

Longevity of *Lucilia sericata* (Meigen, 1826) used as pollinator

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Highlights

- 1) Crop growers reliant on insect pollination need additional pollinating species.
- 2) The longevity of *Lucilia sericata* as a pollinator was compared under different enclosure conditions.
- 3) Provisioning greenhouses with a sugar + water solution increased fly longevity.

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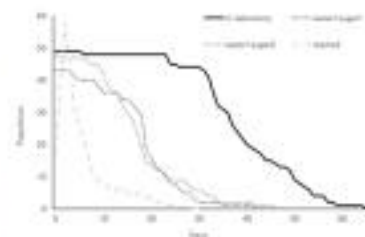
Back ground: Crop growers reliant on insect pollination need additional pollinating species.

Methods

The longevity of *Lucilia sericata* as a pollinator was compared under different conditions. Four sets of rearing cages each containing 50 adult flies were given four different treatments, 1) no food (control), 2) only water (water), 3) only sugar (sugar), and 4) water and sugar (water + sugar).



Findings



Provisioning greenhouses with a sugar + water solution increased fly longevity.

CONCLUSION

We compared the longevity of *L. sericata*, which is used as a pollinator, in cages in the laboratory and in a strawberry greenhouse under controlled feeding conditions. Better understanding the longevity of *L. sericata* under variable cage, laboratory and greenhouse conditions will improve their management as pollinator.

Abstract

Pollinators play an important role in the production of many agricultural products. Honeybees, *Apis mellifera* L., are leading pollinators, but the number of honeybees in the world is declining. Finding alternatives is beginning to be important. In the present study, we compared the longevity of *Lucilia sericata* (Meigen, 1826), which is used as a pollinator, in cages in the laboratory and in a vinyl greenhouse under controlled feeding conditions. First, we showed that the longevity of the flies was significantly extended (c.a. 40 days) in the laboratory when water and sugar were supplied compared to the cases without sugar and/or water. Second, we found that the average longevity was 20 days with water and sugar in cages kept in a vinyl greenhouse during the summer. Finally, we released marked flies into a vinyl greenhouse where strawberries were cultivated without feeding water or sugar in the spring. As a result, fewer than 10% of the flies survived 10 days after release. Based on the result, we discuss the use of this species as a pollinator in the context of its survival rate.

Keywords Diptera • lifespan • pollination • quality control

Introduction

Pollinators play an important role in the production of many agricultural products (Aizen et al. 2009), and honeybees are heavily relied upon to provide this service (Yoshiyama 2010). Globally the number of honeybees has been declining due to the inter-relationship between the stresses caused by climate change and pests and diseases (e.g., Conte and Navajas 2008; Yoshiyama 2010; Taniguchi et al., 2012). Growers of crops reliant on insect pollination also want to introduce additional pollinating species to improve crop yields and to reduce their dependence on honey bees (Osterman et al. 2021a). Currently there are at least 66 potential manageable pollinators (Osterman et al. 2021b). Although most of these are bees, Calliphorid and Syrphid flies have also received attention as manageable pollinators (Howlett 2012; Howlett and Gee 2019; Rader et al. 2020). These insects can efficiently pollinate a wide range of crops, often complimenting honey bees by being active under conditions not suitable to honey bees (Howlett et al. 2013; Cook et al. 2020; Howlett et al 2021). Calliphorid flies in particular, are effective pollinators for crops in enclosures or under nets (Howlett 2012), conditions not favoured by honey bees (Evans et al. 2019).

The common green bottle fly *Lucilia sericata* (Meigen, 1826) is one species gaining attention as a new pollinator (Hanada et al. 2016). Currently, *L. sericata* is being used as a pollinator of strawberries in enclosed cultivation on several farms in Japan, and its effectiveness as a pollinator has been confirmed (Hanada et al. 2016). In particular, strawberry plants pollinated by *L. sericata* produce fewer deformed fruits compared to plants not exposed to insect pollination (Herman et al. 2019). Although Hanada et al. (2019) note that fruits pollinated by orchard bee (*Osmia cornuta*

(Latrielle, 1805) were heavier than those pollinated by *L. sericata*, they suggest that this may have been due to lower visitation rates of *L. sericata* compared to the bees in their trials. Given that optimal strawberry pollination by *L. sericata* is likely to require frequent flower visitation by these flies, it is therefore important to understand factors such as adult fly longevity so that adequate numbers can be sustained to ensure adequate flower visitation.

Hanada et al. (2016) reported an 80% normal fruit production in strawberry pollination by *L. sericata* adults, a figure that suggests that *L. sericata* has a pollination rate comparable to that of honey bees. However, unlike the their findings for strawberries, the authors found *L. sericata* to be a less effective pollinator of egg plants, tomatoes, and melons.

Commercially, *L. sericata* used for pollination purposes have been trademarked with the name 'beeflies'. According to the "Beefly User Manual" produced by the Nara Prefecture Agricultural Research and Development Center (2019), referred to henceforth as NPAR (2019), one of the advantages of using *L. sericata* as pollinators is high performance in a wider range of temperatures. That is, honeybees are active at temperatures between 15°C and 25°C, while *L. sericata* are active at temperatures between 10°C and 35°C (NPAR 2019). Moreover, *L. sericata* flies do not require UV light for their activity, which makes them suitable for use in areas with short daylight hours and in the winter season (NPAR 2019). In addition, *L. sericata* does not sting, unlike honeybees, so it does not harm humans, and also it does not require the management of hives. Further, honeybees are used on the premise that they will return to the hive, but *L. sericata* does not need a hive and can be used for short periods. For these reasons, *L. sericata* has recently become more popular in Japan as

a pollinator.

The longevity of a released pollinator is an important characteristic of rearing and releasing. The longevity of *L. sericata* used as pollinators in enclosed greenhouses is estimated to be about two weeks (NPAR 2019). To maintain their effectiveness in strawberry cultivation, new individuals should be released in the greenhouse every 7–10 days according to the user’s manual (NPAR 2019).

Previous studies on the longevity of *L. sericata* particularly regarding their use for pollination in greenhouses, have not been performed, although there are many studies on the lifespan and life cycle in the fly species (Herrmann et al. 2019; Pruna et al. 2019). NPAR (2019) reports the longevity of *L. sericata* in a greenhouse is two weeks. However, 32% of *L. sericata* were still alive at 12 weeks (Davies 2006), and they can survive as long as 16 weeks (Barnes and Gennard 2013) under laboratory conditions. In addition, a comparison of survival rates in post-cold storage conditions in the laboratory showed that *L. sericata* can survive at long as 60 days (Ichikawa et al. 2020). Although several studies have reported the basic biology including longevity of *L. sericata* (e.g., Khater and Geden 2018; Barnes and Gennard 2013; Kim et al. 2007; Smith and Wall 1998; Firoozfar et al. 2011; Zheng et al. 2017, Parry et al. 2017, Kelany et al. 1988), few studies have assessed the longevity of *L. sericata* in greenhouses used for growing vegetables commercially. Furthermore, the longevities of *L. sericata* used as pollinators in the laboratory and greenhouse has been reported.

We first compared longevity under different feeding conditions in a laboratory. If different feeding conditions can prolong the longevity of a *L. sericata* used as pollinators, it will be possible to limit the number released in greenhouses,

improving cost-effectiveness of their management. Moreover, by comparing the longevity of the fly under both laboratory and greenhouse conditions, we can improve our understanding of how to better utilize them for pollination under different conditions.

Materials and methods

1. Materials

In this study, we set up a cage for rearing larvae (height 24.4 cm × width 28.2 cm × depth 38.8 cm) in an incubator (MIR-154, PHC, Tokyo) maintained at 25°C, containing dry dog food (300 g), liver (500 g), water (400 g), and sawdust (necessary for pupation of larvae). The dog food (Field, Petline Ltd., Gifu Prefecture, Japan) contains poultry, pulses, oils and fats, oligosaccharides, yeast, vitamins, and minerals. Eggs of *L. sericata* were donated by Japan Maggot Company (Okayama City, Japan). The larvae were grown in the rearing cage until they became pupae. We placed the adult flies that emerged from pupae in transparent plastic containers (diameter 8.6 cm × height 4.1 cm) in adult-rearing cages (height 25 cm × width 25 cm × depth 30 cm), for experiments on longevity in the laboratory. In addition, we used adult flies of the same age that we received from the Japan Maggot Company reared under the same conditions.

2. Effect of feeding on longevity in laboratory

To assess how feeding affected adult longevity, flies were exposed to different food/no food treatments. Eggs were hatched in the above-mentioned rearing cages in an incubator (MIR-154, PHC, Tokyo) and kept at 25°C. The hatched larvae were

fed the dog food described above (300 g), liver (500 g), and water (400 g) and raised until almost all of them became pupae in the incubator. After sufficient pupation, the sawdust was sieved to collect them.

Four sets of rearing cages each containing 50 adult flies were given four different treatments: 1) no food (control), 2) only water (water), 3) only sugar (sugar), and 4) water and sugar (water + sugar). Pupae were placed in each cage and allowed to emerge. Emerged flies were counted daily in each cage. Dead individuals were removed from each cage daily, and the number was recorded. We continued to count the number of living flies until all individuals had died. Individuals that didn't emerge during this study were excluded from this measurement. All groups were kept at 25°C and 16L:8D.

3. Effect of feeding on longevity in greenhouse

A similar experiment was conducted in adult rearing cages in a greenhouse applying the same treatments as in the laboratory. We prepared two replications for each treatment.

The cages were gently transferred by hand to the greenhouse (90m²: 6 m width x 15 m long) after 50 individuals had emerged. The flies that emerged were reared, and the population in each cage was checked daily. Dead individuals were removed from each cage, and the number of living flies was recorded daily. The experiment was performed between August and September 2021, and the temperature and humidity inside the greenhouse were also recorded during the measurements. We continued to count the numbers until all individuals used in this experiment had died.

4. Survival rates of *L. sericata* used as pollinators released in a greenhouse

The length of survival was estimated by releasing marked *L. sericata* in a strawberry greenhouse where the flies are actually used as pollinators (Field Science Center of Okayama University: latitude 133.91, longitude 34.69). We tracked the number of surviving marked individuals for 31 days. These pupae emerged in the rearing cage as described above. Individuals (n= 120) that emerged in 24 h were randomly selected from the cage and anesthetized with carbon dioxide gas (e.g., Miyatake and Haraguchi 1996). The anesthetized individuals were placed on an Icenon cooling pad (Hakugen Earth, Tokyo, Japan) and marked with a white quick-drying paint (Mitsubishi, Tokyo, Japan). Marked individuals were released into the strawberry greenhouse. The experiment was performed between March 19 and April 18, 2021. The strawberries were planted in the unheated greenhouse. Chemical fertilizer, 10-10-7 g.m⁻² of N-P₂O₅-K₂O, was basically applied, 4 hills of 1.3 m wide were raised, and 120 plants were planted on each hill in 2 rows: 25 cm and 20 cm apart between and within rows, respectively. No insecticides or acaricides were used during the experimental period. The greenhouse was ventilated with a fan when the temperature exceeded 26°C. We counted the number of marked live flies every day during the experiment. We used descriptive analyses (means and standard deviations) and trend-line figures to present our findings.

5. Statistical analysis

We used descriptive analyses (means and standard deviations) and trend-line figures to present our findings.

Results

1. Effect of feeding on longevity in laboratory

Figure 1 shows the length of survival of individuals after emergence for each treatment. All flies died within 7 days after emergence in all treatments except "water + sugar". In the latter treatment, the longest-lived individual was 65 days, and the mean value and SD were 40 ± 10.8 days. The longevity of flies exposed to the other treatments "control", "water" and "sugar" were not observably different (Figure 1).

2. Effect of feeding on longevity in greenhouse

The results of the greenhouse experiment are shown in Figure 2. The order in which all individuals died was "control", "sugar", "water", and "water + sugar", which differs from the laboratory results. Replications were conducted for each treatment group with similar results across replicates. However, the longevity of flies exposed to the "water + sugar" treatment was greatly extended compared to the other treatments (Figure 2) with flies found to survive 20.1 ± 8.28 d (mean $\pm$ SD) with the maximum recorded being 35 days when "water + sugar" were given in the laboratory and thus the extension was shorter than in the laboratory. Although the "sugar + water" treatment extended the longevity of flies surviving in the greenhouse, under these conditions, flies did not survive as long as those in the laboratory exposed to the same treatment. At the time of measurement, the mean $\pm$ SD temperature was $35 \pm 5.78^{\circ}\text{C}$ and the mean humidity was $50.9 \pm 18.1\%$.

3. Longevity in use as a pollinator

The numbers of marked flies in the greenhouse are shown in Figure 3. The first day of the graph is the day of the release, and numbers were counted one hour after marking. Three days after release, about half (57.7 of 120 flies) of the released individuals were found alive, whereas nine days after release, less than 10% (9.67 flies) of the released group were found alive. Most marked flies survived much less than two weeks, and marked individuals were not observed after 31 days. During the experiment, the mean temperature was $17 \pm 2.89^{\circ}\text{C}$ (SD) and the mean humidity was $57 \pm 18\%$ (SD).

Discussion

To our knowledge, this is the first study to describe the longevity of *L. sericata* when used as a pollinator for commercial strawberry crops under greenhouse conditions. We confirmed that feeding a sugar + water solution is one strategy to extend the longevity of *L. sericata* under both laboratory and greenhouse conditions. However, longevity was noted to vary substantially between these conditions (Figure 4). Currently *L. sericata* has been estimated to survive just one to two weeks when used to pollinate strawberries in greenhouses (NPAR 2019). Providing a sugar + water solution may be one way to further extend the longevity of *L. sericata* under these conditions; however, further research is required to assess how this might impact their efficiency as pollinators.

Under laboratory conditions, the longevity of *L. sericata* when fed on a sugar + water solution was found to be similar to that of a study by Ichikawa et al. (2020)

(35.8 days) but longer than a study by Blystone and Hansen (2014) (< 30 days). Although other food substrates that can also extend the longevity of adult *L. sericata* under laboratory conditions include liver (up to 116 days), agar containing 20% horse blood and 5% yeast or 5% blood agar (Barnes and Gennard 2013), these substrates are not suitable for fruit-pollinating flies due to hygiene risks.

L. sericata is capable of surviving for a substantial period of time inside shaded houses under certain conditions. For example, Davies (2006) found that 32% of flies were alive after 12 weeks in semi-protected shaded houses (away from direct sunlight, rainfall and predators) and flies are fed a daily diet of lamb or pig liver. In our study the longevity of flies fed a sugar + water solution was found to be shorter under greenhouse conditions than in the laboratory. The reason for the difference was not determined, but the mean temperature was much higher in the greenhouse (mean $\pm$ SD: 35 $\pm$ 5.78°C) and temperature and humidity fluctuated to a much greater extent. The presence of ants within the greenhouse could also possibly have impacted fly longevity (e.g., preying on the flies and consuming food), but these potential impacts require further study. The order in which all individuals died under greenhouse conditions was reversed between water and sugar only treatments compared to laboratory conditions. Access to water may possibly be more important to fly longevity than access to sugar under hot greenhouse temperatures. Overall, our findings confirm the estimated longevity of *L. sericata* (up to two weeks) when used as pollinators under greenhouse conditions NPAR (2019).

When we released marked flies into a strawberry greenhouse where this fly is typically used for pollination purposes, the number of flies seen at two weeks was greatly reduced (Figure 3). A comparison of all experiments conducted in this study

is shown in Figure 4. An important difference in the observed longevity in these experiments is that the number of individuals in the cage placed in a greenhouse began to decline at two weeks, while the number of marked individuals released in greenhouse had completely declined to zero during the first two weeks. This difference suggests that there may be room to prolong the longevity of *L. sericata* used as pollinators in a greenhouse. One possible reasons for the difference in the results between the experiments conducted in the greenhouse and the experiments in which marked individuals were released is the significant difference in temperature or other conditions due to the difference in the season in which these experiments were conducted. In addition, the difference in humidity, the presence of predators such as frogs and spiders in the greenhouse (they were indeed present in the house for the duration of this experiment: K. Shimomae, personal observation), and the fact that flies cannot consume foods in the greenhouse as consistently as they can in rearing cages may also affect this difference. For example, strawberries themselves could serve as nectar for flies, but this is a subject for future research.

The presence of materials attractive to flies, such as fruit residues and dead animal bodies, is believed to decrease the efficiency of flower visitation by *L. sericata* (NAPAR 2019). Similarly, feeding *L. sericata* with water + sugar solution in greenhouses could interfere with its effectiveness. Furthermore, the balance between fly pollination effectiveness and longevity requires additional evaluation to determine whether feeding flies a sugar + water solution is worthwhile.

Our study provides information to further understand and develop *L. sericata* as a managed crop pollinator. Such knowledge will assist growers to further diversify their pollinating species for crop pollination, reducing reliance on only honey bees

(Osterman et al. 2021a). Calliphorid flies, including *L. sericata*, are common pollinators of a range of crops (Howlett et al. 2021; Rader et al. 2020) and offer significant potential as managed crop pollinators. However, to development management strategies for such species requires a much greater understanding of their life-cycle requirements.

Conclusion

We compared the longevity of *Lucilia sericata*, which is used as a pollinator, in cages in the laboratory and in a strawberry greenhouse under controlled feeding conditions. Better understanding the longevity of *L. sericata* under variable cage, laboratory, and greenhouse conditions will improve their management as an alternative pollinator to honey bees.

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Legends of Figures

Fig. 1 Effect of feeding on longevity of *Lucilia sericata* in the laboratory. Solid lines indicate controls, dotted lines (circles) indicate rearing with only water, dotted lines (squares) indicate rearing with only sugar, and wavy lines indicate rearing with water and sugar. All groups were kept at 25°C and 16L:8D light-dark conditions. The vertical line shows the number of surviving individuals, and the horizontal line shows the number of days since the measurements were started.

Fig. 2 Effect of feeding on longevity of *Lucilia sericata* in the greenhouse. The allocated number in each treatment group indicates the number of repeated trials. Solid lines indicate controls, dotted line (circles) indicates rearing with only water, dotted line (squares) indicates rearing with only sugar, and wavy lines indicate rearing with water and sugar. The vertical line shows the number of surviving individuals, and the horizontal line shows the number of days after the measurements were started.

Fig. 3 Longevity of flies estimated by tracking marked *Lucilia sericata* adults. Anesthetized marked flies (120) were released in a greenhouse where strawberries were growing. One hour after the release, we circled the greenhouse and counted the number of marked individuals. This was repeated three times on the first day. Thereafter, we counted marked individuals every other day. The number of observed individuals represents the average of three measurements.

Fig. 4 Comparison of results from the laboratory, greenhouse, and release of marked *L. sericata* individuals into the greenhouse. The solid line indicates flies fed with water and sugar (water + sugar1) in the laboratory, dotted line indicates flies fed with water and sugar (water + sugar2) in rearing cages in the greenhouse, and wavy line indicates the marked individuals released in the strawberry greenhouse.

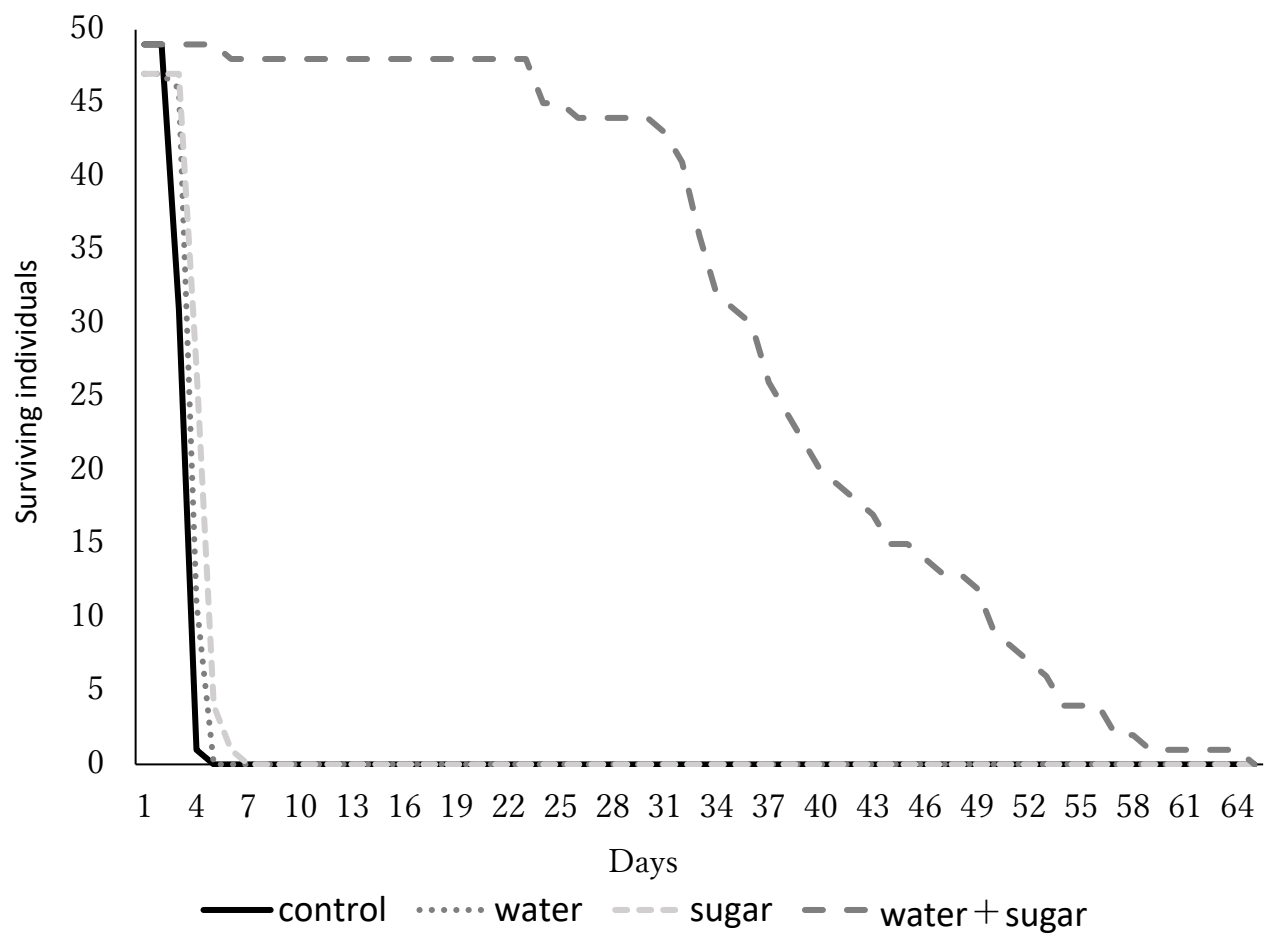


Figure 1

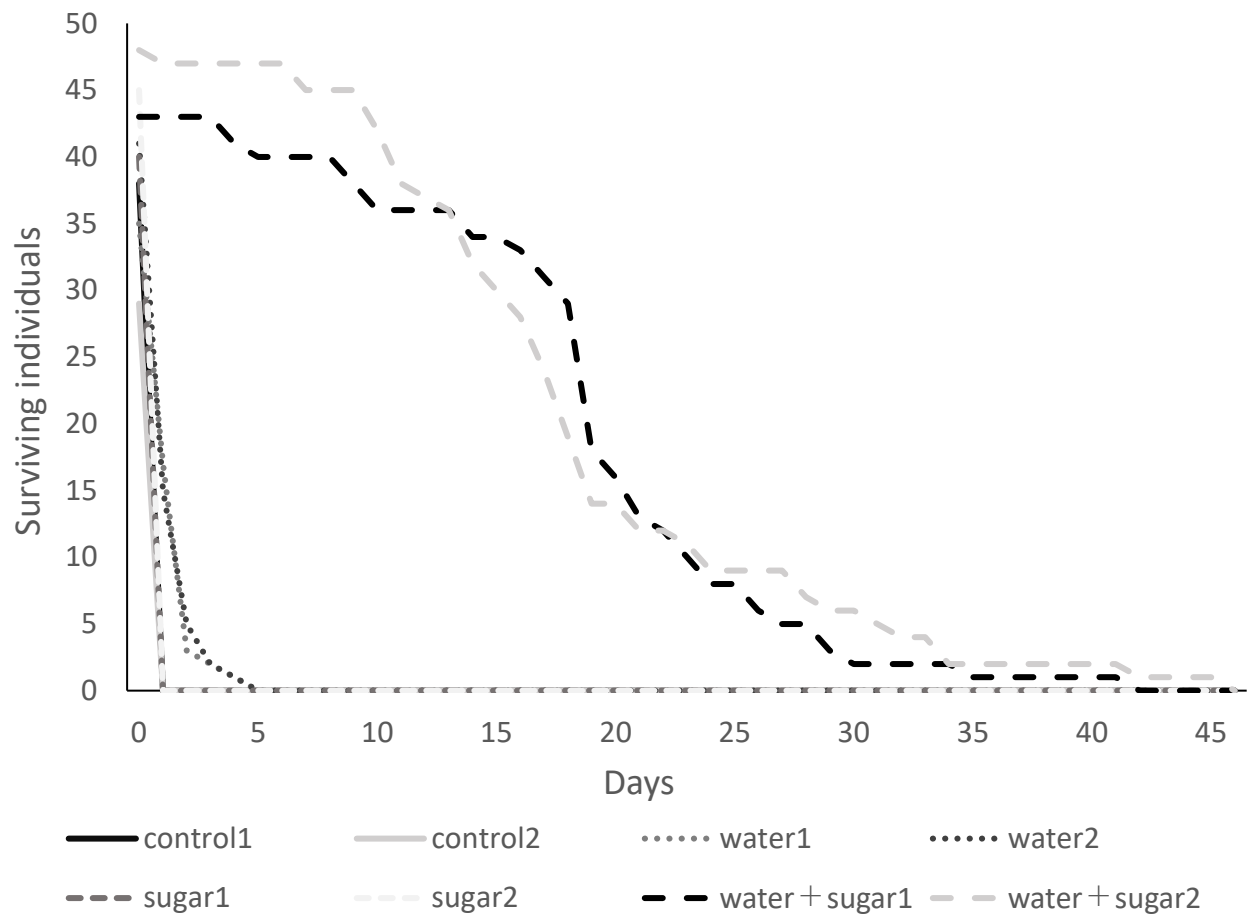


Figure 2

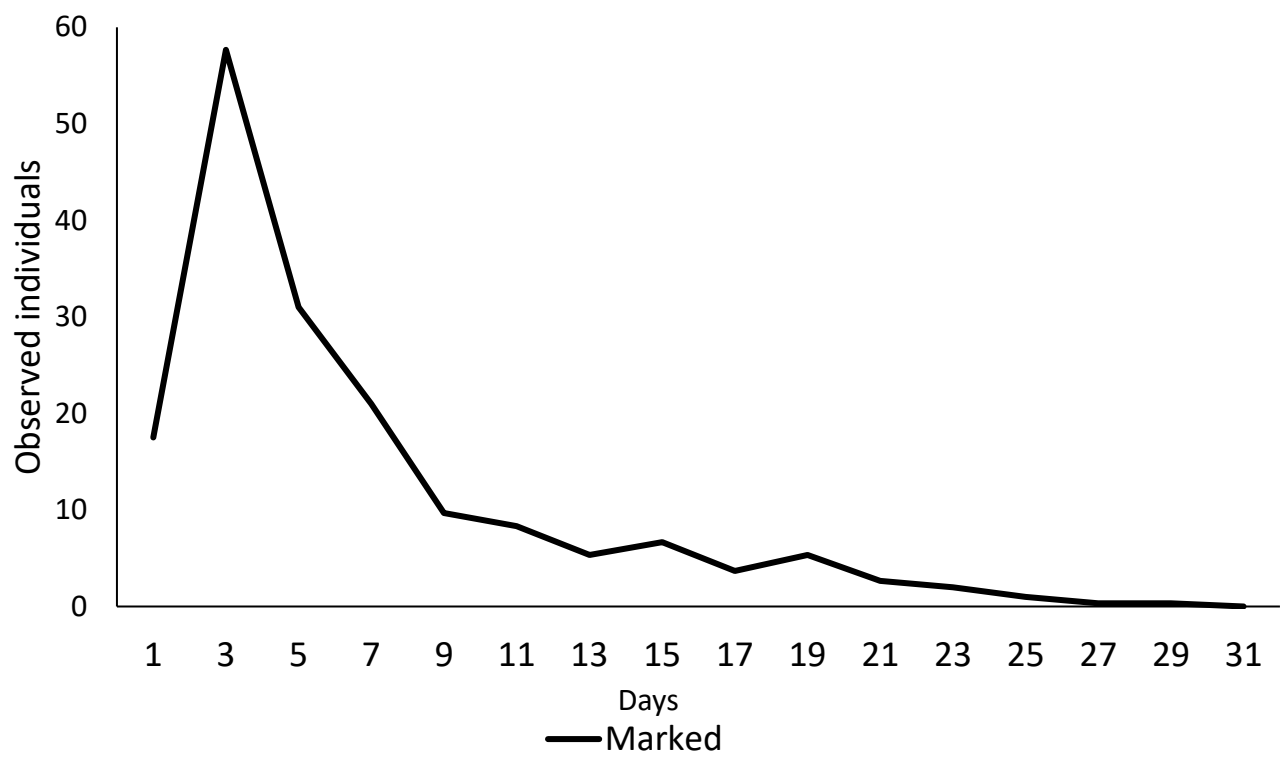


Figure 3

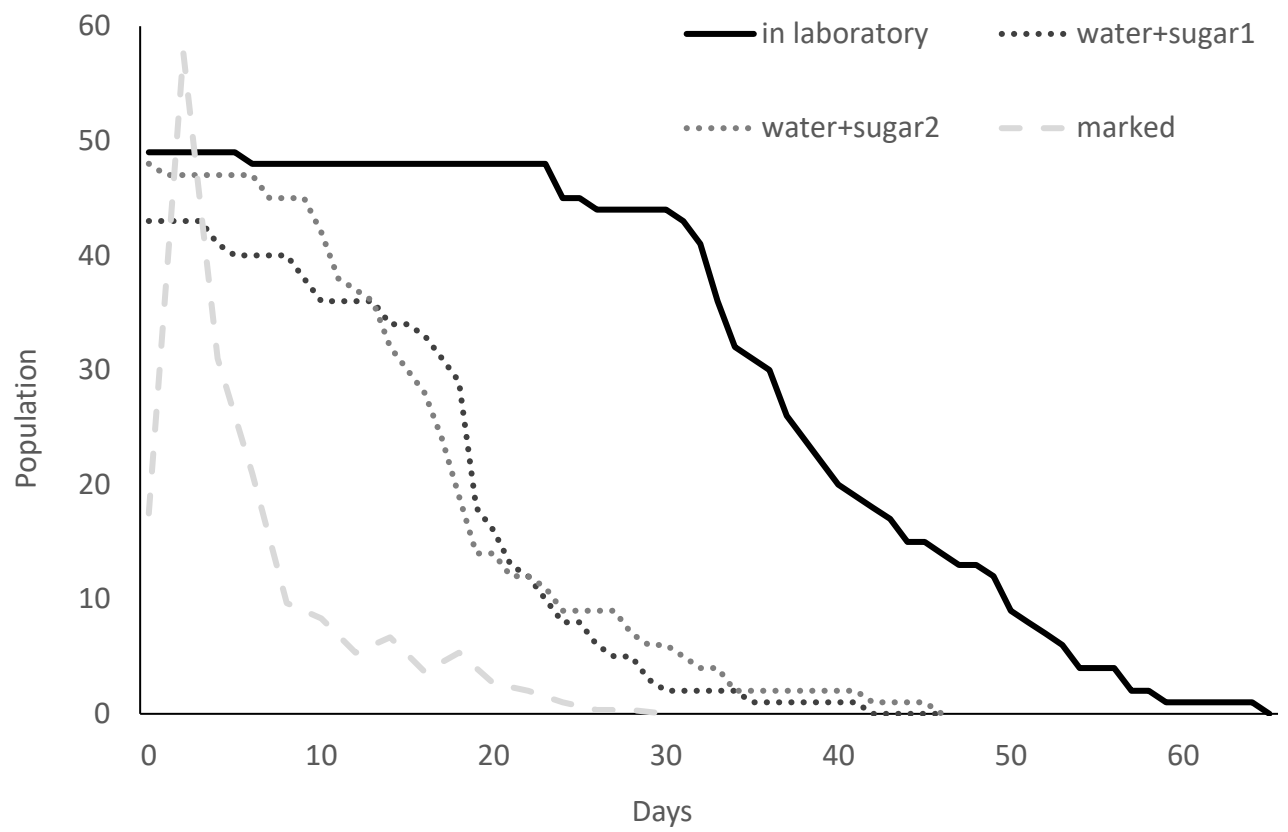


Figure 4