# Technique Notes

## Creation of artificial berries as a tool to study reproduction in *Drosophila suzukii.*

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**Abstract**

*Drosophila suzukii* is an invasive fruit fly species that has spread from Southeast Asia to North America and Europe. The introduction of the species has resulted in massive crop loss among soft skinned fruits due to the species' rare ability, among *Drosophila*, to lay eggs directly into ripe fruit. Chemical and biological control methods have not shown clear indications of successfully suppressing population growth. In an attempt to better understand what properties make cultivars of fruits either more or less susceptible to parasitism by *D. suzukii*, artificial berries were created as a means to provide uniform berries in which a single chemical or morphological feature could be manipulated. By controlling the medium and incubation periods, chemical composition and berry firmness, respectively, were successfully varied. Artificial berries showed attractiveness and reproductive viability similar to those of real blueberries. Further, artificial berries in which sugar content was manipulated indicated that sugar content is important for berry selection; however, sugar is not the only factor that effects selection. Key Words: *Drosophila suzukii*; Oviposit; Fecundity; Artificial Berry; Crop Protection

**Introduction**

The spotted wing fruit fly, *Drosophila suzukii,* is an invasive pest that targets thin-skinned fruits such as blueberries, raspberries, cherries, and grapes (Lee *et al.*, 2011; Walsh *et al.*, 2011; Asplen *et al.*, 2015). While most other drosophilids lay their eggs in rotting fruit, the ability of *D. suzukii* to use ripe fruit, via a specialized serrated ovipositor, makes them a serious problem for market growers (Bellamy, Sisterson, and Walse, 2013; Kinjo *et al.*, 2013; Steffan *et al.*, 2013). The commercial impact of *D. suzukii* has been considerable, with estimated crop losses of 20-50%, and the potential for upwards of $500 million in lost revenue annually (Bolda, Goodhue, and Zalom, 2010; Walsh, *et al.*, 2011; Cini, Ioriatti, and Anfora, 2012). Efforts to develop control methods have included baits (Cha *et al.*, 2012; Hamby *et al.*, 2013; Cha, Landolt, and Adams, 2017), insect predators (Mazzetto, 2016, Woltz *et al.*, 2014), and pesticides. However, it is unclear if baits can depress populations sufficiently to protect crops, biological controls have not been proven effective, and the pest's use of ripe fruit makes pesticide application problematic (Bruck *et al.*, 2011).

Determining which factors play significant roles in oviposition choice and reproductive success may aid in applying current, and developing new, detection and management tools. Head to head tests have indicated that fruits such as raspberries are more attractive as oviposition sites than other potential targets (Bellamy *et al.*, 2013). Further, characteristics such as firmness, sugar content, size, and pH have been tested to determine if certain varietals or ontogenic stages of the fruit may be more susceptible than others (Gong *et al.*, 21016, Lee *et al.*, 2015, Kinjo, 2013, Lee *et al.*, 2011). While these studies have provided critical information that growers may be able to use in developing or switching to more resistant crops, it remains difficult to isolate individual characteristics while controlling for others. Using real berries, for instance, makes it possible to compare cultivars for the effect of firmness on oviposition (Kinjo, 2013, Lee *et al.*, 2015), but there are many other differences between varietal treatments that may be affecting these results. Similarly, as fruit ripens, firmness, size, color, and sugar content can all change independently of each other making it difficult to assign significance to a specific factor. The use of artificial substrates whose contents can be controlled and manipulated factors has to date relied on simple sheets that may have their own limitations. *D.*

*suzukii* are known to use visual cues such as color and size in their oviposition choices, both of which are difficult to reproduce using filled petri dishes and other two-dimensional target choices (Kirkpatrick *et al.*, 2016; Takahara and Takahashi, 2017). Finding a way to make an artificial fruit that allows both the physical components (*e.g*., size, shape, and firmness) and chemical components (*e.g*., sugar content, acidity) to be simultaneously and independently manipulated may help us get a better grasp of the relative importance of these factors.

This paper provides a description of a technique, borrowed from molecular gastronomy, to create artificial fruit (in this case blueberries) with defined and controllable physical and chemical characteristics. The effectiveness of these artificial berries was then tested to investigate their attractiveness and viability as oviposition sites.

**Materials and Methods**

*Fly cultures*

We used a laboratory cultured population of *D. suzukii* that was originally started from wild-caught flies on the Saint Joseph's University campus (Philadelphia, PA) in 2014. Stocks were kept in 15 mL vials containing fly food (Formula 4-24 Instant Drosophila Media), yeast, several blueberries, and an aqueous 0.5 % propionic acid solution to hydrate the fly food. Fly stocks were kept at 21$^{o}$C under 16:8 L:D conditions with stocks transferred to fresh food vials weekly.

*Artificial berry production*

Artificial berries can be created through the addition of sodium alginate to a homogenous liquid medium at a ratio of 1 gram of sodium alginate for every 100 mL of medium. Adding the sodium alginate to the medium while it is being mixed (in this case during the blending of the real blueberries) ensures consistent and thorough mixing throughout. The solution is then added, drop-wise with a wide mouthed syringe (the size and amount dispensed determined the size of the resulting berries), into a 1% calcium chloride water bath. The resulting spheres are then left in the bath for 30 minutes, removed, and washed with cold water to remove excess salt from the surface. Spheres may be kept refrigerated and used within two days. Three different berries were produced: pure blended blueberries, blueberries plus 10% additional sugar, and sugar water at equal concentration to the real berries as determined via the USDA's database for food nutrients (Agricultural Research Service 2016). To test our ability to modify the berries firmness, soak times were varied in a separate set of production trials to investigate its effect on resulting berry firmness. Firmness of the berries (both real and artificial) was determined by measuring the maximum force needed to pierce the berry with a thin screw. Force was determined using a Vernier pressure sensor (Vernier Software and technology, Beaverton OR), which was driven into the berries using a Velmex BiSlide (Velmex, Inc., Bloomfield, NY) positioner. Maximum force was determined using LoggerPro (Vernier software and Technology, Beaverton OR).

*Single female assays*

Virgin, two-day-old male and female pairs were placed into vials and observed until they copulated. Directly following the completion of copulation, the females were placed, individually, into a vial containing three treatment berries. Five days later the females were removed from their vials and total cumulative counts of adult offspring were made between day 14 and 21, post-copulation. Differences between treatments were determined *via* a one-way ANOVA with LSD *post-hoc* analysis.

*Population box assays*

To assess both oviposition choice and treatment effect on reproductive success, groups of mixed sex flies were placed in population boxes and presented with a choice of different berry types. Each clear acrylic population box was 46 cm × 21 cm in size, with mesh ports at each end to allow airflow through the box. Each box also contained ten threaded openings in the floor into which 100 mL glass jars could be screwed at an even spacing in a 2 × 5 pattern. To keep the box humid and prevent the berries from drying out, two 500 mL beakers, and the two center jars, were filled with water. The remaining eight jars contained six each of either real blueberries, artificial blueberries, artificial blueberries with an additional 10% cane sugar, or spheres made of a medium of 10% cane sugar solution. Each box contained two jars of each the above treatments. The location of each jar was semi-randomized, with no two jars of the same treatment placed next to each other.

Fifty adult flies were counted under $CO_2$ anesthesia, then spread out evenly throughout the box. The number of flies in each jar was then recorded once/day over five days (and subsequently averaged), after which the jars were removed from the box and the contents (minus flies) were transferred to individual vials. Total cumulative counts of emerging adult offspring were recorded for a week-long period starting 14 days after the flies were initially introduced to the boxes. The percentage of the total population found on each berry type was averaged between replicate jars within each box, as were the offspring counts, producing one data point per box for each treatment. The box trials were replicated eight times. Differences between treatments were determined via a one-way ANOVA with an LSD *post-hoc* test. Percentages of parent flies on each berry type were arcsin transformed before statistical testing to meet the requirements of ANOVA.

**Results**

Changes in soak time in the calcium chloride bath were found to modify the firmness of the berries with increased soaking leading to increased firmness. Soak time and firmness followed a very linear relationship (linear regression, $r^2 = 0.69$, $F = 86.97$, $p < 0.001$) with a 4× increase in soak time leading to an ~4× increase in firmness. However, our firmest artificial berries were 24% less firm than real berries.

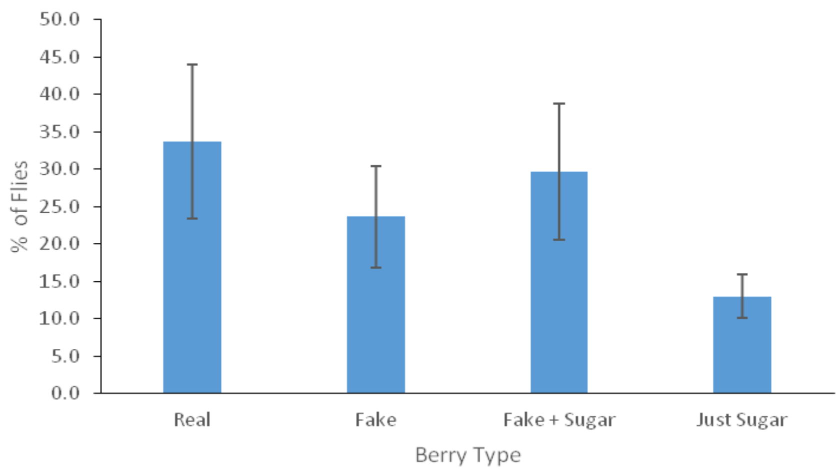


Figure 1. Average (± standard deviation) percentage of fly population found on each type of berry (average of two sub-replicate jars, averaged over five daily observations).

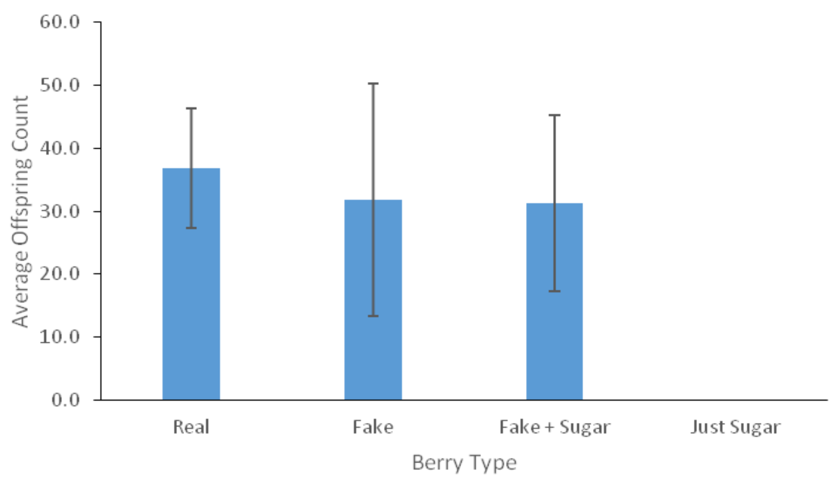


Figure 2. Average (± standard deviation) offspring counts (average of two sub-replicates per box) for each population box berry treatment.

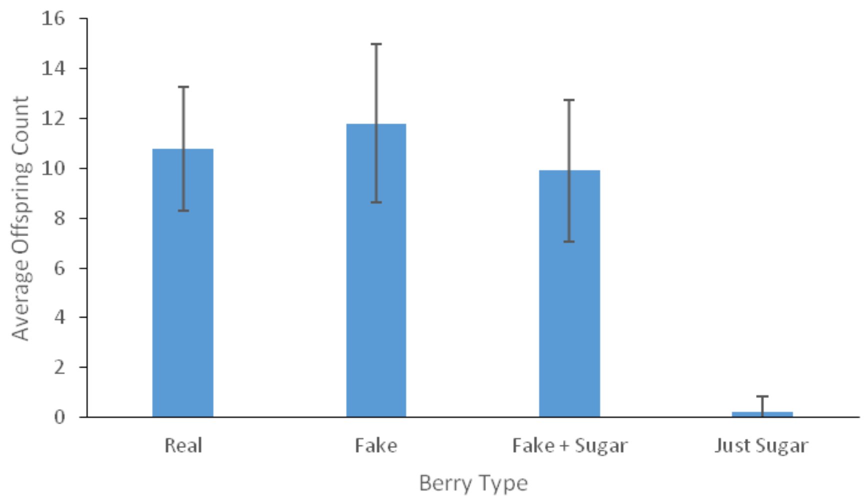


Figure 3. Average (± standard deviation) offspring counts (average of two sub-replicates per box) for individual female berry treatment.

Population box assays showed significant differences among treatments for number of adult flies on each type of berries (one-way ANOVA df = 3, F = 10.7, $p < 0.001$), with significant differences between the real berries and both the fake and sugar water treatments (Figure 1). Offspring counts similarly showed significant differences between treatments (one-way ANOVA df = 3, F = 14.5, $p < 0.001$), but this was driven almost solely by the lack of offspring in the sugar water treatment (Figure 2).

Offspring production from individual females with access to different berry types showed significant differences (one-way ANOVA df = 3, F = 47.9, $p < 0.001$) with the same pattern of production seen in the box assays: no difference in production between treatments except the sugar water berries (Figure 3).

**Discussion**

This paper illustrates a new way to produce artificial berries for studies on the reproductive biology of *D. suzukii*, a species of drosophila that relies heavily on ripe fruit for oviposition. Both the chemical and physical characteristics of these berries were manipulated to isolate specific aspects of oviposition choice and larval developmental success. We have shown that the attractiveness and ability to support eggs and larvae of *D. suzukii* were the same between real and artificial berries.

Comparisons of real, artificial, and sugar water berries indicated that adult flies were using more than sugar content to determine oviposition suitability. While similar numbers of adult flies were found on real and artificial (made with pure blueberry juice) berries, significantly fewer flies were found on the berries made with pure sugar water with a brix level equal to the real berries. Interestingly, while flies preferred artificial berries made with juice over sugar water, the flies preferred artificial berries with added sugar. Therefore, it appears that though flies were influenced by components other than sugar, sugar levels did play a role in their choice. The need for additional (+10%) sugar in the artificial berries to match the attractiveness of real berries may indicate that there is a factor not perfectly recreated in the artificial berries that the sugar-spike helps to overcome. That said, the levels of attractiveness were very similar.

Once oviposition occurred there were no differences among berry types, aside from the sugar water treatment, in terms of offspring production. This indicates that the conditions necessary for egg viability and larval development were successfully recreated in our artificial berries. Further, the same pattern was seen in both the population boxes and the individual female assays.

The attractiveness and reproductive viability of these artificial berries, along with the ability to manipulate many of their characteristics, makes them a useful tool in determining fruit susceptibility and control methodologies for ripe-fruit seeking pests. While this study only investigated the effects of sugar and juice content, it is certainly possible to isolate and manipulate a wide range of other chemical characteristics that may vary among varietals. As the technique described here can be used for any liquid it would be possible to add, subtract, or modify real juice or artificial mixtures. It may be possible, therefore, to determine the exact chemical cues adults use to choose oviposition sites. Further, the same approach can provide information on the environment necessary for egg and larval development, which may in turn provide additional control options.

While such chemical manipulations are possible using 2D petri-dish sheets of juice infused agar or other materials (*e.g.*, Takahara and Takahashi, 2017), our berries look very much like real berries which may provide some advantages. Admittedly, more work remains to increase the firmness of the artificial berries to match that of their real counterparts. We are, however, able to manipulate berry size along with their firmness, providing more realism and the ability to independently vary physical parameters separate from chemical characteristics. This capability may be of particular importance when studying the effect of fruit ontogeny where different aspects may vary at different time points. Overall, we have found that this method of producing artificial berries provides an easy and versatile approach to studying the reproductive and nutritional needs of an important agricultural pest species.

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## Trap model for capturing *Drosophila suzukii* (Matsmura, 1931).

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**Abstract**

The monitoring of the Spotted Wing Drosophila relies on a variety of correlating factors, necessary for an effective detection of population density of a given place. The type of trap is one of said factors, and