi substrat, Einheitserde, Germany) and placed in a room with controlled light (light-dark cycles of 16–8 hr), temperature (24°C) and humidity conditions (35% relative humidity). Plants were checked daily and watered every other day. For each plant, we recorded the germination success and the time to germination (time from sowing the seed to appearance of seedling). After germination, plants were allowed to grow until their fourth trifolium (a leaf composed of three leaflets) was produced. We measured the time for production of the fourth trifolium, the above-ground fresh biomass (i.e. the shoot separated from the root and weighed to the nearest 0.1 mg soon after the plant produced the fourth trifolium) and below-ground dry biomass (roots were cut from the shoot, cleaned under tap water, left to dry at ambient temperature during 48 hr and weighed to the nearest 0.1 mg). Then, 2 months after sowing, we collected the seeds that did not germinate and determined whether they were decomposed or if they were still intact. Finally, we tested whether the hole in the seeds left by the bruchid beetles or their parasitoids after their emergence had a positive effect on the seed germination success. We placed 40 lima bean seeds from one population (AZUL) on wet cotton after slightly scratching half of them in order to mimic mechanical damage done by bruchids and their parasitoids to the seed coat.

2.4 | Effects of seed damage by healthy and parasitized beetles on the performance of the leaf herbivore *Spodoptera latifascia* and on plant defensive chemical compounds

This experiment was conducted in May 2017 with seeds collected from one lima bean population (AZUL). Seeds were kept in plastic containers until insect emergence and were then selected on the basis of different combinations of infestation and parasitism. As in the previous experiment, we used seeds infested by one, two or three healthy/parasitized beetles, as well as control seeds. In total, we used 137 seeds (number of replicates for control seeds: $n = 22$; seeds damaged by one, two or three beetles: $n = 21, 19, 11$, respectively; seeds damaged by one, two or three beetles with at least one parasitized: $n = 21, 20, 23$, respectively). To increase the germination success of control seeds, we lightly scratched their seed coat by removing approximately 0.5 mm^2 of the seed coat with fine point forceps, before sowing all seeds under the same conditions as those used in the previous experiment. The experiment started when all plants had grown at least two trifolia. Second instar caterpillars (*S. latifascia*) were weighed to the nearest 0.01 mg and placed on one leaflet (of the second or third trifolium, depending on plant growth) using plastic clip cages [3 cm in diameter, covered with a nylon mesh screen (Shlichta et al., 2018)] to prevent them from leaving the leaf. Caterpillars were left on the plants for 48 hr, after which they were weighed to calculate their relative growth rate [(larval mass 2 – larval mass 1)/days]. Damaged leaves were scanned and the quantity of leaf material eaten was measured in number of pixels with Photoshop CS4 (Shlichta et al., 2018). Immediately after removing the caterpillars, two undamaged leaflets of the same trifolium were collected, submerged in liquid nitrogen and stored at −80°C until chemical analyses were performed. Samples were ground using cold mortars and pestles. Approximately 0.02 mg of fine powder was placed in screw cap tubes and then 1 ml of 70% methanol was added. Samples were heated at 90°C for 10 min and then placed in an ultrasonic shaker (Branson 2210) for 15 min before centrifuging at 2000 *g* for 10 min. The supernatant was transferred to a new tube and separated in two parts for chemical analyses. An Acquity ultra-high-pressure liquid chromatography (UPLC) system coupled to a Synapt G2 QTOF mass spectrometer (Waters) was used to measure the concentration of CNGs (linamarin and lotaustralin) and flavonoid compounds (Moreira et al., 2018; Shlichta et al., 2014).

2.5 | Effects of seed damage on seed germination, plant growth and reproduction, and resistance to herbivores in the field

This experiment was conducted to determine the fate of plants produced by damaged seeds in a natural field setting in southern Mexico. The experiment took place in 2016 at the Experimental Campus of The Universidad del Mar, located 15 km northwest of the city of Puerto Escondido (Oaxaca, Mexico, 15°55′27.9″N 97°09′04.3″W) where wild lima bean occurs naturally (Hernández-Cumplido et al., 2016). To account for natural variation between populations, we used seeds of two wild lima bean populations (INK and AZUL, Table S1). Seed-infestation categories consisted of undamaged seeds and seeds with one beetle exit hole. Because a large number of seeds were used in the lab experiments, we were not able to replicate all insect-damage combinations used in the laboratory experiments. Three seeds were sown in individual biodegradable pots (8 × 8 cm), with 19 pots per seed-infestation categories. The pots were placed in a field cage to protect plants from herbivory. After recording seed germination, seedlings were randomly thinned, leaving only one plant to grow in each pot. Plants were watered every other day. Two weeks later, plants were transplanted to soil in a common garden setting where they were exposed to natural populations of herbivores (number of replicates per seed-infestation categories: $n = 25$ for undamaged seeds and $n = 24$ for damaged seeds). Plants from the two seed-infestation categories for each of the two populations were randomly distributed among four rows. Plants within the same row were separated by 1 meter, and plants between 2 rows were separated by 2 m. Plant size (total number of trifolia) and leaf damage (percentage of damaged leaves, calculated as the number of damaged leaves divided by the total number of leaves and multiplied by 100) were recorded 1 month after plants were transferred to the common garden. Later in the season, when the first plant produced its first flower (counted as day one), we started recording the number of days to the first flower per plant and counted the number of seeds produced by each plant.

2.6 | Statistical analyses

All statistical analyses were performed using R (ver. 4.0.2; R Core Team, 2013).

2.6.1 | Seed selection, morphological and chemical characterization

We used one-way ANOVA with the infestation categories of the seed (no insect, only healthy beetles or healthy and parasitized beetles) as explanatory fixed factors.

2.6.2 | Effects of seed damage by healthy and parasitized beetle larvae on seed mass, germination and seedling growth

Seed, shoot and root mass were analysed using linear mixed models. Generalized linear mixed models following a binomial distribution (for binary variable) were used to assess the effect of seed-infestation categories on the probability of seed germination. Time variables (time to germination and to production of the fourth trifolium) were analysed using cox models. For all the models, lima bean populations were used as random factors and the initial number of host beetles (one, two or three), parasitoid presence (yes or no) and their interaction were used as explanatory fixed factors. Pairwise comparisons between seeds with and without parasitoids were performed with Tukey's post hoc tests (only between seeds with the same number of initial beetle infestation). The effect of manual seed scratching on seed germination was tested using a chi-square analysis, and we used a logistic regression to evaluate the probability of germination (binary variable) as a function of seed mass for seeds infested by at least one insect.

2.6.3 | Effects of seed damage by healthy and parasitized beetles on the performance and damage of the leaf herbivore *Spodoptera latifascia* and on plant chemical compounds

The relative growth rate [(larval mass 2 – larval mass 1)/days] of *S. latifascia* as well as the leaf concentrations in cyanogenic glycosides (linamarin and lotaustralin) and flavonoids were analysed with linear models. Caterpillar leaf damage was analysed with a generalized linear model with a gamma distribution (for non-normal continuous data). For all these models, the initial number of host beetles (one, two or three), the presence of parasitoids (yes or no), their interactions as well as the trifolium position were used as explanatory fixed factors.

2.6.4 | Effects of seed damage on seed germination, plant growth and reproduction, and resistance to herbivores in the field

Time to germination and leaf damage were analysed using a linear model while a generalized linear model following a binomial distribution was used to test the effect of seed-infestation categories on the probability of seed germination. A generalized linear mixed model following a Poisson distribution (ideal for discrete variables, such as counts) was used to analyse the number of trifolia, with an observation-level random effect in order to correct for overdispersion. Finally, we used a cox analysis for the time taken to the production of flowers. In all the models, seed infestation status (control or one beetle) was used as an explanatory fixed factor and the two lima bean populations were used as cofactors.

3 | RESULTS

3.1 | Preliminary seed analysis

The preliminary seed analysis revealed no significant differences in seed size and chemical profile among intact seeds and seeds attacked by healthy and parasitized beetles (Figure S1).

3.2 | Effects of seed damage by healthy and parasitized beetle larvae on seed mass, germination and seedling growth

3.2.1 | Seed mass

We found that seed beetle infestation significantly reduced seed mass compared to control seeds ($\chi^2 = 88.17$, $df = 3$, $p < 0.001$; Figure 2a). The presence of parasitoids significantly mitigated the seed weight loss due to beetle infestation ($\chi^2 = 11.1$, $df = 1$, $p < 0.001$). However, there was an interaction between beetle infestation and parasitoid presence ($\chi^2 = 7.6$, $df = 2$, $p = 0.02$): the positive effect of parasitoids on lessening the amount of seed damage was stronger when there were more beetles per seed. Indeed, the effect of parasitoids was non-significant with only one host beetle, but when three beetle larvae developed inside a seed, seeds with parasitized beetles (with parasitoid holes) were on average 20% heavier than seeds with healthy beetles.

3.2.2 | Seed germination

The probability that an infested seed will germinate was significantly associated with seed mass ($\chi^2 = 32.0$, $R^2 = 0.15$, $p < 0.001$, Figure S2). In addition, germination success (proportion of germinated seeds over the duration of the study) of control seeds was improved by seed infestation, but tended to decrease with the number of beetles per seed ($\chi^2 = 41$, $df = 3$, $p < 0.001$; Figure 2b). On average, parasitoids increased seed germination (within 2 months after sowing) by 70% ($\chi^2 = 12$, $df = 1$, $p < 0.001$). No significant differences were found in the time to seed germination between control seeds and seeds attacked by beetles ($\chi^2 = 0.08$, $df = 1$, $p = 0.78$). Similarly, no effect of parasitism was found ($\chi^2 = 1.11$, $df = 1$, $p = 0.29$). At the end of the experiment (2 months), the soil was searched for non-germinated seeds. All the undamaged seeds were recovered, compared to none from all other seed-infestation categories, which suggests that these seeds had died and decomposed in the soil. Consequently, we estimate that the mortality of non-germinated infested seeds was 100%. Furthermore, the germination of artificially scratched seeds was three-fold higher than that of control seeds ($\chi^2_{1,38} = 15.55$, $p < 0.001$, Figure S3).

3.2.3 | Time for production of the fourth trifolium

Plants that developed from undamaged seeds grew significantly faster than plants from seeds infested by beetles ($\chi^2 = 31.45$, $df = 1$, $p < 0.001$; Figure 3a). Parasitoid presence did not significantly affect the time of production of the fourth trifolium ($\chi^2 = 2.46$, $df = 1$, $p = 0.12$).

3.2.4 | Shoot mass

Plant shoots developed from undamaged seeds were significantly larger than those from seeds infested by healthy beetles ($\chi^2 = 60.53$,

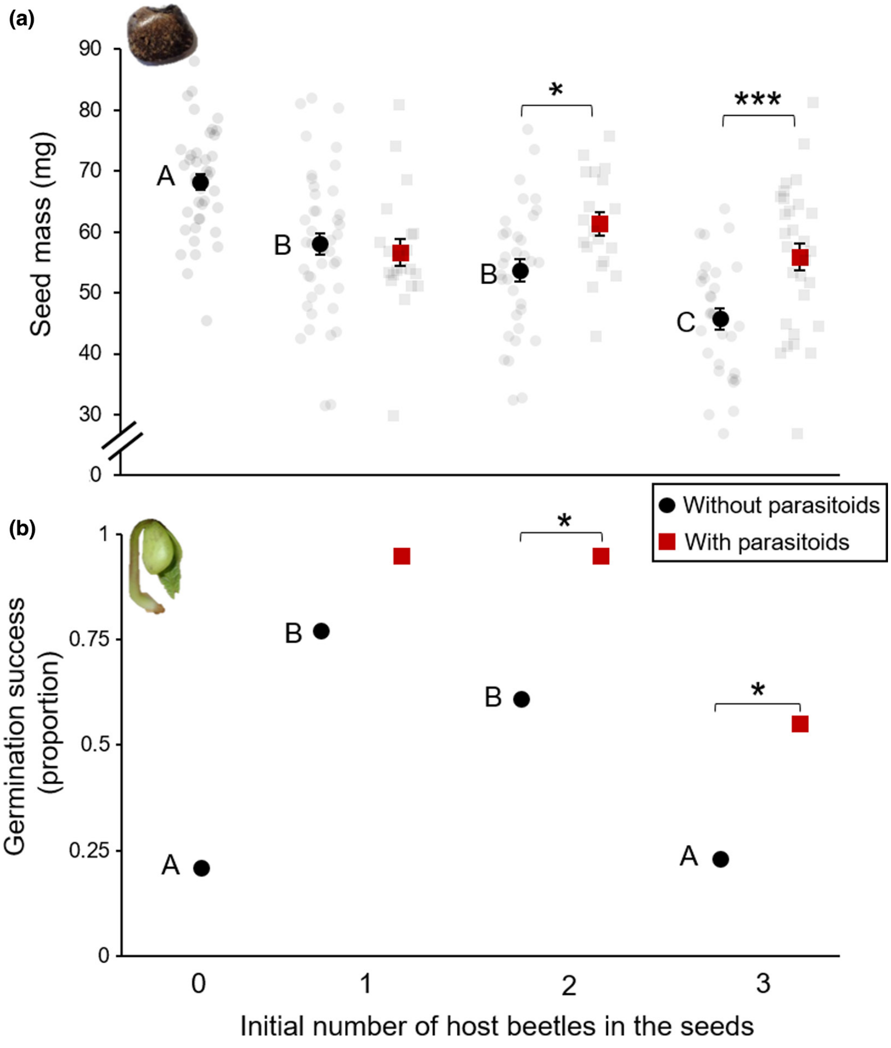

FIGURE 2 Impact of insect infestation by healthy and parasitized beetles on *Phaseolus lunatus* (a) mean seed mass (mean ± *SEM*) and (b) mean seed germination success. Different upper-case letters indicate a significant difference among seeds without parasitoids (circles). Stars indicate pairwise differences between seeds without versus seeds with (squares) parasitoids with the same initial number of host beetles in the seeds (*$p < 0.05$; ***$p < 0.001$). Small circles and squares in light grey represent the distribution of jittered raw data

df = 3, $p < 0.001$; Figure 3b). Overall, parasitoids significantly increased plant shoot mass ($\chi^2 = 9.33$, *df* = 1, $p = 0.002$) but this effect was only significant for plants that developed from seeds infested by two beetles (Tukey's post hoc: $p = 0.009$).

3.2.5 | Root mass

We found a significant negative effect of seed beetle infestation on plant root dry mass compared to plants from control seeds ($\chi^2 = 99.96$, *df* = 3, $p < 0.001$; Figure 3c). Parasitoids significantly increased root mass ($\chi^2 = 5.83$, *df* = 1, $p = 0.016$) but here, the effect was only significant for plants that developed from seeds infested by three beetles (Tukey's post hoc: $p = 0.02$).

3.3 | Effects of seed damage by healthy and parasitized beetles on the performance and feeding damage of the leaf herbivore *Spodoptera latifascia* and plant chemical defensive compounds

We found that the relative growth rate of the leaf herbivore *S. latifascia* was significantly different on plants produced by seeds with or without beetle damage ($\chi^2 = 9.44$, *df* = 3, $p = 0.02$). Plants from seeds infested by three beetles allowed a faster caterpillar growth than plants from undamaged seeds (Tukey's post hoc, $p = 0.037$; Figure 4a), but we did not find significant effects of parasitoids ($\chi^2 = 0.1$, *df* = 1, $p = 0.8$). Accordingly, plants from seeds infested by three beetles also tend to receive more damage than undamaged seeds, but this effect was only marginally significant ($\chi^2 = 7.4$, *df* = 3, $p = 0.052$). We found no effect of parasitoids on plant damage ($\chi^2 = 0.39$, *df* = 1, $p = 0.56$). Overall, seed beetle infestation altered the content of CNGs in leaves (linamarin: $\chi^2 = 7.14$, *df* = 3, $p < 0.001$; lotaustralin: $\chi^2 = 15$, *df* = 5.03, $p = 0.003$; Figure 4b,c). Plants that developed from seeds previously infested with at least two beetles had a lower concentration of linamarin and lotaustralin, the two predominant cyanogenic glycosides in lima bean. Similarly, flavonoids were lower in leaves of plants from seeds attacked by two beetles than from undamaged seeds ($\chi^2 = 18$, *df* = 5.55, $p = 0.002$; Figure 4d). The presence of parasitoids in the seeds did not have any effect on CNGs or flavonoid content in leaves (all $p > 0.5$).

3.4 | Effects of seed damage on seed germination, plant growth, reproduction and resistance to herbivores in the field

Germination success (proportion of germinated seeds over the duration of the experiment) in the field was not affected by seed damage ($\chi^2 = 0.03$, *df* = 1, $p = 0.86$; Figure 5a). However, we found a significant effect of seed infestation on the time to germination ($\chi^2 = 12.42$, *df* = 1, $p = 0.001$), with beetle infested seeds germinating faster than undamaged seeds (Figure 5b). Plants that developed from seeds attacked by one beetle produced on average 25% fewer trifolia than plants from undamaged seeds, but the difference was only marginally significant ($\chi^2 = 1.12$, *df* = 1, $p = 0.07$; Figure 5c), and for these latter plants, the time of production of the first flower was 30% shorter than for plants from damaged seeds ($\chi^2 = 7.72$, *df* = 1, $p = 0.005$; Figure 5d). Leaf damage by the leaf herbivore (percentage of damaged leaves) was double on plants from damaged seeds than on control plants grown from undamaged seeds ($F = 16.14$, *df* = 1, $p < 0.001$; Figure 5e). Finally, the total number of seeds produced per plant at the end of the season by plants germinated from undamaged (control) or damaged seeds was not significantly different ($F = 0.11$, *df* = 1, $p = 0.74$; Figure 5f).

4 | DISCUSSION

We found that pre-dispersal seed damage by seed-feeding beetles strongly affects plant performance in wild lima bean, from germination and early plant development to chemical defence and resistance to a generalist herbivore. Germination success of infested seeds was directly correlated with seed mass, implying that the impact of beetle larvae is dependent on the magnitude of the damage. Minor beetle damage facilitated seed germination, but high levels of damage reduced germination and caused poor plant performance. We also found that the negative effects of seed beetle damage on plant performance are partially ameliorated by parasitoids. Furthermore, we showed that seed damage had long term effects on plant growth and defence. In the lab, plants produced by damaged seeds had lower levels of CNGs and flavonoid compounds, which resulted in higher growth rate of caterpillars on these plants. These results were further supported by a field experiment, where plants produced by beetle-damaged seeds grew slower and suffered more herbivory than plants from undamaged seeds.

4.1 | Top-down effects of pre-dispersal seed damage on germination and seed viability

Seed mass loss due to feeding by beetle larvae was directly proportional to the number of larvae inside the seed, as previously reported by Fox et al. (2012). Results from our study as well as others show that: (a) the presence of holes in the seed coat facilitates rapid seed germination (Baskin & Baskin, 2004; Degreef et al., 2002; Han et al., 2018; Takakura, 2002), and (b) we also show that seed viability is inversely correlated to seed damage. Germination success was significantly lower for seeds infested by three beetles compared to seeds infested by one or two beetles, while germination of undamaged seeds was similar to that of seeds infested with three beetle larvae. We could not determine the degree of damage to the embryo, but clearly, the greater the seed damage, the greater the chances that the embryo will be compromised, which would make the seed unviable. We found similar results from the field experiment, where infested seeds germinated faster than undamaged seeds, which

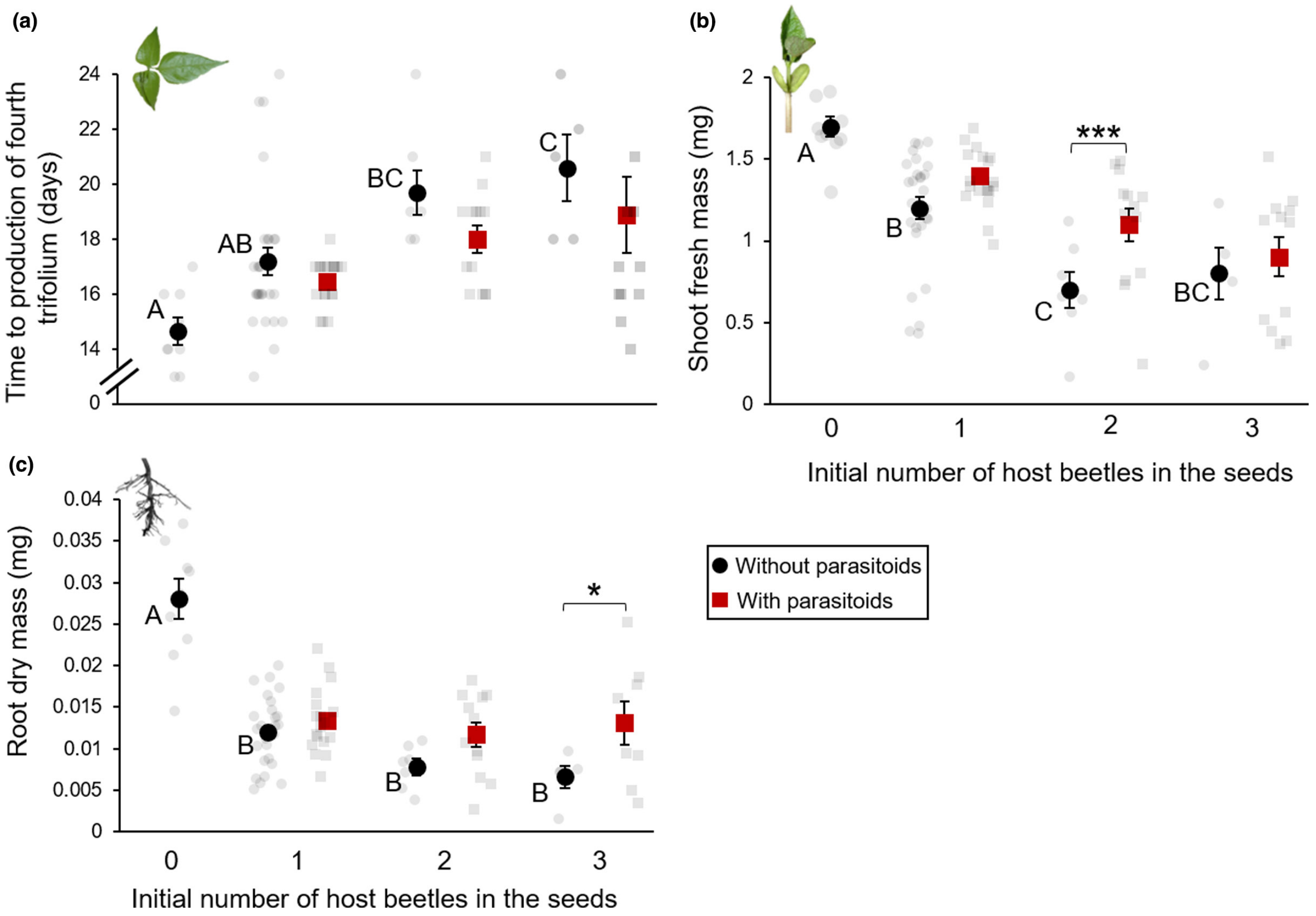

FIGURE 3 Laboratory experiments showing the effects of *Phaseolus lunatus* seed infestation by healthy and parasitized beetles. (a) Number of days from seed germination until the production of the fourth trifolium. (b) Fresh shoot mass of *P. lunatus* seedlings that had just produced their fourth trifolium. (c) *P. lunatus* root mass estimated just after the plants had produced their fourth trifolium, left to dry one night at ambient temperature. Different upper-case letters indicate statistically significant differences among seeds without parasitoid (circles), while stars indicate pairwise comparisons between seeds with (squares) and without parasitoids (*$p < 0.05$; ***$p < 0.001$). Big circles and squares indicate means (±*SEM*), while small circles and squares in light grey represent the distribution of jittered raw data

has been shown to be an important fitness trait for plants (Verdú & Traveset, 2005). An explanation for this is that beetle damage to the seed coat enables water to enter the seed and breaks dormancy (Degreef et al., 2002). We confirmed this by placing 40 lima bean seeds on wet cotton after lightly scratching half of the seeds. The mean seed germination rate of the scratched group was threefold higher than that of the non-scratched group.

Yet, the presumed benefits of germination as a result of seed damage may be overestimated. Undamaged seeds that did not germinate during our lab experiment were all recovered at the end of the experiment, whereas beetle-damaged seeds that did not germinate were not recovered, which indicates that they had most likely rotted in the soil (Mateus et al., 2011). This implies that in nature, infested seeds that do not immediately germinate will decompose in the soil (Chang et al., 2011; Mateus et al., 2011). Thus, it is likely that only undamaged seeds contribute to the seed bank, ensuring the long-term persistence of a plant species in an ecosystem (Alexander & Schrag, 2003) as well as allowing its survival during unfavourable periods (Moody-Weis & Alexander, 2007). A study on lima bean in Costa Rica showed that factors that affect the size of the seed bank (e.g., unusual weather conditions) can have dramatic consequences for future colonisation and plant population densities (Degreef et al., 2002). In this context, previous studies reported relatively high levels of seed infestation that are both spatially and temporally dynamic (Alvarez et al., 2006; Moreira et al., 2015; Zaugg et al., 2013). Consequently, we expect seed insects to play a significant and dynamic role in driving the demography of bean plant populations and the evolution of plant resistance to herbivores.

4.2 | Top-down effects of pre-dispersal seed damage on plant performance

We found strong negative effects of beetle infestation on plant performance (plant growth, shoot and root biomass), both in the laboratory and in the field. Plant growth speed can be an important fitness trait as it may confer a competitive advantage (Allcock & Hik, 2004). Shoot biomass is often used as a proxy for plant fitness, with the assumption

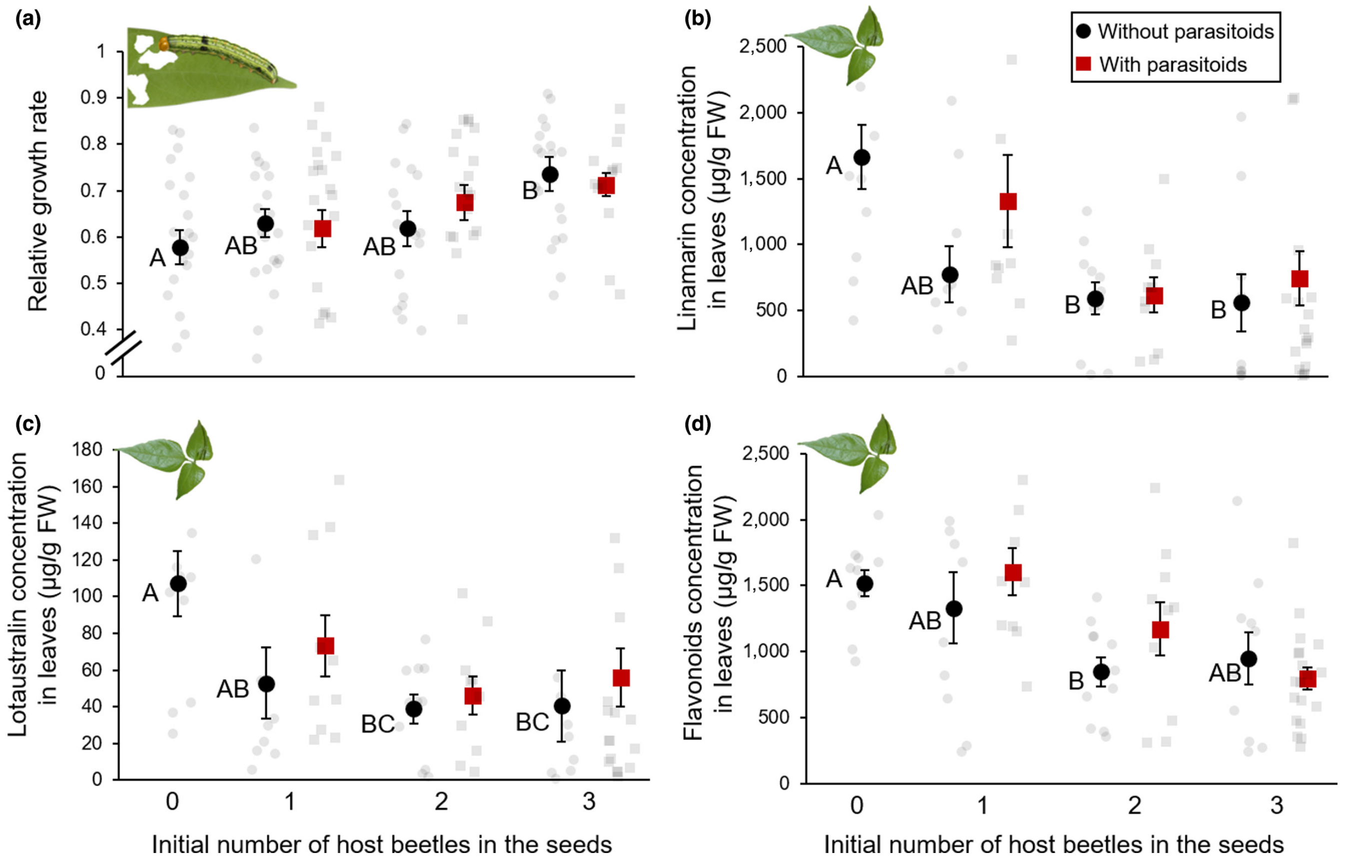

FIGURE 4 Effect of initial seed infestation by beetles and parasitoids on the (a) relative growth rate of a leaf herbivore and on the leaf concentration in the following plant chemical defensive compounds: (b) linamarin, (c) lotaustralin and (d) flavonoids. Different upper-case letters indicate statistically significant differences among seeds without parasitoid (circles). No significant differences were found among seeds with (squares) and without parasitoids. Big circles and squares indicate means (±*SEM*), while small circles and squares in light grey represent the distribution of jittered raw data

that larger plants will produce more and/or larger seeds (Vega, 2000), although we did not find any effect of seed damage on plant fitness in our field experiment. The few other studies that have assessed seedling performance following seed predation mostly found negative effects of seed damage (Fox et al., 2010; Mack, 1998; Mateus et al., 2011), but did not consider the interactions with subsequent herbivores.

4.3 | Top-down effects of pre-dispersal seed damage on plant resistance and herbivore performance

One of the most novel results from this study is the finding that seed damage resulted in a decrease in leaf defensive compounds and an increase in damage by leaf herbivores on plants produced by these seeds (both in the laboratory and in the field). One explanation is that beetle damage reduces the resources available for the seedling; thus, a trade-off between plant growth and plant secondary metabolism impairs the biosynthesis of defensive compounds.

Previous studies have examined the effect of plant-mediated interactions between leaf herbivores early in the season and subsequent seed-feeding insects later in the season (Bustos-Segura et al., 2020; Hernández-Cumplido et al., 2016; McArt et al., 2013). Overall, results show that early-season leaf herbivory increases seed resistance against seed-feeding insects, which may have a positive effect on plant fitness. Here, we went a step further and examined the plant-mediated interactions between seed-feeding insects and subsequent leaf herbivores. We found that the effects of seed-feeding insects go beyond seed damage and can continue through plant development, whereby plants produced from damaged seeds had lower levels of leaf defence compounds (cyanogenic compounds and flavonoids) and suffered more damage than plants produced by control undamaged seeds. We propose that damage caused by seed-feeding insects may be an underestimated cause of intraspecific variation in plant traits related to plant growth and resistance. The negative consequences of seed damage on plant traits will be dependent on the plant system and how prevalent is the damage of seeds in the population.

4.4 | Top-down effects of parasitoids on seed germination, plant growth and herbivore performance

The parasitism of beetle larvae in the seeds was, overall, beneficial for the plant. Parasitoids were especially important at high

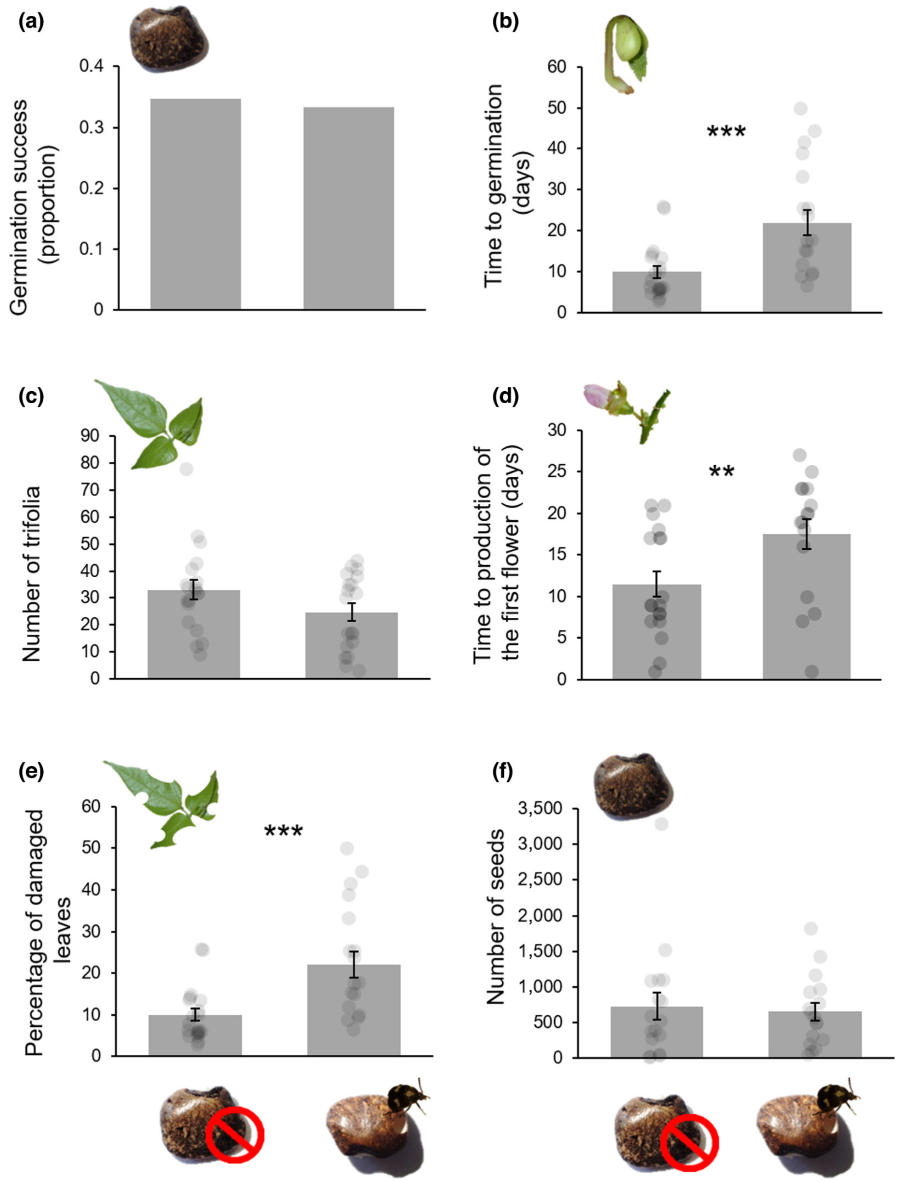

FIGURE 5 Effects of seed beetle infestation on *Phaseolus lunatus* plant performance under field conditions. (a) Proportion of seeds that germinated. (b) Time between seed sowing and germination. (c) Number of trifolia per plant after 1 month of transplantation. (d) Number of days to produce the first flower. (e) Percentage of damaged leaves per plant (number of damaged leaves/total number of leaves). (f) Number of seeds produced per plant. Stars indicate significant differences between undamaged and damaged seeds (*$p < 0.05$; **$p < 0.001$; ***$p < 0.001$). Bars show means (±*SEM*) and small circles and squares in light grey represent the distribution of jittered raw data

beetle infestation when seeds were infested by two or three larvae. Evidently, the parasitoids reduce the consumption of seed tissue by the beetle larvae (Martins, 2013; Nakai et al., 2011), resulting in increased seed germination, as much as threefold for highly damaged seeds. Hence, parasitoids indirectly affect plant fitness, potentially saving a significant number of seeds. Similar results were found elsewhere (Gómez & Zamora, 1994). The beneficial effects of parasitoids on seeds continued after seed germination. Overall, plants that germinated from seeds damaged by parasitized beetle larvae had more shoot and root mass than those that germinated from seeds infested by healthy beetle larvae. Another study, however, found that garden peas damaged by parasitized and unparasitized beetles (*Bruchus pisorum*) showed no difference in germination rate or seedling growth (Mateus et al., 2011). Parasitoids of seed feeders can even negatively affect plant fitness by stimulating their host to consume more (Xi et al., 2015). Hence, the impact of parasitoids of seed beetles on plant fitness may vary for different systems. Our results also show that despite reducing seed damage caused by beetles, parasitoids did not affect the defence chemistry in plants from damaged seeds nor leaf herbivore performance. The evidence that parasitoids affect herbivore-induced chemical changes in plants is mixed. In a recent study, we found that leaf-herbivore parasitoids reduced leaf damage but did not affect the changes in seed resistance against seed beetles (Bustos-Segura et al., 2020). Other studies show that parasitism can reduce plant resistance against herbivory mediated by physiological changes in the herbivore's saliva (Tan et al., 2018; Zhu et al., 2018) with consequences for plant fitness (Tan et al., 2020). However, plant damage by caterpillars of *Trichoplusia ni* parasitized by a polyembrionic parasitoid (*Copidosoma floridanum*) induced a higher production of glucosinolates and higher resistance in *Brassica oleraceae* than damage caused by unparasitized caterpillars (Ode et al., 2016). In our study the beneficial effects of parasitoids on beetle-infested seeds are likely due to the lower amount of seed tissue being damaged and not due to physiologically or chemically induced changes in the seeds. Parasitization by *S. bruchivora* immediately halts the development of beetle larvae and prevents further feeding.

5 | CONCLUSIONS

We found that the impact of seed-feeding beetles on lima bean plants extends beyond their net effect on the pre-dispersal seed set. Damaged seeds either perished or germinated, whereas most undamaged seeds survived and did not germinate, thus potentially contributing to the seed bank. The laboratory and field assays revealed that seedlings originating from severely damaged seeds grew slower, had lower levels of chemical defences and, as a result, were less resistant to leaf herbivores compared to seedlings from undamaged seeds. As a consequence, plants originating from damaged seeds may act as refuges (defence-reduced space) for herbivores that would normally be impacted by lima bean defences, which may in turn affect the herbivores' population dynamics with community-wide ecological consequences. We show that, in parallel, parasitoids play a crucial role in mitigating the negative effects of seed-feeding beetles by reducing the amount of damage caused by beetle larvae, but they did not lessen the reduced seedling resistance caused by the seed damage. The overall outcome of the study illustrates how a seed herbivore and its parasitoid can significantly impact plant resistance and multitrophic interactions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

M.A.C.C. had the original idea; M.A.C.C., B.B. and G.A.D. designed the experiment; M.A.C.C. and D.l.F. performed the lab experiments; G.G. performed the chemical analyses; M.A.C.C. and C.B.-S. performed the field experiment; C.B.-S., M.A.C.C. and G.A.D. performed the statistical analyses; M.A.C.C. and B.B. wrote the manuscript and all the authors contributed to several earlier versions of the manuscript.

DATA AVAILABILITY STATEMENT

Our data is archived in the Dryad Digital Repository https://doi.org/10.5061/dryad.7wm37pvvv (Cuny et al., 2022).

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