(*Fragaria* x *ananassa*), surinam cherry (*Eugenia uniflora*), strawberry guava (*Psidium cattleianum*), and guava (*Psidium guajava*) crops (Figure 2).

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Rearing method for *Drosophila suzukii* and *Zaprionus indianus* (Diptera: Drosophilidae) on artificial culture media.

Schlesener, Daniele Cristine Hoffmann*[1], Jutiane Wollmann[1], Alexandra Peter Krüger[1], Liliane Nachtigall Martins[1], Fernanda Carla Santos Geisler[2], and Flávio Roberto Mello Garcia[1,2]. [1]Crop Protection Postgraduate Program, Eliseu Maciel School of Agronomy at the Federal University of Pelotas (UFPel), Pelotas, RS, Brazil; [2]Entomology Postgraduate Program, Biology Institute at the Federal University of Pelotas (UFPel), Pelotas, RS, Brazil. *Corresponding author: mity_dani@yahoo.com.br

Introduction

The Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae), is an alien species whose main characteristic is an ability to infest intact fruit, perforating it in order to lay eggs and allowing the possibility of larval development.

Since *D. suzukii* was identified in a Californian raspberry field in 2008 (Hauser, 2009), it has spread rapidly throughout North America (Walsh *et al*., 2011; Hauser, 2011), Europe (Cini *et al*., 2012), and South America (Deprá *et al*., 2014), causing damage to a series of commercial fruit crops, wild fruits, and ornamental plants (Lee *et al*., 2015; Schlesener *et al*., 2015; Arnó *et al*., 2016). A recent study indicates environmentally adequate areas in Oceania and Africa where a particular predisposition to the occurrence of *D. suzukii* is found, although there has been no record of the species occurring in those continents. Models indicate that -

due to the environmental conditions - those areas are prone to the establishment of this species in case of future invasion (Dos Santos *et al.*, 2017).

The SWD displays a preference for soft, fragile tegument fruits such as blackberries (*Rubus* sp.), cattley guava (*Psidium cattleianum* Sabine), cherries (*Prunus* sp.), raspberries (*Rubus idaeus* L.), strawberries (*Fragaria x ananassa* Duch.), surinam cherries (*Eugenia uniflora* L.), grapes (*Vitis* spp.), amongst others (Bolda *et al*., 2010; Burrack *et al*., 2013).

Drosophila suzukii presents some morphological characteristics that allow it to be easily identified from other *Drosophila* species of the *melanogaster* species group. Males display a dark spot along the front edge of each wing and two rows of combs on the first and second tarsal segments of the first pair of legs, whilst females have a doubly serrated and narrow ovipositor, lined with robust sclerotized teeth (Kansawa, 1939; Walsh *et al.*, 2011; Vilela and Mori, 2014). The species has a short biological cycle, with high biotic potential and overlapping generations (Emiljanowicz *et al.*, 2014) and a preference for mild weather (Mitsui *et al.*, 2010).

The African fig fly, *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae), is also an invasive species with a high colonization potential, spreading rapidly throughout tropical regions, probably due to the intensification of world fruit trade (Stein *et al.*, 2003). This species presents a significant capacity for survival in anthropized settings, with a high biotic potential, and it is considered to be one of the most abundant species amongst drosophilid community members in Brazil (Silva *et al.*, 2005). The African fig fly has a yellow/brownish color, and it can be identified by the presence of longitudinal white stripes with narrow black borders over the head and thorax (Vilela, 1999).

Zaprionus indianus presents a generalist feeding behavior, where most of its nutrition comes from bacteria and yeast found in damaged and/or decomposing fruit, particularly the *Candida tropicalis* (Commar *et al.*, 2012) yeast. This substrate is used in feeding for adults, oviposition, and larval development (Vilela *et al.*, 2000), thus considered a secondary pest. Nevertheless, this species is the main pest to fig (*Ficus carica* L., var. Roxo de Valinhos) crops, since it lays eggs through ostiole of the fruit, while it has just started ripening. Hence the name 'African fig fly' (Vilela *et al.*, 2000).

The aforementioned species are of great importance in fruit production, since both cause damage to economically significant crops. In order to perform studies in the species mentioned before, it is essential to use a defined, low cost, fly culture medium to keep these species under laboratory conditions, and obtain a large number of flies all year around. In this context, we present a recipe of food media and raising protocol to breed *D. suzukii* and *Z. indianus* in the laboratory.

Materials and Methods

Drosophila suzukii and *Z. indianus* stocks were established in the Insect Ecology Lab, in the Ecology, Zoology, and Genetics Department from the Biology Institute in the Federal University of Pelotas (UFPel). Fly cultures were conditioned in B.O.D. (Biochemical Oxygen Demand) climate chambers at 23 $\pm$ 1ºC for *D. suzukii* and 25 $\pm$ 1ºC for *Z. indianus*, 70 $\pm$ 10% relative humidity (RH), and 12:12h photoperiod.

Collecting flies

Adults of *D. suzukii* were obtained from infested fruits. Blackberry fruits (*Rubus* spp.) were collected in a rural property located in Rincão da Caneleira, Pelotas, Rio Grande do Sul, Brazil (31°38'20"S and 52°30'43"W). After collection, fruits were placed in a thermal box and sent to the lab, where they were weighed and individualized in plastic containers (150 mL) with a screened lid and a fine layer of vermiculite (1 cm). Fruits were kept in a climate-controlled room at 23 $\pm$ 2°C temperature, 70 $\pm$ 10% RH, and a 12:12 h photoperiod, until adult flies emerged. After emerging, assumed *D. suzukii* couples were kept in glass vials (8.5 × 2.5 cm) containing the food media (see below) and covered by hydrophilic cotton plugs (Figure 1 A). Couples were kept for a 10 day period, so they could copulate and oviposit, followed by specific confirmation of specimens by diagnosed taxonomic characteristics (Vlach, 2013), aided by an optical stereomicroscope.

The flies that originated from the *Z. indianus* stock came from infested strawberry fruits collected in Pelotas, Rio Grande do Sul state, Brazil (31°40'49"S and 52°26'14"W).

Table 1. Fly food ingredients to culture *D. suzukii* and *Z. indianus*

Ingredients	Quantity
Distilled Water	1000 mL
Bacteriological Agar	8 g
Brewer's Yeast	40 g
Cornmeal (medium sized grains)	80 g
Fine sugar	100 g
Propionic acid	3.0 mL
Methylparaben (10%)*	8 mL

*Methylparaben: 0.8 g dissolved in 8 mL of ethanol 99,9%

Fly medium recipe and medium preparation

The fly medium recipe used to culture *D. suzukii* and *Z. indianus* described here (Table 1) is a modified fly medium referred to as standard cornmeal-yeast-glucose-agar medium by Matsubayashi *et al.* (1992), traditionally used to culture *Drosophila* species at several *Drosophila* labs in Japan.

The preparation of the fly medium has the following steps: 1) add 1/3 of the distilled water (330 mL) to the cornmeal and leave it to rest; 2) mix the brewer´s yeast, agar, and the remaining water (2/3 of water), and cook at high heat, constantly stirring, until boiling; 3) add the hydrated cornmeal and keep stirring until boiling; 4) finally, adding the fine sugar and keep stirring over mild heat until it boils once more; 5) remove the pot from heat and immediately add the propionic acid and methylparaben solution. Allow the solution to cool slightly; 6) dispense the media into the glass vials (8 mL medium per vial); 7) wait for cooling and vapor release of excess humidity of the vials under room temperature. Cover the vials with a fine cotton cloth, to avoid contamination by other drosophilids. 8) Afterwards, plug the vials with hydrophilic cotton. If necessary, the fly media can be stored in hermetically sealed plastic bags in refrigerator at 5 $\pm$ 2°C temperatures for up to 30 days.

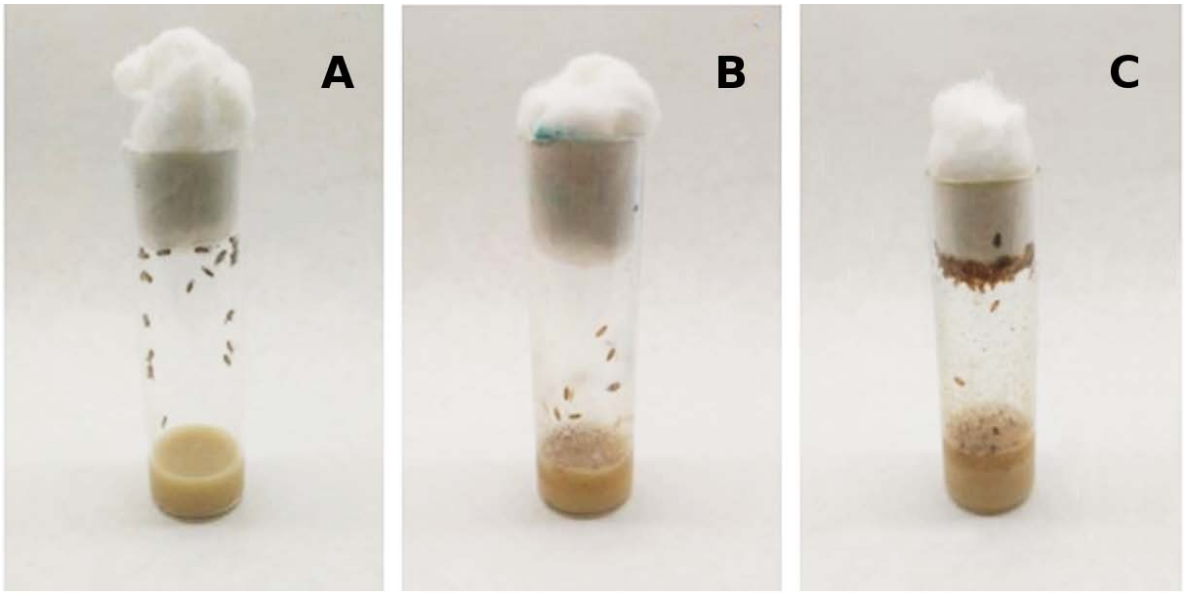

Figure 1. Rearing *D. suzukii* at the laboratory. A) Adults in oviposition stage; B) fly culture media with early stages of development, larvae, pre-pupae and pupae; and C) fly culture showing empty pupae.

Culturing fly

To keep *D. suzukii* and *Z. indianus* cultures in good health, maintenance ought to be done twice a week, consisting in the transference of recently emerged flies, those still on a pre-oviposition period or on early stages of laying eggs, into new vials (Figure 1A), and eliminating senile subjects and tubes where the

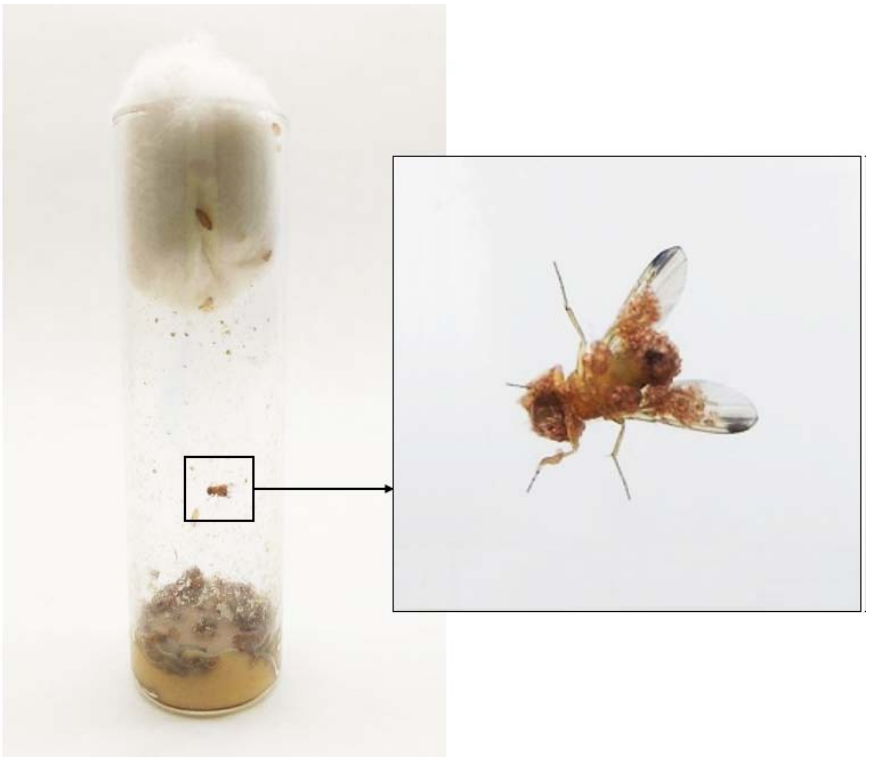

Figure 2. Rearing *D. suzukii* at the laboratory. Fly culture infected with mites (left). In detail, a male infected with mites.

majority of flies has already emerged. At each of the removal of flies it is important to properly mark the tubes, and the recipients of new subjects dated accordingly, as to maintain an organized culture.

Flies as old as three days are placed into new food vials (Figure 1A), which will be a source of humidity, substrate for egg-laying, and food for larvae and adults. Flies will remain in this environment for up to seven days, after which they can be transferred or discarded (Figure 1B). Keeping flies inside the vials for a longer period is not recommended, because it will be overcrowded, with consequent competition for food and loss of insect quality. The optimum density of adults per vial will be that in which the number of flies may occupy, at most, half the surface of the medium. If the aim is to get a lot of progeny or to obtain vials showing the developmental life cycle, make a new replica every 3-5 days.

Cultures that do not present further adult emergence must be discarded (Figure 1C). The discarding must also be done when the presence of mites adhered to the flies body is identified (Figure 2). The occurrence of mites is ordinary in colonies, but that may lead subjects to be not as fit, because of the stress induced by the mites. The presence of mites can be minimized by eliminating contaminated culture vials and cleaning the benches and instruments with alcohol 70%. The use of cotton plugs will minimize the dissemination of mites to the laboratory; make sure that the plugs fit tight to the culture vial.

Cleaning the glass vials must be performed on a weekly basis. The remainder of the fly media is removed with tweezers and spatula, and the vials are soaked into a hot sodium hypochlorite (3%) solution for up to 24 h. Afterwards, the vials are rinsed with boiling water and placed on test tube racks to drain, and later dried in lab stoves (at 35°C), where they remain until they are once again used.

Results and Discussion

Drosophila suzukii average development time (egg to adult) was 11 days at 23°C. Emiljanowicz *et al.* (2014) have observed a longer period (12.8 days), most likely due to the lower temperature (22°C) and medium composition. Andreazza *et al.* (2016) used a modified medium from Emiljanowicz *et al.* (2014) and obtained adults in 11 days at 25ºC temperature. In a study that sought to compare natural (blueberry) and artificial yeast based diet, it was observed that the development time was shorter when flies were fed a natural diet, and the average development periods were 10.6 and 11.7 days, respectively (Jaramillo *et al.*, 2015).

The *Z. indianus* species took 14 days to fully develop (egg to adult) at 25°C. The development time in artificial diets based on banana and yeast varied between 12.7 and 28.8 days at 28°C and 18°C, respectively (Nava *et al.*, 2007). With a natural diet (fig), the African fig fly develops in an average of 17 days (Pasini and Lúcio, 2014).

The fly food and rearing method here described is suitable for the rearing of both species, since it provides adequate conditions for biological development, furthermore presenting unlikelihood of contamination by other drosophilids and opportunistic microorganisms (mites, fungi, and bacteria). In the event of contamination, the problem is easily dealt with, by eliminating the contaminated cultures, with no consequence to the remainder culture. Rearing methodology using plastic boxes as cages tends to be more prone to be contaminated by other drosophilids, since there is a larger surface in contact with the external environment. Also, detection and elimination of the contamination demands disposing of many more subjects, since the entire cage ought to be eliminated (Andreazza *et al.*, 2016). Furthermore, compartmentalization of fly cultures in several vials facilitates the removal of different biological stages of the fly for lab bioassays.

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