

Nevertheless, in the case of SCC treatment alone, the frequency of observed lethal frequency did not decrease; it actually increased slightly. However, when besides the SCC there was exposure to radiation, a slight increase was obtained on the frequency of this kind of genes. On the remaining comparisons, to see the effect of chlorophyllin there are no possible significant differences.

The fact that there was no inhibition effect seen on the previously obtained sex-linked recessive lethals test matches with the effect observed by us on the second chromosome, as a result of this assay and with the one observed on the third chromosome on somatic cells. All the above show a similar action of SCC among sex and autosomal chromosomes against gamma radiation effect.

In virtue of the above data, we can conclude that chlorophyllin's action as pre-treatment against gamma radiation has no response that benefits a protective or inhibiting action as it also happens on sex chromosomes, which indicates chlorophyllin does not work on sex nor autosomal chromosomes against gamma radiation.

Acknowledgments: The author gratefully acknowledges the continuous support of the authorities of the Instituto Nacional de Investigaciones Nucleares during the course of my activities. To Dr. Emilio Pimentel who kindly provided Chlorophyllin.

References: Cruces, M.P., E. Pimentel, and S. Zimmering 2003, *Mutation Res.* 536: 139-144; Graf, U., E. Wurgler, and A. Katz 1984, *Environ. Mutagen.* 6: 153-188; Guerrero, L., 2004, (Thesis), Benemérita Universidad Autónoma de Puebla; Merriam, J., and W. Fyffe 1972, *Mutation Res.* 14: 309-314; Olvera, O, S. Zimmering, M.P. Cruces, E. Pimentel, C. Arceo, J. Guzmán, and M. De la Rosa 1997, In: *Food Factors for Cancer Prevention*. (Ohigasshi, Osawa T. Terao. J. Watanabe S. y Yoshikawa T., eds.). Springer-Verla, Tokio. 567-571; Pimentel, E., M.P. Cruces, and S. Zimmering 1999, *Mutation Res.* 446: 189-192; Pimentel, E., M.P. Cruces, and S. Zimmering 2000, *Mutation Res.* 472: 71-74; Wallace, B., 1956, *J. Genet.* 54: 280-293; Zimmering, S., O. Olvera, M.E. Hernández, M.P. Cruces, C. Arceo, and E. Pimentel 1990, *Mutation. Res.* 245: 47-49.



What happens when exotic species arrive in a new area? The case of drosophilids in the Brazilian Savanna.

Leão, B.F.D.¹, F. Roque², P.H.M. Deus², and R. Tidon¹. ¹Instituto de Biologia, Universidade de Brasília, Brasília, DF – BRAZIL; ²Instituto Federal de Brasília, Planaltina,

DF – BRAZIL; Corresponding author: rotidon@pq.cnpq.br

Abstract

Drosophilids have been widely used as models in scientific research, including the area of biological invasions. In the past two decades, three exotic species of these flies have arrived in the Neotropics. *Zaprionus indianus* was first detected in 1999, and after two years it was widely distributed throughout the Neotropics. Currently, it dominates drosophilid assemblages under specific environmental conditions. *Drosophila suzukii* and *D. nasuta* were recorded in southern Brazil in 2013 and 2015, respectively, and have rapidly expanded their distribution northward. In this paper, we describe the temporal variations in these two recently introduced species in the Brazilian Savanna, a two million-km² biome located in the center of South America, where we have been regularly collecting drosophilids since 1998. *Drosophila nasuta* and *D. suzukii* were first detected in the Brazilian Savanna in December 2013. Two years after their arrival, *D. nasuta* seems to have succeeded in colonizing forests, reaching peak abundance in the rainy season, whereas *D. suzukii* remains rare. We conclude that these exotic species differ in their ability to establish viable populations in natural environments and recommend monitoring them to understand the dynamics of the early stages of biological invasion.

Introduction

The world is becoming biotically homogenized, with cosmopolitan taxa replacing geographically restricted species (McKinney and Lockwood, 1999). In Brazil, *ca.* 300 species of neotropical drosophilids coexist with 12 species from other biogeographical domains (Tidon *et al.*, 2016). These exotic species have probably been arriving since at least the 16th century, transported by ships and airplanes. However, in the late 20th century, the establishment and spread of *Zaprionus indianus* Gupta (Vilela, 1999) caught the attention of the scientific community. In addition to economically impacting fig plantations, it provided an informative model system for understanding the ecological and evolutionary processes of invasions (Gilbert *et al.*, 2016).

Drosophila suzukii Matsumura and *D. nasuta* Lamb have arrived in the Neotropics in the 21st century. The former is a widespread species known as the spotted-wing *Drosophila* due to the prominent dark spot on the wings of males (Walsh *et al.*, 2011). The female has a long and narrow ovipositor with many teeth that is used to infest soft-skinned fruit crops, causing enormous economic damage on commercial plantations (Walsh *et al.*, op cit.). *Drosophila suzukii* is endemic to southeastern Asia and has spread to western regions; it was found in Hawaii in the early 1980s and more recently in North America and Europe, where it has reached invasive pest status (Asplen *et al.*, 2015). In Brazil, it was first detected in the southern region in February 2013 (Deprá *et al.*, 2014). *Drosophila nasuta* is a tropical species characterized by a brownish stripe on the pleurae, an iridescent silvery-whitish frons, and a row of cuneiform setae on the first femur (Vilela and Goñi, 2015). It probably originated in southeastern Asia (Kitagawa *et al.*, 1982) and spread by passive transportation to Africa, the Oriental region, the Pacific Islands, and western North America (David and Tsacas, 1981; Brake and Bächli, 2008). In Brazil, it was first recorded in the southeastern region in March 2015 (Vilela and Goñi, op cit.).

The Brazilian Savanna, locally known as Cerrado, is the second-largest South American biome. Its vegetation is highly variable on the well-drained interfluvies, while gallery forests follow the watercourses (Oliveira and Marquis, 2002). The predominant vegetation type, called *cerrado sensu stricto*, harbors a unique array of drought- and fire-adapted plant species. The climate of the region is seasonal with an average annual rainfall of 1500 mm concentrated between November and March (Eiten, 1972), and mean temperatures range from 22°C to 27°C. The drosophilid communities in the Brazilian Savanna have been intensively studied since 1998 and currently include 122 Neotropical and eleven exotic species (Roque *et al.*, 2016).

Here, we provide the first record of *Drosophila nasuta* in the Brazilian Savanna and describe temporal fluctuations in *D. suzukii* and *D. nasuta* populations at two sites in this biome. Therefore, this study contributes to the understanding of exotic species establishment in new areas.

Materials and Methods

The flies were collected in two areas near Brasília, the capital of Brazil. The first area was the IBGE Ecological Reserve (IBGE) (15°56'S; 47°53'W), where ten sites were sampled bimonthly. The collections occurred from October 2013 to April 2016 (from five sites in gallery forests) and from October 2013 to February 2016 (from five sites in *cerrado sensu stricto*). At each site, nine retention traps (Roque *et al.*, 2011) were distributed in three non-spatially autocorrelated sample units (SUs), corresponding to a 1,345-trap sampling effort. The second sampled area was a gallery forest located approximately 60 km from IBGE at the *Campus Planaltina* of the *Instituto Federal de Brasília* (PLA) (15°38'S; 47°41'W). This forest was sampled monthly from September 2015 to April 2016 using five retention traps (Roque *et al.*, op cit.) in each collection expedition, resulting in a total 40-trap sampling effort. The traps were baited with bananas fermented with dried baker's yeast (*Saccharomyces cerevisiae* Hansen) and left in the field for four consecutive days.

Captured flies were stored in 70% ethanol and identified using taxonomic keys and descriptions. Based on their biogeographical origin, all specimens were classified into four categories: *D. suzukii*, *D. nasuta*, other exotic species (EXO), and native Neotropical species (NEO). Voucher specimens were deposited in the Collection of the *Laboratório de Biologia Evolutiva* of the *Universidade de Brasília* and the *Laboratório de Biologia Animal* of the *Instituto Federal de Brasília* (*Campus Planaltina*).

Table 1. Abundance of drosophilids in gallery forest and *cerrado sensu stricto* of the IBGE Ecological Reserve between October 2013 and April 2016. NEO: Neotropical species, EXO: other exotic species. Gray columns indicate the core rainy season.

	2013		2014					
	Oct	Dec	Feb	Apr	Jun	Aug	Oct	Dec
Forest IBGE								
NEO	26	654	11080	4379	323	110	32	123
EXO	25	441	1613	146	34	1948	326	411
<i>D. suzukii</i>	0	0	0	0	0	0	0	0
<i>D. nasuta</i>	0	1	1	0	0	0	0	0
Total forest	51	1096	12694	4525	357	2058	358	534
Cerrado sensu stricto IBGE								
NEO	27	375	1847	318	246	80	54	397
EXO	14	10685	2133	114	118	156	59	3053
<i>D. suzukii</i>	0	3	0	1	0	0	0	5
<i>D. nasuta</i>	0	0	0	3	0	0	0	0
Total cerrado	41	11063	3980	436	364	236	113	3455

(Table 1 continues), Last column represents totals for each species in each region.

2015							2016		Total
	Feb	Apr	Jun	Aug	Oct	Dec	Feb	Apr*	For each species 2013- 2016
Forest IBGE									
NEO	2488	17567	1363	205	53	80	2455	348	41286
EXO	164	367	123	1581	258	940	298	31	8706
<i>D. suzukii</i>	0	10	1	0	0	0	0	0	11
<i>D. nasuta</i>	0	6	0	0	0	228	260	8	504
Total forest	2652	17950	1487	1786	311	1248	3013	387	50507
Cerrado sensu stricto IBGE									
NEO	311	2454	397	198	40	78	567	-	7389
EXO	2337	3093	105	329	47	4415	172	-	26830
<i>D. suzukii</i>	14	12	0	0	0	6	0	-	41
<i>D. nasuta</i>	0	2	0	0	0	4	6	-	15
Total cerrado	2662	5561	502	Aug	87	4503	745	-	34275

Results and Discussion

Among the 86,658 drosophilids collected, 52 specimens were identified as *Drosophila suzukii* (Table 1). The male collected at IBGE in December 2013 by Paula *et al.* (2014) was the first record of this species in the Brazilian Savanna, but in reviewing the collections made by these authors, we identified two female

spotted-wing *Drosophila*. Therefore, ten months after the first record in South America (Deprá *et al.*, 2014), we found *D. suzukii* in the Brazilian Savanna. Since then, periodic collections have caught only a few individuals, primarily during the rainy season and in savanna vegetation.

Benito *et al.* (2016), based on the thermal requirements of the spotted-wing *Drosophila*, suggested that it prefers a moderate climate. In fact, the temperate southern region of the country is where invasive populations are spreading and causing fruit production losses. According to scenarios modeled by these authors, most of the Brazilian Savanna is not favorable for the establishment of this species except the area surveyed in the present study. It is worth noting that *D. suzukii* females survived an average of 20 or more days at 94% relative humidity (RH) in the laboratory, whereas below 20% RH they could not reproduce (Toshen *et al.*, 2016). As rains in this tropical biome are heavily concentrated between November and March, the stressful dry seasons probably cause local extinctions of *D. suzukii*. On the other hand, if Southern immigrants transported by the fruit trade arrive during the rainy season, these propagules will be able to establish new populations. Therefore, it is too early to say whether *D. suzukii* will establish in central Brazil. Continued monitoring of drosophilids in preserved and converted areas will contribute to answering this question.

We primarily captured *Drosophila nasuta* in forests and throughout the rainy season (Tables 1 and 2). The first five specimens were collected in IBGE in December 2013 and early 2014, but we were not able to identify them until after the publication of the paper by Vilela and Goñi (2015). These authors recorded *D. nasuta* in southeast Brazil (São Paulo city) in March 2015 and provided an accurate species description that allowed us to identify it among our samples. The first peak in the abundance of *D. nasuta* in the Brazilian Savanna was observed in both study areas in December 2015.

Table 2. Abundance of drosophilids in a gallery forest of the *Campus Planaltina* of the *Instituto Federal de Brasília* between September 2015 and April 2016. NEO: Neotropical species, EXO: other exotic species. Gray columns indicate the core rainy season.

	2015				2016				Total
	Sep	Oct	Nov*	Dec	Jan	Feb	Mar	Apr	
NEO	11	7	0	24	10	127	167	75	421
EXO	48	2	0	639	68	285	131	84	1257
<i>D. nasuta</i>	0	0	0	8	73	66	33	18	198
Total	59	9	0	671	151	478	331	177	1876

* In November 2015, the traps were placed in the field, but no drosophilids were captured.

Drosophila suzukii and *D. nasuta* seem to differ in their ability to establish viable populations in the natural environments of the Brazilian Savanna landscape. Two years after it was first recorded, *D. nasuta* succeeded in colonizing forests; in both areas surveyed (IBGE and PLA), it corresponded to approximately 11% of the drosophilids collected in the rainy season. In contrast, the spotted-wing *Drosophila* has never reached a relative abundance of 1% in a single collection and so can be considered a rare species in these communities. These results strongly contrast with those obtained for *Zaprionus indianus*. When first recorded in the Brazilian Savanna, the African fig fly corresponded to 7% of the drosophilids collected (February 1999), and a year later (February 2000), its relative abundance had reached almost 90% of the drosophilids in savanna-type vegetation (Tidon *et al.*, 2003).

It is challenging to predict the fate of an introduced propagule in a new area, and we are aware that extensive monitoring is required to estimate the spread and population growth of *Drosophila suzukii* and *D. nasuta*. For example, the exotic species *D. ananassae* Doleschall and *D. busckii* Coquillett have been recorded in Brazil for decades but remain rare in most collections (Tidon, 2006; Roque *et al.*, 2013), whereas *D. simulans* Sturtevant and *Zaprionus indianus* respond rapidly to favorable environmental cues, undergoing extensive population growth and dominating drosophilid assemblages under specific circumstances (Döge *et al.*, 2015). Therefore, studies documenting the establishment of recently introduced species, as presented here

for *D. suzukii* and *D. nasuta*, are fundamental to understanding the dynamics of biological invasions.

Acknowledgments: We thank *Reserva Ecológica do IBGE, Instituto Federal de Brasília (Campus Planaltina)* and *Universidade de Brasília* for logistical support. This research was funded by the Conselho Federal de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação para Aperfeiçoamento de Pessoal de Ensino Superior (CAPES/PROEX/PPG-Ecologia).

References: Asplen, M.K., G. Anfora, A. Biondi, D.S. Choi, D. Chu, K.M. Daane, P. Gilbert, A.P. Gutierrez, K.A. Hoelmer, W.D. Hutchison, R. Isaacs, Z.L. Jiang, Z. Kárpáti, M.T. Kimura, M. Pascual, C.R. Philips, C. Plantamps, L. Ponti, G. Véték, H. Vogt, V.M. Walton, Y. Yu, L. Zappalà, and N. Desneux 2015, *J. Pest Sci.* 88: 469–494; Benito, N.P., M.L. Silva, and R.S.S. Santos 2016, *Pesqui. Agropecu Bras.* 51: 571–578; Brake, I., and G. Bächli 2008, *World catalogue of insects 9: drosophilidae (Diptera)*. Stenstrup, Appolo Books; David, J.R., and L. Tsacas 1981, *C.R. Soc. Biogéog.* 57: 11–26; Deprá, M., J.L. Poppe, H.J. Schmitz, D.C. De Toni, and V.L. Valente 2014, *J. Pest Sci.* 87: 379–383; Döge, J.S., H.O. Valadão, and R. Tidon 2015, *Biol. Invasions* 8: 2461–2474; Eiten, G., 1972, *Bot. Rev.* 38: 205–341; Gilbert, P., M. Hill, M. Pascual, C. Plantamp, J.S. Terblanche, A. Yassin, and C.M. Sgrò 2016, *Biol. Invasions* 18: 1089–1103; Kitagawa, O., K. Wakahama, Y. Fuyama, Y. Shimada, E. Takanashi, M. Hatsumi, M. Uwabo, and Y. Mita 1982, *JPN. J. Genet.* 57: 113–141; McKinney, M.L., and J.L. Lockwood 1999, *Trends Ecol. Evol.* 14: 450–453; Oliveira, O.S., and R.J. Marquis 2002, *The cerrados of Brazil: ecology and natural history of a neotropical savanna*. New York, Columbia University Press; Paula, M.A., P.H.S. Lopes, and R. Tidon 2014, *Dros. Inf. Serv.* 97: 113–115; Roque, F., R.A. Mata, and R. Tidon 2013, *Biodivers. Conserv.* 22: 657–672; Roque, F., L. Mencarini, B.F.D. Leão, M.D. Delgado, and R. Tidon 2016, *Dros. Inf. Serv.* 99: 48–49; Roque, F., S.C.F. Oliveira, and R. Tidon 2011, *Dros. Inf. Serv.* 94: 140–141; Tidon, R., 2006, *Biol. J. Linn. Soc.* 87: 233–247; Tidon, R., M.S. Gottschalk, H.J. Schmitz, M.B. Martins 2016, *Drosophilidae in Catálogo Taxonômico da Fauna do Brasil. PNUD*. Available: <http://fauna.jbrj.gov.br/fauna/faunadobrasil/183241> (Accessed 02 November 2016); Tidon, R., D.F. Leite, and B.F.D. Leão 2003, *Biol. Conserv.* 112: 299–305; Tochen, S., J.M. Woltz, D.T. Dalton, J.C. Lee, N.G. Wiman, and V.M. Walton 2016, *J. App. Entomol.* 140: 47–57; Vilela, C.R., 1999, *Dros. Inf. Serv.* 82: 37–39; Vilela, C.R., and B. Goñi 2015, *Rev. Bras. Entomol.* 59: 346–350; Walsh, D.B., M.P. Bolda, R.E. Goodhue, A.J. Dreves, J. Lee, D.J. Bruck, V.M. Walton, S.D. O’Neal, and F.G. Zalom 2011, *J. Integ. Pest Man.* 106: 289–295.



A burst of spontaneous mutations in isofemale lines of *Drosophila melanogaster* from Senegal.

Cossard, Raynald^a, and Stéphane R. Prigent^b. ^aInstitut de Biologie Intégrative de la Cellule (I2BC) UMR 9198 CEA/CNRS/UPSUD, Gif-sur-Yvette, France; ^bInstitut

Systématique Evolution Biodiversité (ISYEB) UMR 7205 CNRS-MNHN-UPMC-EPHE, PSL University, Paris, France. E-mail: stephane.prigent@mnhn.fr

Introduction

With more than a century of use *Drosophila melanogaster* has proven to be a good model for genetic studies (Green, 2010). In addition to the ease of handling the species in laboratory, it is particularly interesting because of its ability to produce spontaneous mutants (Lindsley and Zimm, 1992). These mutants have played a fundamental role in understanding genetic mechanisms and most of them are now characterized at the molecular level and the function of the affected genes is known. Therefore, recovering classical mutant phenotypes is today of little value as they are not going to improve our knowledge much. However, recovering these phenotypes might be relevant for indicating genetic instability in natural populations. Indeed a large proportion of natural mutations is due to mobile element activity, and this mutagenic activity is associated with stress (Capy *et al.*, 2000) or possibly with the admixture of divergent populations as in the P-M system (Kidwell *et al.*, 1977).

From the biogeographical distribution of species closely related to it, *D. melanogaster* probably