

## SEVEN DECADES OF STUDIES WITH ASOPINAE PREDATORS IN BRAZIL (1933●-○2014)

## SETE DÉCADAS DE ESTUDOS COM ASOPINAE PREDADORES NO BRASIL (1933●-○2014)

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**ABSTRACT:** Studies of Asopinæ predators in Brazil were initiated in the 1930's, focusing on the use of these insects in biological control programs. However, the beginning of a period of major contributions to the knowledge on Asopinæ predators started in the year 1986, with the release of the first research results, in the Proceedings of the X Brazilian Congress of Entomology in Maceió, Alagoas, Brazil. In 1991 the first scientific paper was published on the predator *Podisus connexivus* Bergroth [= *Podisus nigrispinus* (Dallas)] (Hemiptera: Pentatomidae), with study of the biological parameters of this predator for alternative feeding of the caterpillars *Bombyx mori* Linnaeus (Lepidoptera: Bombycidae) and larvae *Musca domestica* (Linnaeus) (Diptera: Muscidae). The first master thesis studying Asopinæ predators in Brazil was defended in 1990 at the Federal University of Lavras, which focused was on biology and predatory capacity of *Podisus nigrolimbatus* [= *Brontocoris nigrolimbatus* = *Brontocoris tabidus*] and *Podisus connexivus* [= *Podisus nigrispinus*] in the laboratory. Throughout all these years of research and investigations, advances with Asopinæ predators occurred in several fields that resulted in a better understanding of these insects, making them an effective tool for controlling insect pests in the agriculture and forestry sectors.

**KEYWORDS:** Biology. Biochemistry. Ecology. Mass rearing. Taxonomy. Toxicology.

### INTRODUCTION

Stink bugs of the subfamily Asopinæ are highlighted in biological control programs of agricultural and forest pests because they are generalist predators, feeding on a large number of insects from different orders. Due to characteristics including natural occurrence, aggressiveness and voracity, these insects have been widely studied in Brazil for Integrated Pest Management (IPM) programs. These natural enemies contribute to the population balance of phytophagous insects, mainly defoliating caterpillars, permitting reduced use of pesticides in agricultural systems and planted forests, and consequently the conservation of the environment (DE BORTOLI et al., 2011; TORRES et al., 2006a; VACARI et al., 2007; 2004).

Studies using Asopinæ predators in Brazil were initiated in the 1930's, where the first results of a Brazilian study were published in the paper entitled "Contribution to the biological study of three pentatomids and potential use of these insects to control harmful species" (SILVA, 1933). This article was published in the journal "O Campo" (The Field), Rio de Janeiro, by the researcher Aristóteles Godofredo de Araujo e Silva who studied the biological parameters of the predator Asopinæ *Oplomus* (*Catostyrax*) *catena* (Drury)

feeding on the caterpillar *Actinote pellenea* (Acraeidae) on bonesets (*Eupatorium* sp.); and *patetius* (*sic*) (*Eupodisus*) *mellipes* (Bergroth) and *Alcaeorhynchus grandis* (Dallas) preying on the caterpillar *Dione juno* (Heliconiidae) in passion fruit, with a detailed description of the morphology of these insects.

In 1940, Professor Ângelo Moreira da Costa Lima reported that Asopinæ predators may be considered useful for agriculture, since they generally prey on eggs, larvae and adults of pest insects. The best known species attack larvae of Lepidoptera and eggs, larvae and even adult beetles of the superfamily Chrysomeloidea (COSTA LIMA, 1940).

After these first reports, Asopinæ predators were targeted for study by a group of taxonomists who began a painstaking investigative Systematic and Taxonomy study of the species of this group in Brazil. In 1951, the researcher Romeu de Tella published the second article on Asopinæ predators by a Brazilian in the Journal *Bragantia*, entitled "Bionomic data of a predatory stinkbug", in which the morphological description was presented for the life cycle phases of *Tynacantha marginata* (Dallas) (Heteroptera: Pentatomidae: Asopinæ) (TELLA, 1951).

With the exception of the first article published with Asopinae, whose main focus was to study the biology of predators, including *A. grandis*, and which despite this presents an important morphological description of this insect, all publications in nearly five decades of research considered taxonomy of great importance for the continuation of studies in other segments.

In 1976, for the first time in the X Brazilian Congress on Entomology in Maceió, Alagoas, Brazil, results were presented of a research entitled “Biology of *Podisus* sp. (Pentatomidae: Asopinae)” (MORAES et al., 1976).

In 1990 the first Master’s dissertation was defended on the theme “Biology and predatory capacity of *Podisus nigrolimbatus* Spinola, 1832 and *Podisus connexius* Bergroth, 1861 (Hemiptera: Pentatomidae: Asopinae) in laboratory conditions”, developed at the Federal University of Lavras (UFLA), which focused on predatory stinkbugs (FERNANDES, 1990). The species studied are today referred to as *Brontocoris tabidus* and *Podisus nigrispinus*, respectively.

In 1974, at the Luiz de Queiroz School of Agriculture (Escola Superior de Agricultura Luiz de Queiroz - ESALQ) the first doctoral thesis was defended entitled “Biology of *Thyriniteina arnobia* (Stoll) (Lepidoptera, Geometridae) and observations on the occurrence of natural enemies”, which evaluated the predator Asopinae (*Alcaeorrhynchus grandis*). However, the focus of this thesis was not the stinkbug, but the caterpillar *Thyriniteina arnobia* (Lepidoptera: Geometridae) (BERTI FILHO, 1974). In 1995, at the Federal University of Viçosa (UFV), the first doctoral thesis was defended which dealt specifically with Asopinae predators whose theme was “Artificial diet for *Podisus nigrispinus* (Dallas) and influence on its ability for predation of *Anticarsia gemmatalis* Hubner” (SAAVEDRA, 1995).

Through years of research and investigations, advances with Asopinae predators have resulted in various studies of biology and plant health such as: increasing the number of species reported (THOMAS, 1992), definition and presentation of the main species for biological control programs (PIRES et al., 2009a; THOMAS, 1992), increase in the number of institutions collaborating in this line of research (Federal University of Viçosa - UFV, Federal University of Lavras - UFLA, Federal University of Mato Grosso - UFMT, Federal University of Vales do Jequitinhonha and Mucuri - UFFVJM, Federal University of Paraíba - UFPB, Federal University of Grande Dourados - UFGD, Federal University of

Rio Grande do Sul – UFRGS, Federal University of Minas Gerais – UFMG, Federal University of Pará – UFPA, Federal Rural University of Pernambuco – UFRPE and EMBRAPA), evolution of equipment for mass rearing (ZANUNCIO et al., 1994a) and techniques and form of management (MOLINA-RUGAMA et al., 1998; PELÚZIO, 2012; PIRES et al., 2006; ZANUNCIO et al., 1994a), accommodation and ambiente (MEDEIROS et al., 2003; SANTOS et al., 2005), physiology and internal and external morphology (AZEVEDO et al., 2007; GUEDES et al., 2007; LEMOS et al., 2006; 2005a;b;c; SOARES et al., 2011; SÁ et al., 2013), studies on sensory and (ALDRICH et al., 1997; CORACINI et al., 1999; TORRES et al., 1997a) behavioral aspects (CARVALHO et al., 1995; 1994; FERREIRA et al., 2008; LEMOS et al., 2005d; PIRES et al., 2010a; 2009b; SAAVEDRA et al., 1997; SILVA et al., 2012; VIVIAN et al., 2002), toxicology, with focus on studies on selectivity of insecticides (BATALHA et al., 1995; CASTRO et al., 2013; 2012; GONRING et al., 2003; GUEDES et al., 1992; PIKANÇO et al., 1997; SUINAGA et al., 1996; ZANUNCIO et al., 2003) and herbicides (CAMILO et al., 2012; MENEZES et al., 2012), implementation of Asopinae predators in biological control programs in genetically modified plants, tolerant to glyphosate (LACERDA, 2008), ability to associate predators with parasitoids and entomopathogenic organisms in the control of pests (CARVALHO et al., 2012; HOLTZ et al., 2006a; SOARES, 2010; TORRES; Ruberson, 2008), liberation of these natural enemies (NEVES et al., 2009; PIRES, 2010; TORRES et al., 2002) and breeding of individuals for mass rearing to increase the reproductive potential (PELÚZIO, 2012).

Stinkbugs predators, which are highlighted as biological control agents in Brazil, are of the genera *Brontocoris* and *Podisus* (Pentatomidae: Asopinae), most aggressive and encountered in practically the entire country. Besides species of these genera, *Alcaeorrhynchus*, *Supputius* and *Tynacantha* have species with potential for control of agricultural and forest pests.

The Asopinae species most studied and recommended for biological control in IPM programs in Brazil are *Alcaeorrhynchus grandis* (Dallas), *Brontocoris tabidus* (Signoret), *Podisus distinctus* (Stal), *Podisus nigrispinus* (Dallas), *Supputius cincticeps* (Stal) and *Tynacantha marginata* (Dallas), highlighting *P. nigrispinus* due to its voracity and aggressiveness (De BORTOLI et al., 2011; VACARI et al., 2007; 2004; MENEZES et al., 2013).

The advancement of studies with Asopinae predators in Brazil contributes to the understanding of various aspects related to these insects and makes them an efficient tool for the management of agricultural and forest pests. This study aimed to perform a literature review on Asopinae produced by Brazilian researchers over seven decades of scientific contributions.

## DEVELOPMENT

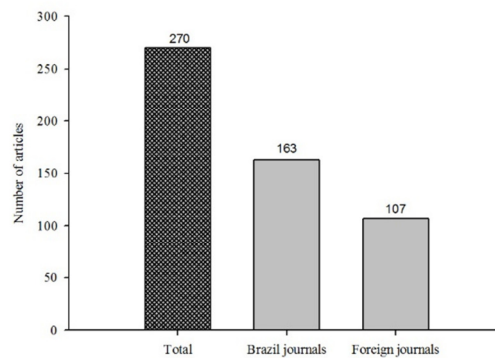
The search and acquisition of publications was performed with aid of library searches of institutions including the Federal University of Mato Grosso, Federal University of Viçosa, Federal University of Lavras, Federal Rural University of Rio de Janeiro, Federal University of Rio de Janeiro (National Museum of the Federal University of Rio de Janeiro), Federal University of Paraná, Federal University of Rio Grande do Sul, Federal University of the Vales do Jequitinhonha and Mucuri, University of São Paulo, Library of Embrapa

Amazônia Oriental, National Agricultural Library, Library of the Oswaldo Cruz Institute, as well as the personal collection of Professor Dr. Jocélia Grazia (UFRGS).

## Historical contextualization

Brazilian researchers have contributed to studies on Heteroptera: Asopinae over the last 70 years. Since 1933, 270 scientific papers were published with predatory stinkbugs. Of this total, 163 were published in Brazil journals and 107 in foreign journals, corresponding to 60.38 and 39.62%, respectively (Figure 1).

This scientific production is mainly due to the efforts of researchers from Brazilian Federal Universities and Embrapa. The UFV is a Brazilian institution with great contribution to this line of research and is considered a great multiplier of these studies. This has been done directly with scientific production and training of researchers, now dispersed throughout Brazil and abroad, continuing studies on Asopinae.

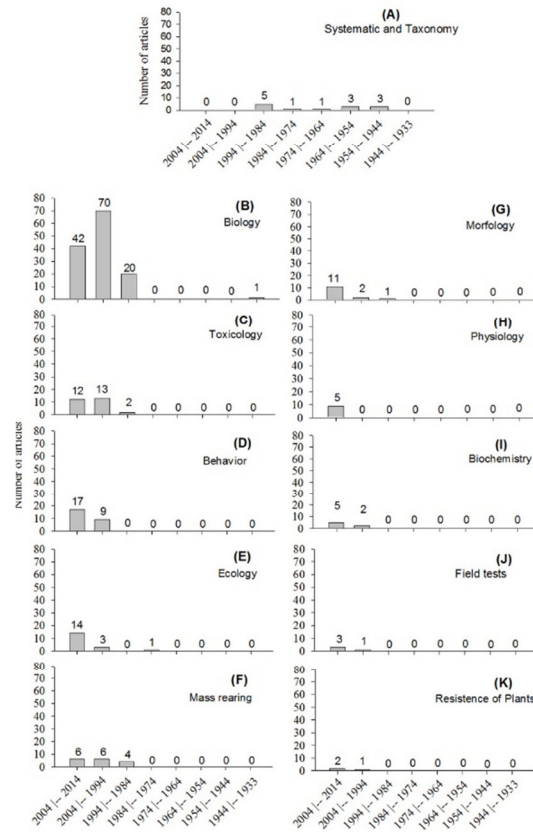


**Figure 1.** Total number and number of Brazilian and foreign journals in which articles were published by Brazilian researchers over seven decades of research with Asopinae predators.

## The importance of Systematics and Taxonomy on the history of research with Asopinae in Brazil

The Systematics and Taxonomy of Asopinae is highlighted at the beginning of studies with these predators and for practically five decades contributed decisively to the establishment of this line of entomological research. With the exception of the first article (1933), all publications until 1982 were along these lines, and only in 1982 was the study published entitled “Bioecology of *Podisus nigrolimbatus* Spinola (Heteroptera: Pentatomidae), predator of *Pyrrhalta luteola* (Mülher) (Coleoptera: Chrysomelidae)” in an different from taxonomy

(ARTOLA et al., 1982). The scientific contribution provided by systematists and taxonomists totals 13 papers, corresponding to 4.81% of the scientific contribution with Asopinae produced by Brazilian researchers over these 70 years (Figure 2A). This line of research was fundamental for understanding this group of insects by identifying the main species occurring in Brazil (BUCKUP, 1961; 1960; 1952; COSTA LEITE, 1957; COSTA LIMA, 1940; GRAZIA et al., 1987; 1985; 1980; LIMA, 1948; SILVA, 1965; TELA, 1951). The knowledge generated was key to the development of applied studies, which were and continue to be developed.



**Figure 2.** Retrospect per research area over 70 years of studies with Asopinae predators in Brazil.

**Scientific contributions in the field of biology (biological and reproductive parameters)**

Studies on the biology (biological and reproductive parameters) of Asopinae predators were the most numerous, totaling 133 publications over these 70 years of research and correspond to 49.26% of the scientific contribution (Figure 2). Of this total, only the article by SILVA (1933), a pioneer on these predators in Brazil, was not produced in the last three decades (1984-1994; 1994-2004; 2004-2014) (Figure 2B). The biology of these predators was really developed after 1984.

Some publications in this area can be considered classics due to the relevance of their contributions. Among these, the works involving the use of artificial diets can be highlighted (Saavedra et al 2001; 1999; 1996; 1995; 1992a; 1992b), which were important for understanding the nutritional adequacy of predators produced on a large scale. Their focus was to obtain a food capable of substituting the natural and alternative preys, which makes rearing of the stinkbug predator an onerous process. Also in this line of study, a positive contribution was knowledge on the beneficial effects of using plant material (plant leaves) as a

food supplement for these insects, which can improve the biological and reproductive parameters of these predators (ASSIS JR. et al., 1998; MOREIRA et al., 1997; OLIVEIRA et al., 2002).

The studies showing the effects of climate conditions on the biology of Asopinae also allowed for understanding the ideal temperature conditions, as well as the extreme limits (maximum and minimum) that compromise the reproduction and development of these insects. These studies may aid in the implementation of biological control programs with Asopinae, since thermal requirements can predict the unfeasibility of such programs in certain locations in Brazil and worldwide (DIDONET et al., 1995; MEDEIROS et al., 2004; 2003; SANTOS et al., 2005; TORRES et al., 1998).

The justification for this large number of publications in the field of biology of Asopinae may be explained by several factors, however, principally due to the fact that these predators have generalist feeding habits, which permitted the generation of large amounts of information in basic studies. The need to understand factors such as adequate food conditions (use and adequacy of natural, alternative and artificial diets and food

supplements) resulted in performance improvements of mass rearing in the laboratory and in the field (PIRES et al., 2010a; SAAVEDRA et al., 1997; 1996; VACARI et al., 2007; ZANUNCIO et al., 2001; 1996; ZANUNCIO et al., 1993a; 1991).

### Scientific contributions in the field of Toxicology

Research on the toxicology of insecticides, seeking to understand the effects of chemical compounds on Asopinae resulted in publication of 27 scientific articles by Brazilian researchers, corresponding to 10.00% of production on Asopinae in Brazil (Figure 2C). The first article published on this subject was in 1992, where the subject of the study was “Selectivity of the insecticides deltamethrin, fenvalerate and fenitrothion for *Podisus connexivus* Bergroth [= *P. nigrispinus*] (Heteroptera: Pentatomidae)” (GUEDES et al., 1992).

From this work, the main segment of research sought to determine aspects associated with effects of insecticides on the predator (CASTRO et al., 2012; PEREIRA et al., 2009; PICANÇO et al., 1997; TORRES; RUBERSON, 2004; TORRES et al., 2003; 1999; ZANUNCIO et al., 2003; 1993b), which can be regarded as basic research, since from these results other studies with wider applicability were initiated. These include studies on selectivity of insecticides, which aim to understand compounds with toxicological effect on pests, but which preserve the natural enemy (GONRING et al., 2003; SUINAGA et al., 1996; TORRES, 2012; ZANUNCIO et al., 1998). This information contributed to the implementation of management tactics, such as chemical control associated with the use of Asopinae predators, which can increase efficiency for IPM in the agriculture and forestry sectors (BATALHA et al., 1995; CASTRO et al., 2013; 2012; GUEDES et al., 1992; PICANÇO et al., 1997; ZANUNCIO et al., 2003).

In this line of research, researchers confirmed the selectivity of insecticides by evaluating mortality or otherwise of non-insect predators. Moreover, they showed the ability of predators to develop and reproduce when submitted to sub-lethal doses of chemicals as one of the important milestones for toxicology research in Brazil (LEMONS et al., 2005c; MAGALHÃES et al., 2002; ZANUNCIO et al., 2013; ZANUNCIO et al., 2005). Hormesis, where a determined concentration of a chemical compound with insecticide characteristics promotes a positive responses in the biology of predators, was a big surprise to researchers in the area (ZANUNCIO et al. 2011b; ZANUNCIO et al. 2003).

The discovery that herbicides used in agroecosystems for weed control and which act on metabolic pathways that insects do not have could be toxic to natural enemies led to the need to test the selectivity of these compounds against Asopinae. Negative effects on the development, survival and reproduction of Asopinae predators exposed to commercial herbicide concentrations were reported (CAMILO et al., 2012; MENEZES et al., 2012). These studies led to a reflection on the need to align, through the selection of products less aggressive to natural enemies, Weed Management with IPM.

### Scientific contributions in the field of investigating behavioral aspects

The main focus of this line of research was behavioral aspects of Asopinae predators, with the publication of 26 articles on these predators in Brazil, accounting for 9.63% of scientific production (Figure 2D). Dissemination of the results of these surveys was initiated in 1994 with publication of the article entitled “Pace of mating behavior and sexual activity of *Podisus connexivus* Bergroth [= *P. nigrispinus*] (Heteroptera: Pentatomidae: Asopinae) (CARVALHO et al., 1994a).

From these works, studies on this subject have shown the attack strategy of Asopinae predators on pests, defense mechanisms of herbivores in predation conditions and acceptance of food in the form of natural or alternative prey or artificial diets (CARVALHO et al., 1995; 1994a; CORACINI et al., 1999; FERREIRA et al., 2008; GROSMAN et al., 2005; LEMOS et al., 2005d; PIRES et al., 2009b; SAAVEDRA et al., 1997; SILVA et al., 2012; SOARES et al., 2009; VIVIAN et al., 2002). This helped to understand the occurrence of cannibalism in mass rearing and reduced losses caused by this behavior (PIRES et al., 2010a).

Observations on the behavior of courtship and copulation helped to understand aspects associated with the acceptance or rejection by his co-specific female (CARVALHO et al., 1994; MENDONÇA et al., 1997) and provided information on the mating dynamics of these insects. Thus, it was discovered that the reproductive success of *P. nigrispinus* is associated with conducting multiple and lasting copulations (RODRIGUES et al., 2008; SOARES et al., 2011).

Other papers on this subject provide detailed information on the defense of prey against the predator (PIRES et al., 2009b; SOARES et al., 2009; ZANUNCIO et al., 2008) and that temperature influences the predation ability of

Asopinae (AZEVEDO et al., 1999a; b). Others evaluated the behavior of searching for prey (GROSMAN et al., 2005), attack and predation (FERREIRA et al., 2008; LEMOS et al., 2005d; VACARI et al., 2012) and feeding in function of prey density (OLIVEIRA et al., 2008; VIVIAN et al., 2002). This improved the conditions for delivery of food in mass rearing, where excess food can impair nutrition of predators because they present extra-oral digestion and the beginning of food intake occurs sometime after attack of the prey. Thus, excessive supply of food causes the insect, which has predatory instincts, to constantly attack the moving prey so that it spends too much time performing this activity without eating properly.

### Scientific contributions in the Ecology field

Studies on ecological interactions of Asopinae contributed to the production of 18 scientific articles throughout these 70 years of studies in Brazil, corresponding to 6.67% of production (Figure 2E).

The first article on ecology published by a Brazilian researcher was in 1982, entitled "Bioecology of *Podisus nigrolimbatus* Spinola (Heteroptera: Pentatomidae) predator of *Pyrrhalsa luteola* (Müller) (Coleoptera: Chrysomelidae)". This study demonstrated the predation ability of this species on a chrysomelid (ARTOLA et al., 1982). From these results, other studies contributed to the understanding of issues such as the interaction of Asopinae eggs with parasitoid wasps (ZANUNCIO et al., 2000), which may hinder the implementation of biological control programs using these natural enemies. The predator studied is today known as *Brontocoris tabidus*. On the other hand, association of the Asopinae predator with a parasitoid of the genus *Trichogramma* showed satisfactory results. There was no emergence of *Trichogramma* adults, but the viability of eggs of Asopinae was not affected, therefore the associated use of these two natural enemies may be recommended (HOLTZ et al., 2006a). The interaction of *P. nigrispinus* with the parasitoid *Palmistichus elaeisis* (Delvare; LaSalle) (Hymenoptera: Eulophidae) was also observed in the laboratory, with results indicating that this predator is able to recognize, through chemical and morphological signals, previously parasitized prey and avoid them, reducing intraguild predation rates in the field (SOARES, 2010).

Other ecological studies were performed with life tables that sought to provide information on the population dynamics of a species, highlighting issues such as mortality rates and estimates of breeding in age intervals of a

population under specific conditions (MEDEIROS et al., 2003b; MOREIRA et al., 1995; VACARI et al., 2009; ZANUNCIO et al., 2006).

Studies on the response of Asopinae predators reared in the laboratory with alternative prey (*Tenebrio molitor* pupae) led researchers to question the success of this natural enemy in the field, since it could lose the ability to locate its natural prey. This led to the development of studies to verify localization capabilities of these prey in the field. Results showed that Asopinae expressed greater preference to move in the direction of eucalyptus plants injured by the attack of *Thyrintea arnobia* in relation to those semi-injured (HOLTZ et al., 2006b).

The relationship of Asopinae predators with bacteria was also studied. The bacterium *Bacillus thuringiensis*, used in transgenic cotton plants, was expressed in the predator Asopinae which fed on the caterpillar that attacked the plant, but caused no negative effect on the predator (TORRES; RUBERSON, 2008; TORRES et al., 2006b). However, long-term effects of this interaction indicated decrease in offspring of females of *P. nigrispinus* over generations, resulting in a lower population growth rate for those that daily consumed the infected larvae (GOULART et al., 2010). Effects of the fungi *Metarhizium anisopliae* (Metsch.) Sorok. and *Beauveria bassiana* (Bals.) Vuill., on the Asopinae predator were satisfactory and they can be used together. Nymphs of the predator had higher mortality when exposed to *M. anisopliae*, but adults were not affected (FRANÇA et al., 2006).

With regard to the host plant, the predator *B. tabidus* showed no preference among the tested plants (*Amaranthus lividus*, *Gossypium hirsutum* and *Phaseolus vulgaris*), which favors its occurrence in different ecosystems using plants as a source of water and possibly nutrients (COELHO et al., 2009).

### Scientific contributions in Mass Rearing studies

Contributions to the knowledge and conduct of mass rearing of Asopinae predators throughout these 70 years of research in Brazil was presented in a total of 16 articles published by Brazilian researchers, corresponding to 5.93% of publications (Figure 2F). The first article published on this subject was in 1992, whose theme was "Methods for rearing of Hemiptera predators of caterpillars" (ZANUNCIO et al., 1992).

Studies on mass rearing methods have permitted improved management of these insects (DE BORTOLI et al., 2011; PIRES et al., 2006;

TORRES et al., 2006a; 1997b; ZANUNCIO et al., 2011a; 1994a; 1994b) and development of equipment and techniques for improved production of these natural enemies with higher quality and lower cost (OLIVEIRA et al., 2001; OTUKA et al., 2006; PIRES et al., 2009a; ZANUNCIO et al., 1994a).

Currently, companies operating in the forest sector, mainly with *Eucalyptus* spp., use Asopinæ predators as biological control agents against defoliating caterpillars, which can aid in reducing mega populations of these pests and also reduce the use of insecticides.

The advances acquired from studies in this sector have improved quality and increased production of these insects in the laboratory for subsequent release during periods of Lepidoptera defoliator outbreaks. The company Vallourec Florestal annually produces about 500,000 adult predators of the species *Podisus nigrispinus* in conditions of release, for use in their plantations, which can control or even prevent outbreaks of these pests.

#### **Scientific contributions in the field of Morphology**

Studies on the morphology of Asopinæ predators resulted in the publication of 14 scientific articles produced by Brazilian researchers, corresponding to 5.19% of scientific production with Asopinæ (Figure 2G). The first work in this line of study was published in 1994, entitled “Morphological characterization of the sex pheromone gland of the Neotropical predator *Podisus nigrispinus* (Dallas)” (CARVALHO et al., 1994b).

From this research, the studies on this subject allowed for understanding details on the effects of the diet (CUNHA et al., 2012; LEMOS et al., 2010a; b; 2005a; b; MOREIRA et al., 1996) and the methods of its administration (FIALHO et al., 2009), affecting the internal morphology of these insects. Studies on the possibility of mating (copulation duration and successive copulations) provide morphological changes of the spermatheca and ducts showed that the spermatheca of Asopinæ presented elastic characteristics and that the ducts were not affected (“swollen”) after 30 min copulations (RODRIGUES et al., 2008). Morphology of the spermatozoa of Asopinæ was observed and results may help systematists and taxonomists identify insects of this group (ARAÚJO et al., 2011).

#### **Scientific contributions in the field of Physiology**

Studies on physiological issues of Asopinæ resulted in publication of nine scientific articles by Brazilian researchers representing 3.33% of production during these 70 years of research (Figure 2H). The first results published in this line of research occurred in the last decade (2004–2014), in 2004, with the work entitled “Weight gain of the predator *Podisus distinctus* (Heteroptera: Pentatomidae) in combinations with the prey *Tenebrio molitor* (Coleoptera: Tenebrionidae) y *Musca domestica* (Diptera: Muscidae)”, where the researchers verified weight gain of the predator Asopinæ in function of different combinations of alternative prey (MATOS-NETO et al., 2004). In this line of research, other studies have shown that the type of diet is an important nutritional factor to increase the body weight of Asopinæ (LEMOS et al., 2009a; b).

Other studies has shown that the physiological response of Asopinæ predators, through weight gain and rate of consumption relative to the cotton bollworm *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae), is inversely proportional to the amount of food offered (PEREIRA et al., 2008).

Alterations in the reproductive structures of males and females of *B. tadius* were also observed in insects of different ages and diets, showing that those ingesting plants showed morphologic changes in the reproductive tract (LEMOS et al., 2011; 2010c).

#### **Scientific contributions in the field of Biochemistry**

Biochemistry fills a large gap and contributes substantially to the knowledge generated by research with Asopinæ predators. This line of research further aids in clarifying questions since the conclusions of several studies showed the need for further clarification. Studies involving biochemistry associated with predatory stinkbugs resulted in the publication of seven articles by Brazilian researchers over the 70 years, corresponding to 2.59% of production (Figure 2I). The first article published on this subject was in 1997, entitled “Extract of the pheromone gland in attracting and stimulation of feeding of *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stal)”, where the effect of the extract from dorsal-abdominal glands, producers of sex pheromone of *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae), was observed when added to artificial diet as an attractant and feeding stimulant (TORRES et al., 1997a).

In this line of research, other studies have characterized the enzyme pattern of the content of salivary glands and the midgut of the predator *Brontocoris tabidus*, indicating that this insect may be considered an obligate zoophytophagous species (AZEVEDO et al., 2007; GUEDES et al., 2007; OLIVEIRA et al., 2006). Regarding the same subject, other studies have shown that Asopinae predators present a “standard” enzyme in the salivary gland in function of the feeding behavior. The presence of pectinase can be observed in phytophagous species, phospholipase and trypsin as indicators of insects adapted to predation, and amylase and peptidase for omnivorous insects (phytophagous and predators) (TORRES; BOYD JR., 2009).

A study with the predator *P. nigrispinus* showed that its enzymes differ along the anterior, middle and posterior regions of the midgut (FIALHO et al., 2013).

### Scientific contributions in the field investigating Field Tests

Field tests resulted in the production of four articles with Asopinae by Brazilian researchers, corresponding to 1.48% of scientific production (Figure 2J).

The first study published was “Field tests of predaceous pentatomid pheromones and semiochemistry of *Podisus* and *Supputius* species (Hemiptera: Pentatomidae: Asopinae)” (ALDRICH et al., 1997).

Other studies have been performed to verify the dispersion ability of nymphs of the predator *P. nigrispinus* in greenhouses (TORRES et al., 2002); and of adults with sliced or intact wings, seeking to understand the dispersion ability and effect of these conditions on the number of eggs per female of the insects (NEVES et al., 2009).

Pre-release strategies can provide insects used in the field with a faster response in foraging activity. Some care must be taken with respect to this period, since long periods without feeding can cause losses in mass rearing by cannibalism (PIRES et al., 2010a).

### Scientific contributions in the field investigating Resistance of Plants

Studies focusing on understanding the mechanisms that give the plant the ability to defend itself (plant resistance), with Asopinae predators involved in this interaction, resulted in the production of three scientific articles by Brazilian researchers, corresponding to 1.11% of

contributions in these 70 years of research (Figure 2K).

The first article published on this line of research was in 2000 with the theme “Tritrophic interaction between *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae), *Eucalyptus* and caterpillars of *Thyrinteina arnobia* (Stoll) (Lepidoptera: Geometridae): I- visitation”. In this work it was found that the presence of caterpillars on the plant resulted in a greater number of visits by the predator, which may be due to release of kairomones in interaction of the host plant with the herbivore (CAVALCANTI et al., 2000).

After this study, Brazilian researchers focused on studies regarding the response of the predator Asopinae to the plant defense induced by the herbivore (HOLTZ et al., 2008), and in studies on antibiosis showed that the predator is not fully adapted to the exotic host plant, as demonstrated in its biological and reproductive characteristics (HOLTZ et al., 2010).

### CONCLUSIONS

In the decades of 1994-2004 and 2004-2014, numerous lines of study with Asopinae were highlighted involving various segments.

The importance of these two decades may be due to an increased number of researchers, multiplying forces and therefore increasing the number of publications on Asopinae. Technological development of equipment and techniques is another factor related to these advances, by allowing better investigative and thorough conditions in the studies. Equipment such as high-precision microscopes, scanning electron microscopy, olfactometers, chromatographs, microtomes, etc., along with easily and rapid access to the latest technologies through the global network of computers, has contributed to constant improvement in quality of the research and permitted that results are accessible to scholars and professionals.

Even with the many years of research, predatory stinkbugs of the subfamily Asopinae appear to be the most well studied, especially regarding field research, including dynamic dispersion, migration and foraging of these insects, and the expanded use of these predators in the biological control of agricultural and forestry pests.

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**RESUMO:** Estudos dos predadores Asopinae no Brasil foram iniciados na década de 1930 focando o uso desses insetos em programas de controle biológico. Entretanto, o início de um período de grandes contribuições sobre o conhecimento de predadores Asopinae iniciou-se a partir do ano de 1986, com a divulgação do primeiro resultado de pesquisa, nos Anais do X Congresso Brasileiro de Entomologia em Maceió, Alagoas. Em 1991, foi publicado o primeiro artigo científico sobre o predador *Podisus connexivus* Bergroth, 1891 [= *Podisus nigrispinus* (Dallas, 1851)] (Hemiptera: Pentatomidae), com o estudo dos parâmetros biológicos desse predador em alimentação alternada de lagartas de *Bombyx mori* Linnaeus, 1758 (Lepidoptera: Bombycidae) e larvas de *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae). A primeira dissertação de mestrado com predadores Asopinae no Brasil, foi defendida em 1990, na Universidade Federal de Lavras, onde o foco foi a biologia e a capacidade predatória de *Podisus nigrolimbatus* [= *Brontocoris nigrolimbatus* = *Brontocoris tabidus*] e *Podisus connexivus* [= *P. nigrispinus*] em laboratório. Ao longo de todos esses anos de investigações, os avanços com predadores Asopinae ocorreram em vários ramos que resultaram em um melhor entendimento desses insetos, tornando-os uma eficiente ferramenta no controle de insetos pragas nos setores agrícola e florestal.

**PALAVRAS-CHAVE:** Biologia. Bioquímica. Ecologia. Criação massal. Taxonomia. Toxicologia.

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