Road to ruin: Herbivory in a common tropical weed (*Turnera subulata*) along a rural-urban gradient

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HIGHLIGHTS

- Higher arthropod herbivory in cities can drive the evolution of urban plant defence
- We found increased herbivory in urban habitat, mostly by lace bugs
- We found a significant positive effect of impervious surface on herbivory
- Extensive impervious surfaces and stressful urban environment facilitate herbivory, but do not lead to the expression of HCN, a antiherbivore defense chemical

Abstract

Urbanization is associated with numerous changes to the biotic and abiotic environment, many of which degrade biodiversity. For example, cities often have increased pollution levels that harm wildlife. Nevertheless, the elevated concentration of some gases can fertilize urban plants, with concomitant positive effects on herbivore populations that could select defence phenotypes in plants. However, actual evidence supporting increased herbivory in urban environments remains scarce and contradictory. Giving the gaps in our knowledge, particularly in tropical cities, we evaluate herbivory on *Turnera subulata*, a common weed, along a rural-urban gradient in Joao Pessoa, Brazil. We expected higher rates of herbivory in urban areas and we predicted these populations to evolve cyanogenesis. We quantified herbivory and screened for cyanogenesis in 32 populations (617 plants) along the urbanization. Our results show that urban populations had significantly higher herbivory rates than rural populations. The likelihood of herbivory was positively correlated to increased amount of impervious surface, although there was no difference in amount of leaf loss to herbivory. Despite elevated herbivory, we found no evidence for the evolution of cyanongenesis in any of the populations. Habitat loss, predator release, and nutrient enrichment likely act in concert to benefit of leaf-eating arthropods, influencing the herbivory patterns observed in our study.

Key words: plant defence, urban ecology, cyanogenesis, insect herbivory.

Introduction

Urbanization is associated with numerous changes to the biotic and abiotic environment, which can have large impacts on urban biodiversity (Grimm et al., 2008;

Fenoglio et al., 2020). An often-identified source of harm to wildlife are the elevated chemical pollution levels found in cities (Grimm et al., 2008; Parris, 2016). However, other studies have indicated that the increased levels of certain pollutants in the urban environment can positively benefit some species. For instance, elevated nitrogen oxides (NOx) from vehicle emissions can increase the nitrogen content and the nutritional quality of the foliage of certain species of plants (Bell et al. 2011; Honor et al, 2009; Spencer et al. 1988). This may in turn favour increased abundance of some herbivorous insect arthropods in urban environments (Dale & Frank, 2018; Raupp et al. 2010).

Insect arthropod abundance can also be affected through changes to their community structure (reviewed in Miles et al. 2019). Urbanization can lead to a decline in arthropod predators through habitat fragmentation and degradation, which offers a release from predation from their insect prey (Faeth et al 2005). As a result, the abundance of herbivorous arthropod species may be greater in cities relative to natural habitats. This increase in herbivorous species, and concomitant increase in the rate of herbivory, can compromise the growth, reproduction and survival of plants (Mauricio & Rausher, 1997; Crawley, 1989), with important consequences in the evolution of the defence mechanisms (Hanley et al. 2007; Mauricio & Rausher, 1997; Miles et al., 2019). Increased arthropod herbivory can drive the evolution of plant defence mechanisms (e.g. Johnson et al. 2018; Mauricio & Rausher, 1997; Züst et al. 2012). For instance, invasive wild parsnip rapidly reacquired its chemical defences when its specialist herbivore was introduced into its range (Zangerl and Berenbaum 2005; Zangerl et al. 2008). Sufficient herbivory pressure along urbanization gradients may trigger the evolution of chemical defence mechanisms in urban plant populations (Miles et al., 2019; Raupp et al. 2010), however little work has been done to investigate this possibility.

Studies that have estimated herbivory rates along urban-rural gradients have shown that there is considerable spatial variation in herbivory across the urbanization gradient.

However, studies differ in the results on herbivory rates between urban and rural areas; some suggest an increase in herbivory with increased urbanization (Cuevas-Reyes et al. 2013, Dale & Frank, 2017; Turrini et al. 2016) while others indicate a reduction in herbivory (Kozlov et al. 2017; Moreira et al. 2019). In addition, most studies evaluating urban-rural gradients in herbivory are limited to cities in temperate regions (Miles et al. 2019). To our knowledge, the only study carried out in a tropical region found increased herbivory rates of *Solanum lycorcapurm* in habitats with greater levels urbanization (Cuevas-Reyes et al. 2013). Recent reviews on the topic (Miles et al. 2019; Rivkin et al. 2019) emphasize the urgent need for studies in tropical areas.

We assessed the effects of urbanization on herbivory and the evolution of antiherbivore defence in *Turnera subulata* in Joao Pessoa, Brazil. This species is a useful system to test the effects of urbanization on plant-herbivore interactions for three reasons. First, it is native to Central and South America (Schlindwein and Medeiros, 2006), and commonly found across many regions of the tropics. Second, it thrives in urban habitats, despite elevated pollution and habitat fragmentation. Third, its close relative, *T. ulmifolia*, produces the antiherbivore defence chemical hydrogen cyanide (HCN; Schappert & Shore, 1995). In cyanogenic plants, HCN is released following tissue damage, and is toxic to insect herbivores (Hughes, 1991). Herbivory may select for the evolution of cyanogenesis in *T. ulmifolia* if HCN reduces their susceptibility to herbivores (Schappert & Shore, 1999). Although *T. subulata* has not yet been documented to express cyanogenesis (Shore & Obrist 1992), it is possible that under elevated herbivory pressure, this trait may evolve. In our study, we sought to answer the following questions: (1) Is there an effect of urbanization on herbivory rates on *T. subulate*? (2) Has HCN expression evolved in urban populations as a result of increased herbivory pressure? Addressing these questions will help to fill the gaps in the field surrounding the effects of urbanization on species interactions in the tropics.

Methods

Study system

Turnera subulata (Passifloraceae) is a perennial herb/subshrub with distylic flowers, native to Brazil and common in cities and rural areas (Fig. 1). In urban areas, *T. subulata* are typically found in dense populations alongside pavement, lawns, and dry fields (Fig. 1). Individuals reproduce via outcrossing and is an important nectar resource for many species of bees (Schlindwein & Medeiros, 2006). *Turnera subulata* is depredated by arthropod herbivores from different feeding guilds; most leaf herbivory is caused 23 herbivore species (Cruz et al. 2019; Schappert & Shore, 1999). The most prevalent herbivores were the specialist butterfly *Euptoieta hegesia* cateppilars, Coleaptora in the genii *Disonycha* and *Parchicola*, aphids, and leaf miners (Schappert & Shore, 1999).

Sampling design

In December 2018, a total of 38 sites were selected, along an east-west transect spanning a gradient of urbanization in the city of Joao Pessoa (>800,000 people) capital of Paraiba, north-eastern Brazil (Fig. 1). We considered a site a viable population if they consist of at least 16 *T. subulata* plants within a 100 m radius of the centre of the population. Each site in the city was at least 400 m away from the next and in the rural area they were at least one kilometre part (see Fig. 2). We collected 20 plants per population, except in sites which had fewer than 20 plants (N = 8 sites). To avoid sampling close relatives, we sampled individual plants spaced at least 2 m apart, except in sites with fewer than 20 individuals. We quantified the degree of urbanization at each site by calculating the amount of impervious surface within a 100 m radius of the population (sensu Rivkin et al., 2020).

We measured the amount of herbivory by calculating the percent area of each leaf that was damaged by herbivory. We selected the largest four leaves on up to three ramets per

plant (N = 12 leaves per plant), and measured the amount of damage on each using the software application BioLeaf (Machado et al. 2016). This method is an effective and provide reliable measurements of leaf damage (see details in Machado et al. 2016). We also recorded whether the leaves had experienced herbivory from sap-suckling lace bugs, which turn the leaves a white-yellow and black speckled colour (Fig S1; Guidoti et al. 2015).

Cyanogenesis assays

We screened each plant for the presence of HCN using the Feigl-Anger assay, which use a color change reaction to indicate the presence (blue) or absence (white) of HCN (Shore & Obrist, 1992). We collected two to three young leaves from each plant, froze them at -20 ^{o}C for at least 48 hours to lyse the cell wall and induce the formation of HCN. We then followed the same protocol used by Thompson et al. (2016) to determine the frequency of cyanogenic plants in each population.

Statistical analysis

We tested for the effects of urbanization on herbivory in two different ways. First, we estimated broad-scale patterns in herbivory by using a two-sided t-test to compare percent herbivory rate per plant between urban and nonurban populations. We also used t-test to identify differences in lace bug feeding (white-yellow and black speckled leaves) between urban and nonurban populations.

Second, we estimated fine-scale patterns in herbivory by running two generalized linear regression models to determine how herbivory varies among populations across the urbanization gradient using R version 4.0.2 (R Core Team 2018). The first model tested for differences in the percent herbivory rate experienced by each plant averaged across the population. The second model tested for differences in the presence or absence of herbivory experienced by a plant averaged across the population. Each response variable was run against the same predictor variable: the amount of impervious surface in a 100 m radius,

which was our estimate of urbanization. Each model was run on a quasibinomial distribution to account for the data being distributed between 0 and 1. Lastly, we assessed the significance of impervious surfaces from both models using Analysis of Variance (ANOVA) implemented using the *Anova* function in the *car* v. 3.0-6 package (Fox and Weisburg 2011) to calculate Wald chi-square test statistics with Type II sums of squares.

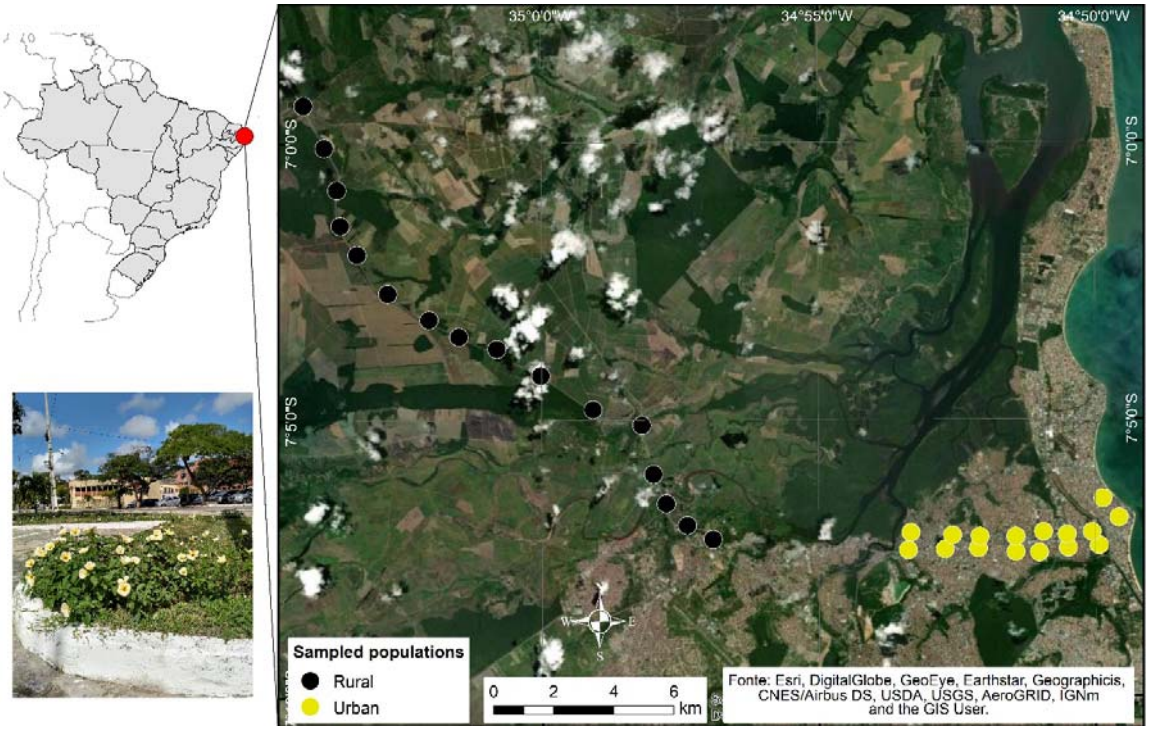

Figure 1. Location of Joao Pessoa city in NE Brazil, the sampled rural & urban populations and photo of a population of *Turnera subulata* in urban environment with low level of urbanization.

Results

We found increased herbivory in urban habitats. Over 42% of the urban plants had leaf damage due to herbivory, whereas in non-urban plants 20.9% showed signs of herbivory ($t= 3.97$; $df = 30$; $p< 0.001$, Fig 2). The average rate of leaf damage was also significantly higher in individuals from urban areas ($t= 2.04$; $df = 615$; $p= 0.042$). Lastly, 33% of plants in urban populations exhibited evidence of lace bug (*Gargaphia* sp) herbivory, whereas only 4.6% of plants in rural populations exhibited lace bug damage ($t = 3.98$; $df = 30$; $p < 0.001$, Fig. 3).

When we tested for fine-scale patterns of herbivory along the urbanization gradient, we found no detectable effect of amount of impervious surface on average percent herbivory per population ($\chi^2_1 = 2.13$, $p = 0.145$). However, we found there was a significant positive effect of impervious surface on the presence of herbivory ($\chi^2_1 = 12.87$, $p < 0.001$), where urban populations were more likely to experience herbivory than nonurban populations (Fig 3).

Lastly, we assayed five rural populations (97 plants) and seven urban populations (138 plants) for HCN. We detected no evidence of HCN production in any of the populations we assayed, suggesting that this chemical defence has not evolved in these populations.

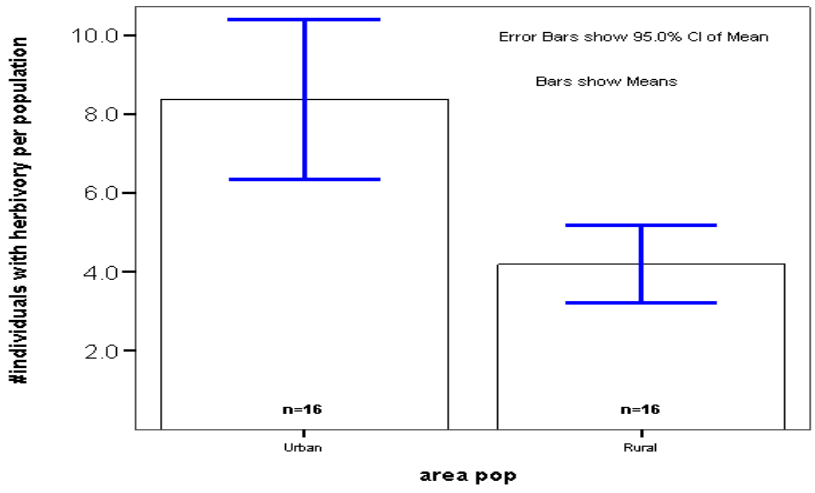

Figure 2. Average number of individuals that showed signs of herbivory in the rural and urban population. Herbivory was significantly more often in individuals from urban populations (t= 3.97; df = 30; p< 0.001.)

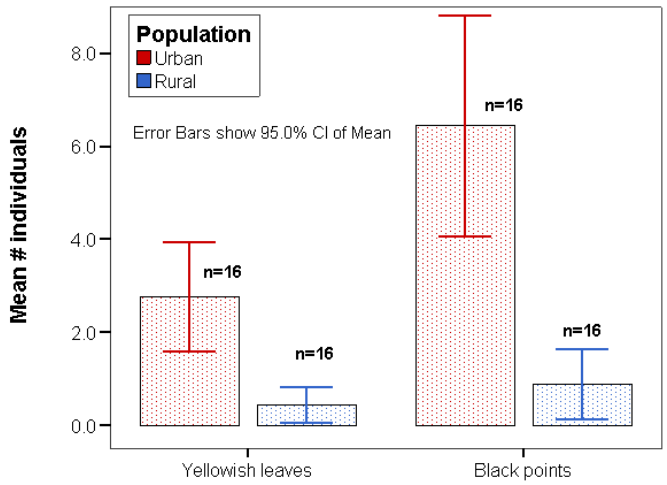

Figure 3. Average number of individuals showing evidence of lace bug herbivory. Differences were significant both for yellowish leaves (t = 3.98; df = 30 ; p < 0.001) and individuals with leaves with presence of black points (t = 4.76; df = 30; p < 0.001).

Discussion

As predicted, we found that urban plants experienced higher levels of herbivory, however we did not find expression of HCN in any of the plants we tested. Our results reinforce earlier findings reporting that *T. subulata* has not evolved cyanogenesis (Shore & Obrist, 1992). Arthropod herbivory seems to be the main selective driver for cyanogenesis in the closely related *T. ulmifolia* (Schappert & Shore, 1999) and this herbivore-plant interaction can drive fast evolutionary changes in plant chemical defences (e.g. Agrawal et al. 2012; Zanger et al., 2008). Nevertheless, the absence of HCN in *T. subulata* populations might be

linked to more efficient mechanical protection (e.g. pilosity in leaves) and mutualistic interactions in this species that could fend off herbivory without need of HCN. Moreover, the most damaging effects of herbivory in urban plants were caused by lace bugs (*Gargaphia* sp, see fig.S1 & S3) and their feeding methods probably does not cause cell rupture and release of HCN, therefore minimizing selection for this type of chemical defence.

The lace bugs (Tingidae) feed on sap of living plants using specialized buccal apparatus that pierce the cellular tissue to extract the sap and cause serious damage in the foliage (Guidoti et al. 2015), which lead to some species being used to control population of weed plants. For instance, lace bugs are considered as one of the three most successful biological control agents against the invasive *Lantana camara* (Guidoti et al., 2015; Baars & Heystek 2003). The impact of lace bugs on urban plant population could be quite high since they feeds gregariously and cause heavy damage in the leaves (Guidoti et al. 2015, see figs S1 & S3). At a community level their herbivory on *T. subulata* may be regulating urban community structure by inflicting reduction on competition within and among plant species, although speculative, this possibility deserves further study.

City life: a road to ruin?

We observed greater herbivory in urban populations. This trend may be the results of increased vehicular pollution, a ubiquitous feature of urbanization. Despite its negative impacts on different organisms (Durrani et al. 2004; Mazzoli-Rocha et al. 2014; Rai, 2016), pollutants like NOx can increase the nitrogen content in leaves and the nutritional quality of plant foliage (e.g. Bell et al. 2011; Moreira et al. 2019; Spencer et al. 1988; Truscott et al. 2005). This high foliage quality has been associated to high arthropod herbivore abundance in urban environment because many arthropods are nitrogen limited (e.g. Dale & Frank, 2018;

Jones & Leather, 2012; Raupp et al. 2010) and may be capitalizing on the increased nitrogen in roadside plants. Although we collected rural plants along a highway with high traffic load, rural plants were surrounded by fewer impervious surfaces, possibly reducing their exposure to pollutants and decreasing their nitrogen content. This trend is supported by our results showing increased likelihood of herbivory in habitats that were more urbanized (i.e., surrounded by more impervious surfaces).

Rural populations may also harbour a greater diversity of predators than urban areas (e.g. Raupp et al. 2010; Turrini et al., 2016) hence a more diverse predatory guild in rural areas could be exerting greater top-down control compared to urban sites. A number of studies indicate a reduction of predators and parasitoid species in more urbanized areas, which has been suggested to cause increased herbivory from lower-guild species (e.g. Burkman & Gardner, 2014; Denys & Schmidt 1998; McIntyre 2000; Rocha & Fellowes, 2020). The decline in richness at high trophic levels in urban environment seem to be pervasive and has been associated with increased herbivory (Cuevas-Reyes et al. 2013; McIntyre 2000; Raupp et al. 2010; Turrini et al. 2016).

The complex relation of abiotic environmental factors interfering with biotic interactions, thus regulating the strength of the trophic effects on food webs in urban environment, is yet not well understood (e.g. El-Sabaawi 2018; Lagucki et al. 2017; Turrini et al. 2016). For example, habitat fragmentation and increased impervious surface in urban areas could reduce natural enemy abundance and diversity by reducing connectivity (Peralta et al. 2011; Rocha & Fellowes, 2020) and the spatial distribution and patches size of plants could affect the abundance of parasitoid species (Fenoglio et al., 2010; Peralta et al. 2011; Raupp et al., 2010). The mechanisms driving levels of herbivory in urban areas are intricate and more than one can be the key drivers. We suggest that the extensive impervious surfaces and highly stressful urban environment causes significant shifts in arthropods' communities,

releasing populations of herbivorous from top-down control, facilitating their increased abundance in cities. However, whether plants will evolve increased herbivory defence in response to elevated herbivory in urban environments remains unclear.

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