Seed predation food web, nutrient availability, and impact on the seed germination of *Senegalia tenuifolia* (Fabaceae)

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Abstract: Food web studies, which include qualitative and quantitative information about species interactions for all trophic levels in a specific community, are important tools to characterize the community structure, and to understand the role of species interaction in natural communities. With this aim, we collected fruits of *Senegalia tenuifolia* from a total of nine sites from three different areas in Minas Gerais, from June to August 2011. Fruits were collected and taken to the laboratory in special containers, to allow insect development and description. And then, we characterized the food web of insects associated with these seeds, and described interactions between these organisms and the host plant, and assessed the impact of insect attacks on seed germination with an experiment under controlled conditions. Additionally, we estimated the available NPK content and the Carbon:Nitrogen ratio in both fruits and seeds. In *S. tenuifolia* seeds, we have found 937 associated insects of 15 species structured in four trophic levels. We found two Bruchinae seed-feeders species: *Merobruchus terani* and *Stator maculatopygus*. We also have observed the seed-feeding hymenopteran species *Allorhogas* sp. 1 and *Prodecatoma* sp. 1, and hymenopteran parasitoids species such as *Eupelmus* sp.1, *Lyrcus* sp.1, *Eurytoma* sp.1, *Horismenus* sp.1, *Chelonus* sp.1, *Aprostocetus* sp.1, *Eiphosoma* sp.1 and an unidentified Pteromalinae. Considering quantitative food web metrics, our data have suggested low values of generality and high values of trophic vulnerability, besides others, which demonstrated how species interactions are specialized. The impact of seed-feeding insects on seeds was found severe and prevented their germination. Lastly, seeds contained higher NPK content than fruits. In conclusion, the seed predation food web supported by *S. tenuifolia* has depicted two different features: specialized species interactions, considering parasitoids and seed-feeding species, and, generalized species interactions, considering host-plant and seed-feeding species. Indeed, seeds are resources with high nutrient concentration supporting a rich and complex food web. Finally, *S. tenuifolia* seeds are directly impacted by seed-feeding insects, preventing them from germination, which could result in a negative effect on host-plant population. Rev. Biol. Trop. 63 (4): 1149-1159. Epub 2015 December 01.

Key words: Bruchinae, seed-feeders, host-parasitoids, insect-plant interactions, quantitative food web.

Food web studies, which focus mainly on trophic interactions, are important tools for characterizing community structure, understanding the role of species interaction and determining the influence of these interactions on observed patterns and dynamic properties of natural communities (Paine, 1992; Hall & Raffaelli, 1991). A complete food web study should include qualitative and quantitative information about species interactions for all trophic levels in a specific community (Lewis et al., 2002). However, the effort to explore a complete food web is daunting, especially for tropical ecosystems, and an alternative approach is to focus on community subsets (Huxham, Raffaelli, & Pike, 1995; Lewis et al., 2002). Quantitative analyses are important to distinguish important and frequent species associations from those that are casual and rare, which is not possible when considering only

qualitative data. In this light, host-parasitoid interactions and guilds of herbivorous insect species have been shown to be good models, since the host-parasitoid interactions are strictly quantitative – i.e., the interactions can be expressed in the same units (Memmott & Godfray, 1994; Lewis et al., 2002).

The insects associated with fruits and seeds are interesting systems to study host-parasitoid interactions, because these systems are rich and easily sampled-seed-feeders and their parasitoids are quite static, and live within seeds throughout their larval phase. In this way, we studied the interactions among fruits and seeds of the plant *Senegalia tenuifolia* (L.) Britton & Rose (Fabaceae: Mimosoideae) and its associated insects.

Senegalia tenuifolia is locally known as *unha-de-gato*, and it is a pioneer species broadly distributed in South America (Queiroz, 2009). Other names that have been used for this species are *Acacia tenuifolia* (L.) Willd, *Mimosa tenuifolia* L., and *Acacia paniculata* Duss. (tropicos.org, 2011), and has been characterized as a liana (Brandes & Barros, 2008) or as a shrub (Lima, Mansano, & Araújo, 2012). However, the best characterization is a scandent shrub, i.e., a shrub that is able to become a liana and reach a greater height (Silva, Santos, & Fernandes-Bulhão, 2007). *Senegalia tenuifolia* flowering occurs from November to January, and the fruit ripening occurs from January to August. After this phase, fruits open and seeds begin to fall on the ground.

Seeds are one of the most nutritive plant parts, because they determine the initial seedling success (Woodwell, Whittaker, & Houghton, 1975), and, consequently seed-feeding organisms are common. Bruchinae is a beetle subfamily which feed on a single seed during their entire larval phase (Southgate, 1979). These seed-feeding interactions may directly affect plant reproduction, influencing seed fecundity and viability, and seedling establishment (Fox, Wallin, Bush, Czesak, & Messina, 2012). Therefore, studies on interactions between seed predators and plants are essential to the development of a better understanding

of community dynamics in natural habitats. Interactions between seed predators and plants may influence recruitment and, as a result, plant population dynamics (Campbell & Clarke, 2006).

Sometimes herbivore impacts are not strongly affecting the host plant, when species at higher trophic levels, such as parasitoids, control herbivore populations (Stiling & Rossi, 1997). Nevertheless, studies commonly ignore parasite and parasitoid species due to methodological and taxonomic difficulties in documenting their interactions (Huxham et al., 1995), or even due to the low abundance of these organisms. Hence, studies on parasites and parasitoids are mostly common in systems with economic importance (Schmale, Wäckers, Cardona, & Dorn, 2002; Bukovinszky, Van Veen, Jongema, & Dicke, 2008). As a result, most species associated with plants that are not economically important remain unknown to science. Assuming the source food web supported by *S. tenuifolia* and its associated insects, our goals were to**:** (*i*) determine the abundance, assign the observed insect species to trophic levels and describe the quantitative food web metrics, (*ii*) estimate NPK content available and the Carbon:Nitrogen ratio in the fruits and seeds, and (*iii*) evaluate seed-feeding insect impacts on seed germination.

MATERIALS AND METHODS

Study area: We carried out our study in Lavras and Luminárias municipalities, Minas Gerais state, Southeastern Brazil (Fig. 1 SM-Supplementary material). We selected three areas in the Southern region of the state where *S. tenuifolia* occurs: Ae, La, and Lu. Within these areas, we established nine sites, or subareas, at least 400 m away from each other, from where we collected *S. tenuifolia* fruits: Ae-1 (21°14'4.57'' S - 44°57'6.38'' W), Ae-2 (21°14'5.71'' S - 44°57'8.66'' W), Ae-3 (21°14'7.87'' S - 44°58'0.06'' W), La-1 (21°18'3.46'' S - 44°58'0.53'' W), La-2 (21°19'4.85'' S - 44°57'8.66'' W), Lu-1 (21°31'1.36'' S - 44°53'1.78'' W), Lu-2
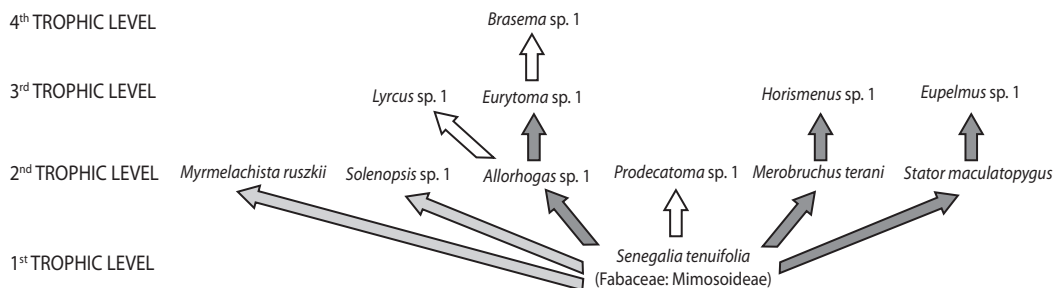
Fig. 1. The food web of insects associated with *Senegalia tenuifolia* fruits and their confirmed and potential trophic interactions. Insects with uncertain relationships were excluded. Black arrows represent confirmed relationships, white arrows represent potential relationships according to the literature and gray arrows represent relationships without consumption.

(21°31'5.13'' S - 44°52'6.32'' W), Lu-3 (21°31'5.31'' S - 44°52'3.84'' W), and Lu-4 (21°31'26.90'' S - 44°51'40.80'' W).

These nine subareas were located at an average altitude of 900 m.a.s.l. The predominant climate in the region is highland tropical, characterized by mild summers and dry winters, the average annual temperature is 19.4°C and the average annual rainfall is 1 530 mm (Sparovek, VanLier, & Dourado-Neto, 2007). The original vegetation in the region is characterized as a seasonal forest and belongs to the Atlantic Forest domain (Veloso, Rangel-Filho, & Lima, 1991) in transition with the Cerrado domain. The vegetation has been severely fragmented by human activities, mainly related to coffee plantations and other crops, as well as pastures. In this fragmented landscape, the studied plants can be frequently found at forest edges.

Fruits, seeds, nutrients and associated insects: From June to August 2011, we sampled eight of the subareas established for the present study (subarea Lu-4 was not included this year) three times, collecting fruits randomly from *S. tenuifolia*. In each subarea, we collected 25 fruits per sampling event, summing up 75 fruits per subarea and 600 fruits in the total. Next, we stored fruits in paper bags identified by subarea and date and took them to the laboratory for subsequent processing.

In the laboratory, we individually stored each fruit in a labeled PVC tube, sealed with voile attached with rubber bands, allowing air exchange between the tube and the laboratory. This storage allowed the complete development of insects contained in the fruits, and promoted their later emergence without loses.

After three months of storage, we analyzed fruits. First, we recorded all Bruchinae egg signal located on the surface of collected *S. tenuifolia* pods to determine egg abundances. It was not possible to identify Bruchinae eggs to species, so we recorded all eggs (without Bruchinae species identification). Next, we classified seeds as aborted (small and black), attacked (with a hole or consumed/deformed by the seed-feeding Hymenoptera, *Allorhogas* sp.), or apparently healthy (without any attack sign), and counted them. Based on these values, we calculated the proportion of seeds attacked (seed consumption rate).

We stored all insects found in *S. tenuifolia* fruits and seeds in 1.5 mL labeled plastic microtubes, containing 70 % alcohol. Next, we morphotyped and sent them to specialists for identification. During larval phase, Bruchinae mouthpart larvae, constituted by keratin, is not absorbed by the adult beetles, parasitoid species are not also able to absorb them when parasitizing its hosts, so these materials help to identify species-specific host-parasitoid relationships. We determined these relationships between Bruchinae and parasitoids based on

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the comparison of Bruchinae larvae remaining mouthparts between those that successfully developed and those that were parasitized. We also used literature data to help us determining these relationships (Janzen, 1969; Southgate, 1979; Johnson & Siemens, 1997; Graham, 1987; Macêdo & Monteiro, 1989; Hanson & Gauld, 1995; Wharton, Marsh, & Sharkey, 1997; Marsh, Macêdo, & Pimental, 2000; Richard , Fabre, & Dejean , 2001; Longino, 2003, 2006; Montoya, Rodríguez, & Hawkins,, 2003; Perioto & Lara, 2004; Perry, Mondor, & Addicott, 2004; Morse & Farrel, 2005; Sari & Ribeiro-Costa, 2005; Askew & Nieves-Aldrey, 2006; Östergård, Hambäck, & Ehrlén, 2007; Triana & Ravelo, 2007; Bonet, 2008; Chavarría, Hanson, Marsh, & Shaw, 2009; Rosumek et al., 2009; Perry et al., 2004; Fusu, 2009; Lanan, Dornhaus, & Bronstein, 2011; Rossi, Rodrigues, Ishino, & Kestring, 2011; Pringle, 2014).

In order to estimate nutrient availability and C:N balance of *S. tenuifolia* seeds and fruits we grounded fruits and seeds and sent them to the Laboratory of Leaf Analysis at the Federal University of Lavras for chemical analysis (NPK-nitrogen, phosphorus, and potassium). For each of the eight subareas, we classified analysis of chemical elements according to five categories: infested fruits (IF), uninfested fruits (UF), unattacked seeds of infested fruits (USIF), attacked seeds of infested fruits (ASIF), and unattacked seeds of uninfested fruits (USUF). It was not possible to analyze fruit and seed categories for each sampling event alone due to the reduced *S. tenuifolia* seed biomass. Lastly, we have estimated carbon:nitrogen ratio (C:N) for all fruit and seed categories (for more details please see Supplementary Material).

Seed germination test: In August 2012, in order to carry out germination tests, we collected 25 *S. tenuifolia* fruits in each subarea, except for La-1 and Ae-1. We excluded these subareas from germination tests due to fires that occurred in September 2011 which affected these sites. Next, we stored fruits in paper bags identified by subarea and took them to the laboratory for the tests.

In the laboratory, we have set three seed germination treatments with 15 seeds (N= 15) each treatment and replicated each treatment three times, totalizing 135 seeds. Treatments were categorized as: unattacked seeds of infested fruits (USIF), attacked seeds of infested fruits (ASIF), and unattacked seeds of uninfested fruits (USUF). We have selected randomly 45 seeds of each category and placed each seed on an individual 9 cm diameter Petri dish, having as the substrate filter paper soaked with distilled water and sealed the dishes with PVC wrap. Next, we randomly placed the petri dishes with seeds of different treatments into B.O.D. incubator chamber with photophase 12:12 h and constant temperature at 25 ºC. Over a period of 15 days, we daily monitored germination and considered germinated seeds those that developed approximately 2 mm of root (Rehman, Harris, Bourne, & Wilkin, 1996).

For data analysis, we built two generalized linear models (GLM) to compare insect species abundances belonging to second (seed-feeders) and third (primary parasitoids) trophic levels separately. We included in models only species presented in Fig. 1. In the first model, total abundance of each seed-feeding species was the response variable and seed-feeding species identity was explanatory variable. The second model was similar, but made for primary parasitoids. We also have built four GLM to compare chemical element contents and C:N ratio between fruit and seed categories and one GLM to compare germination rates between the three categories (for more details please see supplementary material). In each model, we used the most suitable distribution, according to the data type: Gaussian distribution for continuous, Poisson for count and Binomial for percentage data (McCullagh & Nelder, 1989). In addition, we made a contrast analysis to test differences between insect species abundance and seed categories.

Lastly, we have calculated basic quantitative food web metrics –please see Jordán and Scheuring (2004)– to our source food

web supported by *S. tenuifolia*. The species included in the analysis are represented in the Fig. 1. It is interesting to observe that three rare hymenopteran species were excluded since we were not sure about their trophic position and links. Quantitative food web analyses were performed using R package *cheddar* (Hudson, 2014). All analyses were performed in the software R 2.14 (R Development Core Team, 2011).

RESULTS

We assessed a total of 598 fruits and 4 352 seeds of *S. tenuifolia*. Fruits had a maximum of 14 locust seeds (8.13 ± 2.30) and 14 seeds (7.29 ± 2.64). Among all seeds assessed, 23.7 % had been attacked, 7.8 % aborted, and 68.5 % were apparently healthy. Hence, 25.7 % of seeds that completed their development, i.e., non-aborted seeds, had been attacked.

Insects associated with seeds and their interactions: In 598 *S. tenuifolia* fruits, we have found 937 associated insects of 15 species. Among these, we found two coleopteran species (708 individuals) and 13 hymenopteran species (229 individuals) (Table 1 Supplementary Material), which included two ant species (43 individuals). We found the ants *Myrmelachista ruszkii* (Forel, 1903) and *Crematogaster* sp.1, which occurred in nine fruits and had summed abundances of 34 and seven individuals, respectively.

Apart from seed-feeding adults, we counted 2 192 Bruchinae eggs on fruits and found that egg abundance increased as the host plant reproductive season advanced (Table 1). Among the three most abundant seed-feeding insects, *Merobruchus terani* (Kingsolver, 1980) (Chrysomelidae: Bruchinae) was most abundant (P<0.001, F=17.0) followed by *Stator maculatopygus* (Chrysomelidae: Bruchinae) (Pic, 1930) and *Allorhogas* sp. 1 (Braconidae), which had similar abundances. Furthermore, it is interesting to notice that two Bruchinae species represented more than 90% of the total seed-feeding insect abundance, and that among parasitoids, two hymenopteran species corresponded to 85% of the total abundance of this guild. In addition, parasitoid species abundances from lowest to highest were: *Eupelmus* sp. 1 (Eupelmidae) = *Lyrcus* sp. 1 (Pteromalidae) < *Eurytoma* sp. 1 (Eurytomidae) < *Horismenus* sp. 1 (Eulophidae) (P<0.001, F=24.0). In addition, in comparison to seed predators, parasitoids were rare in the system and showed low total and relative abundance (Table 1 Supplementary Material).

A summary of each species feeding habits is presented in Table 1 of the Supplementary material. Based on the feeding habits listed in Table 1SM, we constructed a diagram with probable feeding interactions of insects associated with *S. tenuifolia* fruits (Fig. 1). Species with uncertain relationships were not included in Fig. 1. Considering the food web depicted at

TABLE 1

Abundance of eggs of seed-feeding Bruchinae laid on pods of *Senegalia tenuifolia*
in the region of Lavras, Southeastern Brazil

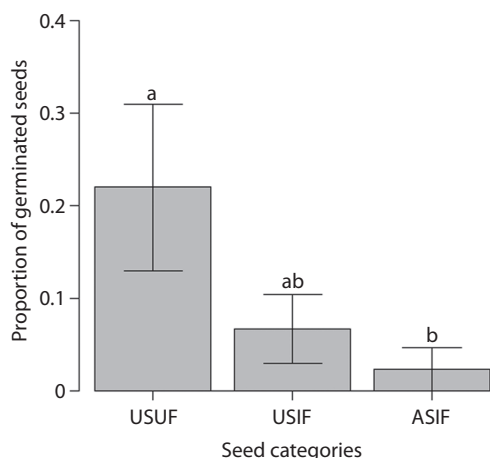| Subarea	Sampling			Total
	June 2011	July 2011	August 2011	
La-1	115	40	42	197
La-2	49	74	60	183
Ae-1	121	80	103	304
Ae-2	64	88	111	263
Ae-3	142	204	262	608
Lu-1	88	93	101	282
Lu-2	10	23	49	82
Lu-3	51	60	162	273
Total	640	662	890	2 192

Fig. 2. Proportion of different categories of *Senegalia tenuifolia* germinated seeds in the region of Lavras, Southeastern Brazil. USUF = unattacked seed of an uninfested fruit, USIF = unattacked seed of an infested fruit, and ASIF = attacked seed of an infested fruit. Different letters represent statistically distinct categories.

Fig. 1, quantitative food web metrics were estimated as follow: 12 species or nodes *(S)*, linkage density of 0.92 (*LS*), 0.08 of connectance (*C*), proportion of basal, intermediate and top species of 0.08, 0.33 and 0.58 respectively, 2.20 of predator-prey ratio, 1.00 and 2.20 of trophic generality and trophic vulnerability respectively, and lastly, an average chain length of 0.76.

Nutrient availability and C:N balance of *Senegalia tenuifolia* seeds and fruits: As general outcomes, chemical analyses have suggested seeds had higher nutrient contents than fruits. Both infested fruits and attacked seeds tended to have a higher P and K contents than uninfested fruits and unattacked seeds. In respect to C:N ratio, fruits depicted eight times higher ratio than seeds, however, when considering both fruit categories and three seed categories the C:N ratio were similar. Further, despite the C:N ratio importance to live organisms, it did not influence the associated insects richness. Nutrients availability and C:N balance have suggested no connection to any of the seed predator data so we have decided to include the details as supplementary material, including statistical test and figures.

Germination test of *Senegalia tenuifolia* seeds: Of the 135 seeds of *S. tenuifolia* that we tested, 15 germinated (11.11%). Among these, ten belonged to the seed category USUF, four to USIF, and only one to ASIF. Seed germination rates were ranked as follows: USUF > USIF > ASIF. However, the germination rate differed significantly only between USUF and ASIF seed categories (P=0.043, F=6.6, Fig. 2). Despite this, we observed lower germination rate in both seed categories that came from infested fruits (USIF and ASIF) compared to seeds of uninfested fruits (Fig. 2). Therefore, *S. tenuifolia* seed consumption by insects has a direct negative effect on seed germination through ASIF seeds.

DISCUSSION

In the studied food web, associated with *S. tenuifolia* seeds, we found 15 different insect species, spanning three trophic levels: seed-predating insects and their primary and secondary parasitoids. This food web is a relatively complex system composed of three compartmentalized subwebs involving two Bruchinae species and *Allorhogas* sp. 1, and their parasitoids. The attacks by seed-predating insects were influenced by chemical seed and fruit characteristics, at the same time seeds had lower C:N ratio than fruits. Further, seed-feeding insects of the Bruchinae subfamily had a negative effect on *S. tenuifolia* seed germination.

Among all food web species, seed-predating Bruchinae were the two most abundant species, with *Merobruchus terani* being eight times more abundant than *Stator maculatopygus*. Seed-predating Bruchinae, such as those found in the present study, spend their entire larval phase feeding within seeds (Southgate, 1979) and are very common in the Fabaceae family (Janzen, 1969; Southgate, 1979; Östergård et al., 2007; Rossi et al., 2011). The beetles *M. terani* and *S. maculatopygus* attack seeds throughout the reproductive season, and we observed the highest Bruchinae egg abundances at the end of *Senegalia tenuifolia* reproductive season. *Merobruchus terani*

attacks fruits while they are still attached to the mother-plant, and only one Bruchinae individual develops inside each seed (Johnson & Siemens, 1997). *Merobruchus terani* is considered a specialist on *Acacia* (Johnson & Siemens, 1997). *Stator maculatopygus* has previously been reported consuming *Senegalia bonariensis* (Gillies ex Hook. & Arn.) Seigler & Ebinger seeds, and was described as having the habit of ovipositing directly into dispersed fruits – i.e., secondary predation (Morse & Farrel, 2005). However, we found all the *S. maculatopygus* individuals in *S. tenuifolia* pre-dispersed fruits.

We also observed a braconid, *Allorhogas* sp. 1 as a *S. tenuifolia* seed predator. According to the literature, this genus is exclusively composed of seed-predating and gall-inducing insects (Marsh et al., 2000; Chavarría et al., 2009). In *S. tenuifolia*, *Allorhogas* sp.1 feeds from the extremities, causing deformities in developing seeds. Each seed may contain one or several individuals, similar to *Allorhogas* species observed by Macêdo and Monteiro (1989). We also found a *Prodecatoma* (Braconidae) individual, species of this genus have been found in fruits and seeds of several families, among them Fabaceae (Perioto & Lara, 2004).

Among primary parasitoids, the most abundant was *Horismenus* sp.1 (Eulophidae), which was identified as a Bruchinae *Merobruchus terani* parasitoid. In the present study, we found one *Eupelmus* species, which is a Bruchinae *Stator maculatopygus* parasitoid. According to the literature, *Eupelmus* are coleopteran larvae and pupae ectoparasitoids (Fusu, 2009) and may also be Bruchinae parasitoids (Sari & Ribeiro-Costa, 2005; Bonet, 2008). One Pteromalinae species that occurred only once into the collected fruit and was not identified may be a Bruchinae larvae parasitoid or even a hyperparasitoid (Hanson & Gauld, 1995). *Lyrcus* was another genus found, of which only one species has been cited as a possible *Allorhogas* parasitoid (Marsh et al., 2000).

We found *Eurytoma* sp. 1 located at seed extremities attacked by *Allorhogas* sp.1, and suggest that it is a parasitoid on seed-predating hymenoptera. This is supported by *Eurytoma* species classification as *Allorhogas* larval ectoparasites by several authors (Macêdo & Monteiro, 1989; Marsh et al., 2000). An interesting component of the studied food web is the hymenopteran *Brasema* sp.1. Insects of this genus can be *Eurytoma* parasites (Askew & Nieves-Aldrey, 2006). Therefore, if the *Brasema* species found in *S. tenuifolia* is *Eurytoma* sp.1 parasitoid, then it is a secondary parasitoid. We also observed another possible secondary parasitoid: *Aprostocetus* sp.1 (Eulophidae: Tetrastichinae), a genus that comprises egg parasitoids and hyperparasitoids of several insect orders, including hymenopterans of the genus *Eurytoma* (Graham, 1987). Higher hyperparasitism occurrences in a system decrease parasitism and herbivore mortality rates (Montoya et al., 2003). It may suggest a top-down cascade effect releasing herbivorous species to consume seeds, so increasing the impact on *S. tenuifolia*.

Due to their low abundance, it was not possible to determine relationships of some insects within the studied food web. We found insects of the genus *Chelonus*, which is usually a lepidopteran parasitoid (Wharton et al., 1997), as is the genus *Eiphosoma* (Triana & Ravelo, 2007). Hence, the absence of lepidopterans from the collected insects explains the very low abundance of these parasitoids.

We also found two ant species, *Myrmelachista ruszkii* (Longino, 2006) and *Crematogaster* sp. (Longino, 2003), which belong to exclusively arboreal genera. Frequently, *Crematogaster* species are observed forming small satellite colonies on plants to keep themselves close to ephemeral food sources, such as extrafloral nectaries (Lanan et al., 2011), organs that are present on the studied plant species. Some studies have detected a positive effect of ants on plants (Rosumek et al., 2009), frequently because ants play an indirect defense role, driving off organisms that could feed on the host plant's seeds (Perry et al., 2004; Pringle, 2014). Thus, *M. ruszkii* and *Crematogaster* sp. may be using *S. tenuifolia* fruits as a shelter, protecting fruits from attacks by seed-feeders, since *Crematogaster* ants are very aggressive (Richard et al., 2001; Longino, 2003).

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After we excluded three hymenopteran species by lacking robustness on trophic position and links, structural source food web were described in respect to quantitative metrics to better understand it. We have found striking differences between *S. tenuifolia* data and the results presented by Tylianakis, Tscharntke and Lewis (2007). Most of our estimated metrics showed lower values, except trophic vulnerability. It reveals how simple our seed predation food web is. Further, considering the host-parasitoid species specificity here ascribed, source seed predation food web supported by *S. tenuifolia* has a higher vulnerability to secondary extinction. In respect to connectance, our results showed similar values to those observed in other food webs (Dunne, 2006; Tylianakis et al., 2007), corroborating with Martinez's idea (1992) in which connectance values are likely to be similar throughout natural food webs.

Chemical analysis carried out with different fruit and seed categories revealed a pattern of higher nitrogen, phosphorus, and potassium content in *S. tenuifolia* seeds than in fruits. Higher NPK content is essential for insects which feed on a single seed during their whole larval phase. Then, seed-feeding strategy may have advantage in terms of limiting factor such as nitrogen (Huberty & Denno, 2006). Further, this kind of resource – e.g., seeds – can support a complex food web composed by different species and interactions as observed here.

Based on the germination test carried out in 2012, we observed that *S. tenuifolia* seeds attacked by insects had reduced germination rates. This reduction happens because seed consumption by insects at the ripening phase makes seeds hollow and destroys their germination potential (Scherer & Romanowski, 2005). Another relevant factor is that seed predators frequently kill the embryo or remove part of the seed endosperm, which makes germination impossible (Southgate, 1979; Tomaz, Kestring, & Rossi, 2007; Fox et al., 2012).

The present study shows that resources with high nutrient concentration, such as seeds, may sustain a relatively complex food web, involving species of several taxonomic groups

and trophic levels. In addition, based on the natural history of involved species and their roles in the food web, this system is an interesting case for the study of complex ecological interactions and their structural and functional patterns. The structural characterization of the source food web of insects associated with *Senegalia tenuifolia* seeds showed an interspecific interaction frequency with few species presenting high interaction frequencies and strong interaction strength, but most species presenting low interaction frequency and weak interaction strength. This food web is complex, it is unravelling through the direct and indirect relationship explorations among different trophic level species will advance our understanding of interaction ecology.

RESUMEN

Estudios de redes alimentarias, que incluyen información cualitativa y cuantitativa sobre las interacciones entre especies para todos los niveles tróficos en una comunidad específica, son herramientas importantes para caracterizar la estructura de la comunidad, y para comprender el papel de la interacción de las especies en las comunidades naturales. Con este objetivo, se recolectaron frutos de *Senegalia tenuifolia* de un total de nueve sitios de tres áreas diferentes, en Minas Gerais, entre junio y agosto 2011. Los frutos

fueron recolectados y llevados al laboratorio en contenedores especiales, para permitir el desarrollo y descripción del insecto. Luego, se caracterizó la red alimentaria de los insectos asociados a estas semillas, se describieron las interacciones entre estos organismos y la planta hospedera, y se evaluó el impacto del ataque de insectos en la germinación de semillas mediante un experimento en condiciones controladas. Además, se estimó el contenido de NPK y la relación carbono:nitrógeno en frutos y en semillas. En semillas de *S. tenuifolia*, se encontraron 937 insectos de 15 especies estructuradas en cuatro niveles tróficos. Se encontraron dos especies de Bruchinae que se alimentan de semillas: *Merobruchus terani* y *Stator maculatopygus*. También se observaron especies de himenópteros que se alimentan de semillas: *Allorhogas* sp. 1 y *Prodecatoma* sp. 1, y especies de himenópteros parasitoides como: *Eupelmus* sp.1, *Lyrcus* sp.1, *Eurytoma* sp.1, *Horismenus* sp.1, *Chelonus* sp.1, *Aprostocetus* sp.1, *Eiphosoma* sp.1 y un Pteromalinae no identificado. Nuestros datos sugieren valores bajos de generalidad trófica y valores altos de vulnerabilidad trófica, además de otros, lo que demuestra cómo las interacciones entre especies son especializadas. El impacto en las semillas de los insectos que se alimentan de estas es severo e impidió su germinación. Por último, las semillas tenían un mayor contenido de NPK que los frutos. En conclusión, la red alimentaria de depredación de semillas con *S. tenuifolia* tiene dos características: interacciones de especies especializadas, considerando parasitoides y especies comedoras de semillas, e interacciones generalizadas entre especies, teniendo en cuenta la planta hospedera y las especies comedoras de semillas. De hecho, las semillas son recursos con alta concentración de nutrientes que proveen una rica y compleja red trófica. Por último, las semillas de *S. tenuifolia* se ven directamente afectadas por los insectos que se alimentan de semillas, que les impide la germinación, lo que podría resultar en un efecto negativo en la población planta-hospedero.

Palabras clave: Bruchinae, comedores de semillas, hospedero-parasitoides, interacción planta-insecto, cuantificación de redes alimentarias.

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