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Diversity of Stink Bugs (Pentatomidae) Associated with Canola: Looking for Potential Pests

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Abstract

Canola (Brassicaceae: *Brassica* spp.) is an important feedstock for biodiesel production and a potential ingredient for use in the food industry. In different continents, various arthropod pests damage canola plants. Stink bugs (Heteroptera: Pentatomidae) are present in all zoogeographical regions, and many species are recognized by their economic importance as crop pests. Our aim was to describe the composition, structure, and diversity of the assemblage of stink bugs sampled on canola in southern Brazil. A total of 878 pentatomids were captured, belonging to 27 species. The dominant species were *Euschistus heros* (F.) ($n = 439$), *Dichelops furcatus* (F.) ($n = 160$), and *Nezara viridula* L. ($n = 79$). The species richness estimators indicated the samples correspond from 79.7 to 93.1% of the richness estimated. Comparing canola to other monocultures, the richness of Pentatomidae was much superior. Otherwise, when compared to studies conducted in native vegetation and urban fragments, the richness is similar, and sometimes higher. This relatively high number of associations brings out the vulnerability of the canola fields according to the expansion of its cultivated area. Nine species of Pentatomidae are recorded on canola for the first time in Brazil. The most abundant species reported here are often stressed as stink bugs of economic importance in agro-ecosystems. The economic importance of each species varies greatly depending on the plant attacked. Detailed studies are needed to evaluate the damage caused to canola by stink bugs.

Introduction

Canola is a commercial name for cultivars of oilseed rape, *Brassica* spp. (Brassicales: Brassicaceae), considered the second most abundant source of edible oil in the world (Aider & Barbana 2011). It contains around 40% of oil and about 38–43% protein (Shahidi 1990). For this reason, canola is an important feedstock for biodiesel production (Gonçalves *et al* 2017) and, at the same time, a potential ingredient for use in the food industry (Aider & Barbana 2011). The commercial production is broadly supplied by two species: *Brassica napus* L. and *Brassica rapa* L. (Raymer 2002).

Canola is the primary oilseed crop in Europe, and biodiesel is the main purpose of its oil production (U.S. Department of Agriculture [USDA] 2017), while in Brazil, the oil production is mostly for the food market (Bergmann *et al* 2013). In 2017, Rio Grande do Sul State (Brazil) had the largest cultivated area (90%) and the largest production (34.6 thousand tons of grains) (Companhia Nacional de Abastecimento [CONAB] 2018).

In different continents, many arthropod pests damage canola plants. Local pests colonizing canola differ in compositions and abundance (Gu *et al* 2007). Stink bugs (Pentatomidae) are the fourth most numerous family within

Heteroptera, including more than 4700 species (Grazia *et al* 2015a). They are present in all zoogeographical regions, and many species are recognized by their economic importance as crop pests (Panizzi *et al* 2000). Most stink bugs are herbivorous, and nymphs and adults may damage plants by piercing the plant tissues and sucking nutrients (Grazia *et al* 2015b). In the Neotropical region, stink bugs are often reported causing damage to plants belonging to Asteraceae, Brassicaceae, Fabaceae, Poaceae, and Solanaceae, among others (Smaniotto & Panizzi 2015).

In Canada, one of the biggest producers of canola around the world, the mirids from *Lygus* (e.g., *Lygus keltoni* Schwartz, *Lygus lineolaris* (Palisot de Beauvois), *Lygus elisus* Van Duzee, and *Lygus borealis* (Kelton)) are the only Heteroptera reported causing damage (Canola Grower'S Manual 2018). In the USA and Mexico, *Bagrada hilaris* (Burmeister) (Pentatomidae) is calling attention for its potential of damage in canola (Palumbo *et al* 2016). *Lygus* spp. are also important pests of canola in Turkey and, among the Pentatomidae, *Eurydema ventralis* Kolenati and *Peribalus strictus* (F.) appear in moderate abundances (Demirel 2009). Although there are no studies evaluating the abundance and damage caused by stink bugs in Brazil, species such as *Nezara viridula* (L.), *Euschistus heros* (F.), *Piezodorus guildinii* (Westwood), and *Neomegalotomus parvus* (Westwood) (Alydidae) have been associated with canola fields (Dias 1992, Tomm *et al* 2009, 2014). Currently, 17 phytophagous stink bugs are reported on canola in Brazil (Marsaro Júnior *et al* 2017).

This booming of agribusiness has increased population of stink bugs on several crops, and their economical injuries, as well (Panizzi 2015). The recent and constant expansion of canola fields in South America (Shahidi 1990) contributes to the establishment of many pests. The knowledge of associated species feeding and reproducing on a particular culture is the first step to identify crop pests before developing an appropriate insect management system.

In this study, our aim was to evaluate and describe the composition, structure, and diversity of the assemblage of phytophagous stink bugs (Pentatomidae) sampled on canola plant. Moreover, the identification of highly abundant species may indicate potential pests for this crop, aiding to prevent agricultural damages and losses.

Material and Methods

The study was carried out at the Centro Nacional de Pesquisa de Trigo – Embrapa Trigo experiment station, located in Passo Fundo, Rio Grande do Sul State, Brazil (28°14´S; 52°24´W; 638 a. s. l.). The climate of the region is classified as Cfa (Köppen classification system), the average annual temperature ranges between 16 and 18°C (Alvares *et al* 2013).

The sampling site was constituted by plots of canola (*Brassica napus* L. var. *oleifera*, cv. Hyola 433) (comprising ~ 800m^2) sown on May 2015, 2016, and July 2017. The surrounded matrix was composed of other plots of crops, such as wheat (*Triticum sativum* L.), rye (*Secale cereale* L.), and oat (*Avena sativa* L.). Natural vegetation was also present in the area nearby the canola plots. During the canola plant development, all cultural practices were applied, except that no pesticides were sprayed.

Samples were taken mostly during the reproductive period of the crop when the incidence of stink bugs is intensified (Marsaro Júnior *et al* 2017). In total, 28 samples unequally spaced during the canola plant cultivation along the 3 years were taken (2 samples on September and October 2015, 3 samples from September to December 2016, and 23 samples from September to December 2017). Stink bugs were sampled with an entomological net and by visual inspection on the plants. Both adults and nymphs were captured. Adults were slain in the field with killing jars and preserved in 95% ethanol. Nymphs were kept in pots with wet paper, taken to the laboratory, and reared with canola pods in an environmental chamber (25 ± 1°C temperature, 65 ± 10% RH and L14:D10 photoperiod) until they reached adulthood for proper identification. Identifications reached the species level by the use of literature (e.g., Scwertner & Grazia 2007, Weiler *et al* 2016, Bianchi *et al* 2017) and direct comparison with specimens deposited in the collection of the Laboratório de Entomologia Sistemática da Universidade Federal do Rio Grande do Sul (UFRGS). Voucher specimens are deposited at the UFRGS and preserved in 95–100% ethanol under − 20°C.

The quantitative data were analyzed for alpha diversity (species diversity, species abundance distribution—SAD) using the software PASt 3.18 (Hammer *et al* 2001). The fitness of the SAD was tested in four models: geometric, broken-stick, log-series, and log-normal. Sampling sufficiency curve for the richness of pentatomids associated with canola was obtained with 999 randomizations using the EstimateS 9.1.0 software (Colwell 2006) and compared with the data from the non-parametric estimator Chao 1, Chao 2, Jacknife 1, and Jacknife 2 to determine the efficiency of the sampling. Each one of these estimators of richness bases its calculation on a distinct parameter (i.e., occurrence of singletons, doubletons, unique, and duplicates). Intending to indicate potential crop pest, we calculated the Simpsons dominance index. Species belonging to the subfamily Asopinae were removed from the analyses due to their predacious habits (but see ahead).

Results

A total of 878 individual phytophagous pentatomids were captured, belonging to 27 species in 12 genera (Table 1).

Table 1 Composition, abundance (*n*) and frequency (*F*) of stink bugs (Heteroptera: Pentatomidae) sampled on canola (*Brassica napus* L. var. *oleifera*, cv. Hyola 433) in Passo Fundo, Rio Grande do Sul State, Brazil.* New record on canola in Brazil

Species	*n*	*F* (%)
Adustonotus irroratus (Bunde, Grazia, & Mendonça)	31	3.53
Chinavia difficilis (Stål)*	1	0.11
Chinavia erythrocnemis (Berg)	13	1.48
Chinavia herbida (Stål)	26	2.96
Chinavia impicticornis (Stål)*	2	0.23
Chinavia nigrodorsata (Breddin)*	3	0.34
Chinavia obstinata (Stål)	3	0.34
Chinavia pengue (Rolston)	4	0.46
Dichelops furcatus (F.)	160	18.22
Dichelops melacanthus (Dallas)	6	0.68
Edessa meditabunda (F.)	6	0.68
Edessa piperitia Westwood	2	0.23
Edessa rufomarginata (De Geer)	2	0.23
Euschistus heros (F.)	439	50.00
Euschistus picticornis Stål	39	4.44
Euschistus riograndensis Weiler & Grazia*	1	0.11
Euschistus triangulator (Herrich-Schäffer)	14	1.60
Mormidea cornicolis Stål*	3	0.34
Mormidea sp.	1	0.11
Mormidea v-luteum (Lichtenstein)*	4	0.46
Nezara viridula (L.)	79	9.00
Oebalus poecilus (Dallas)*	1	0.11
Pallantia macula (Dallas)*	1	0.11
Piezodorus guildinii (Westwood)	2	0.23
Thoreyella brasiliensis Spinola*	17	1.94
Thyanta humilis Bergroth	5	0.57
Thyanta perditor (F.)	13	1.49
Total	878	100

Nine out of these species are recorded on canola for the first time in Brazil. Pentatominae presented higher abundance ($n = 868$) and species richness ($S = 24$) than the other sampled subfamily, Edessinae ($n = 10$; $S = 3$). Five singletons and four doubletons were captured; and six unique and five duplicates were reported in our samples. The overall species abundance distribution (SAD) for the assemblage significantly fits the geometric model ($k = 0.1708$; chi^2 = 1413; $p < 0.0001$) (Fig. 1). The Simpson dominance index was 3.59, and the most abundant species were *E. heros* ($n = 439$), *Dichelops furcatus* (F.) ($n = 160$), and *N. viridula* ($n = 79$). These three species sum more than 75% of the sampled specimens. The following most abundant species is *Euschistus picticornis* Stål ($n = 39$) and together to the remaining 23 sampled species sum 22.8% of the total abundance (Table 1). Besides phytophagous stink bugs, we collected three species of predacious pentatomids (Asopinae): *Podisus crassimargo* (Stål), *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stål).

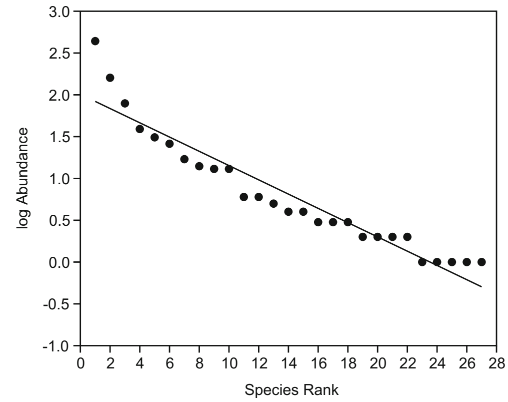


Fig. 1 Species abundance distribution of Pentatomidae sampled on canola (*Brassica napus* L. var. *oleifera*, cv. Hyola 433) in Passo Fundo, Rio Grande do Sul State, Brazil

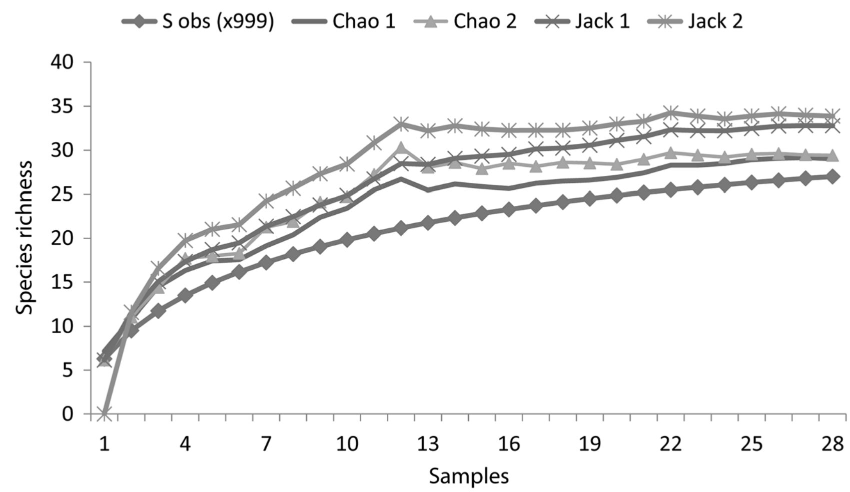
The species accumulation rarefied curve (Fig. 2) shows a tendency toward the asymptote, suggesting the number of sampled species is close to stabilization. The species richness estimators indicated the samples correspond from 79.7% (Jacknife 2; $S = 33.89$) to 93.1% (Chao 1; $S = 29$) of the richness estimated (Fig. 2).

Discussion

The fields of canola are increasing in size every year, being cultivated in distinct geographical regions, and crossing the borders toward different countries (Shahidi 1990). Pentatomidae have a worldwide distribution, and the damage caused by the stink bugs is reported for many crop plants in all continents (Grazia *et al* 2015a). The knowledge and recognition of potential crop pest in local scale are important steps to labor-saving pest management (Panizzi 2013), and it is a steppingstone to controlling pest infestations. Here, we present a comprehensive evaluation of the assemblage of stink bugs associated with canola crops in Southern Brazil.

Comparing our samples to other monocultures, the richness seems to be much higher in canola than in soybean in Brazil (e.g., Corrêa-Ferreira & De Azevedo 2002) and in wheat and cotton in the USA (Reay-Jones 2010, Reeves *et al* 2010). Otherwise, the richness in canola crops of southern Brazil is similar, and sometimes higher, when compared to studies conducted in native vegetation and urban fragments (e.g., Campos *et al* 2009, Firmino *et al* 2017). This relatively high number of associations brings out the vulnerability of the canola fields according to the expansion of its cultivated area. Pentatomids became pest to many crops during the last century, among other reasons, due to the enlargement of cultivated fields (Panizzi 1997), and the agricultural area in Brazil is increasing annually (Instituto Brasileiro de Geografia e

Fig. 2 Sample-based rarefaction of species richness and estimated species richness of Pentatomidae sampled on canola (*Brassica napus* L. var. *oleifera*, cv. Hyola 433) in Passo Fundo, Rio Grande do Sul State, Brazil



Estatística [IBGE] 2004). Stink bugs are the major pests of soybean, summing more than 50 species worldwide feeding on this plant (Kogan & Turnipseed 1987) and also pests of several other leguminous and non-leguminous crops (Panizzi *et al* 2000).

The rarefaction curve shows the tendency to stabilize, and the observed richness ($S = 27$) is close to the estimated values (Chao 1: $S = 29.00$; Jacknife 2: $S = 33.89$). It indicates a local sample sufficiency, or something close to that. The richness is an absolute value and may be compared directly to other researches. On the other hand, we expect the composition of pentatomids associated to canola must change in other regions, increasing the list of stink bugs feeding on canola. Even close places may present a significant difference between the compositions of stink bug assemblage (Bianchi *et al* 2014).

The geometric model of SAD points the prevalence of *E. heros* sampled, representing more than twice the abundance of *D. furcatus* and four times of *N. viridula*. In this SAD model, the most abundant species is virtually responsible to occupy a large portion of the resource, followed by the second most abundant species, representing in abundance at maximum the half of the most abundant sampled, and then occupying a smaller fraction of the resource (Magurran 1988, Krebs 1989). It is common to communities of phytophagous insects bearing low richness of host plant, as monocultures or areas close to (e.g., Klein *et al* 2013), on the other hand, studies in natural ecosystems the SAD of pentatomids tends to fit in log-series model (e.g., Schmidt & Barcellos 2007, Mendonça *et al* 2009). It suggests the abundance of each pentatomid species is closely related to the environmental and its heterogeneity of recourses.

The most abundant species reported here, the Neotropical brown stink bug (*E. heros*), the green-belly sting bug (*D. furcatus*), and the southern green stink bug (*N. viridula*), are often addressed as stink bugs of economic importance in agro-ecosystems (for a review of these and other stink bugs species see Smaniotto & Panizzi 2015). Those species have a broad distribution in South America, *N. viridula* being cosmopolitan (Panizzi 2015). They can be listed as potential pests to canola in Brazil.

Canola field is largely affected by the presence of the painted bug, *B. hilaris* (Palumbo *et al* 2016). Recently, the painted bug was reported for the first time in South America, in Chile (Faúndez *et al* 2017). *Bagrada hilaris* can be considered an invasive species and its distribution has increased every year in America (Palumbo *et al* 2016). Among a broad set of host plants, summing more than 20 botanical families, the painted bug causes plenty of damages on Brassicaceae crops (Palumbo *et al* 2016). The damage caused only on brassicas in Arizona and California states by painted bug was evaluated at over a billion dollars in 2010 (Arizona Agricultural Statistics 2011, California Department of Food and Agriculture [CDFA] 2012). The painted bug was not recorded in our samples; however, once *B. hilaris* cross the Andean boarder breaking out this region, the canola produced can be under risk. Here, we warn the dangerous potential of *B. hilaris* to canola crops, and its likely soon invasion in Brazil.

Although sometimes overlooked due to the hard species identification, the presence of immature stages brings valuable information to ecological studies. Nymphs can represent a significant amount of the sampled fauna, both in crop fields (Reeves *et al* 2010) or natural ecosystems (Mendonça *et al* 2009). It means the nymphs are also responsible for the total damage to the crops (Reay-Jones 2010). The presence of immature stages indicates reproductive hosts. During our samples, the immature stages of the following species reached the adulthood feeding on the canola pods: *Chinavia erythrocnemis* (Berg), *Chinavia herbida* (Stål), *D. furcatus*, *Edessa meditabunda* (F.), *N. viridula*, and *Thyanta perditor* (F.). It means that canola pods provide enough nutrition to these species to complete their life cycle. Other studies could be designed to elucidate the importance of canola as host plant and its effect on nymph and adult biology of these stink bug species.

The economic importance of each species of stink bug varies greatly depending on the plant attacked (Panizzi *et al* 2000). Many studies have evaluated the damage and the decrease in yield caused by stink bugs for umpteen crops, such as soybean (Corrêa-Ferreira & De Azevedo 2002), cotton (Soria *et al* 2017), rice (Tindall *et al* 2005), and many others (Panizzi *et al* 2000). Detailed studies are needed to evaluate the damage caused to canola by *E. heros*, *D. furcatus*, *N. viridula*, and other species sampled here. These studies will provide important data for the implementation of best pest management for canola fields.

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Authors' Contributions ALMJ, PRVSP, and ARP planned, designed, and executed field work; FMB and JG made the taxa identification; FMB analyzed the data; FMB and ALMJ wrote the first draft; all authors contributed to the final version of the paper.

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