SHORT COMMUNICATION



Anthropogenic disturbance may promote the invasion of forest landscape by an open-habitat specialist introduced dung beetle species in Brazil

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Received: 26 January 2020 / Accepted: 7 September 2020 / Published online: 7 October 2020 © Institute of Zoology, Slovak Academy of Sciences 2020

Abstract

Digitonthophagus gazella, a dung beetle species introduced into many countries as a biological control agent for cattle dung removal, is adapted to open pastures and avoid shaded areas such as forests. With a short development period, high fecundity, and long dispersion capability, *D. gazella* is an effective competitor that is considered an invasive species in many countries. Until recently, only one study evaluated its possible presence in a small forest area, suggesting an invasion process might be occurring. We report here the presence of *D. gazella* in five distinct forest landscapes (FLs): a regenerating native forest, a reforested native forest, and three forests with exotic plant species. The FLs are embedded in an area with several other FLs, which is surrounded by a river and a pasture. However, cattle have access to these FLs. We found that the anthropogenic disturbances may be facilitating the species dispersal to shaded habitats as there was a higher abundance of *D. gazella* in areas where cattle were more active. The possibility of this species in invading FLs is worrisome because it has been shown that *D. gazella* can cause significant negative impacts to the native dung beetle community, which is of great ecological importance for the ecosystem.

Keywords Scarabaeidae · Introduced non-native species · Invasion process · Digitonthophagus gazella · Bovine

Introduction

Insect species introduced as biological control agents rarely become invasive. However, the few exceptions remind us that extreme care must be taken to avoid such possibility due to significant ecological consequences. For example, *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae), was introduced as a biological control agent of pest aphids but is now considered invasive worldwide due to its negative impact on the indigenous coccinellid community (Roy and Wajnberg 2008).

In 1990, the African dung beetle species *Digitonthophagus gazella* (Fabricius, 1787) (sensu Génier and Moretto 2017) was allegedly introduced and released in Brazil by the Brazilian Agricultural Research Corporation (EMBRAPA) to promote

the biological control of the horn fly (Haematobia irritans (Linnaeus, 1758) (Diptera: Muscidae)). By burying cattle dung, D. gazella also buries the larvae of the horn fly which resides in the dung and enhances the biological control of this pest species (Bianchin et al. 1992). However, as in many other countries, no studies regarding its possible impacts on the native dung beetle community or its potential for invasion into novel ecosystems were conducted prior to its introduction. As a species with high fecundity, short development period (Blume and Aga 1975) and high dispersal range (Barbero and Lopez-Guerrero 1992), D. gazella has all the desirable characteristics for a successful biological control agent (Kimberling 2004). However, these factors also contributed to the species being a successful invader (Sakai et al. 2001). Indeed, few years after its release into the U.S. (Texas) the species invaded Mexico (Barbero and Lopez-Guerrero 1992) and is now considered an invasive species in almost all Neotropical countries (Kohlmann 1994; Ivie and Philips 2008; Vidaurre et al. 2008; Alvarez et al. 2009; Noriega et al. 2010, 2011; Boilly and Vaz-de-Mello 2013; Noriega et al. 2017; Pablo-Cea et al. 2017).

Regarding the impacts on the indigenous community, Howden and Scholtz (1986) showed significant differences in the Mexican dung beetle community after the invasion by

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D. gazella, although due to limitations in their methodology, their findings were inconclusive. In Brazil, Mesquita Filho et al. (2018) showed that the invasion of a pasture area by *D. gazella* had a significant impact on the local dung beetle community with certain native functional groups being more affected. For example, there was a continuous decrease in the tunneler species, the ones responsible for cattle dung removal in the study area (Flechtmann et al. 1995), after the invasion by *D. gazella*.

Until recently, concerns about the possible impacts and invasion by D. gazella were only limited to pasture and savannah areas (Matavelli and Louzada 2008; Noriega et al. 2017) because the species is adapted to the microclimatic conditions in this areas, such as higher luminosity, lower relative humidty and higher and more extreme temperatures compared to forest habitat (Doube 1983; Angotti 2014). This preference has been showed in field experiment where the species avoid shaded habitats (Lobo and Montes de Oca 1994). Even dung pads placed in shaded areas inside open field, like the shade of a mango tree in pastures, were not colonized by the species (Lobo and Montes de Oca 1994). However, although D. gazella is apparently not able to invade and establish in forest landscapes, it may be the case that it is in the beginning of a process of invasion, as suggested by Angotti (2014). Therefore, studies and reports on its presence in forest landscapes are needed as the species can pose serious threats to the native community (Howden and Scholtz 1986; Mesquita Filho et al. 2018).

During an experiment aimed at collecting bark and ambrosia beetles in several forest formations in a location in Brazil, white-fluorescent-light flight intercept traps were employed as a sampling method, and *D. gazella* was collected in all forest areas sampled. In the location, cattle from neighboring pasture have free access to the areas as a natural method to control grasses. Based on the habitat specificity of *D. gazella*, we hypothesized that the presence of cattle might be an explanation for finding the species in the forest formations as resource (dung pad) were available, likely promoting the invasion of such habitat by *D. gazella*.

Material and methods

Insect collection was conducted in five distinct forest type in the Experimental Farm of ESALQ/USP (22°40' S, 48°10' W and altitude of 455 m a.s.l.) which is located in Anhembi, State of São Paulo, Brazil (Fig. 1a). Collection was made monthly using a flight intercept trap model "Luiz de Queiroz" (Matioli and Silveira-Neto 1988), equipped with fluorescent OSRAM® cool daylight L 15 W/765, installed at each site 10 m from the edge (Fig. 1b).

The vegetation types sampled were two areas of native species: A a 25-year-old area of a native Cerrado *stricto* sensu (Brazilian savannah, 16 ha) regenerating area; **B** a 8-year-old area reforested with 20 species native to the Atlantic forest (5.4 ha); and three areas planted with exotic species: **C** a 132-year-old *Pinus oocarpa* and *P. caribea* var. *hondurensis* (11 ha); **D** a 10-year-old *Eucalyptus grandis* area (22 ha); and **E** a 32-year-old area with *Eucalyptus urophylla* (40 ha). Distance between areas ranges from 500 m to 1500 m (Fig. 1c).

The experimental farm has several other species planted and is surrounded by pasture areas on its west side and by planted forest on the southern side. The Tietê River on its northern side divides the total area in almost two small peninsulas (Fig. 1). Cattle have free access to the areas, except to the reforested area (E), which is the only fenced area. However, they graze more frequently in areas A and E, than C and D (ENLF, pers. observ.).

Daily mean maximum and minimum air temperature (°C), mean relative humidity (RH) and rainfall (mm) were recorded at the experimental farm from an electronic weather station. Generalized linear models (GLM) with a quasi-Poisson error distribution (Poisson distribution corrected for overdispersion) was used to evaluate differences in abundance of *D. gazella* among vegetations types, the influence of the climatic variables and presence of cattle (McCullagh and Nelder 1989). Linear regression was performed to evaluate the relation between *D. gazella* abundance and distance from the pasture.

Results

A total of 77 *D. gazella* adults were collected. We found a sex ratio (total females/ total) of 0.56 though 17% of the collected individuals were not identified to sex (Table 1).

There was a significant difference in the abundance among vegetation types ($F_{5,64} = 4.10, p < 0.05$), with the highest abundance on *E. urophylla* stand (estimate = 1.25, *t*-value = 3.96, Pr(>|t|) < 0.001) and the lowest in *E. grandis* stand, where only two individuals were collected (Fig. 2). Abundance varied significantly between months ($F_{1,11} = 10.74, p = 0.01$). Mean minimum air temperature was the only climatic variable to have a significant influence on the abundance of *D. gazella* ($F_{1,9} = 10.11, p = 0.01$). Higher abundance occurred when minimum air temperature was higher (Fig. 3).

Distance from pastureland had a positive significant relation with *D. gazella* abundance (coefficient = 0.022, *t*-value = 5.35, p = 0.01), indicating higher abundance in the areas farther from the pasture (Fig. 1c). Cattle grazing intensity showed a significant positive relationship with *D. gazella* abundance as more beetle were collected in the areas where cattle graze more frequently, Cerrado regenerating area (A) and *E. urophylla* stand (E) ($F_{3.64} = 5.6, p < 0.01$).

Canopy cover was lower in the regenerating area, followed by the reforested and the *Pinus* stand; the higher cover was

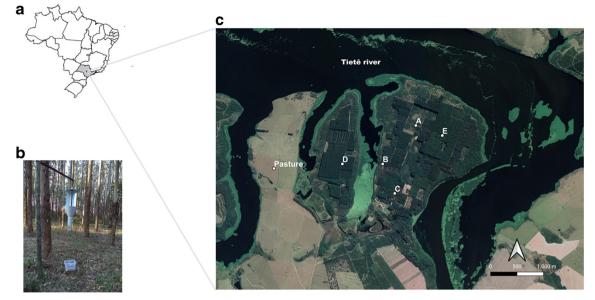


Fig. 1 Map of the study area and sampling method. The figure is composed by three panels. In the upper-left corner (a) there is the location of São Paulo state in Brazil and the location of the Experimental Farm of ESALQ, located in Anhembi (black dot). The white-fluorescent-light trap employed to collect insects is shown at the bottom-right panel (b). The sampling area and sampling points are displayed on the right panel (c).

observed in the *E. urophylla* and *E. grandis* stands, respectively (Fig. 1c).

Discussion

Digitonthophagus gazella, an open-habitat specialist dung beetle species which avoids shaded areas (Doube 1983; Lobo and Montes de Oca 1994), was found inside forest formations. Previous work found *D. gazella* to be able to colonize savannah areas inside forest formations like the Amazon forest (Matavelli and Louzada 2008). Angotti (2014) collected three specimens inside (60 m from the edge) a small fragment of Atlantic forest. However, *D. gazella* has never been collected inside planted forests of *Eucalyptus* and *Pinus* species and reforested areas as the ones sampled in this study.

A: a 25-year-old area of native Cerrado *sctricto* sensu (Brazilian savannah) regenerating area; **B**: a 8-year-old area reforested with 20 species native to the Atlantic forest; **C**: a 32-year-old planted area *Pinus oocarpa* and *P. caribea* var. *hondurensis*; **D**: a 10-year-old *Eucalyptus grandis* planted area; E: a 32-year-old area planted with *Eucalyptus urophylla*

In accordance with our assumption, the higher abundance of *D. gazella* was observed in the *E. urophylla* (**E**) and regenerating (**A**) areas, the sites where cattle graze more intensively. Furthermore, the lower abundance of the beetles was observed in sites closer to pasture, but less grazed by cattle, showing that distance from the pasture was not influencing the abundance of *D. gazella*.

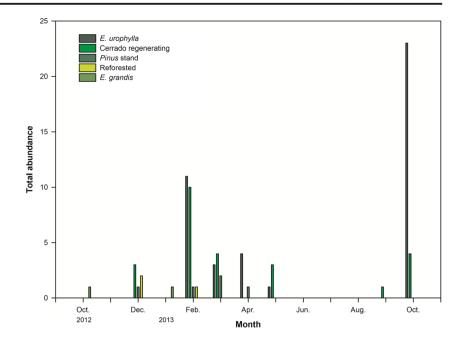
As *D. gazella* was highly active during the warmer and rainy months, environmental condition can be improving the dispersion of the species to the forest habitats. Although the local community (14 native dung beetle species) can be a barrier to the establishment of *D. gazella* (Matavelli and Louzada 2008), disturbed areas like planted and regenerated forests are usually impoverished in the diversity of dung beetle species compared to other vegetations, such as primary forests (Gardner et al. 2008). Moreover, since *D. gazella* is a good competitor species (Bornemissza 1970), the presence of

Table 1Total abundance and sexratio of Digitonthophagus gazellacollected by a light trap in fivedistinct forest landscapes in theExperimental Farm of ESALQ,locate in Anhembi, São Paulo,Brazil

Vegetation type	Total	Males/ females ^a
25-year-old native forest regeneration (Cerrado stricto sensu)	26	13/13
10-year-old Reforested area	3	1/1
32-year-old Pinus oocarpa and P. caribea var. hondurensis	5	0/3
10-year-old Eucalyptus grandis	2	0/2
32-year-old Eucalyptus urophylla	42	11/14
Grand Total	77	25/33

^a not all specimens were sexed

Fig. 2 Total abundance (number of individuals) of *D. gazella* collected in light traps in each of the five vegetation areas sampled in the Experimental Farm of ESALQ, located in Anhembi, São Paulo, Brazil from October 2012 to 2013



cattle can ensure resource availability to support its establishment in those areas, promoting its establishment.

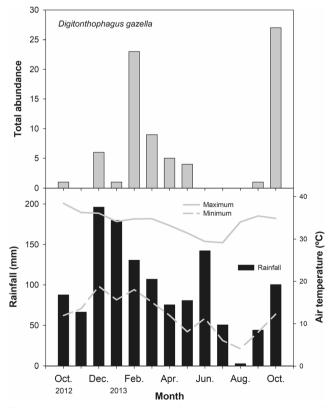


Fig. 3 Total abundance (number of individuals) (upper panel) of *D. gazella* collected in light traps in the Experimental Farm of ESALQ, located in Anhembi, São Paulo, Brazil, from October 2012 to 2013 and mean monthly minimum and maximum air temperatures (°C) and rainfall (mm) (lower panel)

The same proportion of males and females observed is an important characteristic indicating the likely process of invasion. As both sexes were collected, there is a high probability of breeding of *D. gazella*, necessary to initiate a viable population (Sakai et al. 2001). However, we need further studies in order to evaluate if the species has been able to have progeny in the forest areas, which was not possible to be observed in the present study.

Because this study used data from an experiment aimed at collecting bark and ambrosia beetles in the forest areas, there are some limiting aspects that are known to influence dung beetle collection and, consequently, our results. The method of collection (white lamp) may have influenced the low abundance of D. gazella, as thousands of individuals can be collected in light traps in one night, but when equipped with a black light (Mesquita Filho et al. 2018). It is wellknown that white lamps are not the most appropriated one for sampling Scarabaeidae beetles (García-López et al. 2011). Therefore, the influence of such method in attracting D. gazella that were dispersing is not likely in our experimental area because the species avoid shaded areas and the fluorescent white lamp used has a very limited range of attraction. Additionally, in the beginning of the invasion process, it is common that the invasive species will show a low abundance, slowly increasing with time (Strayer et al. 2017). Hence, the low numbers of D. gazella may also be another indicative that the species is in the initial process to invade these areas.

Another limiting aspect is the sampling location at the edge of the areas. Forest edge has a significant impact on dung beetle communities harbouring distinct species and abundance from the interior of forest formations (Spector and Ayzama 2003; Marsh et al. 2018). Hence, there is the need to employ traps inside the areas in order to confirm the presence of a dung beetle species. However, although this could have an influence in our results, it is likely to be less important because the traps were not installed in the edge of a pasture and a forest area, as the case in the studies, but in the border of roads inside the forest plantation surrounded by the same vegetation, and also shaded areas (except in vegetation A and C).

We showed here that given the right conditions, D. gazella may be able to overcome its avoidance of shaded habitats and invade these landscapes. Further studies are needed in order to evaluate if D. gazella may be invading forest areas using proper methodology, because light trap does not allow to evaluate if the beetle has a viable population in the area. Studies applying the methodology employed by Angotti (2014) combining pitfall traps baited with cow dung and sampling from dung pads inside forest areas and pastures need to be conduct in distinct region and vegetation types worldwide. However, our finds are worrisome because the species is already established in the Neotropical region (Kohlmann 1994; Ivie and Philips 2008; Vidaurre et al. 2008; Alvarez et al. 2009; Noriega et al. 2010, 2011; Boilly and Vaz-de-Mello 2013; Noriega et al. 2017; Pablo-Cea et al. 2017) and some agricultural practices like agroforestry are increasing in Brazil (Alves et al. 2015). Therefore, we suggest that researchers conducting studies with dung beetles in forest areas located near pastures include cow pad baited pitfall traps in their surveys to monitor the possible invasion of forest landscapes by D. gazella.

Acknowledgments We thank Fernando Vaz-de-Mello for species confirmation, the several employees of the "Estação Experimental de Ciências Florestais de Anhembi", that provided immensurable help during fieldwork and one anonymous reviewer for thoughtful review. ENLF was supported by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)". WMF thanks Damie Pak very thoughtful and helpful comments and suggestions.

Authors' contributions ENLF, IHC and WACG designed and conducted the experiment. WMF identified the beetles, analyzed the data and wrote the first draft of the manuscript. All authors contributed equally to the final version of the manuscript.

Funding This research was supported by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)", Brazil.

Data availability Not applicable.

Compliance with ethical standards

Conflict of interest There are no conflict of interests.

Code availability Not applicable.

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