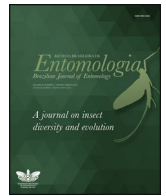




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## Morphological traits, allometric relationship and competition of two seed-feeding species of beetles in infested pods



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### ABSTRACT

Pair-wise competition produces asymmetric consequences for the interacting species, resulting in reduction of species fitness at the individual scale; however, little is known of the effects of competition on the allometric patterns of insects. In this study, we explored how competition, by means of pod infestation, affects the development of female and male individuals in the co-occurring bruchine beetles *Merobruchus terani* and *Stator maculatopygus*. We found differences between *M. terani* and *S. maculatopygus* in all morphometric traits, but no significant differences between males and females in either species. We also found, with an increasing degree of pod infestation, a positive trend in the pronotum, elytron and body weight of *M. terani* and a negative trend in morphological traits and body weight of *S. maculatopygus*. A negative allometry was maintained, suggesting that with increasing body weight, the body structures did not increase proportionally. On the other hand, we found that increasing the degree of pod infestation produced a wider variation in the individuals' body size than in low levels of infestation. Finally, we discuss how pod infestation can trigger competition between species, with both positive and negative impacts, even though the species function similarly in resource exploitation.

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### Introduction

The role of competitive interactions between herbivorous insects as an important community-structuring factor has been debated for 50 years (Kaplan and Denno, 2007). The concept of competition as a central organizing force that prevailed in the early 1960s and 1970s (Denno et al., 1995) changed in the 1980s to considering competition as a weak and infrequent structuring process (Lawton and Strong, 1981; Strong et al., 1984), and more recently to the perspective that herbivorous insects frequently compete (Denno et al., 1995; Kaplan and Denno, 2007). Pair-wise interactions produce asymmetric consequences for the interacting species (Denno et al., 1995; Kaplan and Denno, 2007), resulting in species exclusions at the population level and/or fitness reduction at the individual level. Consequences at regional or local scales result from mortality, niche shifting and avoidance; while fitness reduction results from decreasing survival, fecundity and body size (Denno et al., 1995).

Body size regulates the organism's form under developmental mechanisms (Cariveau et al., 2016; Shingleton et al., 2008),

since most measurable aspects of morphological traits covary with body size (Emlen and Nijhout, 2000). The relationship between the size of one morphological trait and the size of another morphological trait or the body size is termed allometry, a concept widely applicable to ecology and evolution; and is a measurement employed to investigate characteristics of insects and their sexes (Colgoni and Vamosi, 2006; Fox et al., 2003). Still, little is known of the effects of competition on the allometric patterns of insects (Emlen and Nijhout, 2000; Shingleton et al., 2008; Stern and Emlen, 1999).

Members of the coleopteran subfamily Bruchinae are important seed-feeders, capable of damaging edible leguminous seeds and seed stocks used in reforestation programs (Hegazy and Eesa, 1991; Southgate, 1979; Venkataramana et al., 2016). Immature stages live inside seeds that have been excavated by the feeding larvae, while adults live free and feed on pollen and nectar (Southgate, 1979). Usually, seed-feeding, mining and galling insects experience higher levels of competition, since their mobility is constrained by their life-history and resource traits (Denno et al., 1995).

*Merobruchus terani* (Kingsolver, 1980) (Chrysomelidae: Bruchinae) and *Stator maculatopygus* (Pic, 1930) (Chrysomelidae: Bruchinae) are bruchine species that consume seeds of a climbing acacia species, *Senegalia tenuifolia* (L.) Britton & Rose, 1928 (Fabaceae: Mimosoideae), and show similar seed-predation behavior, although *M. terani* has a larger body and occurs in higher

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abundances than *S. maculatopygus* (Tuller et al., 2015). Both larval and pupal stages live inside the seeds, consuming the seeds as they develop (Kingsolver and John, 2004; Johnson and Romero, 2004; Tuller et al., 2015). In terms of resource limitation, we observed that only one seed was sufficient for the immature stages to develop, and two considerations arose: the larvae had no access to other seeds when consuming the original one, and a laboratory experiment had shown that *M. terani* can complete all stages of life inside a single seed (Tuller et al., 2015; Maia et al., 2017). Indeed, these two beetle species are found attacking the same fruit, but not the same seed (Maia et al., 2017), which suggests that they are not competing directly, i.e., interference competition, but rather are competing indirectly, i.e., exploitative competition. Finally, a significant difference between the species in consuming seeds is related to the time when the females lay their eggs. *M. terani* arrives first, while *S. maculatopygus* reaches the fruit just before the pods start to open and after the seeds are released (Johnson and Romero, 2004; Tuller et al., 2015).

These aspects of the biology of the two bruchine species make them an interesting model to better understand the effects of pod infestation (here used as an estimate of indirect competition) on morphological traits and allometry. We examined how competition, through pod infestation, affects the individual morphological traits of *M. terani* and *S. maculatopygus*. We hypothesized that *M. terani*, as a superior competitor, would have a negative impact on the morphological traits of *S. maculatopygus*, and the greater the degree of infestation, the greater the impact would be. Specifically, we measured morphological traits including the pronotum, both elytra, and body size, and evaluated variations between species, males and females, and infestation categories. Finally, we examined the allometric relationships of these morphological traits with respect to the degree of infestation of the pod.

## Methods

### Field samples and beetle measurement

We sampled once a month in July and August 2014 (the ripening period) to collect fruits of *S. tenuifolia*. As *S. tenuifolia* is a liana species, the identification of an individual plant, from its roots to its fruits, is difficult because each plant spreads over other plants, making it difficult to distinguish the number of individuals at a site. We therefore opted to collect the fruits casually through the subareas. We collected 25 fruits per subarea in each sampling event, totaling 350 fruits. The 7 subareas were at least 400 m distant from each other, and located in two municipalities (Lavras and Luminárias) in southern Minas Gerais, Brazil: Aero-2 (S 21°14'5.71" W 044°57'8.66"), Aero-3 (S 21°14'78.7" W 044°58'006"), Lav-1 (S 21°18'3.46" W 044°58'0.53"), Lumi-1 (S 21°31'1.36" W 044°53'1.78"), Lumi-2 (S 21°31'5.13" W 044°52'6.32"), Lumi-3 (S 21°31'5.31" W 044°52'3.84") and Lumi-4 (S 21°31'5.13" W 044°51'40.80").

We stored the pods in labeled paper bags and brought them to the laboratory, where we placed each pod in an individual PVC tube, sealing the ends with voile attached with rubber bands, to allow air exchange. We left the collected pods on a workbench in the same temperature and photoperiod (12:12 h). After three months of storage, we opened the fruits, identified the attacked seeds by species, sex, and larval consumption traits (adults of both species leave a typical hole when emerging from the seeds, while other species that consume the seeds leave no definite visual pattern). By selecting pods containing only attacked seeds with a hole, we ensured that only bruchine species were present, and we could estimate the degree of bruchine infestation as an indirect estimate of competition between species. Further, all attacked seeds held only a single

beetle, and we commonly found both species occupying the same fruit, but in different seeds (Maia et al., 2017). The bruchines were stored in 1.5-mL labeled plastic microtubes containing 70% ethanol. To measure the dry weight, hereafter beetle body weight, of both beetle species, adults emerged or un-emerged from seeds collected in the field (we dissected the un-emerged individuals from inside the seeds) were placed in individual paper bags, dried at 40 °C for 48 h, and weighed using a precision analytical balance (Shimadzu AY220).

In order to estimate the effects of pod infestation on body size, we selected three morphological traits that best explained the body size variation in some species of bruchine beetles (Colgóni and Vamosi, 2006): pronotum length, left elytron length, and right elytron length. These measurements were taken with individuals in dorsal view, using a Leica M205A stereomicroscope equipped with a Leica DFC295 digital camera. Since all the beetles found belonged to the subfamily Bruchinae, we sexed them by examining the width of the last abdominal segment. We deposited voucher specimens in the Entomological Collection of the Laboratory of Ecology and Complexity at the Federal University of Lavras, Minas Gerais, Brazil.

### Statistical analysis

To perform the statistical analyses and isolate the influence of non-bruchid competitors, we considered only pods that were infested exclusively by bruchines (please see Tuller et al., 2015 for more details about the food web). Laboratory measurements after the sampling show lower species abundances than the numbers of seeds predated per pod. That is because some individuals had already emerged from the seeds before the samples were taken. Since all attacked seeds held only a single beetle and we commonly found both species occupying the same fruit, but in different seeds, we assessed the competition as the degree of infestation, i.e. the number of predated seeds in relation to the number of seeds in the pod. We defined three infestation categories: (G1) low, from 0 to 0.30; (G2) medium, from 0.31 to 0.60; and (G3) high, from 0.61 to 0.90. No infestation level above 91% was found.

We evaluated the variation in morphometric traits (left elytron, right elytron, and pronotum) between infestation categories and between males and females. We used general mixed models with a log-normal distribution, and sampling date as a random factor, to reduce the effects of sampling and repeated measures. We also evaluated allometric relationships, estimating the variation rate of the pronotum, left elytron, and right elytron in relation to the individual body weight. In this analysis, we used major axis regressions (i.e., geometric mean), which are considered more appropriate when both  $x$  and  $y$  variables are measured with error (Sokal and Rohlf, 1995). The values of slopes and confidence intervals from the analyses were used in a single plot to illustrate the allometric relationships between species and between infestation categories. All analyses were performed using R 2.15.3 (R Development Core Team, 2013).

## Results

We evaluated 152 individuals of *M. terani*: 38 in G1, 81 in G2 and 33 in G3, totaling 78 females and 74 males. The body weight ranged from 0.4 mg to 3.8 mg, pronotum from 0.56 mm to 1.70 mm, left elytron from 1.45 mm to 2.65 mm, and right elytron from 0.96 mm to 2.60 mm. We also evaluated 24 individuals of *S. maculatopygus*: 18 in G1 and 6 in G2, totaling 5 females and 19 males. We did not find individuals of *S. maculatopygus* in infestation category G3. The body weight ranged from 0.4 mg to 2 mg, pronotum from 0.53 mm to 0.86 mm, left elytron from 1.39 mm to 1.87 mm, and right elytron from 1.39 mm to 1.84 mm. We found high correlations between the

**Table 1**

Female and male species abundance, the abundance of each species for each infestation category, and estimates of body weight and morphological traits.

	<i>Merobruchus terani</i>	<i>Stator maculatopygus</i>
Female abundance	78	5
Male abundance	74	19
G1 abundance	38	18
G2 abundance	81	6
G3 abundance	33	0
Body weight (min–max, mg)	0.4–3.8	0.4–2
Pronotum (min–max, mm)	0.56–1.70	0.53–86
Left elytron (min–max, mm)	1.45–2.65	1.39–1.87
Right elytron (min–max, mm)	1.40–2.60	1.39–1.84

G1, G2 and G3 abundance, abundance for each category of pod infestation; G1, low infestation (0–0.30% of attacked seeds); G2, medium infestation (0.31–0.60% of attacked seeds); G3, high infestation (0.61–0.90% of attacked seeds); min–max, minimum and maximum value.

left and right elytra (Spearman correlation of 0.97 for *M. terani* and 0.98 for *S. maculatopygus*). Thus, below we discuss only the results for the left elytron (Table 1).

We found a significant variation between *M. terani* and *S. maculatopygus* for all morphometric traits. However, we did not observe a significant variation between males and females for either species (Fig. 1). The body weight of *M. terani* was significantly larger in the higher-infestation categories ( $T = -3.47$ , D.F. = 170,  $p < 0.01$ ). The estimated difference was  $0.27 \text{ mg} \pm 0.07 \text{ mg}$  between G1 and G3. We did not find significant values for the pronotum and elytron. We did not observe significant variations in morphometric traits of *S. maculatopygus* between infestation categories (Fig. 2).

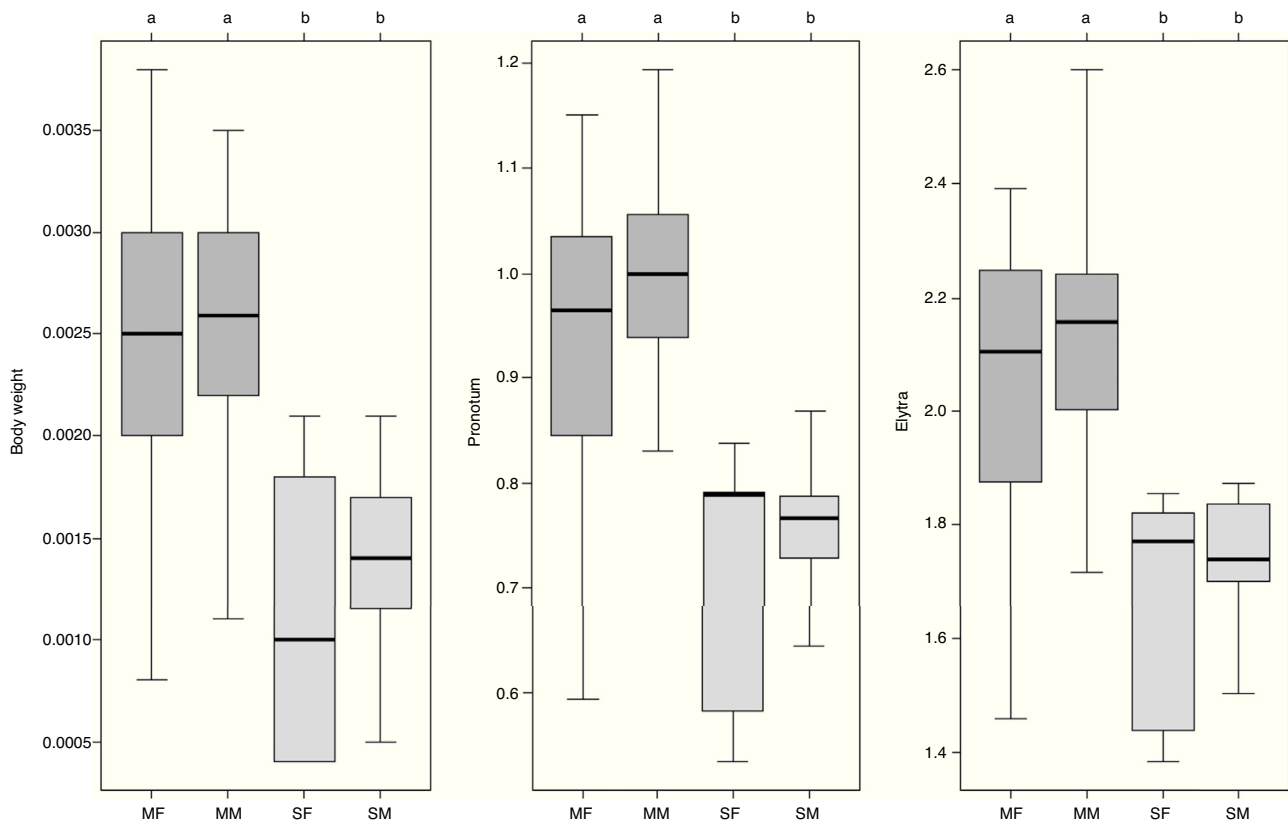
Regarding allometric relationships, our results showed negative allometry for all relationships investigated. The slope of

variation was 0.257 in G1, 0.258 in G2, and 0.541 in G3 between the pronotum and body weight (pronotum allometry) in *M. terani* individuals. However, we observed confidence intervals ranging to zero in G3, indicating a lack of relationship between these morphometric traits. We found a similar pattern for the pronotum allometry of *S. maculatopygus*. However, apart from the lack of individuals in G3, we found in G2 confidence intervals ranging to zero (Fig. 3). The results for allometric relationships between the elytron and body weight (elytron allometry) were identical to the results for pronotum allometry. We observed slopes of variation of 0.19, 0.15, and 0.40 in G1, G2, and G3 respectively for *M. terani*; and 0.09 in both G1 and G2 for *S. maculatopygus*. Again, we observed confidence intervals ranging to zero in the higher infestation categories (Fig. 3).

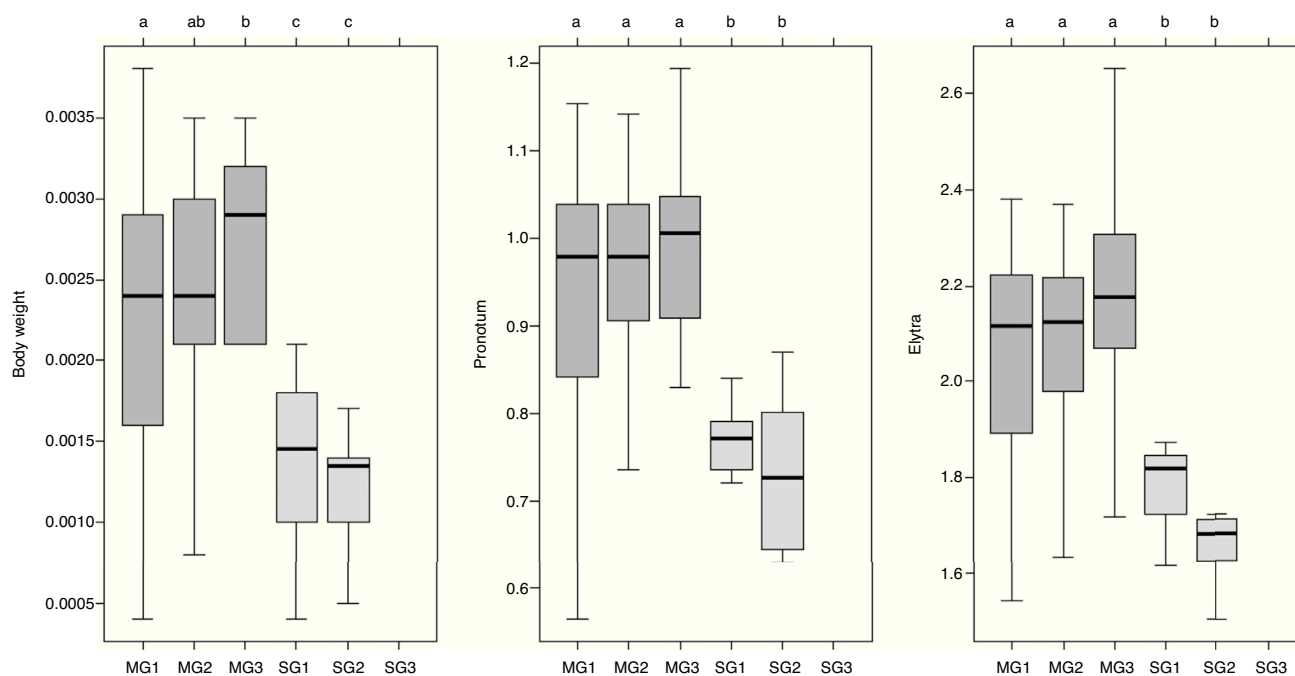
## Discussion

We have demonstrated some consequences from pod infestation for the morphological traits, sexes and their allometric relationships, of the seed-feeding beetles *M. terani* and *S. maculatopygus*. Males and females of both species showed similar morphological traits. For *M. terani*, the higher was the infestation level, the larger the body parts and body weight. *S. maculatopygus* showed the opposite trend, i.e., the higher the infestation level, the smaller the body parts and body weight. Last, a negative allometry pattern was estimated for both species regardless of the level of infestation, although increases in the infestation level resulted in significant variation in body size and morphological traits.

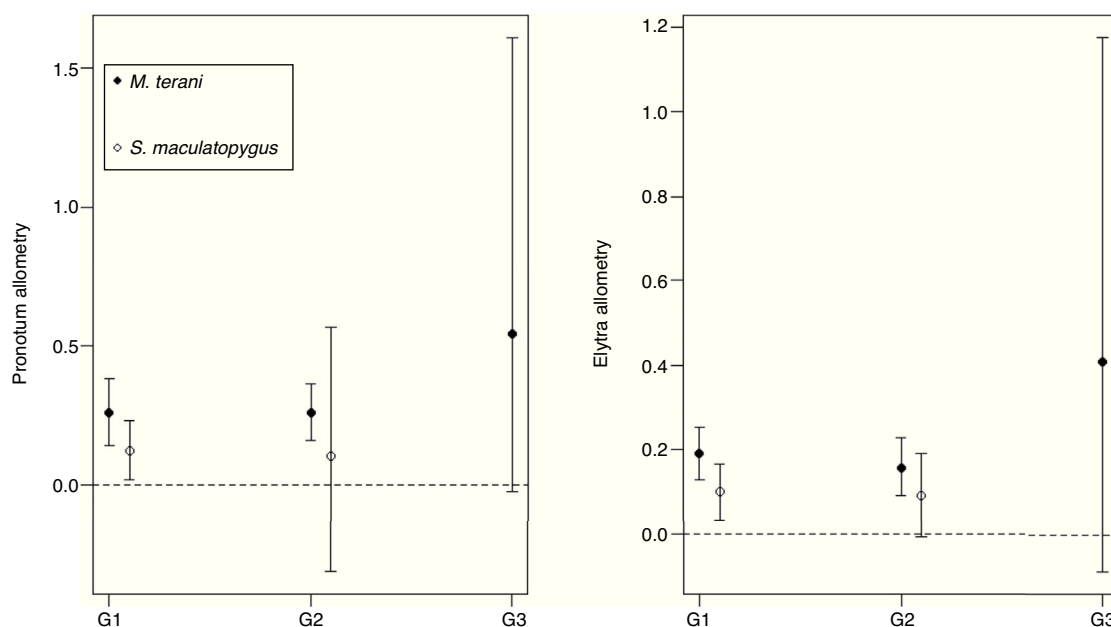
Although males are larger than females, we found no statistically significant sexual dimorphisms for morphometric traits (e.g., pronotum and elytron) in either species. Sexual size dimorphism reflects different male and female adaptations (Fairbairn,



**Fig. 1.** Variations in body weight, pronotum and elytron length between *Merobruchus terani* and *Stator maculatopygus* and for males and females. The analyses used a linear mixed model with a log-normal distribution and Tukey's pairwise comparison. MF, *M. terani* females; MM, *M. terani* males; SF, *S. maculatopygus* females; SM, *S. maculatopygus* males.



**Fig. 2.** Variations in body weight, pronotum and elytron length for *Merobruchus terani* and *Sator maculatopygus* in each infestation category. The analyses used a linear mixed model with a log-normal distribution and Tukey pairwise comparison. M, *M. terani*; S, *S. maculatopygus*. G1, low infestation (0–0.30% of attacked seeds); G2, medium infestation (0.31–0.60% of attacked seeds); G3, high infestation (0.61–0.90% of attacked seeds).



**Fig. 3.** Negative allometry depicted by the slopes and their confidence intervals for the pronotum and elytra allometry (pronotum length and elytra length in relation to body weight) between infestation categories for both bruchine species. G1, low infestation (0–0.30% of attacked seeds); G2, medium infestation (0.31–0.60% of attacked seeds); G3, high infestation (0.61–0.90% of attacked seeds).

1997). It is a common pattern for females to be larger than males; larger females are able to produce and lay more eggs, which results in higher fecundity (Honek, 1993). However, sexual dimorphism is contingent on environmental and biological conditions. Environmental conditions such as the host's defense mechanisms, temperature, size of the host seed, and food shortages affect the larval development and promote the development of adaptive strategies, such as reduction in body size (Carroll and Hoyt, 1986; Center and Johnson, 1974; Honek, 1993). On the other hand, females may be smaller than males in response to biological

factors, such as male–male competition for sexual selection of females (Fox et al., 2003; Savalli and Fox, 1998).

Tuller et al. (2015) found that *M. terani* was five times more abundant than *S. maculatopygus* in pods of *S. tenuifolia*, and proposed that *M. terani* is competitively dominant. Maia et al. (2017) observed dominant species with a positive relationship to their resource quantity, whereas *S. maculatopygus* did not show such a relationship. Our results concord with these findings. The higher body weight and the morphometric traits of *M. terani* could provide a competitive advantage and greater access to the food resource;

while the increase of body weight with infestation levels indicates that *M. terani* may not be limited by competitive intra- and interspecific interaction. On the other hand, *S. maculatomygus* may be limited by interspecific competition, since this species was not observed at the highest level of pod infestation and its body parts and weight became smaller. Therefore, *S. maculatomygus* may reduce competition by avoiding pods with high levels of infestation, or even fail to survive in this condition. Bruchine beetles have modified developmental strategies, such as altered feeding and pupating behaviors, and reduction in body size (Center and Johnson, 1974). Additionally, they show temporal differentiation in oviposition and host specialization (Center and Johnson, 1974). These behavioral responses to selective pressures act to reduce the effect of competitive pressures between species, such as the effects on development time, weight and probability of emergence (Fox et al., 2003; Messina, 1991).

In order to understand the problem of how body parts vary with total body weight or other parts in respect to pod infestation, we employed the allometric approach. We chose the morphological traits that best capture the effects of body variation (elytron width and length and pronotum length) as representative measurements, commonly used to investigate the development of insects and their morphological characteristics (Colgoni and Vamosi, 2006; Fox et al., 2003). The general outcomes demonstrate that the negative allometry, maintained through the different infestation levels, suggests that increasing body weight did not increase proportionally with the body structures. However, the highest level of infestation experienced by *M. terani* increased the relationship (slope value is larger), increasing the size of morphological structures with respect to body weight. On the other hand, we showed that increasing the degree of pod infestation produced a wider variability in the individuals' body size, making the populations of both species more heterogeneous in terms of morphological traits. Further, the stress caused by the infestation levels may increase the instability of the development of the individuals, affecting the relationship among body parts.

In conclusion, we tested the hypothesis that *M. terani* is a superior competitor, negatively affecting *S. maculatomygus*; and an increase in the degree of pod infestation augmented its impact. In general, we corroborated the hypothesis even though the statistical analyses did not support it. *M. terani* seems not to have been affected by intraspecific and interspecific competition in our system, while *S. maculatomygus* is impacted by interspecific competition, decreasing its morphological traits and body size. We also observed that the allometry approach applied to infer the effects of competition on these species did not show consistent patterns, despite the increases in the size heterogeneity of individuals of the populations of both species, in some levels of infestation. Finally, we demonstrated that interspecific competition affects these species differently, although they play similar roles in resource exploitation.

### Conflicts of interest

The authors declare no conflicts of interest.

### Acknowledgments

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