Chapter

Feeding by Florivorous Flies (Tephritidae and Agromyzidae) in Flower Heads of Neotropical Asteraceae (Asterales) from Central Brazil

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Abstract

The four following Diptera families are peculiar because they are predominantly phytophagous: Cecidomyiidae, Agromyzidae, Lonchaeidae and Tephritidae; which is uncommon for dipterans. Tephritine's larvae, depending on the species, consumes leaves, stems, flowers or roots of their host plants. Some tephritines feeds on flower heads of weed Asteraceae and can act in population suppression of invasive species in cultivated areas. In Mid-West of Brazil, we investigate Tephritinae and Agromyzinae flies in flower heads of Asteraceae species in three different phytophisiognomies in Dourados region, state of Mato Grosso do Sul. Here, 12 florivore fly species (9 Tephritinae, and 3 *Melanagromyza* spp., Agromizinae, Agromyzidae) are reported for the first time in Mid-West Brazil. We stablish the species of Asteraceae host for Tephritinae (Tephritidae) and for some species of *Melanagromyza* (Agromyzinae) in environments of Cerrado, Semideciduous Forest, and agroecosystem at Dourados-MS region. The inflorescences of Asteraceae species (± 500 capitula/species) were kept in containers to the emergence of the florivorous flies or their parasitoids. The adult insects after 48 hours were fixed in 80% ethanol for later identification. A total 36 species of Asteraceae were evaluated in the three regions of Dourados-MS, Brazil. Were obtained 120,031 flower heads of Astereceae, emerging 2,698 adults of insects: 833 Tephritinae (Tephritidae), belonging to 7 genera and 9 species; 1,089 *Melanagromyza* spp. (Agromyzidae) and 776 parasitoids (Hymenoptera) from the tephritines and agromyzines. We found that some florivore fly species needs to be better studied to employ in suppression programs of invasive Asteraceae population in the Neotropical Region.

Keywords: florivory, *Melanagromyza* spp., compositae, tephritinae, weed biocontrol

1. Introduction

Diptera is the second Order on diversity in the Class Insecta, but few families are predominantly phytophagous (feeds on plants), such as the ancient Cecidomyiidae, with almost 5,400 species worldwide (Bibionomorpha, Sciaroidea), and the three derived families of Brachycera: Agromyzidae (near 3,000 spp.) (Opomyzoidea), Lonchaeidae [around 600 spp.], and Tephritidae (about 5,000 spp.) (Tephritoidea). From the flies that feed on plants, these are the four families with higher diversity, totaling around 14,000 species.

The Cecidomyiids make galls in plants (like tumors) and feed inside these tissue that grows around their larvae. The three-following family of flies, are of higher economic importance: Agromyzids feed inside different parts of plants, being several species leaf or stem miners, with many pests (*e.g. Liriomyza* spp.) on cultivated vegetables and other crop plants. However, the larvae of species in the genus *Melanagromyza* Hendel 1920 feeds on the seeds of Asteraceae flower heads.

The Agromyzidae are represented by tiny phytophagous flies. Many species are known as leaf miners, with several species characterized as pests of some agricultural crops. Currently, there are almost 3,000 species described worldwide, being about 90 species reported in Brazil. Agromizids are generally species-specific in their host plants. They usually live and breed in a single plant genus, or at most in a family, except for a few pest species such as those of the genus *Liriomyza* which are very polyphagous.

Tephritidae is the most speciose family of fruit flies, with around 5,000 described species, in six subfamilies (Tachiniscinae, Blepharoneurinae, Phytalmyiinae, Trypetinae, Dacinae, and Tephritinae); about 500 genera, and probably many undescribed species worldwide. Tephritids are peculiars because they are among the few groups of dipterans strictly phytophagous, except the Tachiniscinae, which are thought be parasitoids of Lepidoptera, and at least, some species of Phytalmyiinae feed on live or dead bamboos (Poaceae), on fallen or dead trees of other plant families. Blepharoneurinae feed in flowers, fruits, and make galls in Cucurbitaceae; Trypetinae and Dacinae feed in fruits or in seeds of a wide range of plant families, and Tephritinae eat in flowers, make gall, or are leaf-miners in several plant families, such as: Aquifoliaceae, Scrophulariaceae, Verbenaceae, but mainly in flower heads of Asteraceae. Tephritinae is the biggest subfamily of Tephritidae, with around 1,840 described species (valid names) in 11 tribes and 211 genera [1–3].

Some tephritines (Tephritidae) genera are stems gall makers, such as *Eurosta* Loew 1873, *Procecidochares* Hendel 1914, the Neotropical *Tomoplagia* Coquillett 1910; others are florivorous like the species of Neotropical genus *Blepharoneura* Loew 183, that breed specifically in Cucurbitaceae's flowers or fruits. Many other genera (*e.g. Dictyotrypeta* Hendel 1914, *Dioxyna* Hardy 1988, *Cecidochares* Bezzi 1910, *Tetreuaresta* Hendel 1928, *Trupanea* Schrank 1795, *Xanthaciura* Hendel 1914), feed and breed in flower heads of daisis (Asteraceae). In the Neotropical Region, several tribes of Tephritinae and some species of *Melanagromyza* Hendel 1920 (Agromyzinae, Agromyzidae), breed manly upon the flower heads of Asteraceae - the most speciose plant family worldwide.

Florivorous flies are here defined as the dipterans that in their larval phase feed in the flowers of Angiospermae. The larvae of these flies, like other higher Brachycera, undergo through three instars (L1, L2 and L3), generally, doubling in size and weight after each molting of exoskeleton. After completing the third instar, larva expand their exoskeleton, assuming a barrel shape, being the pupa formed inside this last skin. These set (L3 exoskeleton + skin of the pupae) is called puparium. When the pupa inside the puparium is completely formed, that take a few days (around 2 weeks), the inner fly makes a circular hole in the cephalic pole of puparium, and emerges as an adult. Pupation of florivorous flies (Tephritinae and Agromyzinae) on the Neotropical Region, generally, happens inside the flower heads of Asteraceae.

The true flies differ from other groups of insects in this aspect: the skin of their larval body (exuviae) at the end of the third (last) instar is not lost, becoming expanded and hardened, and the pupa is formed internally, having in these phase, two protections. The beginning of this stage is recognized as the **pre-pupa phase**. Like in all other holometabolous insects, when the larva has completed the last instar, starts the phase of pre-pupae, which is characterized by the ending of juvenile feeding activity, expelling all hindgut feces, and starting the organogenesis to build up the pupa.

The adults of Tephritidae, differently of their larvae, which have a restrict kind of food (tissue of the host plant), they feed in different materials found in their environments. Probably they drink fruit juices, extrafloral exudates, nectar, honeydew produced by ancient Hemiptera, microorganisms and other sources of carbohydrates and aminoacids, such as exudates of plant (alive or dead), bird faces, etc. By other hand, Norrbom [1] has pointed out that adults of some Blepharoneurinae species, have labellar teeth in their mouth, being able to rasp plant tissue.

Asteraceae (Asterales) is the most specious family of Angiospermae, with around 24,000 species worldwide [4], having a great diversity of species in Brazil. These plants are adapted to stressful water regimes, hence their great capacity to colonize the most different ecosystems.

Female of florivorous flies (Tephritinae and Agromyzinae) after mate, lay their eggs over the flower buds or inside the young flowers of host plants. After 3–7 days the eggs hatch and larvae start eating, mainly the seeds, to complete their larval phase, emerging from the inflorescences as adult.

2. Protocol to evaluation the diversity of florivorous flies in nature

The sample the diversity of florivorous flies (FF) and their host plants, one can start collecting flower heads of Asteraceae. The inflorescences (capitula) can be held in small containers with same substrate that can keep humidity, such as toilet paper, sterile sand or vermiculite. Depending on the proposal of research, it is possible put the flower heads (around 500) in a container or individually (a flower head or a capitula in each container).

To sample the pattern of diversity for both: Host plants and FF is possible to employ different methodologies. For example, it's possible to collect samples of Asteraceae flower heads by transect; quadrants; in different sub-environments (Phytophysiognomies); using points determined by GPS, and so on. But some procedures are essential: All the plant from which flower heads were sampled must be exsiccated and identified by botanist specialist to have accurate information. The FF needs to be held alive for a couple of days (2 or 3), to allow that adult can acquire specie-specific coloring patterns of body and wings (important to specific and secure species identification).

Below we will present a characterization of some Tephritinae sampled in our recent researches, a synopsis on the main results on the Neotropical FF, their host plants and natural enemies (parasitoids) in natural environments and agroecosystem.

3. Characterization of sampled neotropical Tephritinae

Trupanea Schrank 1795 (**Figure 1a**), with 226 described species, is the largest genus of Tephritinae. It occurs in all biogeographic regions. Some Neotropical species have been reviewed and included in keys by Hendel, Malloch, Hering, Aczel, Frias, and Foote, but all are obsolete by now. The genus *Trupanea* is in need of review [1, 2].

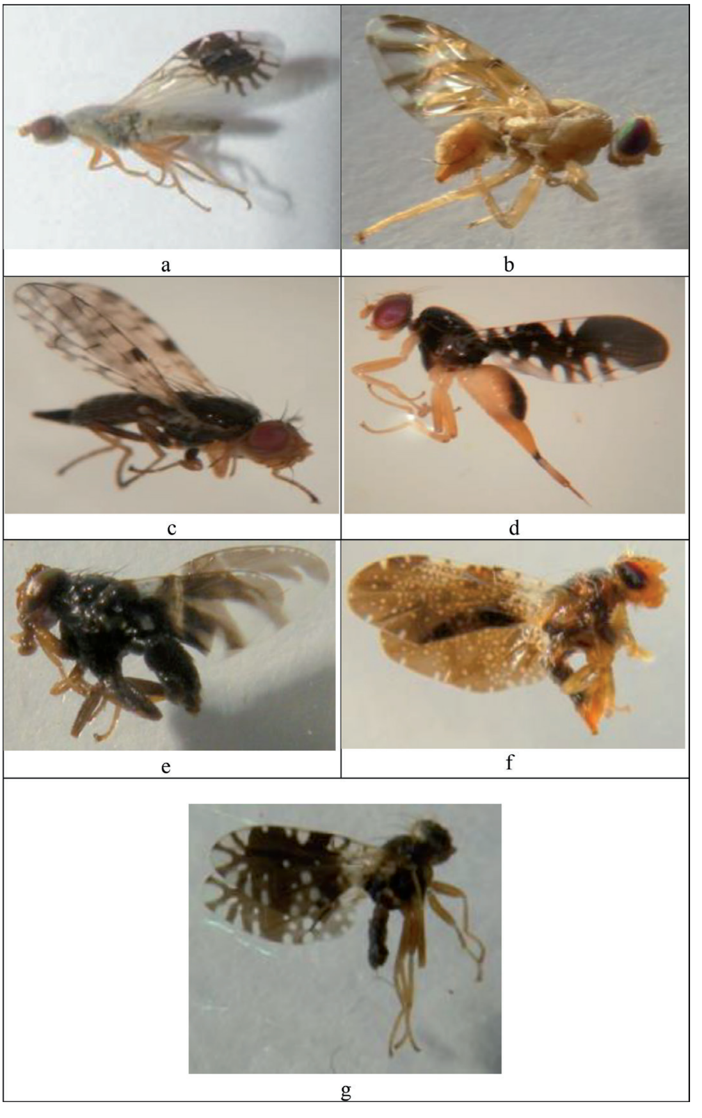

Figure 1.
Some Neotropical genera of Tephritinae (Diptera: Tephritidae) breeding in flower heads of Asteraceae species from Central Brazil: a): Male of Trupanea *Schrank 1795; b): Female of* Tomoplagia *Coquillett 1910; c): Female of* Dioxyna *hardy 1988; d): Female of* Xanthaciura *Hendel 1914; e): Male of* Cecidochares *Bezzi 1910; f): Female of* Dictyotrypeta *Hendel 1914; b): Male of* Tetreuaresta *Hendel 1928.*

The genus *Tomoplagia* Coquillett 1910 (**Figure 1b**), with 61 described spcies and several one not described. From this total, at least 15 species are reported in the Central American region (including Neotropical Mexico). *Tomoplagia* species have interactions with Asteraceae, mainly in the genera of Vernonieae and Mutiseae, Heliantheae and other tribes. Most species reproduce in flower heads, but a South American species forms galls [1–3].

The genus *Dioxyna* Hardy 1988 (**Figure 1c**), has a wide distribution, and 11 described species. In the New World their hosts plants are mainly species of the tribes Heliantheae and Heleniae [1, 2].

The genus *Xanthaciura* Hendel 1914 (**Figure 1d**), includes 17 described species, and several not described ones. At least 10 occur in the Central American region (including Neotropical Mexico). Most species were reviewed by by Aczel.

Xanthaciura has species that consume flowers of several genera of Asteraceae, mainly in the tribes Eupatorieae, Coreopsideae and Heliantheae [1, 2].

The genus *Cecidochares* Bezzi 1910 (**Figure 1e**), includes 13 described and numerous not yet undescribeds. At least three undescribed species occur in the Central American region (and Neotropical Mexico). Species of *Cecidochares* are mainly related to plants of the Eupatorieae tribe (Asteraceae). They make galls on stems, and occur with low incidence on flower heads. *Cecidochares connexa* has been introduced in several Old World countries for the biological control of the important weed: *Chromolaena odorata* [1, 2].

Dictyotrypeta Hendel 1914 (**Figure 1f**) is a Neotropical genus in need of revision. Currently it includes six species and many undescribed ones. It is not clear if all *Dictyotrypeta* species form a monophyletic group. Several of them, such as *Dictyotrypeta incisa* (Wulp) from Central America, South America, Mexico, Guatemala, and *Dictyotrypeta crenulata* (Wulp) from Mexico (Sinaloa, Guerrero, Veracruz), were only tentatively included in this genus. In addition, to the two species above, the fauna from Central America also includes two undescribed species. The known host plants for *Dictyotrypeta* spp. are mainly Asteraceae species in the tribes Heliantheae and Vernonieae [1, 2].

Tetreuaresta Hendel 1928 (**Figure 1g**), a Neotropical genus with 19 species described, six of which occur in the Central American Region (including Neotropical Mexico), and numerous undescribed species. Steyskal provided a key for 5 species, but the genus is in need of revision. The biology of most species is unknown, but five species consume flower heads of Vernonieae (Asteraceae) species. *Tetreuaresta obscuriventris* (Loew) has been employed for biological control of *Elephantopus mollis* in Hawaii, and other Pacific Islands [1, 2].

4. Aims

The main objectives of this paper, are: 1.To investigate the occurrence of Florivorous fly (FF) species associated with Asteraceae flower heads in three phytophysiognomies from Dourados Region; 2. To verify which Asteraceae species are hosts of Tephritidae and Agromyzidae (Diptera) in Cerrado, Semideciduous Forest and Agroecosystem; 3. To quantify the patterns of occurrence of those dipterans and their parasitoids.

5. Material and methods

The work was developed in a tropical region, marked by a landscape dominated by Cerrado Biome, with patches of Semideciduous Forest (Atlantic Forest), and a matrix area of monocultures. The area has three biodiversity hotspots (Cerrado, Atlantic Forest and Chaco). The Cerrado that represents the phytophysiognomy of Savana Parque is located in the district of Itahum (22° 05 '21.8" S and 55°21′11.9" W), Dourados-MS, with 412 m of altitude. The second environment is a Seasonal Semideciduous Forest, belonging to the Atlantic Forest biome and represented by a fragment of native vegetation, with about 35 ha (22° 12 '46.7" S and 54° 54 '53.5" W) and altitude of 452 m. The third environment is a mixed fruit orchard at the *Universidade Federal da Grande Dourados* (UFGD), located at km 12 of the MS-162 Highway (22° 11′ 46.8" S, and 54° 56′ 12.2" W), at 425 m altitude, in the municipality of Dourados-MS.

Asteraceae flower heads were collected and kept in two transparent plastic cups (500 ml) with the openings juxtaposed and secured by adhesive stick tape.

The bottom cup had a layer of dry sterile sand to absorb excess moisture to allow the emergence of flies and/or their parasitoids, since the identification of species of tephritids is based on the morphology of adults. The sampled material was kept in the laboratory under a 12 h photophase, controlled by a timer.

After emergence, adults were fed an artificial diet composed of brown sugar (100 g), 50 g of beer yeast, a tablespoon of honey from *Apis mellifera* L., and 100 ml of sterile water. All adults were kept alive for at least 48 hours for complete pigmentation of the body and wings. After this period, they were killed and kept in bottles with 80% ethanol for specific identification and analysis of qualitative and quantitative data. In this research, 1 ha quadrant was established as the sample area in each evaluated environment. Sampling was repeated every 15 days, and collections were performed on the same day in the three different environments. Tephritids and agromyzids were identified in the *Laboratório de Sistemática e Taxonomia de Tephritidae* (LabTaxon)-UFGD.

The collected plants were processed for species identification by Dr. Nádia Roque, *Universidade Federal da Bahia* (Salvador, BA, Brazil). All exsiccates were coded for each individual and location, and duplicates of these were deposited as voucher specimens at the CGMS Herbarium of the *Museu da Biodiversidade, Faculdade de Ciências Biológicas e Ambientais (FCBA), Universidade Federal da Grande Dourados* (UFGD), Dourados-MS, Brazil, and at the Alexandre Leal Costa Herbário (ALCB) Institute of Biology, Universidade Federal da Bahia (UFBA, Salvador, Brazil). The level of infestation of each host plant was calculated according to the number of flower heads and the biomass of each plant species.

The analyzes were made by analysis of variance (ANOVA and F test), when the data meet the assumptions of normality and homogeneity, using, in this case, the means comparison tests (Tukey, Duncam or t test) at 10% of significance. When the assumptions were not met, Kruskall-Wallis and Mann–Whitney methods were used, respectively. The level of infestation of each host plant was calculated according to the number of flower heads and the biomass of each species of plant. Species richness, abundance, and frequency among the three different environments were evaluated.

5.1 Cerrado biome

Cerrado is an International Biome, important hotspot on biodiversity. Is present from Central Brazil, Bolivia, extending to Southern to north of Amazonia; across the isthmus of Central America, including the Caribbean region; Belize, Guatemala, Southern Mexico, and north of the Mexican Plateau. The Cerrado is the second largest Neotropical biome (behind Amazon = Tropical Andes), and fight an important hotspot of biodiversity worldwide. The Cerrado biome is located mainly in Central Brazil, with approximately 2,000,000 km^2 (25% of Brazilian territory), with different Phytophysiognomies. Many vegetal formations are similar to savanna formations, but some are similar to tropical forests, and riparian forests. This biome has a great diversity of plants and animals, with high rates of plant endemisms, with around 2% of global diversity [5]. Nowadays, the Cerrado biome is recognized as the sixth hotspot of the planet, among 34 listed in order of priority for conservation of habitat [6].

5.2 Atlantic forest biome

The Atlantic Forest is characterized by on of the highest rates of biodiversity and endemism at Neotropical region end worldwide. It's an international biome restricted to three countries in South America: Brazil, Argentina and

Paraguay. It follows the coastal region of the Atlantic Ocean along Brazil and part of Argentina (Province of Misiones), and by land, a part to the southwest of Paraguay. It is characterized by an extensive forest that varies from humid to dry type (plants with broad leaves), tropical forests (desciduous, semi-desciduous and sub-montana), fields of tropical and subtropical pastures, arboreal savanna, steppe savanna and grassy-wood savanna and mangrove. Brazil is home, by far, to the largest stretch of the Atlantic Forest, with an extension of over 4,000 km, extending from the Rio Grande do Norte to the Rio Grande do Sul states. The Atlantic Forest is subdivided into ecoregions that are characterized by housing a huge biodiversity with high rates of endemism of animals and plants. Currently it is very devastated. Of the more than 1,315,000 km2 original from the time of the discovery of Brazil in the year 1,500 by the Portuguese colonizers, there are currently about 8.5% left, having already led several species of animals and plants to extinction, and with about 1,990 species at risk of extinction, according to IBGE [6].

5.2.1 Semideciduous Forest

It's a phytophysiognomy sub-type of the Atlantic Forest, characterized by plant species with senescence of their leaves, which are partially lost on the cold-dry seasons, like end of fall and start of the winter. Their leaf lost is dependent on the weather (coldness-dryness), but the tree renew their leaves/when the climate is mild dor worm or do not lost their leaves when/where the climate keeps constant.

6. Results and discussion

A total 36 species of Asteraceae (13 tribes) were sampled in three environments from regions of Dourados-MS, Mid-West of Brazil, looking for florivorous flies in their flower heads. Eighteen Asteraceae species hosted Florivorous flies (Tephritidae and/or Agromyzidae), and some of their parasitoids (Hymenoptera). From 18 sampled asteraceae species, neither Tephritidae nor Agromyzidae emerged. Twenty seven, species of Asteraceae were recorded in the Cerrado environment (Itahum District), 24 in the Semideciduous forest (phytophisiognomy of the Atlantic Forest, located at Fazenda Coqueiro), and 8 Asteraceae species were sampled in the Agroecosystem (a diversified orchard of fruit trees at UFGD campus) (**Table 1**).

The percentage of frequency of Tephritinae species associated with the species of Asteraceae were evaluated. In the Cerrado, we found higher diversity of Asteraceae (S = 27), being 11 of them host for florivorous flies (FF), emerging 12 species (9 Tephritinae and 3 Agromyzinae) from 374 adults recovered. In the Semideciduous forest, 24 species of Asteraceae (S = 24) were found. Ten of them were hosted by 9 florivorous fly species. Finally, in the agroecosystem, five Asteraceae species hosted four FF species (**Table 2**).

Eighteen Asteraceae species hosted the florivore fly species. Seven astereces had their flower heads coninized simultaneously by tephritines plus agromyzines: *Baccharis triplinervis*, *Bidens pilosa*, *Chaptalia integerrima*, *Chromolaena arnottiana*, *Porophyllum ruderale*, *Vernonia cognata*, and *Vernonia polyanthes*. Fom six host plants (*Chromolaena ivifolia*, *Eupatorium multicrenulatum*, *Praxelis pauciflora*, *Pterocaulon virgatum*, *Vernonia bardanoides* and *Zinnia elegans*) only species of tephritine emerged. Five asteraces were excluvive hosts for agromyzines of the genus *Melanagromyza* (*Aspilia latissima, Bidens sulphurea*, *Emilia fosbergii*, *Lourteigia ballotifolia*, and *Sonchus oleraceus* (**Table 2**).

Asteraceae Taxa (Tribes and Species)	Plant Status	Environment
Anthemideae *Tanacetum vulgare* L.	Nonhost	Agroecosystem (= Orchard)
Astereae *Baccharis linearifolia* (LAM.) Pers.	Nonhost	Cerrado Semideciduous Forest
Baccharis triplinervis (Spreng.)	Host	Cerrado
*Conyza bonariensis (*L.) Cronquist	Nonhost	Agroecosystem, Semideciduous Forest
Solidago microglossa DC.	Nonhost	Cerrado Semideciduous Forest
Cichorieae *Sonchus oleraceus* L.	Host	Cerrado Semideciduous Forest
Cynareae *Arctium lappa* L.	Nonhost	Semideciduous Forest
Eupatorieae *Chromolaena arnottiana* (Griseb.) R.M.King & H. Rob.	Host	Cerrado Semideciduous Forest
Chromolaena ivifolia (L.) R.M.King & H. Rob	Host	Cerrado
Eupatorium macrocephalum (Less.) DC.	Nonhost	Cerrado
Eupatorium multicrenulatum Sch. Bip. ex Baker	Host	Cerrado
Eupatorium odoratum (L.) King & H.E. Robins	Nonhost	Semideciduous Forest
Lourteigia ballotifoli (Kunth) R. M. King & H. Rob. N. V.	Host	Cerrado
Mikania hastato-cordata Malme	Nonhost	Cerrado Semideciduous Forest
Praxelis pauciflora (Kunth) R.M.King & H. Rob.	Host	Agroecosystem
Gnaphalieae *Achyrocline satureioides* (LAM.) D.C.	Nonhost	Cerrado
Heliantheae *Aspilia elata* Pilg.	Nonhost	Cerrado Semideciduous Forest
Aspilia latissima Malme	Host	Cerrado Semideciduous Forest
Bidens pilosa L.	Host	Agroecosystem, Semideciduous Forest
*Bidens sulphurea (*Cav.) Sch. Bip. N. V.	Host	Cerrado Semideciduous Forest
Salmea scandens (L.) DC.	Nonhost	Cerrado Semideciduous Forest
Tridax procumbens L.	Nonhost	Agroecosystem, Cerrado
Unxia kubitzkii H. Robinson	Nonhost	Semideciduous Forest
Zinnia elegans Jacq.	Host	Cerrado
Lactuceae *Hypochaeris brasiliensis* (Less.) Griseb.	Nonhost	Agroecosystem Cerrado Semideciduous Forest
Mutisieae *Chaptalia integerrima* (Vell.) Burkart	Host	Semideciduous Forest
Plucheae *Pterocaulon virgatum* (Lam.) DC.	Host	Semideciduous Forest

Asteraceae Taxa (Tribes and Species)	Plant Status	Environment
Senecioneae *Emilia fosbergii* Nicolson. N.V.	Host	Agroecosystem, Cerrado
Erechtites hieracifolia (L.) Raf.	Nonhost	Cerrado Semideciduous Forest
Targeteae *Porophyllum ruderale* (Jacq.) Cass.	Host	Agroecosystem, Cerrado Semideciduous Forest
Vernonieae *Cyrtocymura scorpioides* (Lam.) H. Rob.	Nonhost	Cerrado Semideciduous Forest
Vernonia bardanoides Less.	Host	Cerrado
Vernonia cognata Less.	Host	Cerrado Semideciduous Forest
Vernonia polyanthes Less.	Host	Cerrado Semideciduous Forest
Vernonanthura brasiliana (L.) H. Rob.	Nonhost	Cerrado Semideciduous Forest
Vernonanthura chamaedrys (Less.) H. Rob.	Nonhost	Cerrado Semideciduous Forest

Table 1.
Status for Asteraceae species to florivorous flies Tephritinae (Tephritidae, and/or Melanagromyza Hendel 1920, Agromyzinae: Agromyzidae), and environment of occurrence in Dourados region, MS, Brazil.

Nine Tephritinae species from seven genera were obtained: The recovered species were: *Trupanea jonesi*, *Tomoplagia brasiliensis*, *Tomoplagia reimoseri*, *Dioxyna chilensis*, *Xanthaciura unipuncta*, *Xanthaciura biocellata*, *Cecidochares fluminensis*, *Dictyotrypeta* sp. and *Tetreuaresta* sp. form 13 Asteraceae species [7]. From the flower heads of 11 species of plants were also obtained three species of *Melanagromyza* (Agromyzina, Agromizidae) from all three environments (**Table 2**).

In the total were obtained 120,031 flower heads of Astereceae, emerging 2,698 adults of insects: 833 Tephritinae (Tephritidae), belonging to 7 genera and 9 species; 1,089 *Melanagromyza* spp. (Agromyzidae) and 776 parasitoids (Hymenoptera) from the tephritines and agromizines. A total of 374 adults of Tephritinae were reared from the flower heads collected on the Cerrado, 269 from the Semideciduous Forest, and 190 fom the Agoecosystem. From the Agroecosystem seven asteraces were sampled (S = 7), 190 individuals of 4 species (2 Tephritinae / 2 Agromyzinae), emerged. In general, the Agromyzidae were more abundant than the Tephritidae (n = 1,089), but the Tephritinae were more biodiverse (nine species) than the Agromyzids, represented by three species. Some 776 adults of Hymenoptera parasitoids emerged from puparium of both families (Tephritidae and Agromyzidae) of florivorous flies (**Tables 1** and **3**).

The collected flower heads give a biomass of 8,202 grams, being 20,766 (5.7%) of the flower heads infested by FF, corresponding to a biomass of 1,587 g (5.16% of total). The species with the highest infestation rates for Tephritinae were: *Chaptalia integerrima*, with 0.500 fly by flower head (FH) and *Chromolaena ivifolia* with 8.09 fly/g. The lowest indexes occurred in *Sonchus oleraceus*, with 0.002 fly/FH and 0,009 individual/g, and *Pteurocaulom virgatum* with 0.010 fly/FH and 0.095 fly/g. For *Melanagromyza* species (Agromyzinae, Agromyzidae), *Bidens pilosa* was the Asteraceae with the highest rate of infestation: 0.079 fly/FH and 0.584 fly/g. The lowest indexes occurred in *Sonchus oleraceus*, with 0.002 fly/FH and 0.009 fly/g

Asteraceae Species	**Environments**		
	Cerrado	**Semideciduous Forest**	**Agroecosystem (= Mixed Orchard)**
Aspilia latissima	—	*Melanagromyza* sp.3	—
Baccharis triplinervis	*Xanthaciura unipuncta*	—	*Melanagromyza* sp.2
Bidens pilosa	*Melanagromyza* sp.1	*Dioxyna chilensis* *Melanagromyza* sp.1	*Dioxyna chilensis* *Melanagromyza* sp.1
Bidens sulphurea	*Melanagromyza* sp.2	—	—
Chaptalia integerrima	—	*Trupanea jonesi* *Melanagromyza* sp.2	—
Chromolaena arnottiana	*Trupanea jonesi* *Tomoplagia reimoseri* *Xanthaciura unipuncta* *Xanthaciura biocellata* *Cecidochares fluminensis* *Melanagromyza* sp.3	*Dioxyna chilensis* – – – –	– – – – –
Chromolaena ivifolia	*Trupanea jonesi* *Xanthaciura biocellata* *Cecidochares fluminensis*	– – –	– – –
Emilia fosbergii	-	—	*Melanagromyza* sp.2
Eupatorium multicrenulatum	—	*Xanthaciura biocellata*	—
Lourteigia ballotifolia	*Melanagromyza* sp.3		
Porophyllum ruderale	*Trupanea jonesi* *Dioxyna chilensis*	*Trupanea jonesi* *Dioxyna chilensis*	*Trupanea jonesi* *Dioxyna chilensis* *Melanagromyza* sp.1
Praxelis pauciflora	—	—	*Trupanea jonesi*
Sonchus oleraceus		*Melanagromyza* sp.3	
Pterocaulon virgatum	– –	*Xanthaciura biocellata* *Tetreuaresta* sp.	– –
Vernonia bardanoides	*Tomoplagia brasiliensis* *Tomoplagia reimoseri*	– –	– –
Vernonia cognata	*Xanthaciura biocellata* *Melanagromyza* sp.3	*Trupanea jonesi* *Xanthaciura biocellata* *Cecidochares fluminensis*	– – –
Vernonia polyanthes	*Tomoplagia brasiliensis* *Tomoplagia reimoseri* *Dioxyna chilensis* *Xanthaciura unipuncta* *Xanthaciura biocellata* *Dictyotrypeta* sp. *Melanagromyza* sp. 3	*Xanthaciura biocellata* *Tomoplagia reimoseri* *Cecidochares fluminensis* – – *Melanagromyza* sp.3	– – – – – –
Zinnia elegans	*Xanthaciura unipuncta* *Xanthaciura biocellata*	— —	— —

Table 2.
Florivorous fly species (Tephritidae and Agromyzidae) associated with flower heads of Asteraceae (Asterales) species in three environments from the region of Dourados-MS, Brazil (January 2011 to august 2012).

Tephritidae	Cerrado	Semideciduous Forest	Agroecosystem	Total
Trupanea jonesi	17	13	30	**60**
Tomoplagia brasiliensis	16			**16**
Tomoplagia reimoseri	16	5		**21**
Dioxyna chilensis	121	239	160	**520**
Xanthaciura unipuncta	70			**70**
Xanthaciura biocellata	130	8		**138**
Cecidochares fluminensis	3	2		**5**
Dictyotrypeta sp.	1			**1**
Tetreuaresta sp.		2		**2**
Subtotal Tephritidae	**374**	**269**	**190**	**833**
Agromyzidae				
Melanagromyza sp.1	73	767	173	**1,013**
Melanagromyza sp. 2	16	32	15	**63**
Melanagromyza sp. 3	10	3	0	**13**
Subtotal Agromyzidae	**99**	**802**	**188**	**1,089**
Parasitoids (Hymenoptera)	259	271	246	**776**
Subtotal	**732**	**1,073**	**632**	**—**
Total of Insects associated to Asteraceae species		**2,698**		

Table 3.
Abundance of Tephritidae and Agromyzidae (Diptera) sampled in the phytophysiognomies: Cerrado, Semideciduous Forest, and agroecosystem in the three subregions of Dourados-MS, Brazil (January 2011 to august 2012).

(**Table 4**). From *Aspilia latissima* only *Melanagromyza* sp.3 emerged, no tephritines were obtained. By other hand, both taxa of florivorous flies: tephritines and some unidentified species of *Melanagromyza* (Agromyzinae) shared the host species of Asteraceae, simultaneously (**Table 4**).

The association of Tephritinae species with Asteraceae species was compared using a symmetric normalization model, validated by chi-square. This association was highly significant {x^2 = 492.72; g.l = 288; ($p < 0.000$)}, explaining 54.8% of the total results. The Tephritidae correlated with the three phytophysiognomies, and with the Asteraceae species. The association of the species of florivorous fly with species of Asteraceae followed the model of symmetric normalization, also validated by chi-square. This relationship was highly significant {x^2 = 93.407; g.l = 16; ($p < 0.000$)}, explaining 100% of all results. Emerged some parasitoids from Asteraceae, being associated with the florivorous flies. All of them are Hymenoptera, and still wait for identification (**Table 5**).

There was a significant difference between the species *T. jonesi* with *X. biocellata*, *D. chilensis* and *X. unipuncta*, with a lower average of individuals [7]. *Xanthaciura biocellata*, *D. chilensis* and *X. unipuncta* were the most abundant Tephritinae species, resulting in a standard deviation with little variability in relation to the mean (**Table 5**).

The abundance of six avaluated species of florivorous flies in the Semideciduous Forest environment was significantly lower than in the agroecosystem, and the Cerrado environment did not differ from the other phytophysiognomies (**Table 6**).

ASTERACEAE SPECIES	Nunber of Flower heads	Biomass of Flower heads	Abundance: Tephritinae and Agromyzinae	*IL- FF/Flower heads	**IL-FF/g
TEPHRITINAE					
Baccharis triplinervis	400	13	7	0.017	0.538
Bidens pilosa	3,114	424	248	0.079	0.584
Chaptalia integerrima	18	6	9	0.500	1.500
Chromolaena arnottiana	1,750	48	114	0.065	2.375
Chromolaena ivifolia	500	11	89	0.178	8.090
Eupatorium multicrenulatum	1,000	11	2	0.002	0.181
Porophyllum ruderale	6,228	639	310	0.049	0.485
Praxelis pauciflora	100	30	3	0.003	0.100
Pterocaulom virgatum	186	21	2	0.010	0.095
Vernonia bardanoides	500	70	8	0.016	0,114
Vernonia cognata	2,000	25	9	0.004	0.36
Vernonia polyanthes	3,500	165	15	0.004	0.90
Zinnia elegans	70	107	11	0.157	0.102
AGROMIZYDAE					
Aspilia latíssima	61	108	2	0.032	0.0185
Baccharis triplinervis	2,567	168	13	0.005	0.026
Bidens pilosa	3,688	483	1,012	0.274	2.095
Bidens sulphurea	269	65	9	0.033	0.138
Chaptalia integerrima	180	60	14	0.077	0.233
Chromolaena arnottiana	500	10	1	0.002	0.100
Emilia fosbergii	611	98	2	0.003	0.020
Lourteigia ballotifolia	1,668	56	9	0.005	0.160
Porophyllum ruderale	186	21	1	0.005	0.047
Sonchus oleraceus	447	111	1	0.002	0.009
Vernonia cognata	500	4	2	0.004	0.500
Vernonia polyanthes	2,000	104	22	0.011	0.211

**IL- FF / Flower heads = Infestation Level Florivorous Flies by Asteraceae Flower Head.*
***IL-FF / g (mass) of Asteraceae Flower heads.*

Table 4.
Levels of infestation by florivorous flies (Diptera: Tephridae and Agromyzidae) in Asteraceae species sampled in three different ecosystems in the region of Dourados-MS, mid-west of Brazil (January 2011 to august 2012).

The frequency of the three species of the genus *Melanagromyza* (Agromyzidae) was correlated with their host plants. A significant frequency index of *Melanagromyza* spp. was found in *Bidens pilosa*. To the *Melanagromyza* spp. the *"picão-preto"* (a weed), *Bidens pilosa*, was the Asteraceae species with the highest abundance and frequency of *Melanagromyza* sp.1, representing 73.95% of the Agromyzidae in this Asteraceae species. In others Asteraceae species, the frequency of occurrence to *Melanagromyza* spp. was 7% or less (**Table 7**).

In Neotropical Region there are few reports for occurrence of tephritids and other florivorous flies in Asteraceae. There are some studies in order to inventory

Species de Tephritinae	n	Ȳ ± SD*
Trupanea jonesi	31	1.97[a] ± 2.01
Tomoplagia brasiliensis	5	2.20[abcd] ± 1.79
Tomoplagia reimoseri	9	2.3[abc] ± 2.23
Xanthaciura biocellata	22	6.27[be] ± 6.84
Xanthaciura unipuncta	14	5.36[ce] ± 4.98
Dioxyna chilensis	90	5.79[de] ± 5.74

**Kruskal Wallis Test = {P(x > X^2); (α < 0.01)}. The Mean comparison by Mann–Whitney Test at 5%. Equal lower-case letters to vertical or column and upper-case letters equal to horizontal or line, do not differ significantly; n: number or sample size; Ȳ ± SD: Mean ± Standard Deviation*

Table 5.
Tephritinae (Diptera: Tephritidae) associated with the flower heads of Asteraceae (average and standard deviation) in the Dourados-MS region, mid-west of Brazil (January 2011 to august 2012).

Phytophysiognomies	n	Ȳ ± SD*
Semideciduous Forest	68	3.96[a] ± 4.54
Cerrado	67	4.67[ab] ± 5.27
Agro-ecosystem	44	5.75[bc] ± 6.28

**Average Comparison Test (Duncan = 10%), being letters equal to each other do not differ Significantly; n: number or sample size; Ȳ ± SD: Mean ± Standard Deviation*

Table 6.
Number of individuals of Tephritinae (Diptera: Tephritidae) obtained from Asteraceae flower heads in three phytophysiognomies [comparison of means and standard deviation] in Dourados region-MS, mid-west of Brazil (January 2011 to august 2012).

the number of species associated with the flower heads of Asteraceae in South, and Southeast of the Brazil.

Our recent researches in the Brazilian Mid-West have established the fowling relationships among florivorous fly species and Asteraceae flower heads: *Dioxyna chilensis* reared from *Bidens pilosa*; *Tomoplagia reimoseri* of *Chromolaena arnottiana*, *Vernonia bardanoides* and *Vernonia polyanthes*; *Trupanea jonesi* of *Chaptalia integerrima*, *Chromolaena arnottiana*, *Chromolaena ivifolia*, *Porophyllum ruderale*, *Praxelis pauciflora* and *Vernonia cognata*; *Cecidochares fluminensis* in *Chromolaena arnottiana*, *Chromolaena ivifolia*, *Vernonia cognata* and *Vernonia polyanthes*, *Dictyotrypeta* sp. in *Vernonia polyanthes* [7].

Herein we found three species of florivorous Tephritinae, reared from their host plants: *Trupanea jonesi*, *Dictyotrypeta* sp. and *Tetreuaresta* sp. (**Table 1**), not yet reported to Brazil. Two new species (*Dictyotrypeta* sp. and *Tetreuaresta* sp.) were obtained, which will be later described. Additionally, three species of *Melanagromyza* (Agromyzinae: Agromyzidae) were reared from the sampled host astereces (**Table 6**), probably, are also new species.

The most frequent species of Tephritinae were *Trupanea jonesi* and *Dioxyna chilensis* associated with the Asteraceae: *Porophyllum ruderale*, totaling 41.34% of all florivorous fly species. The least frequent Tephritinae were: *Xanthaciura unipuncta* in *Baccharis triplinervis*, and *Xanthaciura biocellata* in *Eupatorium multicrenulatum*. *Dictyotrypeta* sp. and *Tetreuaresta* sp. were species-specific to flower heads of *Vernonia polyanthes* and *Pterocaulon virgatum*, respectively. The other species of Tephritinae were more generalists, being *Xanthaciura biocellata* the most polyphagous of all the Tephritinae.

Asteraceae Species	Environments			Total %
	Cerrado	Semideciduous Forest	Agroecosystem	
Aspilia latissima		*Melanagromyza* sp.3 (1.04%)		1.04
Baccharis triplinervis			*Melanagromyza* sp.2 (1.04%)	1.04
Bidens pilosa	*Melanagromyza* sp.1 (2.08%)	*Melanagromyza* sp.1 (51.04%)	*Melanagromyza* sp.1 (20.83%)	73.95
Bidens sulphurea	*Melanagromyza* sp.2 (3.13%)			3.13
Chaptalia integerrima		*Melanagromyza* sp.2 (5.21%)		5.21
Chromolaena arnottiana	*Melanagromyza* sp.3 (1.04%)			1.04
Emilia fosbergii			*Melanagromyza* sp.2 (1.04%)	1.04
Lourteigia ballotifolia	*Melanagromyza* sp.3 (4.17%)			4.17
Porophyllum ruderale			*Melanagromyza* sp.1 (1.04%)	1.04
Sonchus oleraceus		*Melanagromyza* sp.3 (1.04%)		1.04
Vernonia cognata	*Melanagromyza* sp.3 (1.04%)			1.04
Vernonia polyanthes	*Melanagromyza* sp. 3 (1.04%)	*Melanagromyza* sp.3 (5.21%)		6.25

Table 7.
Frequency of occurrence of Melanagromyza spp. (Agromyzinae, Agromyzidae) associated with Asteraceae species (Asterales) in three phytophysiognomies in Dourados region-MS, mid-west of Brazil (January 2011 to august 2012).

The Asteraceae were more abundant in the Cerrado biome. This same pattern was also accompanied by the species of florivorous fly (N = 374). In the Semideciduous forest there was a highest abundance of *Melanagromyza* species (Agromyzinae), with 802 adults (of three morphospecies), and also of parasitoids (Hymenoptera), being recovered 1,073 individuals.

The Asteraceae *Pterocaulon virgatum* and the tephritine *Tetreuaresta* sp. presented a highly specie-specific relationship. The Asteraceae species with highest abundance of Tephritidae, were: *Baccharis triplinervis*, *Zinnia elegans*, *Eupatorium multicrenulatum*, and *Vernonia polyanthes* that were associated with the tephritines: *Xanthaciura unipuncta*, *Xanthaciura biocellata*, *Dictyotrypeta* sp., and *Cecidochares fluminensis*. The flower heads of *Chaptalia integerrima*, *Praxelis pauciflora*, *Porophyllum ruderale* and *Bidens pilosa* were infested by *Dioxyna chilensis*, and *Trupanea jonesi*. From the host plant *Pterocaulon virgatum* only *Tetreuaresta* sp. emerged.

The most frequent and abundant Tephritinae species in the Cerrado, were: *Xanthaciura unipuncta*, *Tomoplagia brasiliensis*, *Dictyotrypeta* sp., and *Tomoplagia reimoseri*. In the Semideciduous Forest, occurred: *C. fluminensis*, *D. chilensis*, and *Tetreuaresta* sp. The Agroecosystem had the low diversity, occurring only three florivorous flies: *T. jonesi*, *Melanagromyza* sp.1 and *Melanagromyza* sp.2 (**Table 6**).

Some Asteraceae species, such as *Bidens pilosa*, *Porophyllum ruderale*, *Conyza bonariensis*, are invasive plants ("weeds"), that compete with plants grown in agroecosystems. Thus, this study recorded that species of Tephritinae (Tephritidae) and *Melanagromyza* spp. (Agromyzinae, Agromyzidae) feed on the seeds of these invasive plants in their larval phase, having the potential to act in the biological control of these Asteraceae in agrossilvipastoral (agriculture and pasture) areas.

Bidens pilosa, was the species of Asteraceae with greater abundance and frequency of *Melanagromyza* spp., representing 73.95% of the Agromyzidae in this Asteraceae. In the others Asteraceae species, the frequency of occurrence of *Melanagromyza* species was equal to or less than 7% (**Table 6**).

Herein, three species of florivorous tephritines: *Trupanea jonesi*, *Dictyotrypeta* sp. and *Tetreuaresta* sp., are for the first time reported in Brazil. *Dictyotrypeta* sp. and *Tetreuaresta* sp. are two new species that will be later described. Three *Melanagromyza* species (Agromyzinae, Agromyzidae) were recovered from the sampled hosts (**Table 6**), which are also new records and, probably, new species.

The insects that live in plant flowers represent a very sophisticated interaction, because in addition to obtaining physical protection, they obtain a higher quality food (proteins and carbohydrates). This is the first study of trophic interactions between the tephritines, agromizynes, asteraces and parasitoids in flower heads of asteraceae in the Midwest of Brazil.

7. Conclusions and perspectives

1. In the Midwest Brazil occur, at least, 12 species of florivore fly species (9 Tephritinae, and 3 *Melanagromyza*, Agromizinae, Agromyzidae);

2. All Tephritinae and Agromizinae were reared of their Asteraceae host plant flower heads, from three different Biomes (Atlantic Forest, Cerrado, and Agroecosystem);

3. Cerrado is the biome with higher species richness (S = 11) of florivorous fly species, but in the Atlantic Forest occurred higher abundance of their parasitoids (Hymenoptera).

4. Further researches are in need for a better understanding on the resouce partioning between Tephritinae (Tephritidae), *Melanagromyza* spp. (Agromyzinae, Agromyzidae), their association with Asteraceae and their respective hymenopteran parasitoids.

5. Some florivore fly species, such as *Trupanea jonesi*, *Tomoplagia brasiliensis*, *T. reimoseri*, *Xanthaciura biocelata*, *X. unipuncta, Dioxyna chilensis* (Tephritinae, Tephritidae), and *Melanagromyza* sp.1 (Agromyzinae, Agromyzidae), need more research on their biology and behavior to be employed in biological control programs against invasive Asteraceae species.

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Conflict of interest

The authors declare no conflict of interest.

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