Discussion

This study shows that the invasive *Drosophila suzukii* is able to use native species of Brazilian savannas as larval resources. As expected, the samplings occurred from the beginning of the rainy season, because it is a season where breeding sites are more available to drosophilid populations (Paula *et al.*, 2014). Annual precipitation is an important abiotic variable for the establishment of populations of *D. suzukii* (dos Santos *et al.*, 2017), and the southern region of Brazil is thought to have more favorable climatic conditions to the establishment success of *D. suzukii* than the study areas (Benito *et al.*, 2016). However, our findings indicate that this species is not occurring occasionally, but already established in the study region. As most crop pests can sustain their populations within agricultural regions, such establishment is likely a spill-over effect from fruit production areas in Cerrado region, and have the potential of threatening native plant populations.

Studies indicate that the greatest losses on fruit production through oviposition of *D. suzukii* occur in species of plants with small fruits (Werts and Green, 2014). Plants of the genus *Miconia* (Melastomataceae), characterized by their wide distribution in the cerrado biome, usually have small and fleshy fruits, which would explain the greater number of flies emerging from their fruits in this work. These results reinforce the need to consider the management of exotic pests not only in agricultural systems but also in natural ecosystems given the potential for invasion *of D. suzukii* and its negative effect on the reproductive success of native species. More surveys of the occurrence of this species in Cerrado biome should be done to better evaluate its current distribution in that region. Finally, the identification of spotted wing drosophila' parasitoids in Cerrado areas would be helpful for the creation of efficient management strategies including these potential biocontrol agents.

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Expression of *UAS-lacZ*$^{4\text{-}2\text{-}4b}$ under the control of *elav-Gal4* significantly reduces lifespan in *Drosophila melanogaster.*

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Introduction

The *UAS/Gal4* system created by Brand and Perrimon (1993) is used extensively in *Drosophila* for the expression of transgenes under specific conditions. This technique can utilize ribonucleic acid interference (RNAi) to post-transcriptionally silence endogenous genes (Fire *et al.*, 1998), in order to determine the phenotypic consequences of loss of function and deduce their functions and mechanisms (Perrimon *et al.*,

2010; Ni *et al*., 2011). Both the presence of *Gal4* and the activity of the responding transgene can influence the phenotypes in *Drosophila melanogaster*; therefore, negative control critical class individuals must be generated using the most benign transgene(s) possible.

While it has been commonly believed that *Gal4* transgenes produce few negative effects in *Drosophila melanogaster*, this is not always true. For example, *GMR-Gal4*^{12} expression can result in an apoptosis-dependent "rough-eye" phenotype (Kramer and Staveley, 2003). In addition, maternally-inherited *arm-Gal4* in combination with *UAS-lacZ*$^{4-1-2}$ reduces the lifespan of critical class males (Slade and Staveley, 2015). Finally, *UAS-GFP*, which has been often utilized as a benign transgene in negative controls, has been shown to reduce lifespan and to impair climbing ability over time when expressed in some neuronal tissues (Mawhinney and Staveley, 2011). As transgenes commonly believed to be benign have been shown to produce subtle negative effects, examination of the commonly-used control transgenes is warranted.

The *UAS-lacZ* transgene is one of the most common negative controls utilized in such experiments. Multiple insertions of this transgene exist, including *UAS-lacZ*$^{4-1-2}$ and *UAS-lacZ*$^{4-2-4b}$. Our research group has conducted a number of experiments using *UAS-lacZ*$^{4-1-2}$ as a negative control, which have shown that this transgene results in a longer median lifespan than RNAi constructs which inhibit endogenous genes such as the autophagy genes, *Autophagy-related 6* or *Atg6* and *Phosphotidylinositol 3 kinase 59F* or *Pi3K59F* (M'Angale and Staveley, 2016a), *CG2076/GHITM* (M'Angale and Staveley, 2016b), *Mitochondrial calcium uptake 1* or *Micu1* (M'Angale and Staveley, 2017a), and *Pdxk* (M'Angale and Staveley, 2017b). However, while several experiments have utilized *UAS-lacZ*$^{4-2-4b}$ as a negative control (Armstrong *et al*., 2002; Elfring *et al*., 1998), a comparison of the effects of this transgene with those of the *UAS-lacZ*$^{4-1-2}$ transgene seemed desirable. The aim of the present study is to determine if the directed expression of *UAS-lacZ*$^{4-2-4b}$ may result in differences in lifespan compared to the standard *UAS-lacZ*$^{4-1-2}$ control.

Materials and Methods

Drosophila stocks and media: *UAS-lacZ*$^{4-1-2}$, *UAS-lacZ*$^{4-2-4b}$, *arm-Gal4*, and *elav-Gal4* lines were obtained from the Bloomington Drosophila Stock Center at Indiana University. Stocks and crosses were maintained on a medium consisting of 65 g/L cornmeal, 15 g/L yeast, 5.5 g/L agar, and 50 ml/L fancy grade molasses. In order to inhibit the growth of mold, 5 mL of 0.1 g/mL methyl paraben in ethanol and 2.5 mL of propionic acid were added to the medium. Stocks were maintained at room temperature (22ºC).

Drosophila crosses: To produce critical class males, *arm-Gal4* or *elav-Gal4* females were crossed with either *UAS-lacZ*$^{4-1-2}$ or *UAS-lacZ*$^{4-2-4b}$ males. Crosses were performed at either 22ºC or 29ºC.

Longevity assays: Longevity assays comparing the lifespan of the critical class progeny of the crosses described above were conducted at 22ºC and 29ºC. Critical class male flies were collected from each genotype and were placed in vials containing no more than 25 flies to avoid overcrowding. Every two days following the initial isolation date, the vials were examined to determine the number of dead flies, and fresh medium was supplied every six days. GraphPad Prism 5.03 was used to create survival curves for the longevity experiment and to conduct the Mantel-Cox test of statistical significance.

Results and Discussion

The expression of *UAS-lacZ*$^{4-2-4b}$ under the direction of maternal *elav-Gal4* resulted in a shorter lifespan compared to that of *UAS-lacZ*$^{4-1-2}$ at both 22ºC and 29ºC, as determined by the Mantel-Cox test ($p < 0.0001$). The median lifespan of the *elav-Gal4/UAS-lacZ*$^{4-1-2}$ flies was 90 days at 22ºC and 56 days at 29ºC, while the *elav-Gal4/UAS-lacZ*$^{4-2-4b}$ flies lived only 72 days at 22ºC, and 40 days at 29ºC. There was no significant difference in the lifespan of flies expressing *UAS-lacZ*$^{4-1-2}$ versus *UAS-lacZ*$^{4-2-4b}$ under the control of *arm-Gal4* at either temperature. The *arm-Gal4/UAS-lacZ*$^{4-1-2}$ flies had a median lifespan of 62 days at 22ºC, and 44 days at 29ºC, while the *arm-Gal4/UAS-lacZ*$^{4-2-4b}$ flies survived for 60 days at 22ºC and 44 days at 29ºC.

From this study, it is evident that *UAS-lacZ*$^{4-1-2}$ and *UAS-lacZ*$^{4-2-4b}$ can have different effects on lifespan, depending on the tissues in which they are expressed. Low-level ubiquitous expression of these transgenes, directed by *arm-Gal4*, does not appear to affect lifespan, whereas expression in the neurons, directed by *elav-Gal4*, caused a reduced lifespan in the flies expressing *UAS-lacZ*$^{4-2-4b}$. From this, we can

conclude that while both *UAS-lacZ* transgenes can result in the same findings when expressed in certain conditions, caution should be exercised when using *UAS-lacZ*$^{4\text{-}2\text{-}4b}$ as a negative control, as the short lifespan that can result may lead researchers to make erroneous conclusions with regards to the effects of certain transgenes on survival. Future studies should investigate whether the differences in lifespan observed between *UAS-lacZ*$^{4\text{-}1\text{-}2}$ and *UAS-lacZ*$^{4\text{-}2\text{-}4b}$ arise when other common *Gal4* lines are used to direct their expression.

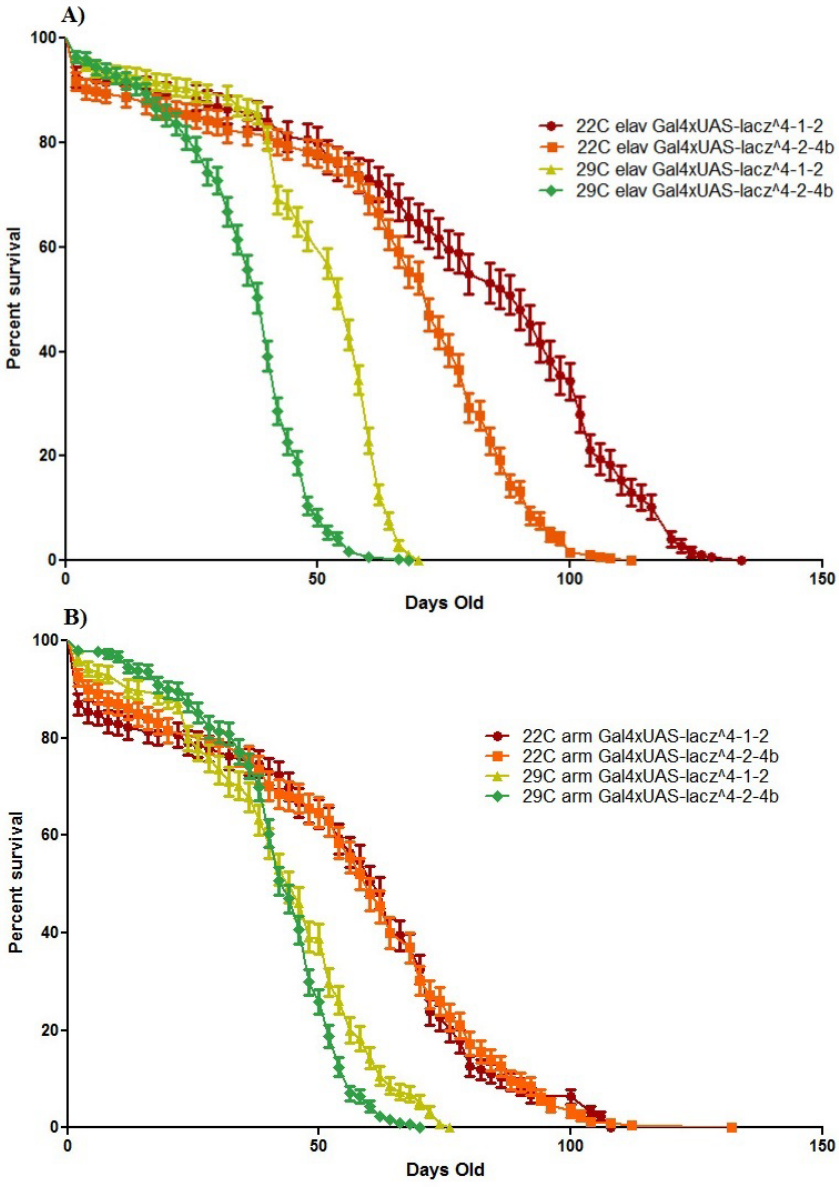

Figure 1. Survival of critical class males possessing either *UAS-lacZ*$^{4\text{-}1\text{-}2}$ or *UAS-lacZ*$^{4\text{-}2\text{-}4b}$ controlled by either A) maternal *elav-Gal4* or B) maternal *arm-Gal4* at 22ºC and 29ºC. Genotypes are *elav-Gal4/UAS-lacZ*$^{4\text{-}1\text{-}2}$ (n = 175 @ 22ºC; n = 301 @ 29ºC), *elav-Gal4/UAS-lacZ*$^{4\text{-}2\text{-}4b}$ (n = 266 @ 22ºC; n = 304 @ 29ºC), *arm-Gal4/UAS-lacZ*$^{4\text{-}1\text{-}2}$ (n = 253 @ 22ºC; n = 245 @ 29ºC), and *arm-Gal4/UAS-lacZ*$^{4\text{-}2\text{-}4b}$ (n = 238 @ 22ºC; n = 298 @ 29ºC). Error bars represent the standard error of the mean.

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