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Influence of dietary yeasts on the fecundity and oviposition of adult spotted-wing drosophila (*Drosophila suzukii*; Diptera: Drosophilidae)

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Abstract

The spotted-wing drosophila (*Drosophila suzukii* Matsumura; Diptera: Drosophilidae), native to Asia and widespread throughout North America, South America and Europe, is an invasive insect pest. *D. suzukii* was first recorded in Europe in Rasquera (Spain) in autumn 2008. The tendency of *D. suzukii* to lay eggs in healthy ripe fruits makes it an important insect pest for soft and stone fruits. Several studies document the attractiveness of yeast volatiles to *D. suzukii* adults and the important role of yeasts in the diet of *D. suzukii* larvae, suggesting that *D. suzukii* adults are also affected by the yeast community in their diet.

Under optimal laboratory conditions (22 °C, 76.4% relative humidity and a photoperiod of 16:8 (L:D) h) the effects of four yeast species, *Saccharomyces cerevisiae*, *Metschnikowia pulcherrima*, *Candida sp.* and *Hanseniaspora uvarum*, in the diet of a South Tyrol ecotype of *D. suzukii* adults were observed. In addition to the tested yeast cultures, two other feeding and oviposition substrates were offered. The cage design was invented for this study, and the construction steps are described in detail.

Among the yeast species known to be associated with *D. suzukii*, the ones used in this study turned out to increase the number of eggs laid compared to the yeast-less control, suggesting that yeast in the diet is beneficial but not essential for *D. suzukii* adults. Significantly more eggs were laid by females fed with *S. cerevisiae* and *H. uvarum* compared to females fed with *Candida sp*. The number of eggs laid, the survivorship, and egg laying behaviors of *D. suzukii* adults were observed simultaneously for each yeast species offered. *D. suzukii* showed a significantly shorter lifetime when fed with yeast species that lead to higher fecundity. Survivorship of *D. suzukii* males showed no significant influences by the yeast diet offered. In the present study, *D. suzukii* female flies showed no oviposition preference for yeast cultures on malt extract agar. The possible reasons for the observed behaviors are discussed in the thesis.

Keywords: *Drosophila suzukii*, spotted-wing drosophila, Drosophilidae, *Saccharomyces cerevisiae*, *Hanseniaspora uvarum*, *Candida sp.*, *Metschnikowia pulcherrima,* yeast, nutrition, oviposition, behavior

Kurzfassung

Die Kirschessigfliege (*Drosophila suzukii* Matsumura; Diptera: Drosophilidae) ist ein invasiver Schädling der ursprünglich aus Asien stammte und sich über Nordamerika, Südamerika und Europa ausgebreitet hat. *D. suzukii* wurde in Europa erstmals im Herbst 2008 in Rasquera (Spanien) nachgewiesen. Die weiblichen Tiere legen ihre Eier in weichschalige Früchte u. a. Steinfrüchte und Beeren. Dies macht *D. suzukii* zu einem wichtigen Schädling. Verschiedene Studien belegen die anziehende Wirkung von Hefen auf *D. suzukii* Fliegen und die wichtige Rolle von Hefen in der Nahrung von *D. suzukii* Larven, daraus lässt sich schließen, dass verschiedene Hefearten auch in der Nahrung von *D. suzukii* Fliegen eine wichtige Rolle spielen.
Unter optimalen Laborbedingungen (22 °C, 67,4% relative Luftfeuchtigkeit und einer Photoperiode von 16:8 (L:D) h) wurden vier Hefearten (*Saccharomyces cerevisiae*, *Metschnikowia pulcherrima*, *Candida sp.* und *Hanseniaspora uvarum)* auf ihre Auswirkungen in der Nahrung eines in Südtirol vorkommenden *D. suzukii* Stammes untersucht. Zusätzlich zu den getesteten Hefekulturen wurden den Fliegen zwei weitere Käfigkomponenten zur Eiablage und als zusätzliche Nahrung angeboten. Die verwendeten Käfige wurden im Rahmen dieser Studie entworfen, auf die Konstruktion wird ausführlich eingegangen.
Von den Hefearten, die mit *D. suzukii* in Verbindung gebracht werden können, führten die in dieser Studie verwendeten zu einer erhöhten Fekundität. Die Ergebnisse zeigen, dass Hefen in der Nahrung positiv aber für die Eiablage nicht essentiell sind. *D. suzukii* Weibchen die mit *S. cerevisiae* oder *H. uvarum* ernährt wurden zeigten eine signifikant höhere Fekundität als jene die mit *Candida sp.* ernährt wurden. Parallel zur Eiablage wurde die Mortalität und das Eiablageverhalten von *D. suzukii* Fliegen in Abhängigkeit von der angebotenen Hefe erhoben. Bei Hefen, die zu einer höheren Fekundität führten, konnte eine signifikant höhere Mortalität bei *D. suzukii* Weibchen beobachtet werden. Auf die Überlebensrate von *D. suzukii* Männchen zeigten die verwendeten Hefen keine signifikante Wirkung. In dieser Studie konnte keine bevorzugte Eiablage auf Malzextrakt Agar Hefekulturen festgestellt werden. Die Ergebnisse werden in der Arbeit diskutiert.

Schlagwörter: *Drosophila suzukii*, Kirschessigfliege, Drosophilidae, Saccharomyces *cerevisiae*, *Hanseniaspora uvarum*, *Candida sp.*, *Metschnikowia pulcherrima*, Hefe, Ernährung, Eiablage, Verhalten

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1. Introduction

1.1. The pest fly *Drosophila suzukii*

Drosophila is a genus of small flies commonly known as vinegar flies or lesser fruit flies. The genus *Drosophila* comprises ≈1,500 species in different areas worldwide (MARKOW and O'GRADY, 2006). *Drosophila* flies are attracted to fermenting organic material such as rotten fruit, on which they feed and in which their larvae develop. Of the known *Drosophila* species, *Drosophila suzukii* Matsumura, (spotted-wing drosophila, German: Kirschessigfliege) is one of only a few species known to oviposit in healthy undamaged fruit on the plant, as opposed to damaged or overripe fruit. The tendency of *D. suzukii* to lay eggs in healthy ripe fruits makes it an important pest fly for soft and stone fruits (MITSUI et al., 2006).

1.1.1. Taxonomic classification

Class:	Insecta
Order:	Diptera
Family:	Drosophilidae
Genus:	Drosophila
Subgenus:	Sophophora
Species group:	*Drosophila melanogaster* species group
Species:	*Drosophila suzukii* Matsumura

1.1.2. Range expansion

Because of globalization and the related export and import of agricultural products, various herbivorous insect pest species are no longer limited to their natural distribution. Many of these species have followed the cultivation of their natural hosts across the globe. One of these invasive insect pests is the pest fly *D. suzukii*, which is native to Asia and has now spread throughout North America, South America and Europe (ASPLEN et al., 2015). *D. suzukii* was first recorded in Europe in Rasquera (Spain) in autumn 2008, where 10 males and two females were collected (CALABRIA et al., 2012). With geographic profiling, an area in the South of France near Avignon was identified as the most likely origination point of *D. suzukii* in Europe before 2008 (CINI et al., 2014).

1.1.3. Description

Adult *D. suzukii* are small flies 2-3 mm in length, with red eyes, a yellow-brown thorax and abdomen and black stripes on the abdomen (WALSH et al., 2011). Male flies are easily identified by the black apical wing spots and the single row of combs on the first and second tarsal segment on the first pair of legs. Females can be identified by the large serrated ovipositor, which enables the female flies to oviposit in the skin of thin-skinned fruits (DEPRÁ et al., 2014; HAUSER, 2011; WALSH et al., 2011). A distinguishing feature of *D. suzukii* females in comparison to females of other *Drosophila* species is the ovipositor, which is 6-7 times as long as one spermatheca, while in most other species the ovipositor is 2-4 times as long as the spermatheca (HAUSER, 2011).

The eggs of *D. suzukii* are milky-white and glossy with two filaments, and the surface of the egg has a honeycomb structure (Figure 1). Larvae are about 0.7 mm in length at hatching (Figure 2) and develop to 3.5 mm before pupating (WALSH et al., 2011).

At present, the only life stage targeted for pest management is the adult stage (WIMAN et al., 2014). Immature stages can be identified by using a real-time PCR assay for the detection and identification of *D. suzukii* (DHAMI and KUMARASINGHE, 2014).

Figure 1. *D. suzukii* egg

Figure 2. *D. suzukii* larvae hatching form egg.

1.1.4. Biology

Continually producing previtellogenic egg chambers at a minimal functional rate make it possible for *Drosophila* species to produce finished eggs relatively quickly when good nutritional resources become available (DRUMMOND-BARBOSA and SPRADLINGER, 2001). *D. suzukii* females oviposit their eggs in the skin of soft and stone fruits (Figure 3). The larvae (Figure 4) develop inside the ripening fruits on the plant. At a single visit *D. suzukii* females can lay more than one egg in a fruit (MITSUI et al., 2006). The larvae hatch after one to two days. The life cycle of *D. suzukii* involves three instars. Larvae development is complete after 6 days and pupae hatch after another 6 days (EMILJANOWICZ et al., 2014). Several larvae can develop to the adult stage in one fruit (MITSUI et al., 2006). The sex ratio in nature is 1:1 (EMILJANOWICZ et al., 2014). On average, females produce 5.7 eggs per day with a total lifetime oviposition of about 630 eggs. The average lifespan for females is 80 days and 94 days for males at 22 °C and about 25% relative humidity (EMILJANOWICZ et al., 2014). EMILJANOWICZ et al. (2014) observed the maximum lifespan for one male reaching 154 days at 22 °C and 25% relative humidity. The numerically highest population developmental parameters were found from *D. suzukii* on 22° C (TOCHEN et al., 2014). TOCHEN et al. (2014) estimated an average of seven generations per season for Corvallis (Oregon). Adult *D. suzukii* are able to survive freezing temperatures during the winter (DALTON et al., 2011).

1.1.5. Host fruits

D. suzukii prefers raspberries, strawberries, blackberries, cherries and blueberries for oviposition (LEE et al., 2011; BELLAMY et al., 2013; REVADI et al., 2015). Other suitable fruits are grapes, apricots, peaches, nectarines, pluots, figs, hardy kiwis, persimmons, plums and pears. If injured, fruits such as apples and oranges and hosts such as dogwood, rose, snowberry, crabapple and flowering cherry may serve as alternate hosts throughout the year (WALSH et al., 2011). Laboratory observations suggest that fruit firmness is one driver of fruit selection (BURRACK et al., 2013). *D. suzukii* prefer the ripe stage of fruits for oviposition. However, as the brix level increases, more *D. suzukii* develop from fruits, confirming an expectation that *D. suzukii* prefer and develop better on riper and hence sweeter fruit. For *D. suzukii* larvae, fruit that is overripe but not spoiled is the optimal habitat (LEE et al., 2011). In addition to the degree of maturity, the penetration force of the fruit skin plays a role. The skin of intact fruit with lower penetration force is preferred for oviposition (LEE et al., 2015).

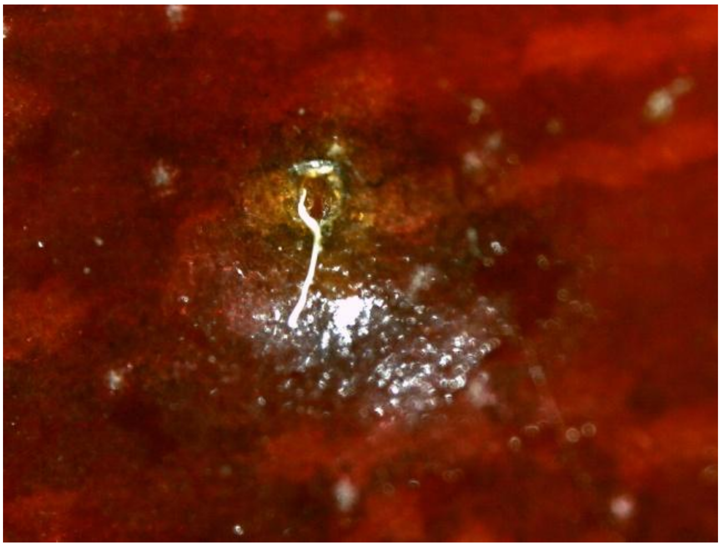


Figure 4. On infested fruits the filaments of the *D. suzukii* eggs are visible on the surface.

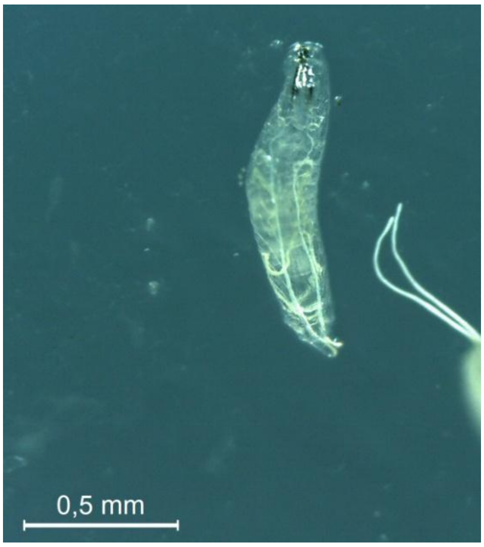


Figure 3. *D. suzukii* larvae

1.2. Yeast-*Drosophila* interactions

Previous studies have shown that yeasts, unlike other fungi, play an important role in the development and behavior of *Drosophila* species. For example, in contrast to *S. cerevisiae*, the fungi *Botrytis cinerea* and *Penicillium sp.* did not increase oviposition on grape juice substrate (BECHER et al., 2012).

1.2.1. Influence of yeast volatiles on *Drosophila* species

It is known that fermentation increases the attraction of adult *D. melanogaster* to their feeding and oviposition source (BECHER et al., 2012; BURRACK et al., 2015; FUYAMA, 1976). Furthermore, *D. suzukii* adults are attracted by many fruit volatiles (REVADI et al., 2015), vinegar and wine volatiles (LANDOLT et al., 2012; CHA et al., 2012) and grape juice (BECHER et al., 2012). Examples of volatiles produced by yeasts are ethanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, ethyl hexanoate, 2-methyl-1-butanol, 3-methyl-1-butanol, 3-hydroxy-2-butanone, ethyl octanoate, acetic acid, 2,3-butanediol, ethyl decanoate and 2-phenyl ethanol (BECHER et al., 2012). Generally, the attractiveness to *D. suzukii* decreases as the chain length of the alcohols and acetates increases (KLEIBER et al., 2014).

PALANCA et al. (2013) showed that *D. melanogaster* show sharply different preferences between volatiles of different yeast species and between different strains from the same yeast species (*S. cerevisiae*). Flies are strongly attracted to most yeast strains of *S. cerevisiae*. Some yeast strains are significantly repellent and some yeast strains have no effect. Interestingly, PALANCA et al. (2013) found no difference in attraction depending on whether or not yeast strains were directly isolated from fruits. However, they observed a significant difference in attraction based on whether or not the strains were isolated from niches associated with fruit-bearing plants.

One reason for the difference in attractiveness between yeast strains is the different respiration of strains of the same yeast species. *S. cerevisiae* cells without functional mitochondria produce different volatiles and are less attractive than cells with functional mitochondria. Furthermore, younger cultures are also more attractive than older cultures (SCHIABOR et al., 2014). In nature, the nutrition of *Drosophila* flies contains a different number of yeast species. Some yeast combinations are more attractive than single yeasts (BARKER and STARMER, 1999). Further, SCHEIDLER et al. (2015) noted differences in the yeast preference by different *Drosophila* species.

1.2.2. Yeasts in the diet of *Drosophila* species

BECHER et al. (2012) showed that yeasts are a limiting factor in the development of *D. melanogaster* and that only living and fermenting yeasts increase oviposition. In their experiments on grapes without yeast, only a very few unusually small flies developed from eggs deposited by yeast-free females on yeast-free grapes. ANAGNOSTOU et al. (2010) found an even greater influence. In their study no increase in size or morphological changes were detected on yeast-free banana medium. Furthermore, they showed that the weight of emerging *D. melanogaster* adults is influenced by yeast community in the diet and by the initial yeast cell mass.

Moreover, larvae develop faster and reach a larger size when two yeasts are present simultaneously in the diet compared to monocultures of either yeast (STARMER and FOGLEMAN, 1986). Even if yeasts have this positive effect on the larvae of *Drosophila* flies, it is not certain whether saprophagous *Drosophila* flies prefer the best yeast for nutrition and for their offspring for oviposition. This seeming “Bad-Mother-Behavior” was described by ANAGNOSTOU et al. (2010). *Drosophila* female yeast choice appears to be a food choice and not a choice of suitable oviposition substrate. Larvae, in contrast, specifically prefer the yeasts with better properties for their own development (ANAGNOSTOU et al., 2010).

1.2.3. Yeast transportation by *Drosophila* species

Symbiosis between fungi and insects is known in a number of cases. Various studies on *Drosophila* species have also shown a beneficial relationship between *Drosophila* and different yeasts (STAMPS et al., 2012; COLUCCIO et al., 2008; HAMBY et al., 2012). In this relationship, *Drosophila* flies and their larvae play an important role in the transmission of these yeasts to host fruits (STAMPS et al., 2012; BECHER et al., 2012).

1.2.3.1. Yeast transportation by *Drosophila* adults

Drosophila flies are vectors of the main yeast flora that typically colonize the surface of sour rotten grapes. Wounded bunches of grapes visited by *Drosophila* flies were characterized by higher total yeast populations and the absence of basidiomycetes (BARATA et al., 2012). The transportation of yeasts gives the adults the potential for parental care (STARMER et al., 1988).

Yeasts were isolated from the alimentary canal of both male and female adult *D. suzukii* flies (HAMBY et al., 2012). The presence of living cells in the alimentary canal of *Drosophila* larvae makes it possible to carry living yeasts through the pupal period and then deposit these yeasts on a new substrate. The extent of survival of yeast cells through the gut depends on the yeast species (STARMER et al., 1988) and strain (COLUCCIO et al., 2008). Spores survive passage through the *Drosophila* digestive tract significantly more efficiently than vegetative cells. The ability to survive passage through the *Drosophila* gut is greatly enhanced by the unique chitosan and dityrosine layers of the spore wall of yeast cells. It is possible that the ascus wall contains nutrients for the fly so that the consumption and dispersal of spores by flies is beneficial for both organisms (COLUCCIO et al., 2008). More suitable pure cultures or mixed cultures of yeasts provide viability benefits to the *Drosophila* larvae. As a result, the likelihood of the yeast of being transported to a new habitat by the emerging flies is increased (STARMER and FOGLEMAN, 1986).

A simpler way for adult flies to inoculate a new substrate is to obtain yeasts by feeding on existing yeast-rich hosts. Transfer of yeast from females to males also takes place during courtship and copulation (head and abdomen), while transfer to the female is most likely to occur during copulation (abdomen) (STARMER et al., 1988).

1.2.3.2. Yeast transportation by *Drosophila* larvae

Larvae transport yeasts in fruit pulp through their movement. Further, the presence of larvae affects the yeast species composition in the fruit pulp by reducing the microbiological diversity in the pulp and the variation in yeast communities from one fruit to the next for their own benefit. Furthermore, the gut of *Drosophila* larvae contains viable yeast cells that help the yeast to spread (STAMPS et al., 2012).
Drosophila larvae produce a suitable microbial environment in the fruit substrates in which they develop. In the fruit pulp, the yeasts reduce the development of molds that might have adverse effects on *Drosophila* larvae (HAMBY et al., 2012; STAMPS et al., 2012). Yeasts showed a positive effect on *D. melanogaster* larvae that were forced to develop in the presence of a competing toxic mold fungus (*Aspergillus nidulans*) (ROHLFS and KÜRSCHNER, 2010).

It can be concluded that both *Drosophila* and yeast receive benefits from this relationship. In this sense, the yeast community can be said to be coadapted to its *Drosophila* vector (STARMER and FOGLEMAN, 1986).

1.2.4. Yeasts associated with *Drosophila* species

The yeast communities of different *Drosophila* species are influenced to a large extent by their diet. Natural *Drosophila* populations carry a very limited number of yeast taxa (CHANDLER et al., 2012). *Hanseniaspora sp.* is the dominant yeast genus associated with many *Drosophila* species (CHANDLER et al., 2012). The yeast species *Candida zemplinina* and *Zygoascus hellenicus* are also found on the body surface of wild *Drosophila* flies (BARATA et al., 2012). *Hanseniaspora uvarum* and *Candida zemplinina,* were recovered from laboratory-reared flies, and thus these species might be part of the natural flora of the surface or digestive tract of *Drosophila* flies (BARATA et al., 2012).
BELLUTTI et al. (2015) isolated *Hanseniaspora uvarum*, *Pichia terricola*, *Rhodotorula mucillaginosa*, *Metschnikowia pulcherrima*, *Saccharomycopsis vini* and *Candida sp*. from the larval feeding grooves of *D. suzukii*-infested Vernatsch grapes in South Tyrol.

Wounded bunches of grapes visited by *Drosophila* flies, were characterized by the typical sour rot colonizing yeasts *Pichia terricola*, *Zygoascus hellenicus* and *Zygosaccharomyces bisporus* (BARATA et al., 2012).

1.2.5. The relationship between *D. suzukii* and yeast

Gravid *D. suzukii* females prefer to find undamaged ripening fruits for oviposition (MITSUI et al., 2006; WALSH et al., 2011) and are attracted to the odor produced by fermenting fruits for feeding (BURRACK et al., 2015). For this reason, the fruit skin is important for *D. suzukii* to find suitable oviposition places. The firmness, brix and pH were observed to correlate weakly with *D. suzukii* infestation (LEE et al., 2011). For larger distances, *D. suzukii* may use leaf volatiles that do not emanate directly from the oviposition source itself, but rather they may signal the presence of ripening fruits (KEESEY et al., 2015).

Wounded bunches of grapes that were not visited by *Drosophila* flies, or other insects, healed their wounds supposedly because the vine plant activated a defense mechanism leading to the healing of skin lesions (BARATA et al., 2012). *D. suzukii*, in contrast to *D. melanogaster* and other *Drosophila* flies, infest healthy fruits. It is possible that the yeasts transferred by the adult female are the first yeasts that grow on fresh wounds and change the decomposer community in the fruit pulp. The fact that a tiny number of yeast cells is sufficient to enable survival of *D. melanogaster* larvae suggests that yeasts transported by female flies is enough to enable offspring to develop (ANAGNOSTOU et al., 2010).

The most commonly identified yeast species on fruits infested by *D. suzukii* is *H. uvarum*, suggesting an association between these two species. Other yeasts frequently isolated from larvae or fruits infested by *D. suzukii* larvae are *Pichia kluyveri (*HAMBY et al., 2012), *P. terricola* and *M. pulcherrima* (BELLUTTI et al., 2015; HAMBY et al., 2012).

1.3. Aim of the study

Although the important role of yeast in the life cycle of saprophagous *Drosophila* species is well known, little is understood about the role of yeast in the diet of adult *D. suzukii* that infest healthy undamaged fruits. The first step in this study was the design of an experimental cage corresponding to the natural feeding and egg-laying behavior of *D. suzukii* adults, which is characterized by visiting distinct oviposition and feeding locations.

The objective of the present study was to determine the influences of the yeast species in the diet of adult *D. suzukii* by addressing following questions in one experiment:

1. Are yeasts in the diet of *D. suzukii* essential for survival and oviposition?
2. Do different yeast species in the diet of *D. suzukii* influence latency until first occurrence of oviposition?
3. Are single yeast species a suitable diet for *D. suzukii* and is the fecundity of *D. suzukii* associated with the yeast species in their diet?
4. Is the life span of *D. suzukii* males and females influenced by different yeast species in their diet?
5. Are *D. suzukii* females attracted to wet yeast cultures on malt extract agar as oviposition locations when *D. suzukii* females have an oviposition choice between different substrates with different ingredients and surface structures?

To answer these questions, *D. suzukii* flies were observed for the number of eggs laid and for mortality when fed with the yeast species *S. cerevisiae*, *M. pulcherrima*, *Candida sp.* and *H. uvarum.* The data obtained were used to estimate the influence of each yeast on the latency until first occurrence of oviposition, the number of eggs laid over the experiment duration, the mean number of eggs laid per day and the mortality of *D. suzukii* females and males during the experiment.
The newly developed experimental cages simulated a natural situation with three distinct substrates that served as feeding, drinking and oviposition locations. To determine influence of the yeast on the egg-laying behavior, the flies were given the choice to oviposit on substrates with or without yeast culture.

2. Material and methods

All preliminary experiments, microbiological work and entomological work were performed between April and July 2015 at the Laimburg Research Centre for Agriculture and Forestry, department of Plant Protection, Pfatten, South Tyrol, Italy.

2.1. Fly cultures

2.1.1. Laboratory rearing

The *D. suzukii* flies used in the bioassay were offspring from a laboratory rearing at the Laimburg Research Centre for Agriculture and Forestry. The laboratory rearing was performed in an accessible climate controlled chamber at 22.0 ± 0.5 °C, 75.0 ± 3% relative humidity and a photoperiod of 16:8 (L:D) h. The laboratory rearing was refreshed on multiple occasions each year, with the latest being in autumn 2014. To refresh the rearing, wild *D. suzukii* pupae from various fruits, such as cherries and grapes, collected in South Tyrol were added to the laboratory population. This was done to decrease the risk of inbreeding and to increase the genetic similarity of the laboratory population to the wild population in South Tyrol. The laboratory rearing contained a standard *D. suzukii* cornmeal diet for feeding and oviposition and a white cup with cotton soaked in a 5% sucrose solution as additional material for feeding and drinking. Tap water was used for the sucrose solution.

The standard *D. suzukii* cornmeal diet used in the laboratory rearing was based on a diet for *Cydia pomonella* and was developed by the Laimburg Research Centre for Agriculture and Forestry. The cornmeal diet contained 2,400 ml distilled water, 25 g Agar-agar, 60 g wheat germ, 50 g cornmeal, 50 g brewer's yeast, 45 g apple pulp, 100 g sucrose, 5 g ascorbic acid, 1.5 g hydroxybenzoate, 1.5 g benzoic acid, 2.25 g Vanderzant vitamin mix and 5 g Wesson's salt. The same cornmeal diet was used to maintain the flies for the bioassay.

To prepare the cornmeal diet, the ingredients were mixed and poured (2 cm high) into sterile boxes; after closing, the boxes with the cornmeal diet were stored at 4 °C and used when needed. Before use the diet was cut into pieces (8 cm long, 5 cm wide and 2 cm high). Shortly before use, dry baker's yeast (*Saccharomyces cerevisiae,* Küchle®) was sprinkled over the surface of the pieces. The cornmeal diet pieces were added to the laboratory rearing cage in white plastic cups (14 cm long, 9 cm wide and 4 cm high). After two days in the cage containing the stock culture, the white cups were removed and closed with clear plastic lids with small holes for air exchange. The offspring were raised under the same conditions and in the same climate-controlled chamber in which the laboratory rearing was performed. When the first flies emerged from the pupal stage, the cups were opened inside the laboratory rearing cage and left there until all flies emerged from the pupal stage. This process ensured that the number of flies in the laboratory rearing remained at a high level.

2.1.2. Fly culture for the bioassay

To obtain a sufficient quantity of eggs, i.e. >400 eggs for one replication, three open cups (14 cm long, 9 cm wide and 4 cm high) with standard *D. suzukii* cornmeal diet (pieces 8 cm long, 5 cm wide, 2 cm high, and sprinkled with dry baker's yeast, *S. cerevisiae* Küchle®) were placed in the laboratory rearing cage for 24 h. During that time, females fed on the diet and oviposited into it. The high number of eggs was necessary because not all eggs hatched, not all larvae survived and not all flies emerged from the pupal stage at the same time. The laboratory rearing contained more than 2,000 flies at the time the eggs were obtained for the bioassay. After removing the cups with the cornmeal diet from the laboratory rearing cage where the *D. suzukii* females had oviposited, the cups were placed in the climate-controlled chamber, where the bioassay was performed. The larvae for the bioassay developed on the cornmeal diet at experimental conditions (22.0 °C (min 21.2 °C, max 23.0 °C), 76.4% mean relative humidity, photoperiod of 16:8 (L:D) h). The cornmeal diet was left in the closed cups until the first flies emerged from the pupal stage (Figure 5). To obtain *D. suzukii* flies of known age, the first flies that emerged from the pupal stage were removed from the plastic cups. After removing these flies, the open cups with the pupae were placed in a simple insect cage (50 cm long, 30 cm wide and 30 cm high; mesh size 1.0 mm × 0.625 mm) to collect the newly emerging flies. The insect cage contained a low cup (diameter 4.8 cm) with cotton, soaked in 5% sucrose solution as food and water source (Figure 6).

After 24 hours the three cups containing the residual larvae and unhatched pupae were removed from the insect cage (Figure 7). The flies in the insect cage were all at the same age, 36 ± 12 h after emergence from pupal stage at this time. To allow for easy selection between males and females, the flies were left for another 24 hours in the cage with a diet of 5% sucrose solution (Figure 8). After this period, the males had developed their characteristic spots on the wings, which simplified distinguishing between males and females.

Finally, the flies were removed from the insect cage using a test tube with a straight rim (12 cm long, diameter 1.6 cm). The division of male and female was conducted with the naked eye inside the test tubes, which were closed with cotton.

Figure 5. Standard *D. suzukii* cornmeal diet with developing larvae.

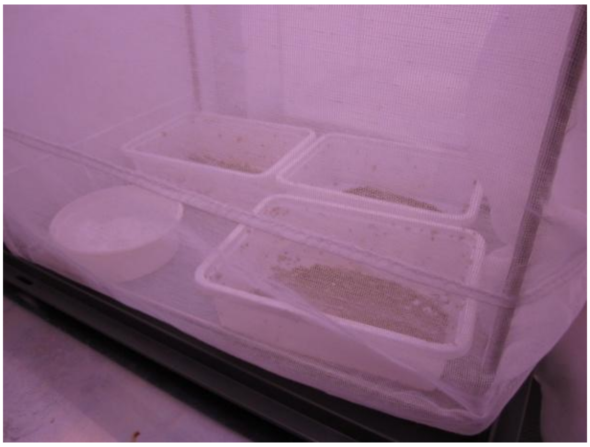


Figure 6. *D. suzukii* flies were collected in an insect cage after emergence from the pupal stage.

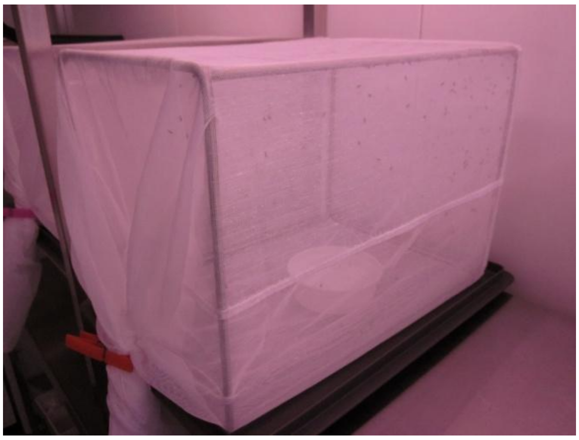


Figure 7. Insect cage containing the emerged *D. suzukii* flies.

Figure 8. *D. suzukii* flies feeding on sucrose solution before the experiment.

2.2. Yeast species and cultivation

2.2.1. Yeast species

The yeasts used in this study were isolated by the Laimburg Research Centre for Agriculture and Forestry from feeding grooves of *D. suzukii* larvae in infested Vernatsch grapes in the field in South Tyrol. The isolation and the species identification were performed by BELLUTTI et al. (2015) and are not part of this study.

After yeast isolation the yeast species were identified by using molecular methods. The DNA was extracted using the NucleoSpin®Tissue (Macheres-Nagel, Italy) standard protocol for cultured cells. The partial sequences of the 28 rDNA were amplified with the universal primer pair NL1, and PCR products were sequenced with NL1/NL4 primers. For long-term storage, purified isolates from the yeasts were maintained frozen at -80 °C in vials containing potato dextrose agar (Merck, Italy) with 15% glycerol, by the Laimburg Research Centre for Agriculture and Forestry, until use.

The yeasts chosen for the bioassay are *Hanseniaspora uvarum* (accession ID: JX423559.1), *Metschnikowia pulcherrima* (accession ID: HM067867.1) and *Candida sp.* (accession ID: JQ917720.1). The *Saccharomyces cerevisiae* (baker's yeast, Küchle®) used in the laboratory rearing was also used in the bioassay as a positive control (Figure 9).

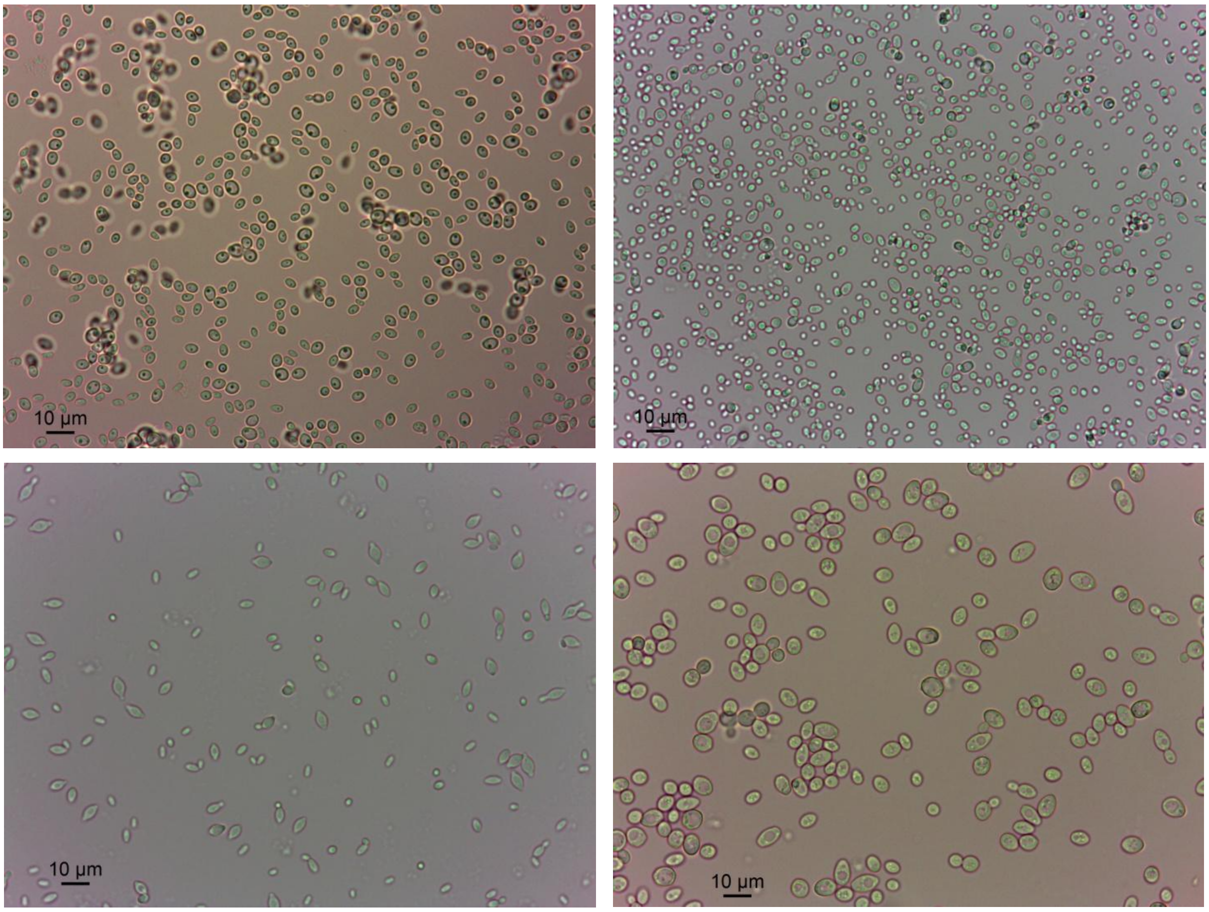


Figure 9. From top left to bottom right: *Metschnikowia pulcherrima*, *Candida sp.*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae.*

2.2.2. Yeast cultivation

All microbiological tasks were performed in the microbiology laboratory under sterile air conditions using a vertical laminar flow hood (ASALAIR 1200) (Figure 10). For yeast cultivation, a malt extract agar (Merck, Italy) was used. Malt extract agar is a solid medium recommended for the detection, isolation and enumeration of yeasts and moulds. The malt extract agar contained 30.0 g/L malt extract, 3.0 g/L peptone from soymeal and 15.0 g/L Agar-agar. Distilled water was used for the malt extract agar. The stated pH value was adjusted before autoclaving. After the malt extract agar was autoclaved and cooled to 50 °C, it was poured into polystyrene Petri dishes (diameter 9 cm or 6 cm). The polystyrene Petri dishes with malt extract agar were prepared several days before use, closed with the original packaging under sterile conditions and stored at 5 °C in the refrigerator for later use.

On the bottom of the Petri dishes, the number of the respective yeast species and the date were noted with a permanent marker before inoculating. From the frozen vials containing pure cultures of the chosen yeast species or from the package containing the Küchle® baker's yeast, one inoculation loop with the stored yeast was transferred from the vials to the polystyrene Petri dishes (ROLL s.a.s. Piove di Sacco, Italy, diameter 9 cm) filled with malt extract agar and then spread on the surface. From the inoculated Petri dishes, after two days, a single yeast colony was transferred with an inoculation loop to a new Petri dish (ROLL s.a.s. Piove di Sacco, Italy, diameter 9 cm) filled with malt extract agar and spread (Figure 11). The Petri dishes that were inoculated with yeast cells from a single yeast colony were used over two weeks as stock culture to inoculate the small polystyrene Petri dishes (ROLL s.a.s. Piove di Sacco, Italy, sterilized by irradiation, diameter 6 cm) filled with malt extract agar for the bioassays.

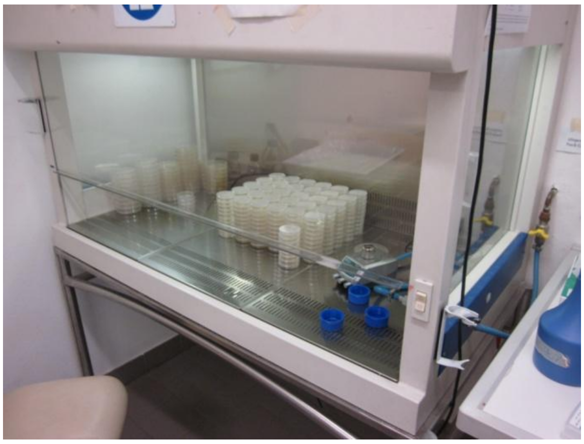


Figure 10. All microbiological tasks were performed in sterile air conditions in a clean bench.

Figure 11. Stock culture of *Candida sp.* on malt extract agar. The Petri dishes were sealed with parafilm.

Small Petri dishes were inoculated every other day in the afternoon after the experimental procedure had been completed. The small Petri dishes were inoculated using a sterilized inoculation loop. With the inoculation loop, one loop full of yeast cells was transferred from the stock culture Petri dish to a 2 ml Eppendorf tube filled with 1 ml 0.9% NaCl (Merck, Italy) solution. Distilled water was used for the 0.9% NaCl solution. The 0.9% NaCl solution had been autoclaved before use. The yeast cell suspension was mixed by a vortex mixer (IKA Minishaker MS1) for 10 seconds at 1800 rpm. With a pipette (Eppendorf Research 3120, 100 µl) 0.1 ml of the yeast cell suspension was pipetted into the small Petri dishes with malt extract agar and spread evenly across the dishes, using a sterilized glass spreader rod. Small Petri dishes for the control treatment were inoculated with 0.9% NaCl without yeast and were otherwise handled like the small Petri dishes for the yeast treatments.

The stock cultures were sealed with parafilm (Parafilm M, Bemis). The small Petri dishes for the bioassay were stored without parafilm sealing. All yeast cultures were stored in an incubator (Heraeus UT6) at a temperature of ≥22 °C. The Petri dishes containing the malt extract agar with 0.9% NaCl solution for the control treatment showed no culture growth of any kind after storage in the incubator. All yeast cultures were observed regularly with the naked eye and under a microscope to guarantee species cleanness (Figure 9).

Every few weeks, the stock cultures were refreshed by using single colonies from the original cultures stored at -80 °C in the freezer. Each yeast species was offered to the flies two or three days after inoculation. Differences in growth period of 24 h were caused by the fact that the small dishes were inoculated every other day. The small Petri dishes offered were completely covered with yeast cultures. Therefore, the amount of yeast offered was not limited.

2.3. Experimental cages

In case the volatiles from the selected yeasts proved minimally attractive to *D. suzukii* flies, the experiments in this study were performed in small experimental cages, which allowed easy location of the yeast diet. However, the cages had to be sufficiently large to allow for natural behaviors such as mating and flying.

2.3.1. Experimental cage design

The experimental cages were made of white polystyrene boxes (CIB Verona, 18 cm long, 18 cm wide and 6 cm high), two polypropylene laboratory cups with threads (Kartell Labware, 200 ml, diameter 6 cm), a polypropylene centrifuge tube with thread (ISOLAB Germany, 50 ml, diameter 3 cm) and a fine insect mesh (mesh size 1.0 mm × 0.625 mm). The laboratory cups were chosen to be the same diameter as the small Petri dishes (6 cm) (Figure 12A).

First, the laboratory cups and the centrifuge tube were closed, and then they were cut three millimeters below the lids using a bread knife.

In the bottom of the plastic boxes, two holes whose diameter was equal to the outside diameter of the laboratory cups (6 cm) and one hole whose diameter was equal to the outside diameter of the centrifuge tube (3 cm) were cut using a pointed nail scissors (Figure 12B). The threads of the laboratory cups without the lids were glued in the two holes with diameter equal to the diameter of the laboratory cups and the thread of the centrifuge tube without the lid was glued in the third hole (Figure 12C). The threads were glued from the outside and from the inside to the plastic box for greater stability. Gluing was performed with a glue gun (BOSCH PKP 18 E). After the threads were glued in the holes, the openings were closed with the corresponding lids (Figure 13). During the experiments the lids contained the three components (yeast diet on malt extract agar, water agar with sucrose solution and paper towel soaked in sucrose solution) (Figure 12C).

The open side was covered with the insect mesh. First, the insect mesh was cut into square pieces (22 cm long and 22 cm wide), then one piece was glued to the cup rim without gaps and the excess mesh was cut off with scissors (Figure 12D). Mesh was more suitable than the transparent lid because it provides a visible barrier in order to avoid confusion by the flies, and allows better air circulation.

If there was space between the laboratory cup and the Petri dish, the flies could crawl under the Petri dishes, where they might die. A small piece of paper between the Petri dish and the bottom of the laboratory cup lid solved this problem.
Using this method, 35 cages (7 replications, 5 treatments) were assembled.

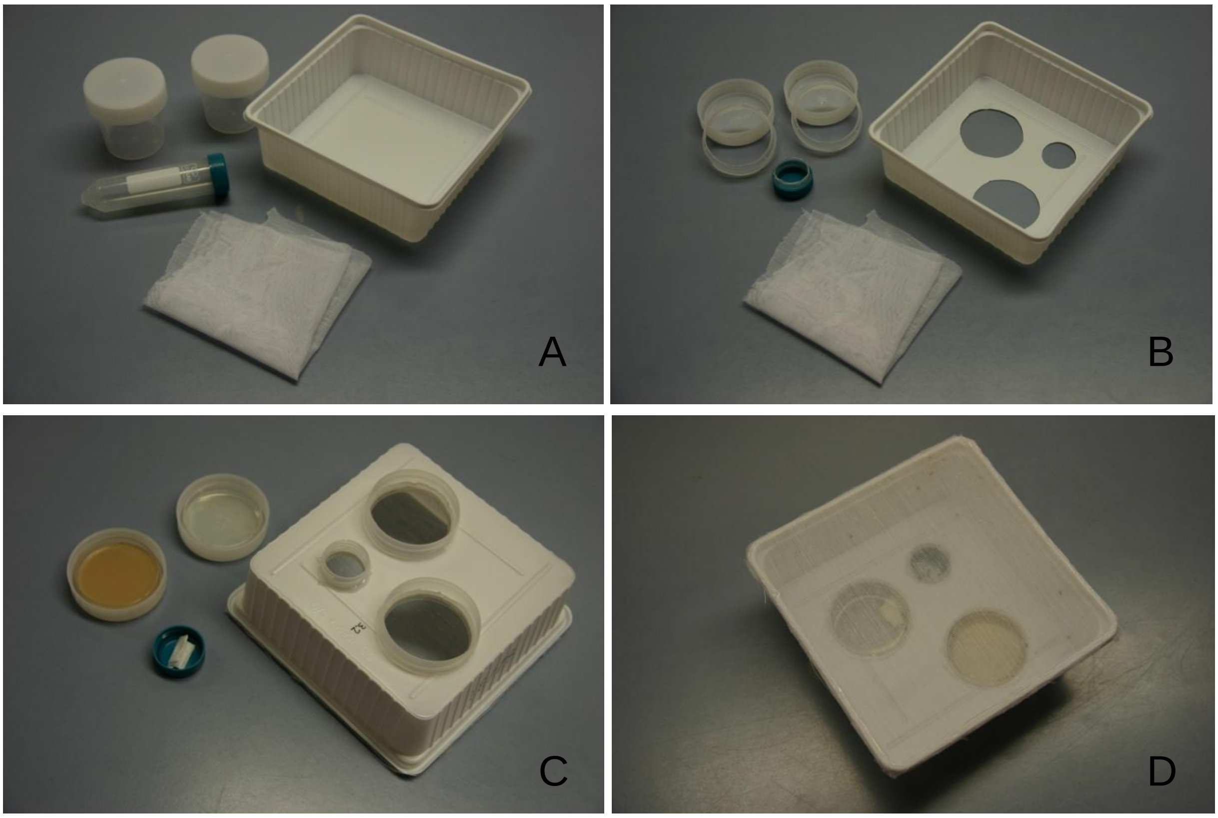


Figure 12. Construction steps for experimental cages for *D. suzukii* flies.

2.3.2. Experimental cage components

Each experimental cage contained one Petri dish filled with malt extract agar covered with yeast (MEA-Y) or malt extract agar without yeast in the control treatment (MEA-C), one Petri dish filled with water agar covered with sucrose solution (WASS) and one piece of paper towel soaked in sucrose solution (PTSS).
The females laid eggs on all three cage components.

2.3.2.1. Yeast culture on malt extract agar (MEA)

The small polystyrene Petri dishes (ROLL s.a.s. Piove di Sacco, Italy, sterilized by irradiation, diameter 6 cm) filled with malt extract agar were inoculated with the respective yeast species on the date indicated in the chapter 'Yeast cultivation' and kept for 48 or 72 h at ≥22 °C in the incubator.

1,750 small Petri dishes were needed for the component MEA to reach 7 replications and 5 treatments. The small Petri dishes filled with MEA were placed in the lids of the laboratory cups with the respective yeast number noted on the bottom. The yeast species had been noted on the bottom of the Petri dish before inoculating. The yeast species was also noted on the lid and close to the thread of the lid on the bottom of the box. On the front of the experimental cage the yeast species and the number of the replication was noted as well. This was done to be certain that the Petri dishes containing the yeasts were placed in the correct experimental cage and remained in the same place in each experimental cage. Thus each experimental cage was assigned its own number, which was easily visible in the climate chamber (Figure 23).

The MEA-Y surface was slightly tacky and not as solid as the WASS and MEA-C surfaces. The surface structure of MEA-Y differed between the yeast species. *H. uvarum* cultures formed a thinner layer compared to the other three yeast species. On the MEA-C, the surface was not influenced by yeast and therefore similar to the WASS surface.

2.3.2.2. Water agar covered with sucrose solution (WASS)

Small polystyrene Petri dishes (ROLL s.a.s. Piove di Sacco Italy, sterilized by irradiation, diameter 6 cm) with 15 g/L Agar-agar (Merck, Italy) were offered to the flies as oviposition locations (Figures 15 and 17). Distilled water was used for the water agar. The water agar was cooled to 50 °C after autoclaving and poured into small Petri dishes. Small Petri dishes filled with water agar were prepared a few days before needed, closed with the original packaging under sterile conditions and stored at 5 °C in the refrigerator until later use. Before the Petri dishes with water agar from the refrigerator at 5 °C were placed into the experimental cages, 0.1 ml 5% sucrose solution was transferred with a pipette (Eppendorf Research 3120, 100 µl) to the surface and spread evenly using a sterilized glass spreader rod. Tap water was used for the sucrose solution. 1,750 small Petri dishes were needed for this component WASS to reach 7 replications and 5 treatments.

The WASS had a solid surface without liquid. The 0.1 ml sucrose solution on the surface was absorbed quickly. Preliminary experiments showed that sucrose on the surface increase the acceptance for the Agar-agar as oviposition place slightly.

2.3.2.3. Paper towel soaked in sucrose solution (PTSS)

As a water supply and additional diet source, a tailored piece of paper towel (≈6 cm long, ≈6 cm wide) soaked in 5% sucrose solution was used. The sucrose solution was prepared daily using cold tap water. The paper towel was folded to the size of the centrifuge tube (diameter: 3 cm) and placed in the clean lid. 1 ml of 5% sucrose solution was transferred using a pipette (Eppendorf Research 3120, 1,000 µl) to the folded paper towel placed in the lid. Paper towel was used because preliminary experiments showed that counting eggs on cotton is very difficult. The component PTSS had a wet liquid surface.

2.4. Experimental procedure

2.4.1. Fly insertion and experimental cage handling

Through the small hole with the centrifuge tube thread, 10 males and 10 females were placed in each experimental cage using the test tube in which the sex of each fly was determined. This task was performed in a larger insect cage with an open side (50 cm long, 30 cm wide and 30 cm high; mesh size 1.0 mm x 0.625 mm) in the entomological laboratory at room temperature (Figure 24).

It is advantageous that the flies prefer to fly upwards and towards the light and therefore towards the mesh. The workplace was located directly under a task light. During the experiments the experimental cages were never turned upside down; this was to prevent flies from escaping. If flies escaped during insertion into the experimental cages, they were caught in the insect cage using a test tube with straight rim (12 cm long, 1.6 cm diameter) and placed back into the experimental cage through one of the cup threads.

Using this process, 35 cages (7 replications, 5 treatments) were filled with *D. suzukii* flies.

When molts became visible on the white inside of the cages (after day 20 at the earliest), all treatments of the same replication were cleaned with a moistened paper towel at the same time to avoid the effects of the molts. All cages were cleaned one or two times during the experimental period. On the three components, microbiological contamination was prevented by daily component changing. The reason for molts on the white boxes was the excretions of the *D. suzukii* flies, which were higher on *S. cerevisiae* and *H. uvarum* than on the other treatments. In the control a very low amount of excrement was noted.

2.4.2. Egg counting and component changing

Egg counting and component changing were performed in the entomological laboratory at room temperature using the same insect cage that was used to prevent flies from escaping when moving them from the test tubes to the experimental cages (Figure 24). If flies escaped from the experimental cages during component changing they were trapped in the insect cage and returned through one of the openings in the experimental cages using the test tubes, which could be closed with cotton.

After 24 h of exposure to the *D. suzukii* flies, the components were changed in succession. The eggs on the three components (MEA, WASS and PTSS) were counted on the same day or the next day (kept in the refrigerator at 5 °C) under the binocular microscope (LEICA MZ6). The components were changed in the morning and the egg counting was performed in the afternoon. Until the counting, the small Petri dishes with the inserted eggs were closed with the lid from the new small Petri dish and kept at room temperature until the afternoon or in the refrigerator until the next day. Only hatched and non-hatched eggs were counted (Figure 20); larvae were not counted. The eggs were counted daily in all 35 cages and on all three components (Figure 14) for 50 days. A different changing method and a different counting method were used for each component.

It was not possible to identify which egg was laid by which female within a single cage. The expectation was that flies that developed over the same time on the same diet would exhibit a qualitatively similar egg-laying behavior. The egg-laying behavior of individual females was not observed. The original data are shown in the appendix of data tables.

To ascertain the number of eggs per female and per day for the calculations, the daily number of counted eggs was divided by the number of remaining flies per cage.

The number of dead male and the number of dead female flies were noted separately for each cage during component changing. Dead flies were removed from the experimental cages with tweezers and preserved in 70% ethanol for further studies.

In the experiment the influence of the different yeasts on the diet was analyzed using the total number of hatched and unhatched eggs from all three components. The influence of the different yeasts on the egg-laying behavior was checked by observing the number of eggs laid on the different components within the experimental cages. The cage components were the same in all treatments and had no influence on the results of the single treatments. All results were measured in the same experiment. In the results, day 0 is the day when the flies were placed in the cages and day 1 is the first day of egg counting and exchange of the three components. After these tasks, the closed Petri dishes with WASS or MEA and the PTSS were autoclaved to kill eggs and hatched larvae.

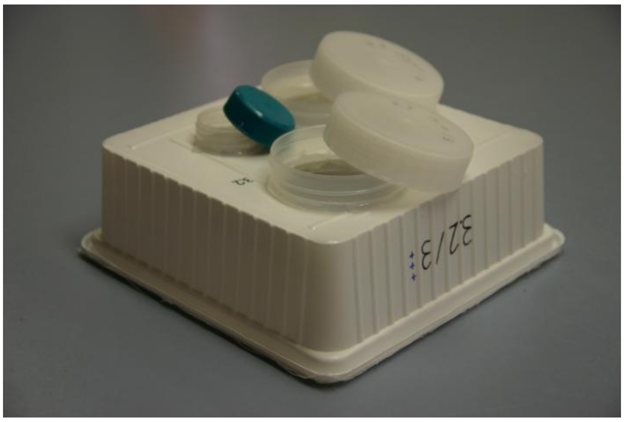


Figure 13. The three lids contained the cage components and allowed for simple changing of the three components.

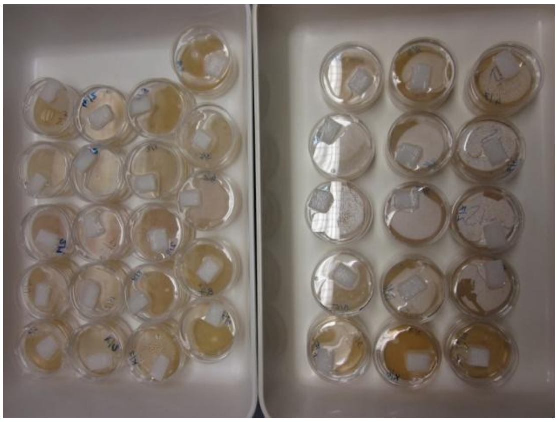


Figure 14. The three components were stored together until the *D. suzukii* eggs were counted.

2.4.2.1. Malt extract agar with yeast cultures (MEA)

Each day the lid from the laboratory cup with the small Petri dish containing MEA-Y or MEA-C from the previous day was opened and the Petri dish from the previous day (Figure 15) was removed and replaced with a fresh Petri dish with MEA from the incubator at ≥22 °C (Figure 16).

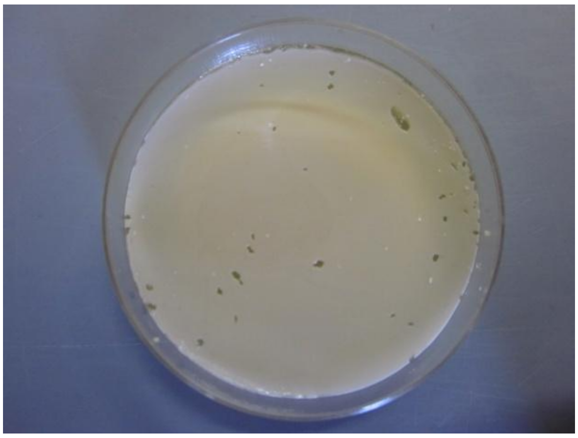


Figure 15. Feeding traces of *D. suzukii* flies on a Petri dish covered with a *S. cerevisiae* culture.

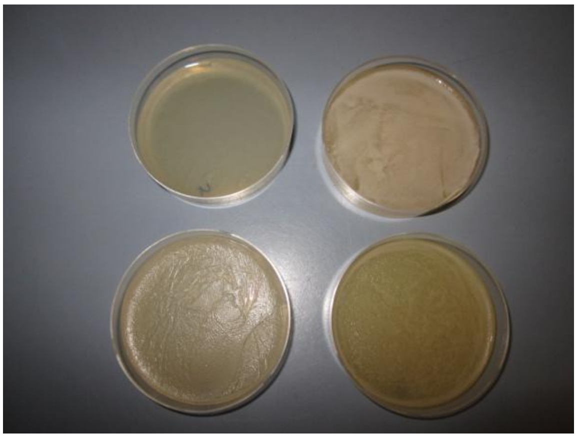


Figure 16. Small dietary plates. From top left to lower right: *H. uvarum*, *Candida sp.*, *S. cerevisiae* and *M. pulcherrima*.

To count the eggs on MEA-Y, the eggs on the yeast surface were first removed with tweezers under the binocular microscope and the number of eggs was noted. Afterwards the yeast colonies were washed under slow running cold tap water. There was no yeast on the surface of the MEA-C, therefore no washing was necessary for this treatment.

Without yeast on the surface of the MEA, it was easy to count the eggs inside the transparent MEA. To count the eggs that were oviposited into the MEA, the uncovered Petri dishes were inverted and the eggs were marked on the bottom of the Petri dishes with a permanent marker under the binocular microscope. After all eggs were marked, the marks were counted with the naked eye.

The number of eggs counted on the yeast surface and the number of eggs counted inside the malt extract agar were added together to determine the number of eggs laid on the MEA.

2.4.2.2. Water agar with sucrose solution (WASS)

Each day the lid from the laboratory cup containing the small Petri dish with WASS (Figure 17 and 19) was opened and the small Petri dish from the previous day was removed and replaced with a new small Petri dish filled with WASS.

To count the eggs, the uncovered Petri dishes were inverted under the binocular microscope and the eggs were marked on the bottom of the Petri dish using a permanent marker. The marks were then counted with the naked eye (Figure 18).

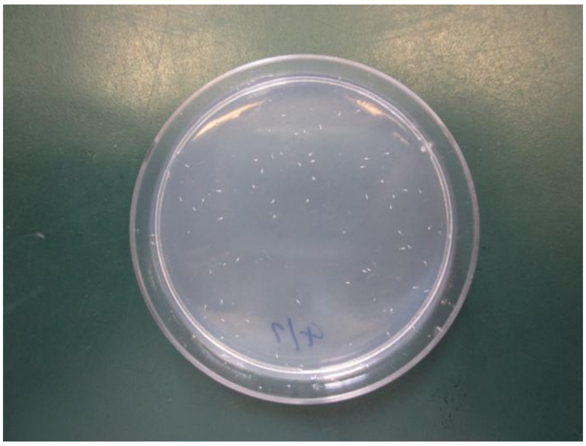


Figure 17. *D. suzukii* eggs oviposited in water agar.

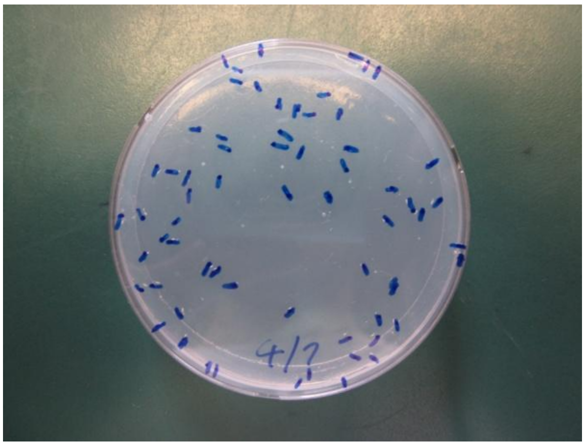


Figure 18. *D. suzukii* eggs were marked on the bottom of the Petri dish with a permanent marker.

Figure 19. *D. suzukii* eggs inserted in water agar and one hatched *D. suzukii* larva on the surface. The photo was taken from the bottom of the Petri dish.

Figure 20. *D. suzukii* eggs: unhatched egg at left and hatched egg at right.

2.4.2.3. Paper towel soaked in sucrose solution (PTSS)

The PTSS was changed daily as were the other two components. First, the paper towel from the previous day was removed with tweezers and placed in the lid of a small Petri dish. The cap of the centrifuge tube was then washed with a squeeze wash bottle to avoid the loss of sticking eggs. The washing water including the eggs was covered in the same Petri dish as the piece of paper towel (Figure 21). For disinfection, the cap was immersed in boiling tap water for a few seconds. The new piece of paper towel was folded to the size of the cap. After placing the folded paper towel in the cap, 1 ml 5% sucrose solution was pipetted onto it. Fresh sucrose solution was prepared daily using tap water.

Figure 21. The washing water including the eggs was covered in the same Petri dish as the piece of paper towel.

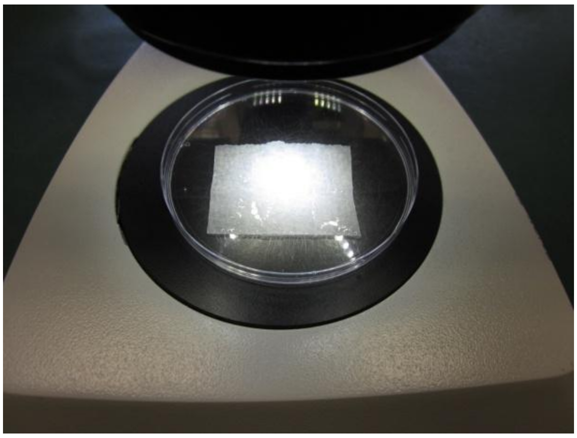


Figure 22. Eggs were counted under the binocular microscope.

To count the eggs, the PTSS was placed in the lid of a Petri dish (diameter 9 cm) and unfolded. First, the eggs left in the lid of the small Petri dish were counted using the binocular microscope. Next, the eggs on the PTSS were removed with tweezers and added to the eggs in the lid of the small Petri dish to avoid counting the same egg twice. The eggs on one side were removed first, and then the PTSS was turned around and the eggs on the other side were removed and counted. This task was performed under a binocular microscope (Figure 22). At the end, the number of eggs counted on the PTSS and the number of eggs in the wash water were added together.

2.4.3. Replications

The 5 treatments were replicated 7 times. The experiments were performed from May 9 to July 18 2015. The replications started (day 0) on different dates (May 8, May 9, May 10, May 23, May 24, May 28, and May 29).

All replications remained in the same accessible climate-controlled room at 22 °C (min 21.2 °C, max 23.0 °C), 76.4% mean relative humidity and a photoperiod of 16:8 (L:D) h.

In the climate cell, the test cages from the same replication (different treatments) were placed side by side (Figure 23). After the eggs were counted each day, the cages were rotated clockwise within the repetitions for one place. This was done to eliminate the influence of lighting, humidification and ventilation between the treatments within one replication.

The sex of the flies that were used was ascertained again at the end of the study to check for mistakes. Unused flies and flies that survived until the end of the experiment were killed by freezing. The experiments were stopped after 50 days, when the number of females in each cage was reduced because of mortality.

Figure 23. Experimental cages in the climate chamber. The yeast species and the number of the replication were noted on the front.

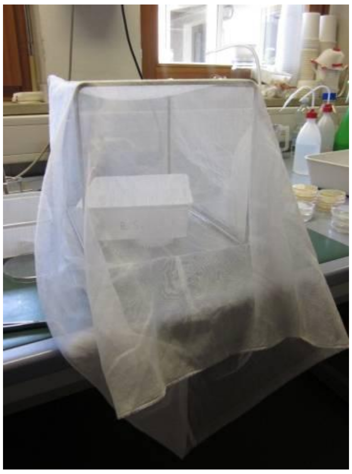


Figure 24. The three components were changed under a fine mesh to prevent flies from escaping.

2.5. Statistical analysis

All statistical analyses were performed using Microsoft Excel 2007 and IBM SPSS Statistics 21.0 for Windows 7 with a confidence limit of 95%.

The influence of different yeasts in the diet of adult *D. suzukii* on the oviposition initiation and the influence of different yeasts in the diet of adult *D. suzukii* on fecundity were analyzed using a general linear model univariate analysis of variance. The observed data were converted to the daily number of eggs per *D. suzukii* female and totaled before being entered into the analysis. The data were log10 transformed in order to achieve homogeneity of variance. Homogeneity of variance was tested with a Levene test. In case of significant effects a post hoc analysis for multiple comparisons was performed using a Tukey's range test.

The mortality of *D. suzukii* females and males was analyzed using a Kaplan-Meier test. For multiple comparisons between the functions a Tarone-Ware test was performed.

The oviposition preference for either of the three cage components was analyzed using a univariate analysis of variance with repeated measures. If the data violated the assumption of sphericity, the values of the Greenhouse-Geisser correction were used. If significant effects were found a Bonferroni adjustment for multiple comparisons was performed. To analyze the observed data, the daily number of eggs on each component was divided by the number of *D. suzukii* females in the respective experimental cage.

3. Results

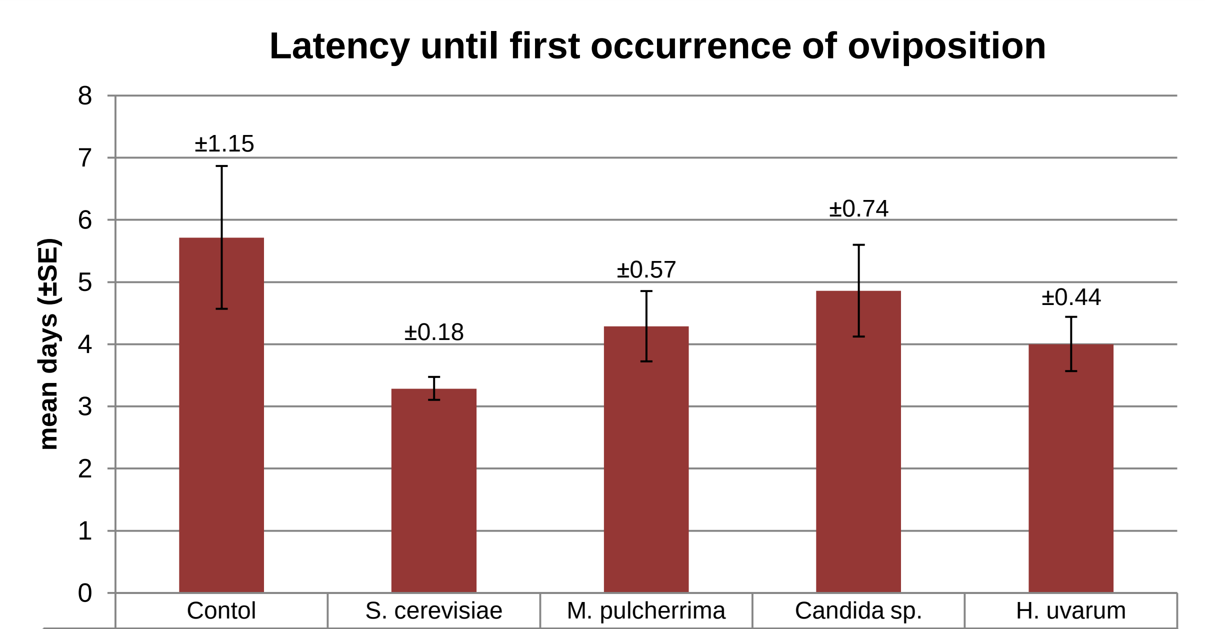
3.1. Influence of dietary yeasts on fecundity and mortality

Different yeasts (*S. cerevisiae*, *M. pulcherrima*, *Candida sp.*, *H. uvarum*) and a control treatment without yeast in the diet of *D. suzukii* adults were used to show the influences of single yeast species on the latency until first occurrence of oviposition, the number of eggs laid and the mortality over the experimental period.

3.1.1. Influence of yeast species on initiation of oviposition

The duration between the release of the newly emerged females in the experimental cage, i.e. day 0, and the first egg laid was on average 4.43 ± 0.61 days.

There was no significant effect of the dietary yeast species on the latency until first occurrence of oviposition by *D. suzukii* females ($F_{(4,30)} = 1.289$, $P = 0.296$) (Figure 25).



	Contol	S. cerevisiae	M. pulcherrima	Candida sp.	H. uvarum
Mean	5.71	3.29	4.29	4.86	4.00

Figure 25. Mean duration in days (± SE; n = 7) from the exposure of 36 ± 12 h old *D. suzukii* adults to cultures of different yeast species until first occurrence of oviposition. The different yeast treatments showed no significant effect on the latency at P ≤ 0.05.

3.1.2. Influence of yeast species on fecundity

During the entire experiment, 62,302 eggs were counted, including all treatments and replications.

The different yeasts in the diet of adult *D. suzukii* had a significant effect on the number of eggs laid during the experimental period, i.e. 50 days ($F_{(4,30)} = 24.067$, $P < 0.0001$). Significantly fewer eggs were laid by *D. suzukii* females in the control treatment compared to *D. suzukii* females fed with the yeast species *S. cerevisiae* ($P < 0.0001$), *M. pulcherrima* ($P < 0.0001$), *Candida sp.* ($P = 0.01$) and *H. uvarum* ($P < 0.0001$). *D. suzukii* females fed with *Candida sp.* laid significantly fewer eggs than females fed with the yeast species *S. cerevisiae* ($P = 0.001$) or *H. uvarum* ($P = 0.001$). No significant differences in the number of eggs laid were found in females fed with *S. cerevisiae* compared to females fed with *M. pulcherrima* ($P = 0.096$); females fed with S*. cerevisiae* compared to females fed with *H. uvarum* ($P = 1.0$); females fed with *M. pulcherrima* compared to females fed with *Candida sp.* ($P = 0.284$) and females fed with *M. pulcherrima* compared to females fed with *H. uvarum* ($P = 0.085$) (Figure 26).

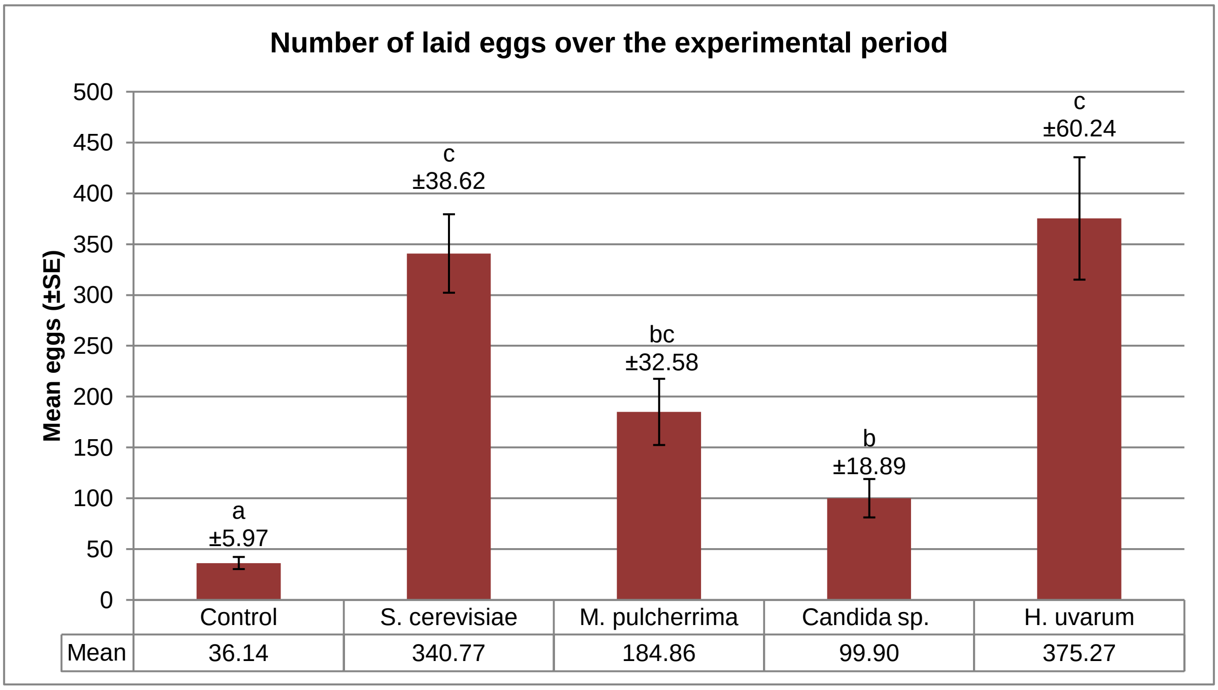


Figure 26. Mean oviposition rate (± SE; n = 7) of *D. suzukii* females during the experimental period, i.e. 50 days. Bars with different letters are significantly different at $P \leq 0.05$.

3.1.3. Influence of yeast species on the daily oviposition rate

The average daily number of eggs oviposited per female was 0.72 ± 0.15 eggs per day in the control (Figure 27); 6.82 ± 1.25 for *S. cerevisiae* (Figure 28); 3.70 ± 0.84 for *M. pulcherrima* (Figure 29); 2.00 ± 0.49 for *Candida sp.* (Figure 30) and 7.51 ± 1.56 for *H. uvarum* (Figure 31). In all 5 treatments the oviposition rate increased until the end of observation.

The highest observed number of eggs laid daily per female was 4.88 eggs in the control (Rep. 4, day 50); 20.0 eggs for *S. cerevisiae* (Rep. 3, day 50); 12.63 eggs for *M. pulcherrima* (Rep. 3, day 48); 10.44 eggs for *Candida sp.* (Rep. 4, day 46) and 21.0 eggs for *H. uvarum* (Rep. 4, day 37).

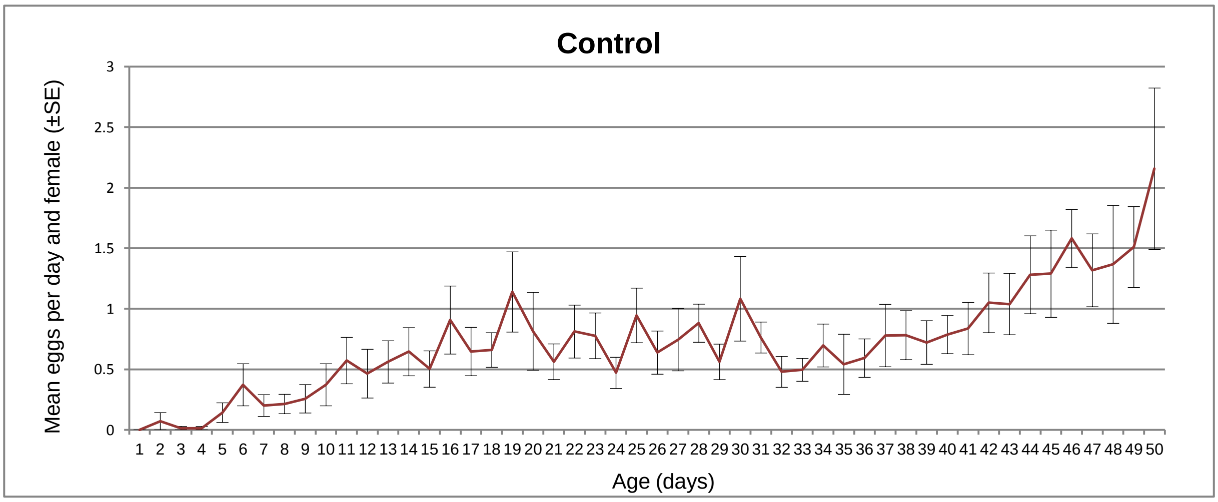


Figure 27. Mean daily oviposition (± SE; n = 7) of *D. suzukii* females in the control treatment without yeast in the diet over the experimental period, i.e. 50 days.

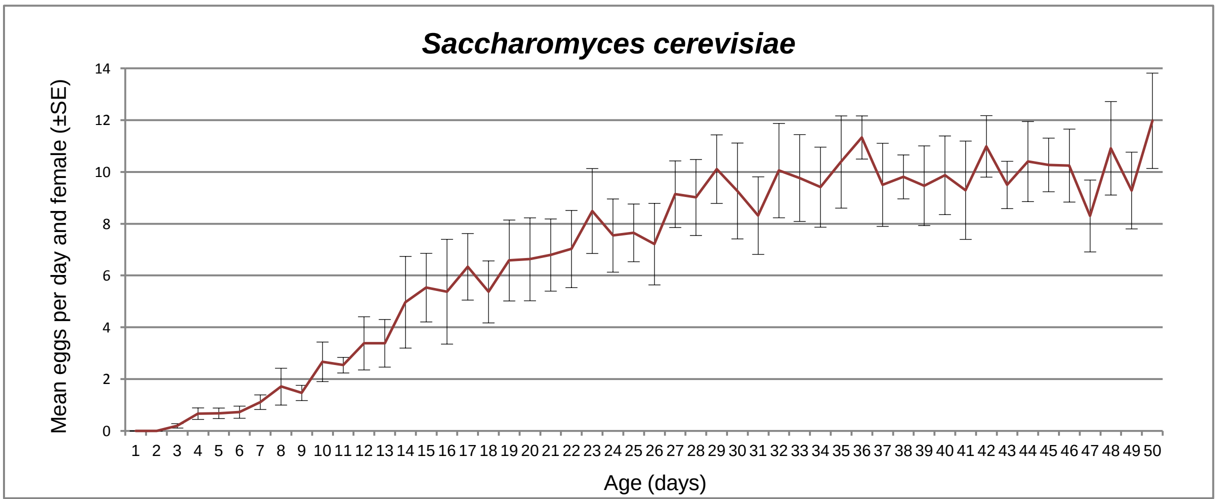


Figure 28. Mean daily oviposition (± SE; n = 7) of *D. suzukii* females fed with *S. cerevisiae* cultures over the experimental period, i.e. 50 days.

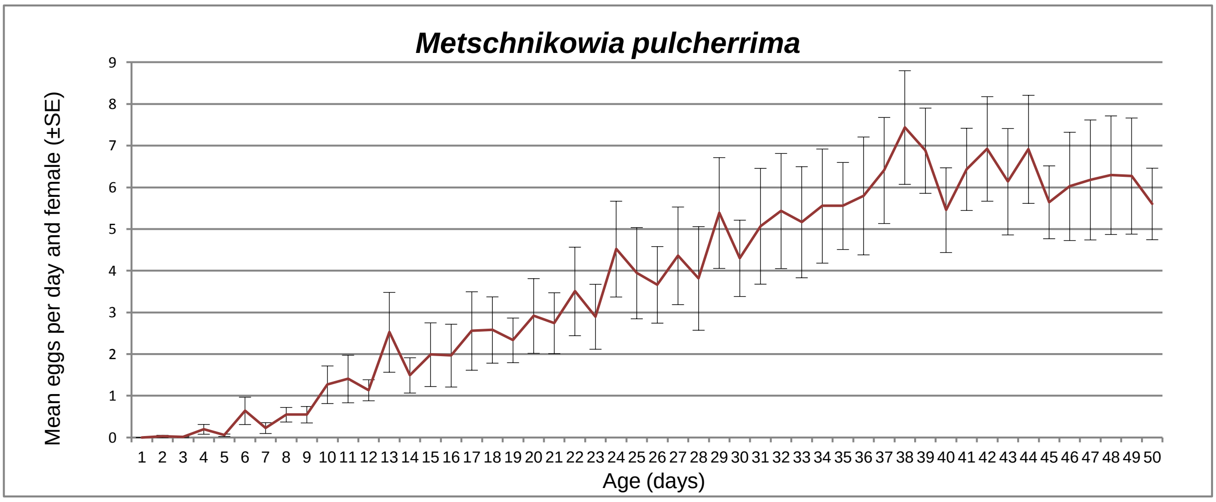


Figure 29. Mean daily oviposition (± SE; n = 7) of *D. suzukii* females fed with *M. pulcherrima* cultures over the experimental period, i.e. 50 days.

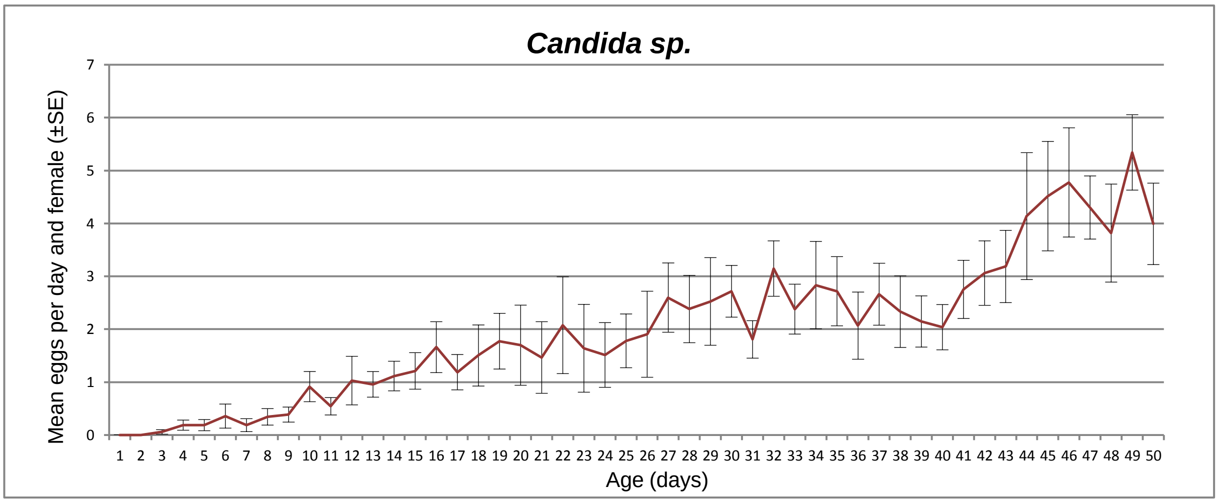


Figure 30. Mean daily oviposition (± SE; n = 7) of *D. suzukii* females fed with *Candida sp.* cultures over the experimental period, i.e. 50 days.

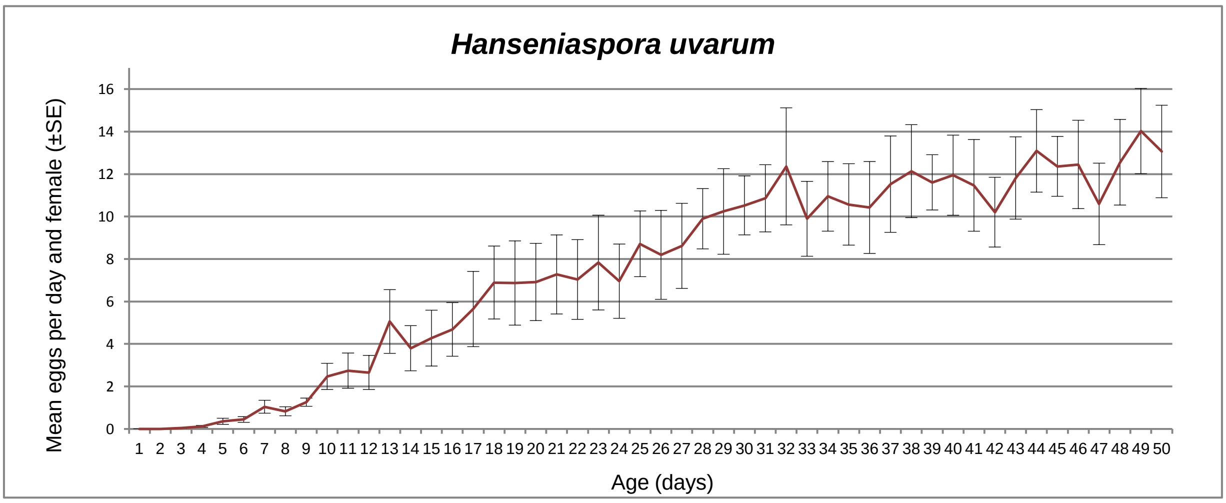


Figure 31. Mean daily oviposition (± SE; n = 7) of *D. suzukii* females fed with *H. uvarum* cultures over the experimental period, i.e. 50 days.

3.2. *D. suzukii* survivorship

3.2.1. Survivorship of *D. suzukii* females

The mean survival of *D. suzukii* females until day 50 was 77.42%. The observed mortalities among the different yeast diets were 14.29% in the control treatment, 35.71% for *S. cerevisiae*, 17.14% for *M. pulcherrima*, 14.29% for *Candida sp.* and 31.43% for *H. uvarum.*

Of the 350 *D. suzukii* females observed in the study, 22.58% (n = 79) died during the experimental period; the mean life span of those females was 32.51 ± 1.42 days. Each treatment contained 70 females at the beginning. In the control 10 females died, and the mean age of death of those females was 34.90 ± 4.54 days; with *S. cerevisiae* 25 females died and the mean age of death was 33.40 ± 2.29 days; with *M. pulcherrima* 12 females died and the mean age of death was 29.92 ± 4.53 days; with *Candida sp.* 10 females died and the mean age of death was 30.80 ± 3.94 days, and with *H. uvarum* 22 females died and the mean age of death was 32.59 ± 2.61 days.

The different yeast species in the diet of *D. suzukii* showed significant influences on the survivorship of *D. suzukii* females ($\chi^2_{(4)}$ = 16.751, P = 0.002). *D. suzukii* females fed with malt extract agar without yeast (control) survived significantly longer than females fed with *S. cerevisiae* (χ^2 = 8.817, P = 0.003) or *H. uvarum* (χ^2 = 6.064, P = 0.014) cultures, while *D. suzukii* females fed with *Candida sp.* cultures survived significantly longer than females fed with *S. cerevisiae* (χ^2 = 8.084, P = 0.004) or *H. uvarum* (χ^2 = 5.542, P = 0.019) cultures. *D. suzukii* females fed with *M. pulcherrima* cultures survived significantly longer than females fed with *S. cerevisiae* (χ^2 = 5.747, P = 0.017) cultures. The survivorship of *D. suzukii* females fed with malt extract agar without yeast (control) was not significantly different from that of females fed with *Candida sp.* (χ^2 = 0.005, P = 0.946) or *M. pulcherrima* (χ^2 = 0.254, P = 0.615) cultures; the survivorship of *D. suzukii* females fed with *M. pulcherrima* cultures was not significantly different from that of females fed with *Candida sp.* (χ^2 = 0.209, P = 0.647) or *H. uvarum* (χ^2 = 3.646, P = 0.056) cultures, and the survivorship of *D. suzukii* females fed with *S. cerevisiae* cultures was not significantly different from that of females fed with *H. uvarum* (χ^2 = 0.265, P = 0.607) cultures (Figure 32).

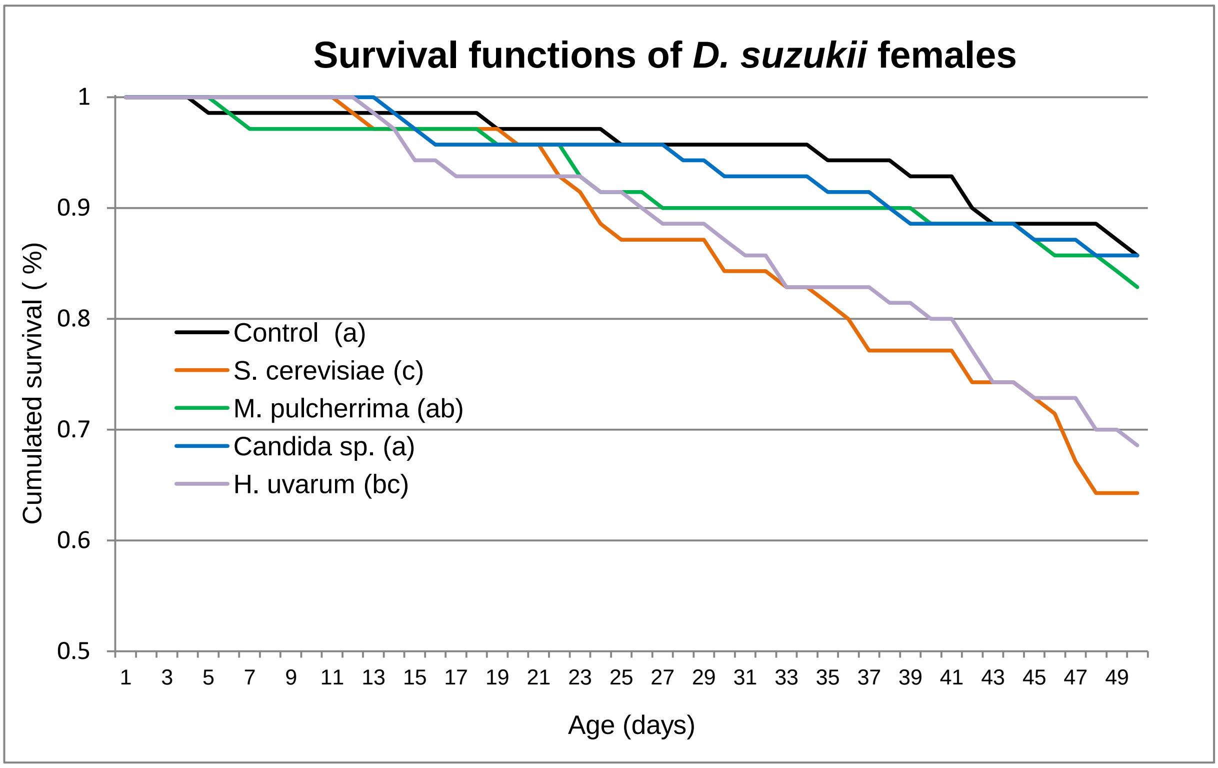


Figure 32. Mortality of *D. suzukii* females (n = 70) on different yeast diets. Treatments followed by the various letters are significantly different at $P \leq 0.05$.

3.2.2. Survivorship of *D. suzukii* males

The mean survival of *D. suzukii* males until day 50 was 88.86%. The observed mortality on the different yeast diets was 18.57% in the control, 11.43% for *S. cerevisiae*, 8.57% for *M. pulcherrima*, 4.29% for *Candida sp.* and 12.86% for *H. uvarum*.

Of the 350 *D. suzukii* males used in the present study, 11.14% died (n = 39), and the mean life span of the dead flies was 29.53 ± 2.31 days. Each treatment contained 70 *D. suzukii* males. In the control treatment 13 males died and the mean age of death was 37.54 ± 3.79 days; with *S. cerevisiae* 8 males died and the mean age of death was 22.25 ± 2.29 days; with *M. pulcherrima* 6 males died and the mean age of death was 20.33 ± 5.64 days; with *Candida sp*. 3 males died and the mean age of death was 38.67 ± 2.03 days, and with *H. uvarum* 9 males died and the mean age of death was 27.70 ± 4.32 days.

The different yeast species and the absence of yeast showed no significant influences on the survivorship of *D. suzukii* males ($\chi^2_{(4)}$ = 7.488, P = 0.112) (Figure 33).

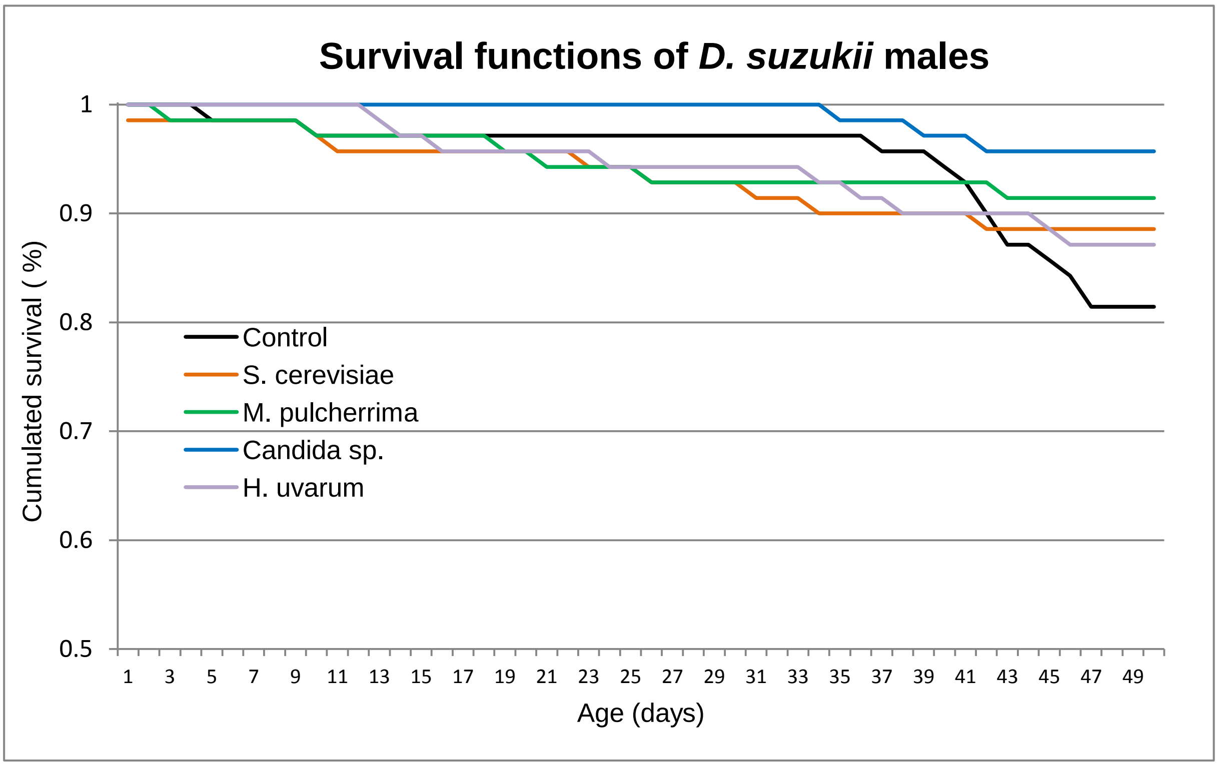


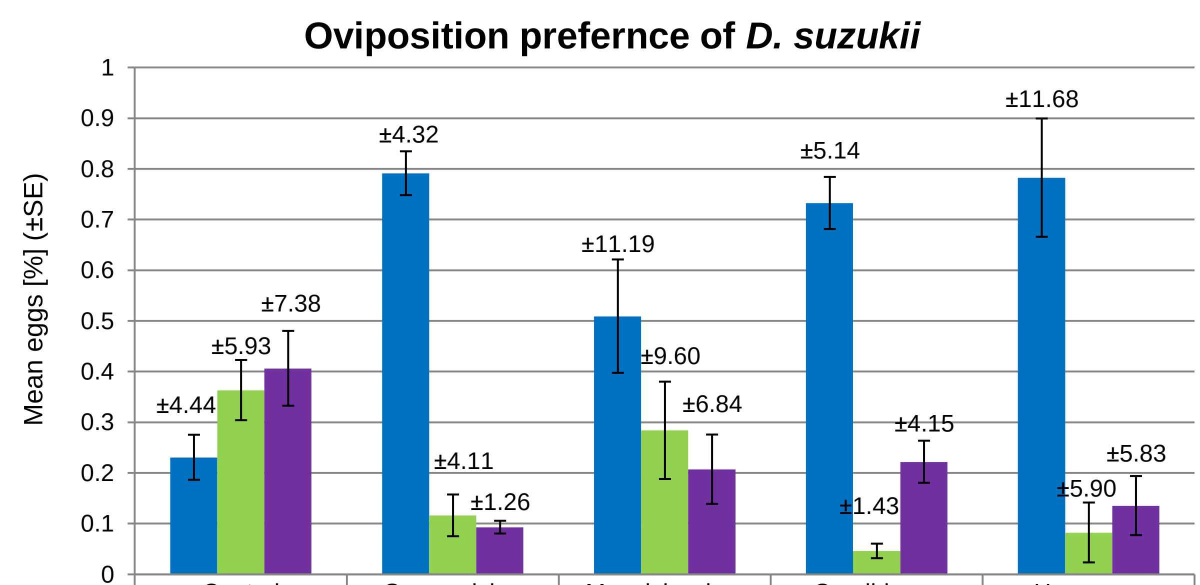
Figure 33. Mortality of *D. suzukii* males (n = 70) on different yeast diets. The survival functions are not significantly different at P ≤ 0.05.

3.3. Egg-laying behavior

The second part of the experiment was evaluated to clear the influence of the chosen yeast species on the egg-laying preference to the cage components. The *D. suzukii* females were able to choose between WASS, MAE-Y or MAE-C, and PTSS as oviposition locations.

3.3.1. Oviposition preference of *D. suzukii* females

Figure 34 shows the percentage of eggs laid during the entire test period on the different experimental cage components.



	Control	S. cerevisiae	M. pulcherrima	Candida sp.	H. uvarum
WASS	23.06%	79.12%	50.91%	73.24%	78.24%
MEA	36.33%	11.61%	28.38%	4.59%	8.23%
PTSS	40.61%	9.28%	20.71%	22.18%	13.53%

Figure 34. Mean number (± SEM; n = 7) of eggs laid by *D. suzukii females* given the choice between three different components (water agar with sucrose solution (WASS), malt extract agar with one of four yeast cultures or without yeast cultures, i.e. control (MAE), and paper towel soaked in sucrose solution (PTSS)).

3.3.2. Oviposition preference of *D. suzukii* over the experimental period

3.3.2.1. Oviposition preference in the control treatment

The average number of eggs laid per day on the different components was 0.17 ± 0.04 eggs on WASS, 0.25 ± 0.02 eggs on MAE-C and 0.30 ± 0.02 eggs on PTSS. The total number of eggs laid was 8.63 ± 2.59 on WASS, 12.39 ± 2.22 on MEA-C and 15.13 ± 4.30 on PTSS, expressed as the area below the curves in Figure 35. *D. suzukii* females showed no significant oviposition preference for any of the cage components ($F_{(2,12)} = 1.166$, $P = 0.345$).

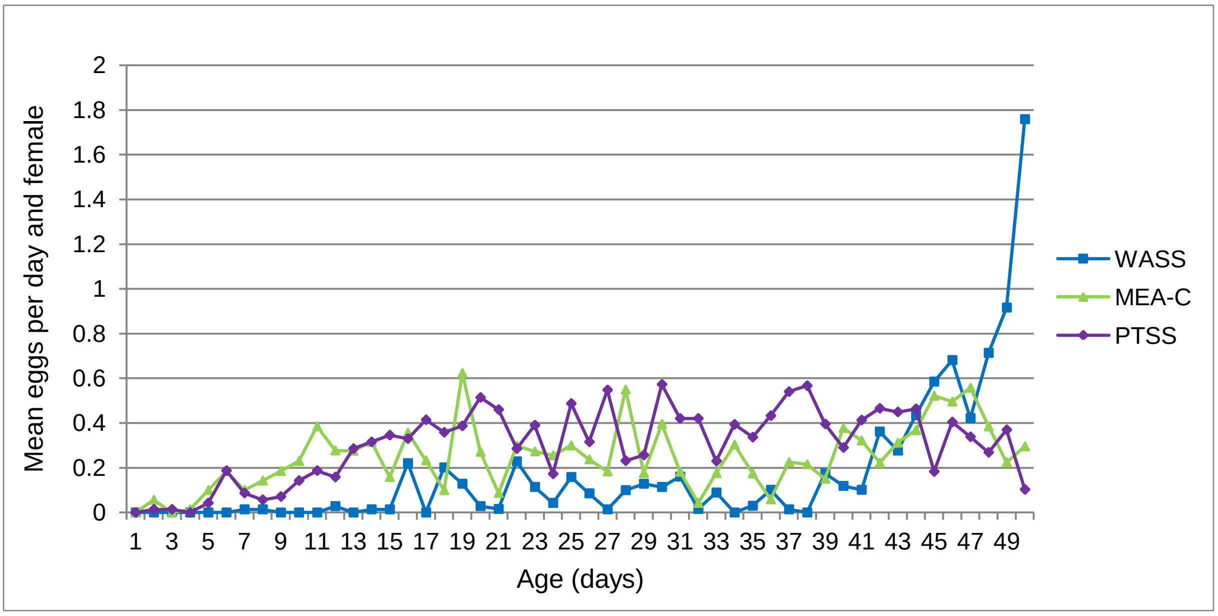


Figure 35. Mean daily oviposition (n = 7) by *D. suzukii* females in the control treatment without yeast in a choice situation over the experimental period, i.e. 50 days. The number of eggs laid on water agar with sucrose solution (WASS), malt extract agar (MEA-C) and paper towel soaked in sucrose solution (PTSS) was not significantly different at P ≤ 0.05.

3.3.2.2. Oviposition choice with *Saccharomyces cerevisiae*

The average number of eggs laid per day on the different components was 5.53 ± 0.52 eggs on WASS, 0.67 ± 0.06 eggs on MEA-Y and 0.62 ± 0.06 eggs on PTSS. The total number of eggs laid was 276.50 ± 43.44 on WASS, 33.33 ± 10.90 eggs on MEA-Y and 30.95 ± 4.40 on PTSS, expressed as the area below the curves in Figure 36. *D. suzukii* showed significantly different oviposition preferences among the cage components (Greenhouse-Geisser, $F_{(1.05,6.305)} = 26.066$, $P = 0.002$). The number of eggs laid on WASS was significantly higher than the number of eggs laid on the MEA-Y ($P = 0.009$) and on the PTSS ($P = 0.004$). No significant difference was found between the number of eggs laid on MEA-Y and PTSS ($P = 1.0$).

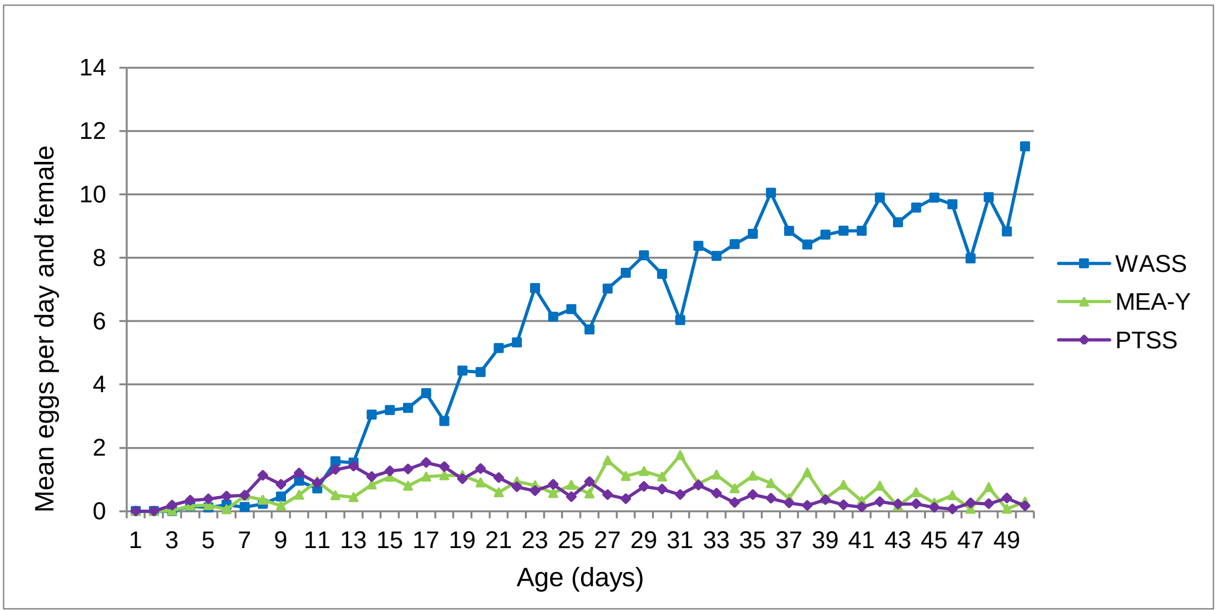


Figure 36. Mean daily oviposition (n = 7) by *D. suzukii* females fed with *S. cerevisiae* in a choice situation over the experimental period, i.e. 50 days. Significantly more eggs were laid on water agar with sucrose solution (WASS) compared to *S. cerevisiae* cultures on malt extract agar (MEA-Y) and paper towel soaked in sucrose solution (PTSS) at $P \leq 0.05$.

3.3.2.3. Oviposition choice with *Metschnikowia pulcherrima*

The average number of eggs laid per day on the different components was 2.15 ± 0.24 eggs on WASS, 1.0 ± 0.12 eggs on MEA-Y and 0.56 ± 0.06 eggs on PTSS.

The total number of eggs laid was 107.27 ± 33.08 on WASS, 49.95 ± 18.65 on MEA-Y and 27.78 ± 6.38 on PTSS, expressed as the area below the curves in Figure 37. *D. suzukii* females showed no significant oviposition preference for any of the cage components ($F_{(2,12)} = 2.984$, $P = 0.089$).

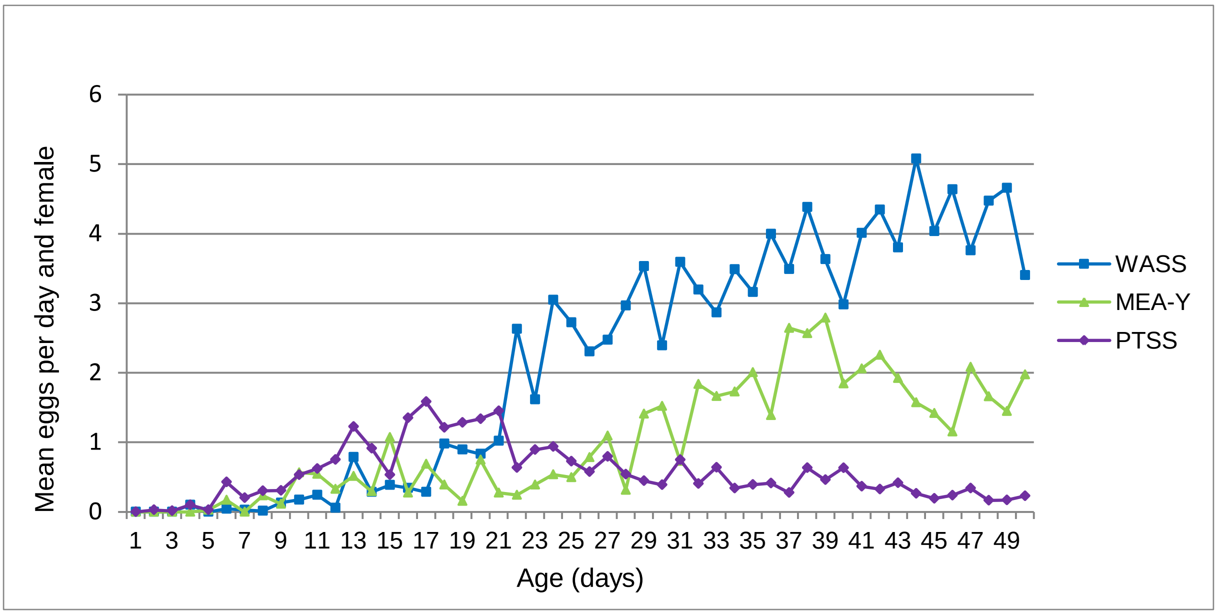


Figure 37. Mean daily oviposition (n=7) by *D. suzukii* females fed with *M. pulcherrima* in a choice situation over the experimental period, i.e. 50 days. The number of eggs laid on water agar with sucrose solution (WASS), *M. pulcherrima* cultures on malt extract agar (MEA-Y) and paper towel soaked in sucrose solution (PTSS) was not significantly different at $P \leq 0.05$.

3.3.2.4. Oviposition choice with *Candida sp.*

The average number of eggs laid per day on the different components was 1.51 ± 0.19 eggs on WASS, 0.07 ± 0.01 eggs on MEA-Y and 0.41 ± 0.04 eggs on PTSS. The total number of eggs laid was 75.66 ± 18.27 eggs on WASS, 3.55 ± 1.07 eggs on MEA-Y and 20.55 ± 5.28 on PTSS, expressed as the area below the curves in Figure 38. *D. suzukii* showed significantly different oviposition preferences among the cage components (Greenhouse-Geisser, $F_{(1.091,6.545)}$ = 11.582, P = 0.012). The number of eggs laid on WASS was significantly higher than the number of eggs laid on MEA-Y (P = 0.026). The number of eggs laid on the paper towel was also significantly higher than the number of eggs laid on MEA-Y (P = 0.031). No significant difference was found between the number of eggs laid on WASS and PTSS (P = 0.082).

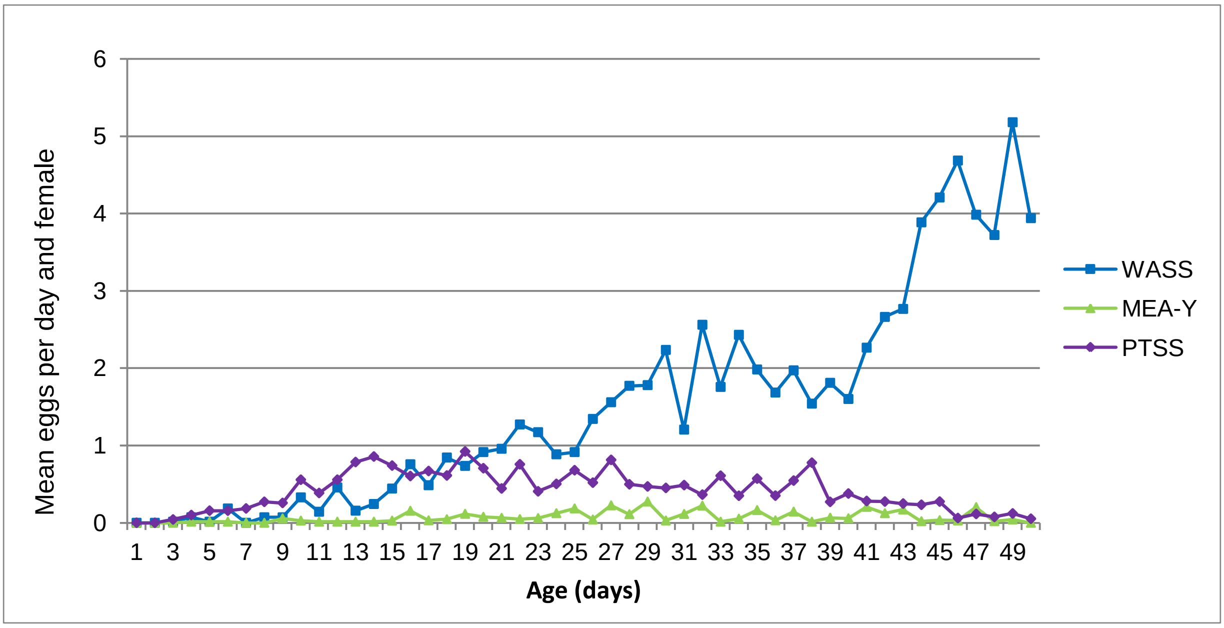


Figure 38. Mean daily oviposition (n = 7) by *D. suzukii* females fed with *Candida sp.* in a choice situation over the experimental period, i.e. 50 days. Significantly more eggs were laid on water agar with sucrose solution (WASS) and paper towel soaked in sucrose solution (PTSS) compared to *Candida sp.* culture on malt extract agar (MEA-Y) at P ≤ 0.05.

3.3.2.5. Oviposition choice with *Hanseniaspora uvarum*

The average number of eggs laid per day on the different components was 6.54 ± 0.65 eggs on WASS, 0.26 ± 0.03 eggs on MEA-Y and 0.70 ± 0.08 eggs on PTSS. The total number of eggs laid was 327.0 ± 64.67 eggs on WASS, 13.19 ± 5.24 eggs on MEA-Y and 35.095 ± 5.63 on PTSS, expressed as the area below the curves in Figure 39. *D. suzukii* showed significantly different oviposition preference among the cage components (Greenhouse-Geisser, $F_{(1.009,6.052)} = 20.251$, $P = 0.004$). The number of eggs laid on WASS was significantly higher than the number of eggs laid on the MEA-Y ($P = 0.012$) and on the PTSS ($P = 0.012$). No significant difference was found between the number of eggs laid on MEA-Y and PTSS ($P = 0.065$).

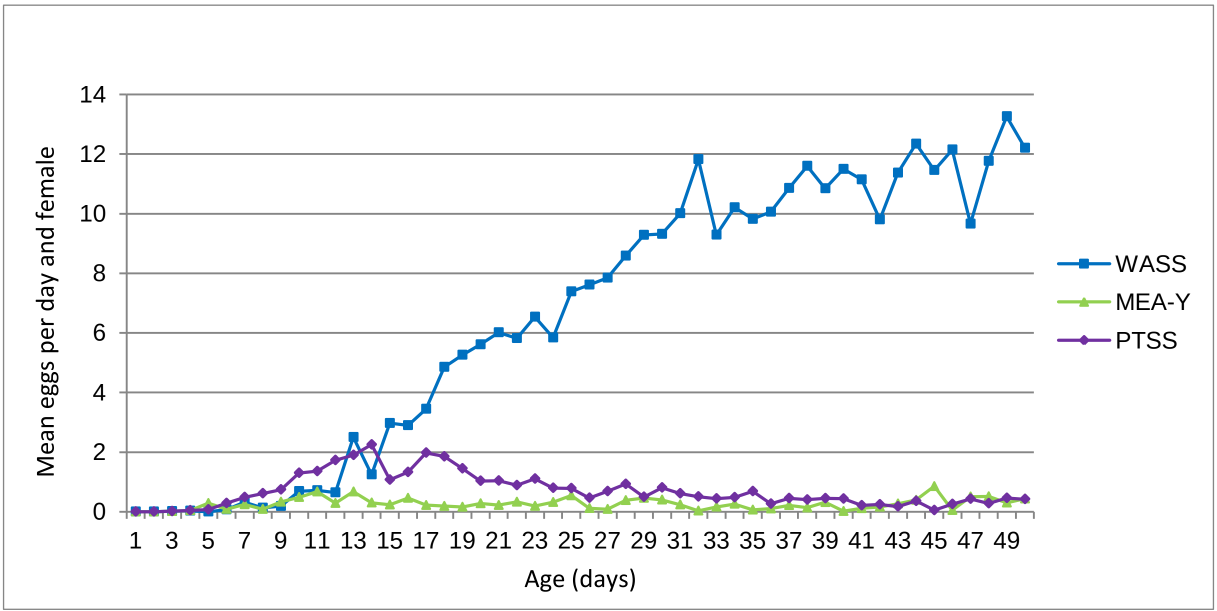


Figure 39. Mean daily oviposition (n = 7) by *D. suzukii* females fed with *H. uvarum* in a choice situation over the experimental period, i.e. 50 days. Significantly more eggs were laid on water agar with sucrose solution (WASS) compared to *H. uvarum* cultures on malt extract agar (MEA-Y) and paper towel soaked in sucrose solution (PTSS) at $P \leq 0.05$.

4. Discussion

4.1. Yeast in the diet of *D. suzukii*

The results of the present study showed no statistical effects of the yeast species on the latency until first occurrence of oviposition of *D. suzukii*, but the observed day of oviposition initiation correlated with the range of the observed total number of eggs laid over the experimental period.

The female flies kept on the control treatment showed a lower fecundity and a longer latency until they laid their first egg. The female flies kept on *H. uvarum* and *S. cerevisiae*, which showed the highest fecundity, had the shortest latency until they laid their first egg. The observed data suggest that the different yeast species had some influence on the latency until first oviposition after emergence from the pupal stage. Further studies could find significant differences by observing oviposition in the first weeks of the adult life span in hourly intervals for individual females. Interestingly, the observed latency until first occurrence of oviposition in the present study, a mean of 6 days after emergence from pupal stage, does not correspond to the results of other studies on *D. suzukii*, which found a high oviposition rate from the very first day of the experiment (EMILJANOWICZ et al., 2014). However, there are some studies of *D. suzukii* that show a slow increase in oviposition and do match the results of this study, for example, the study carried out by JARAMILLO et al. (2015). In addition, previous observations at the Laimburg Research Centre for Agriculture and Forestry on the *D. suzukii* population used showed a similar oviposition initiation as was observed in the present study. KHAZAELI and CURTSINGER (2010) showed that age-specific fecundity is highly heritable in different *D. melanogaster* lines. It might be assumed that oviposition processes are also heritable in different *D. suzukii* lines. Another possible reason for the various latencies until first occurrence of oviposition which was observed in different studies on *D. suzukii* could be the nutrition offered after emergence from pupal stage. In the present study the newly emerged flies were kept for 24 hours in an insect cage with a diet of 5% sucrose solution. The different nutrition offered before starting the feeding experiments with the respective yeast species might have affected the latencies until first occurrence of oviposition. DRUMMOND-BARBOSA and SPRADLING (2001) showed that the *Drosophila* ovary responds to the nutritional value of the diet offered.

The influence of the mating time, which influences the oviposition initiation of *Drosophila* females (KAPELNIKOV et al., 2008), can be excluded because mating was possible from the very first day of the experiments.

D. suzukii females oviposited eggs without yeast cultures in their diet, suggesting that yeast in the diet is not essential for *D. suzukii* adults, but that yeast is beneficial for *D. suzukii* females. Among the yeast species known to be associated with *D. suzukii* larvae or adults, the ones used in the present study turned out to increase the number of eggs laid compared to the yeast-less control. In addition to the significant influences of yeast cultures in the diet compared to the control treatment, the study results showed significantly different effects on fecundity among the individual yeast species. Significantly more eggs were laid by females fed with *S. cerevisiae* and *H. uvarum* than by females fed with *Candida sp*. No significant differences were found between *M. pulcherrima* and the other three yeast species (*Candida sp.*, *H. uvarum* and *S. cerevisiae*). Since the whole surface of the malt extract agar was covered with yeast colonies and the yeast cultures on malt extract agar were offered to the flies two or three days after inoculation, it can be assumed that the amount of yeast offered was not a limiting factor for nutrition.
The reason for the higher fecundity with the different dietary yeast species offered might be the better suitability of respective yeast cultures with regard to nutritional quality or with regard to feeding stimulant activity. As the quantity of excretions of flies were higher on *S. cerevisiae* and *H. uvarum* than on the other treatments, it may be assumed that the flies ingested more of those yeast cultures, because they may complied the taste of *D. suzukii* better compared to the other yeast cultures tested.
The results of the present study add evidence that yeast species in the diet are a determining factor in the number of eggs laid by *D. suzukii* females. Other works suggest *H. uvarum* as a species with a specific association with wild *D. suzukii* populations (HAMBY et al., 2012). The results of the present study showed *H. uvarum* as a highly beneficial yeast species in the diet of adult *D. suzukii*. With *H. uvarum*, the number of eggs laid was higher than with the other yeast cultures and 10 times higher than in the control treatment without yeast cultures. Further studies are necessary to prove how selectively females feed on different yeasts and if a mixture of the yeasts used increases oviposition compared to single yeasts. STARMER and FOLGEMAN (1986) showed that *Drosophila* larvae develop faster and reach a larger

body size when two yeasts are present in the diet compared to monocultures of either yeast species.

The oviposition rate in all treatments tended to increase until the end of the experiment without spikes or breaks in egg-laying. EMILJANOWICZ et al. (2014) found the peak of oviposition for *D. suzukii* at intermediate ages around day 70. It might be that the flies in the present study had not reached their egg-laying peak at the time the experiment ended.

In the present study different yeast species in the diet showed significant effects on the mortality of *D. suzukii* females. No significant effects of the yeast species on the mortality of *D. suzukii* males were found. *D. suzukii* females died earlier when fed with *S. cerevisiae* cultures than females fed with *Candida sp.* cultures, with *M. pulcherrima* cultures or without yeast in their diet. *D. suzukii* females died earlier when fed with *H. uvarum* cultures than females fed without yeast or with *Candida sp.* cultures. The results suggest that the females that laid more eggs died earlier. The reason for this remains unclear.

In the experiments no cause of death was recorded. Most flies probably died due to natural causes, although the lifespan of adult *D. suzukii* was assumed to be longer than 52 days. EMILJANOWICZ et al. (2014) found the average lifespan for females at 80 days and 94 days for males. In the present study 77.42% of the *D. suzukii* females and 88.86% of the *D. suzukii* males survived the experimental period, i.e. 50 days. Since most flies survived the experimental period and the number of eggs laid per female did not decrease, it might be concluded that the lifespan of this South Tyrol ecotype of *D. suzukii* is longer than 52 days.

One explanation for the earlier mortality on *S. cerevisiae* and *H. uvarum* is that a higher number of eggs laid caused a loss of fitness and therefore a higher mortality in an environment without predators and without risk of death by adverse environmental factors. KHAZAELI and CURTSINGER (2010) showed that long-lived genotypes of *D. melanogaster* exhibit low oviposition in the first week of adult life.

4.2. Oviposition choice of *D. suzukii*

In the behavior of *D. suzukii* adults, the visitation of suitable oviposition locations plays a central role and differs from other *Drosophila* species, which lay eggs in their preferred feeding locations (BURRACK et al., 2013, MITSUI et al., 2006). *D. suzukii*, in contrast to most other *Drosophila* species, are attracted to fresh ripe fruits with an intact skin for oviposition (MITSUI et al., 2006). *D. suzukii* prefers fruits that are red in color (LEE et al., 2011) and have a low penetration force of the intact fruit skin for oviposition (BURRACK et al., 2013; LEE et al., 2011). Further, different yeast volatiles are known to play a role in the attraction or repulsion of *Drosophila* adults to yeast-treated sources (BECHER et al., 2012; KLEIBER et al., 2014; PALANCA et al., 2013). Earlier studies on yeasts and *D. suzukii* showed an attraction to yeast volatiles (HAMBY et al., 2012; BELLUTTI et al., 2015; SCHEIDER et al., 2015), but the authors of these studies did not differentiate between egg-laying preference and feeding preference. This differentiation is an important step in the investigation of *D. suzukii* behavior.

In the present study the flies were able to oviposit on three different components. The three cage components were WASS, PTSS and either one of four yeast species on MEA or MEA without yeast as a control. The results showed significantly different preferences for the different cage components for oviposition depending on the yeast treatment. *D. suzukii* fed with *S. cerevisiae* or *H. uvarum* laid significantly more eggs on WASS than on MEA-Y or on PTSS. Females fed with Candida sp. laid significantly more eggs on WASS and PTSS than MEA-Y. *D. suzukii* in the control treatment and *D. suzukii* fed with *M. pulcherrima* showed no significant preference for any of the cage components.

The results suggest that the positive attraction of yeast cultures on malt extract agar to *D. suzukii* in small areas applies only to the feeding source and not to the oviposition location. Yeast cultures, as good source for larval development (LEE et al., 2015), are not preferred for oviposition. This could be part of the evolution of *D. suzukii* from rotten to healthy fruit. In natural conditions, the flies had to leave a food source such as rotten fruit consciously to find suitable fruits for oviposition. This behavior differs from that of *D. melanogaster*, which oviposits on the same yeasts that they prefer for feeding, although the yeast is not the best diet for their offspring (ANAGNOSTOU et al., 2010).

BELLUTTI et al. (2015) observed contrary results with *D. suzukii* for the same yeast strains. In their study, *D. suzukii* females laid more eggs on cherries inoculated with *Candida sp.* when previously fed with *Candida sp.*, and fewer eggs on cherries inoculated with *H. uvarum* when previously fed with *H. uvarum*. One possible reason is the influence of the yeasts on the natural oviposition location, a factor that was eliminated in the present study. Other influences, such as yeast cell concentration, interaction between fruit volatiles and yeast volatiles and the surface structure, cannot be ruled out.

ANAGNOSTOUS et al. (2010) showed that *D. melanogaster* female yeast choice appears to be a food choice rather than a choice of a suitable oviposition substrate. *D. suzukii* oviposit their eggs in the skin of healthy fruit on the plant, which contains a lower number of yeast cells on the surface compared to overripe or damaged fruits (MITSUI et al., 2006). In this study, *D. suzukii* flies chose the surface with the best characteristics for egg insertion. This behavior is surprising, because the larvae of *Drosophila* have no chance for survival on water agar (ANAGNOSTOU et al., 2010). In the present study, in contrast to their study, 0.1 ml 5% sucrose solution was pipetted on the surface of the water agar to increase the acceptability of the water agar as an oviposition site; it remains unclear how strongly the behavior was influenced by the sucrose solution. On MEA-Y and PTSS, most of the eggs were inserted into the surface, as with WASS and MEA-C, in which all eggs were inserted.

The strains of three yeasts in this study were known. Other studies have shown that different yeast strains from the same species have different levels of attractiveness to *Drosophila* species (PALANCA et al., 2013). Particularly for *S. cerevisiae*, different strains have been observed to have different influences (PALANCA et al., 2013). The *S. cerevisiae* used in the present study is a baker's yeast (Küchle®). PALANCA et al. (2013) showed that some strains of baker's yeast are not attractive to *Drosophila* flies. At the Laimburg Research Centre for Agriculture and Forestry, the *S. cerevisiae* strain tested has been used in laboratory rearing with good success. Therefore, it can be assumed either that the strain of *S. cerevisiae* used is suitable for *D. suzukii* or that the laboratory population is adapted to this *S. cerevisiae* strain. It is possible that yeasts with different levels of attractiveness to *Drosophila* also have different influences on fecundity. It is important to understand that the results cannot be generalized for the entire yeast species, but they should help to classify the yeast species used and provide a tentative conclusion regarding the influence of the yeast community on *D. suzukii* diet.

The *H. uvarum*, *M. pulcherrima* and *Candida sp.* yeasts that were used in this study were isolated from *D. suzukii* infested grapes by BELLUTTI et al. (2015). Therefore, it can be assumed that the isolated yeast strains are part of the natural diet of *D. suzukii* larvae.

5. Conclusion

The results of the present study indicate that yeasts in the diet are not essential for *D. suzukii*. Different yeasts in the diet of *D. suzukii* showed no significant effect on the initiation of oviposition, but did show different effects on fecundity. Significantly more eggs were laid by females fed with *S. cerevisiae* or *H. uvarum* than females fed with *Candida sp*., while no significant differences were found between *M. pulcherrima* and the other three yeast species (*Candida sp.*, *H. uvarum and S. cerevisiae*). The South Tyrol ecotype of *D. suzukii* showed no decrease in oviposition in the first 52 days of life. *D. suzukii* females fed with yeast species that led to a higher fecundity also led to higher mortality in the first 52 days of adult lifetime. *D. suzukii* male survivorship was not influenced by the presence of yeasts or the yeast species in the diet. The oviposition choice of *D. suzukii* was influenced by the yeast species in the diet, but *D. suzukii* do not prefer yeast colonies on malt extract agar for oviposition in the presence of an alternative oviposition location with a suitable surface structure.

The results of the present study point to the conclusion that the observed beneficial effects of the wild *H. uvarum* strain used in the study can be applied for laboratory mass rearing, with the advantage that *H. uvarum* corresponds to natural conditions better than artificially altered *S. cerevisiae* cultures.

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8. Appendix data tables

8.1. Replication 1

		1	Control					1	*Saccharomyces cerevisiae*					1	*Metschnikowia pulcherrima*				
		Eggs						Eggs						Eggs					
Date	Day	WASS	MEA-C	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/09	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/10	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/11	3	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
5/12	4	0	0	0	0	0	0	0	4	1	5	0	0	0	0	0	0	0	0
5/13	5	0	0	0	0	0	1	3	5	0	8	0	0	0	0	0	0	0	0
5/14	6	0	0	1	1	0	0	2	0	1	3	0	0	0	0	1	1	0	0
5/15	7	0	0	1	1	0	0	8	15	1	24	0	0	0	0	1	1	0	1
5/16	8	0	0	0	0	0	0	1	8	3	12	0	0	0	1	0	1	0	0
5/17	9	0	0	0	0	0	0	0	6	5	11	0	0	1	0	2	3	0	0
5/18	10	0	1	0	1	0	0	7	4	13	24	0	0	1	0	9	10	0	0
5/19	11	0	0	1	1	0	0	6	3	3	12	1	0	0	0	3	3	0	0
5/20	12	0	4	1	5	0	0	0	15	13	28	0	0	0	1	3	4	0	0
5/21	13	0	3	0	3	0	0	1	2	2	5	0	1	1	0	6	7	0	0
5/22	14	0	1	1	2	0	0	0	15	15	30	0	0	0	0	2	2	0	0
5/23	15	0	1	2	2	0	0	1	21	3	25	0	0	0	0	1	1	0	0
5/24	16	4	0	1	5	0	0	0	23	14	37	0	0	0	4	3	7	0	0
5/25	17	0	3	0	3	0	0	20	27	9	56	0	0	0	1	3	4	0	0
5/26	18	1	0	1	2	0	0	7	51	7	65	0	0	4	0	8	12	0	0
5/27	19	0	15	1	16	0	0	31	47	14	92	0	0	0	1	10	11	0	0
5/28	20	0	0	0	0	0	0	31	34	12	77	0	0	0	1	26	27	0	0
5/29	21	0	1	0	1	0	0	46	3	15	64	0	0	1	0	12	13	0	0
5/30	22	0	8	0	8	0	0	50	30	9	89	0	0	1	0	6	7	0	0
5/31	23	0	9	3	12	0	0	80	2	3	85	1	0	0	1	5	6	0	0
6/01	24	0	8	0	8	0	0	41	2	33	76	0	0	18	0	14	32	0	0
6/02	25	0	8	0	8	0	1	67	1	5	73	0	0	7	1	2	10	0	0
6/03	26	0	2	0	2	0	0	52	13	8	73	0	0	0	6	0	6	0	0
6/04	27	0	3	1	4	0	0	70	1	6	77	0	0	1	0	2	3	0	1
6/05	28	0	10	0	10	0	0	57	32	15	104	0	0	3	0	3	6	0	0
6/06	29	0	1	0	1	0	0	81	0	14	95	0	0	0	2	4	6	0	0
6/07	30	0	7	0	7	0	0	48	27	17	92	0	0	0	11	2	13	0	0
6/08	31	1	3	0	4	0	0	78	17	9	104	0	0	1	0	3	4	0	0
6/09	32	0	0	0	0	0	0	87	3	20	110	0	0	0	1	2	3	0	0
6/10	33	1	1	0	2	0	0	47	0	23	70	0	0	0	2	0	2	0	0
6/11	34	0	1	1	2	0	0	65	6	2	73	1	0	0	1	2	3	0	0
6/12	35	0	1	1	2	0	0	68	1	6	75	0	0	2	1	4	7	0	0
6/13	36	0	0	3	3	0	0	105	17	18	140	0	0	0	1	2	3	0	0
6/14	37	0	3	2	5	0	0	65	1	11	77	0	0	0	1	5	6	0	0
6/15	38	0	0	1	1	0	0	71	47	5	123	0	0	4	2	2	8	0	0
6/16	39	5	1	2	8	0	0	56	1	3	60	0	0	0	1	6	7	0	0
6/17	40	0	4	0	4	1	0	114	18	1	133	0	0	0	1	4	5	0	0
6/18	41	0	0	2	2	0	0	91	0	3	94	0	0	12	1	5	18	0	0
6/19	42	0	0	2	2	0	0	95	35	1	131	0	0	5	0	4	9	0	0
6/20	43	0	0	3	3	1	0	74	0	4	78	0	0	1	1	1	3	0	0
6/21	44	0	5	5	10	0	0	104	25	0	129	0	0	7	6	2	15	0	0
6/22	45	7	8	1	16	0	0	76	12	3	91	0	0	12	0	2	14	0	0
6/23	46	4	6	0	10	0	0	93	21	0	114	0	0	1	1	3	5	0	0
6/24	47	6	1	1	8	0	0	102	0	0	102	0	0	0	1	8	9	0	0
6/25	48	4	1	2	7	0	0	90	38	0	128	0	0	2	1	3	6	0	0
6/26	49	8	0	1	9	0	0	110	0	2	112	0	0	0	2	3	5	0	0
6/27	50	4	4	0	8	0	0	111	18	5	134	0	0	0	0	9	9	0	0
			Remaining flies:			8	8		Remaining flies:			7	9		Remaining flies:			10	8

		1	*Candida sp.*					1	*Hanseniaspora uvarum*				
		Eggs						Eggs					
Date	Day	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/09	1	0	0	0	0	0	0	0	0	0	0	0	0
5/10	2	0	0	0	0	0	0	0	0	0	0	0	0
5/11	3	0	0	0	0	0	0	1	0	0	1	0	0
5/12	4	0	0	0	0	0	0	0	0	0	0	0	0
5/13	5	0	0	0	0	0	0	0	8	0	8	0	0
5/14	6	0	0	0	0	0	0	0	1	0	1	0	0
5/15	7	0	0	0	0	0	0	0	7	1	8	0	0
5/16	8	0	0	3	3	0	0	0	2	2	4	0	0
5/17	9	0	2	6	8	0	0	0	4	4	8	0	0
5/18	10	1	0	3	4	0	0	4	10	3	17	0	0
5/19	11	0	1	1	2	0	0	0	17	4	21	0	0
5/20	12	1	0	6	7	0	0	5	2	3	10	0	0
5/21	13	0	0	6	6	0	0	41	0	7	48	0	0
5/22	14	0	0	11	11	0	0	30	0	5	35	0	0
5/23	15	0	0	3	3	0	0	28	1	7	36	0	0
5/24	16	0	0	6	6	0	0	58	7	2	67	0	0
5/25	17	4	1	13	18	0	0	36	0	11	47	0	0
5/26	18	0	0	4	4	0	0	64	6	1	71	0	0
5/27	19	6	0	5	11	0	0	60	7	3	70	0	0
5/28	20	8	0	4	12	0	0	35	1	12	48	0	0
5/29	21	4	0	3	7	0	0	67	0	4	71	0	0
5/30	22	10	1	1	12	0	0	54	1	3	58	0	0
5/31	23	0	1	4	5	0	0	67	1	2	70	0	0
6/01	24	3	0	5	8	0	0	93	1	4	98	1	0
6/02	25	14	0	7	21	0	0	82	2	5	89	0	0
6/03	26	22	0	2	24	0	0	47	0	3	50	0	0
6/04	27	7	1	4	12	0	0	77	0	3	80	0	0
6/05	28	23	0	0	23	0	0	72	0	17	89	0	0
6/06	29	21	0	3	24	0	0	91	4	1	96	0	0
6/07	30	16	1	4	21	0	0	72	8	0	80	0	1
6/08	31	22	0	0	22	0	0	86	0	3	89	0	1
6/09	32	31	0	1	32	0	0	70	0	2	72	0	0
6/10	33	26	0	1	27	0	0	81	4	3	88	0	0
6/11	34	12	0	2	14	0	0	61	0	3	64	0	0
6/12	35	13	0	6	19	0	1	69	1	1	71	0	0
6/13	36	14	0	2	16	0	0	43	0	1	44	1	0
6/14	37	13	1	2	16	0	0	53	1	2	56	0	0
6/15	38	12	0	12	24	0	0	85	0	1	86	0	0
6/16	39	15	1	4	20	0	0	93	0	2	95	0	0
6/17	40	7	0	3	10	0	0	82	0	0	82	0	0
6/18	41	26	0	0	26	0	0	81	0	1	82	0	0
6/19	42	7	3	0	10	1	0	88	0	1	89	0	0
6/20	43	13	0	6	19	0	0	92	0	0	92	0	0
6/21	44	8	0	1	9	0	0	87	5	0	92	0	0
6/22	45	19	0	0	19	0	0	81	0	0	81	0	0
6/23	46	40	0	0	40	0	0	90	1	1	92	0	0
6/24	47	39	0	2	41	0	0	54	1	1	56	0	0
6/25	48	41	0	0	41	0	0	98	0	1	99	0	0
6/26	49	49	0	0	49	0	0	90	0	0	90	0	0
6/27	50	38	0	0	38	0	0	115	0	0	115	0	0
			Remaining flies:			9	9		Remaining flies:			8	8

8.2. Replication 2

		2	Control					2	*Saccharomyces cerevisiae*					2	*Metschnikowia pulcherrima*				
		Eggs						Eggs						Eggs					
Date	Day	WASS	MEA-C	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/11	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/12	3	0	0	0	0	0	0	0	0	4	4	0	0	0	0	1	1	0	0
5/13	4	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
5/14	5	0	0	0	0	0	0	1	2	4	7	0	0	0	0	0	0	0	0
5/15	6	0	0	0	0	0	0	1	1	3	5	0	0	0	0	1	1	0	0
5/16	7	0	0	0	0	0	0	1	13	2	16	0	0	1	0	0	1	0	0
5/17	8	0	1	0	1	0	0	4	0	7	11	0	0	1	0	3	4	0	0
5/18	9	0	0	0	0	0	0	1	3	12	16	0	0	1	0	2	3	0	0
5/19	10	0	0	0	0	0	0	0	2	7	9	0	0	0	0	0	0	1	0
5/20	11	0	0	0	0	0	0	0	25	3	28	0	0	0	1	4	5	0	0
5/21	12	0	1	0	1	0	0	1	10	5	16	0	0	0	1	3	4	0	0
5/22	13	0	1	1	2	0	0	0	2	36	38	0	0	1	0	5	6	0	0
5/23	14	0	0	0	0	0	0	0	12	8	20	0	0	5	1	5	11	0	0
5/24	15	0	1	0	1	0	0	41	10	26	77	0	0	0	0	6	6	0	0
5/25	16	0	0	2	2	0	0	3	3	6	12	0	0	1	1	3	5	0	0
5/26	17	0	0	2	2	0	0	23	19	23	65	0	0	0	1	4	5	0	0
5/27	18	1	2	0	3	0	0	3	12	51	66	0	0	6	0	2	8	0	0
5/28	19	0	3	0	3	0	1	29	10	17	56	0	0	1	0	4	5	0	1
5/29	20	0	0	0	0	0	0	30	19	9	58	0	0	0	0	4	4	0	0
5/30	21	1	0	2	3	0	0	44	23	20	87	0	0	0	11	2	13	0	0
5/31	22	0	0	0	0	0	0	29	10	5	44	0	0	1	0	6	7	0	0
6/01	23	0	1	0	1	0	0	57	6	18	81	0	0	0	2	9	11	0	0
6/02	24	0	0	1	1	0	0	53	0	6	59	0	0	2	0	8	10	0	0
6/03	25	1	1	1	3	0	0	44	0	1	45	0	0	1	1	1	3	0	0
6/04	26	0	1	1	2	0	0	47	0	5	52	0	0	1	0	5	6	0	0
6/05	27	0	1	1	2	0	0	54	13	6	73	0	0	0	1	6	7	0	0
6/06	28	0	0	2	2	0	0	81	23	5	109	0	0	1	0	5	6	0	0
6/07	29	0	1	0	1	0	0	106	11	16	133	0	0	0	2	9	11	0	0
6/08	30	0	0	1	1	0	0	50	14	3	67	0	0	4	0	4	8	0	0
6/09	31	0	0	4	4	0	0	65	16	14	95	0	0	0	1	10	11	0	0
6/10	32	1	0	4	5	0	0	61	0	3	64	0	0	1	1	3	5	0	0
6/11	33	0	1	1	2	0	0	79	5	0	84	0	0	1	2	13	16	0	0
6/12	34	0	0	3	3	0	0	80	0	0	80	0	0	1	0	7	8	0	0
6/13	35	1	0	3	4	0	0	80	10	3	93	0	0	15	2	8	25	0	0
6/14	36	1	1	3	5	0	0	114	0	1	115	0	0	7	0	12	19	0	0
6/15	37	0	0	5	5	0	0	65	16	1	82	0	0	5	25	5	35	0	0
6/16	38	0	1	5	6	0	0	79	2	1	82	0	0	16	11	14	41	0	0
6/17	39	0	0	3	3	0	0	64	3	4	71	0	0	36	28	9	73	0	0
6/18	40	3	1	1	5	0	0	121	0	2	123	0	0	17	8	11	36	0	0
6/19	41	0	3	0	3	0	0	86	15	3	104	0	0	15	33	6	54	0	0
6/20	42	2	2	5	9	0	0	90	0	0	90	0	0	25	6	3	34	0	0
6/21	43	0	1	0	1	0	0	95	8	0	103	0	0	23	33	9	65	0	0
6/22	44	0	5	1	6	0	0	75	0	2	77	0	0	20	11	6	37	0	0
6/23	45	1	5	1	7	0	0	118	5	0	123	0	0	23	38	2	63	0	0
6/24	46	6	7	2	15	1	0	116	0	0	116	0	0	46	6	0	52	0	0
6/25	47	5	2	2	9	0	0	99	2	1	102	0	0	48	44	1	93	0	0
6/26	48	4	1	3	8	0	0	119	2	0	121	0	0	29	8	0	37	0	0
6/27	49	8	1	2	11	0	0	108	5	0	113	0	0	58	18	0	76	0	0
6/28	50	10	1	0	11	0	0	70	1	1	72	0	0	41	16	1	58	0	0
			Remaining flies:			9	9		Remaining flies:			10	10		Remaining flies:			9	9

		2	Candida sp.					2	Hanseniaspora uvarum				
		Eggs						Eggs					
Date	Day	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	Agar	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/10	1	0	0	0	0	0	0	0	0	0	0	0	0
5/11	2	0	0	0	0	0	0	0	0	0	0	0	0
5/12	3	0	0	0	0	0	0	0	0	0	0	0	0
5/13	4	0	0	0	0	0	0	0	0	0	0	0	0
5/14	5	0	0	1	1	0	0	0	0	0	0	0	0
5/15	6	0	0	1	1	0	0	0	2	0	2	0	0
5/16	7	0	0	0	0	0	0	0	2	2	4	0	0
5/17	8	0	0	2	2	0	0	0	0	5	5	0	0
5/18	9	0	0	2	2	0	0	0	3	6	9	0	0
5/19	10	0	0	1	1	0	0	0	4	2	6	0	0
5/20	11	0	0	2	2	0	0	0	9	2	11	0	0
5/21	12	0	0	1	1	0	0	0	8	2	10	0	0
5/22	13	0	0	8	8	0	0	0	23	2	25	0	0
5/23	14	0	0	3	3	0	0	0	8	5	13	0	0
5/24	15	2	2	0	4	0	0	2	2	7	11	0	0
5/25	16	0	0	2	2	0	0	0	4	7	11	0	0
5/26	17	1	0	2	3	0	0	0	4	9	13	0	0
5/27	18	1	2	4	7	0	0	0	3	21	24	0	0
5/28	19	0	0	4	4	0	0	2	1	10	13	0	0
5/29	20	0	0	5	5	0	0	23	7	4	34	0	0
5/30	21	3	0	1	4	0	0	0	11	10	21	0	0
5/31	22	1	0	1	2	0	0	8	7	8	23	0	0
6/01	23	7	1	3	11	0	0	0	5	4	9	0	0
6/02	24	4	1	1	6	0	0	0	9	3	12	0	0
6/03	25	0	0	4	4	0	0	0	23	17	40	0	0
6/04	26	3	0	4	7	0	0	0	7	10	17	0	0
6/05	27	13	7	8	28	0	0	1	3	15	19	0	1
6/06	28	2	0	7	9	0	0	4	19	24	47	0	0
6/07	29	0	1	9	10	0	0	0	4	13	17	0	0
6/08	30	12	0	3	15	0	0	6	17	27	50	0	0
6/09	31	0	1	8	9	0	0	7	13	8	28	0	0
6/10	32	5	3	5	13	0	0	1	0	9	10	0	0
6/11	33	0	1	10	11	0	0	0	2	8	10	0	0
6/12	34	0	0	6	6	0	0	0	10	16	26	0	0
6/13	35	0	0	8	8	0	0	16	2	19	37	0	0
6/14	36	0	1	8	9	0	0	0	4	7	11	0	0
6/15	37	2	1	9	12	0	0	0	8	15	23	0	0
6/16	38	0	1	4	5	0	0	0	2	11	13	0	0
6/17	39	6	1	2	9	0	0	8	9	22	39	0	0
6/18	40	1	2	6	9	0	0	0	0	15	15	0	0
6/19	41	4	6	1	11	0	0	0	4	7	11	0	0
6/20	42	16	0	3	19	0	0	1	8	9	18	0	0
6/21	43	18	0	4	22	0	0	0	8	6	14	0	0
6/22	44	19	0	2	21	0	0	0	13	11	24	0	0
6/23	45	25	0	2	27	0	0	0	51	1	52	0	0
6/24	46	35	1	1	37	0	0	0	1	8	9	0	0
6/25	47	34	2	2	38	0	0	1	20	7	28	0	0
6/26	48	24	0	2	26	0	0	0	29	8	37	0	0
6/27	49	30	3	5	38	0	0	0	11	21	32	0	0
6/28	50	44	0	1	45	0	0	0	20	18	38	0	0
			Remaining flies:			10	10		Remaining flies:			10	9

8.3. Replication 3

		3	Control					3	*Saccharomyces cerevisiae*					3	*Metschnikowia pulcherrima*				
		Eggs						**Eggs**						**Eggs**					
Date	Day	WASS	MEA-C	PTSS	Sum	Dead M.	Tote W	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/11	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/12	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/13	3	0	0	1	1	0	0	0	0	2	2	0	0	0	0	0	0	0	0
5/14	4	0	0	0	0	0	0	0	0	14	14	0	0	7	0	0	7	0	0
5/15	5	0	2	0	2	0	0	0	3	3	6	0	0	0	1	0	1	0	0
5/16	6	0	0	5	5	0	0	0	1	11	12	0	0	0	12	9	21	0	1
5/17	7	1	0	2	3	0	0	0	1	8	9	0	0	0	0	1	1	0	0
5/18	8	0	1	0	1	0	0	1	2	2	5	0	0	0	12	1	13	0	0
5/19	9	0	1	0	1	0	0	22	0	8	30	0	0	0	0	2	2	0	0
5/20	10	0	0	0	0	0	0	14	1	3	18	0	0	0	14	2	16	0	0
5/21	11	0	4	2	6	0	0	28	1	3	32	0	0	0	1	1	2	0	0
5/22	12	0	1	1	2	0	0	25	5	29	59	0	0	0	17	2	19	0	0
5/23	13	0	0	0	0	0	0	36	0	10	46	0	0	0	9	19	28	0	0
5/24	14	0	2	5	7	0	0	46	8	3	57	0	0	0	6	6	12	0	0
5/25	15	0	2	2	4	0	0	54	1	9	64	0	0	0	37	1	38	0	0
5/26	16	1	1	4	6	0	0	46	20	22	88	0	0	0	7	2	9	0	0
5/27	17	0	2	3	5	0	0	44	0	24	68	0	0	0	28	32	60	0	0
5/28	18	0	2	2	4	0	0	53	0	26	79	0	0	1	3	9	13	0	0
5/29	19	0	3	6	9	0	0	72	1	4	77	0	0	15	7	16	38	0	0
5/30	20	0	0	2	2	0	0	78	0	21	99	0	0	39	21	11	71	0	0
5/31	21	0	2	4	6	0	0	101	1	1	103	0	0	48	0	9	57	1	0
6/01	22	0	2	1	3	0	0	76	7	6	89	0	0	71	0	1	72	0	0
6/02	23	0	3	3	6	0	0	105	0	6	111	0	0	55	0	0	55	0	1
6/03	24	2	0	0	2	0	0	69	11	3	83	0	0	80	0	3	83	0	0
6/04	25	0	2	2	4	0	0	82	0	6	88	0	1	67	0	0	67	0	0
6/05	26	0	3	4	7	0	0	66	5	26	97	0	0	49	0	2	51	0	0
6/06	27	0	1	10	11	0	0	65	0	7	72	0	0	47	6	3	56	0	0
6/07	28	0	3	1	4	0	0	102	0	0	102	0	0	63	0	5	68	0	0
6/08	29	0	2	6	8	0	0	88	0	2	90	0	0	81	0	1	82	0	0
6/09	30	0	0	2	2	0	0	129	1	2	132	0	0	48	3	2	53	0	0
6/10	31	1	0	8	9	0	0	49	0	4	53	0	0	53	0	0	53	0	0
6/11	32	0	0	4	4	0	0	97	3	5	105	0	0	78	0	0	78	0	0
6/12	33	0	2	4	6	0	0	61	0	2	63	0	0	38	0	0	38	0	0
6/13	34	0	1	0	1	0	0	97	0	0	97	0	0	68	1	1	70	0	0
6/14	35	0	1	2	3	0	0	96	0	1	97	0	1	53	0	2	55	0	0
6/15	36	0	0	3	3	0	0	74	1	0	75	0	0	76	0	0	76	0	0
6/16	37	0	0	1	1	0	0	44	0	1	45	0	1	47	1	1	49	0	0
6/17	38	0	2	4	6	0	0	72	0	0	72	0	0	74	5	2	81	0	0
6/18	39	0	1	3	4	0	0	81	0	3	84	0	0	61	0	0	61	0	0
6/19	40	1	1	5	7	0	0	72	6	1	79	0	0	63	0	0	63	0	0
6/20	41	1	1	1	3	0	0	72	0	1	73	0	0	52	3	0	55	0	0
6/21	42	0	1	6	7	0	0	81	1	0	82	0	1	85	0	0	85	0	0
6/22	43	0	2	4	6	0	0	63	0	1	64	0	0	57	1	0	58	0	0
6/23	44	0	3	8	12	0	0	69	0	1	70	0	0	84	0	0	84	0	0
6/24	45	1	0	1	2	0	0	70	0	0	70	0	0	35	0	0	35	0	0
6/25	46	0	1	5	6	0	0	76	0	0	76	0	0	76	14	0	90	0	0
6/26	47	0	0	3	3	0	0	43	1	0	44	0	2	32	3	1	36	0	0
6/27	48	0	1	6	7	0	0	70	0	0	70	0	0	101	0	0	101	0	0
6/28	49	0	1	6	7	0	0	40	0	0	40	0	0	42	1	0	43	0	0
6/29	50	6	1	5	12	0	0	80	0	0	80	0	0	41	1	0	42	0	0
			Remaining flies:			10	10		Remaining flies:			10	4		Remaining flies:			9	8

		3	Candida sp.					3	Hanseniaspora uvarum				
		Eggs						Eggs					
Date	Day	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/11	1	0	0	0	0	0	0	0	0	0	0	0	0
5/12	2	0	0	0	0	0	0	0	0	0	0	0	0
5/13	3	0	0	3	3	0	0	0	0	1	1	0	0
5/14	4	0	0	6	6	0	0	1	0	1	2	0	0
5/15	5	0	0	8	8	0	0	0	0	1	1	0	0
5/16	6	0	1	2	3	0	0	1	1	4	6	0	0
5/17	7	0	0	9	9	0	0	5	1	15	21	0	0
5/18	8	0	0	5	5	0	0	8	2	8	18	0	0
5/19	9	0	2	0	2	0	0	9	1	10	20	0	0
5/20	10	0	1	8	9	0	0	17	1	26	44	0	0
5/21	11	0	0	9	9	0	0	48	1	6	55	0	0
5/22	12	0	0	6	6	0	0	0	2	21	23	0	0
5/23	13	6	0	11	17	0	0	57	1	24	82	0	0
5/24	14	1	0	20	21	0	0	28	0	35	63	0	0
5/25	15	15	0	9	24	0	0	44	2	14	60	0	0
5/26	16	19	0	9	38	0	0	41	0	23	64	0	0
5/27	17	9	0	5	14	0	0	82	1	44	127	0	1
5/28	18	36	0	1	37	0	0	53	0	28	81	0	0
5/29	19	31	1	6	38	0	0	75	1	31	107	0	0
5/30	20	52	1	8	61	0	0	106	0	12	118	0	0
5/31	21	54	0	0	54	0	0	105	0	19	124	0	0
6/01	22	71	0	0	71	0	0	113	0	12	125	0	0
6/02	23	62	0	2	64	0	0	113	1	18	132	0	0
6/03	24	51	0	0	51	0	0	75	2	6	83	0	1
6/04	25	38	0	0	38	0	0	104	0	5	109	0	0
6/05	26	61	0	4	65	0	0	89	0	1	90	0	0
6/06	27	47	0	0	47	0	0	110	0	13	123	0	0
6/07	28	54	1	0	55	0	1	114	0	6	120	0	0
6/08	29	47	0	5	52	0	0	110	0	11	121	0	0
6/09	30	30	0	2	32	0	0	111	0	3	114	0	0
6/10	31	28	0	1	29	0	0	88	1	7	96	0	0
6/11	32	27	0	0	27	0	0	136	1	11	148	0	0
6/12	33	38	0	1	39	0	0	81	0	1	82	0	2
6/13	34	62	0	2	64	0	0	81	0	3	84	1	0
6/14	35	52	0	0	52	0	0	49	0	10	59	0	0
6/15	36	39	0	1	40	0	0	84	1	1	86	0	0
6/16	37	50	0	2	52	0	0	84	1	4	89	0	0
6/17	38	34	0	5	39	0	0	118	0	1	119	0	0
6/18	39	36	0	0	36	0	0	77	0	0	77	0	0
6/19	40	25	1	2	28	0	0	80	0	2	82	0	0
6/20	41	44	0	2	46	0	0	105	0	0	105	0	0
6/21	42	40	0	0	40	0	0	57	0	0	57	0	1
6/22	43	46	0	1	47	0	0	88	0	1	89	0	2
6/23	44	43	0	0	43	0	0	51	1	1	53	0	0
6/24	45	61	0	0	61	0	0	53	0	0	53	0	0
6/25	46	50	0	0	50	0	0	43	0	0	43	0	0
6/26	47	27	4	0	31	0	0	19	0	2	21	0	0
6/27	48	79	0	1	80	0	0	29	0	0	29	0	0
6/28	49	66	0	1	67	0	0	45	0	0	45	0	0
6/29	50	71	0	0	71	0	0	33	1	0	34	0	0
			Remaining flies:			10	9		Remaining flies:			9	3

8.4. Replication 4

		4	*Control*					4	*Saccharomyces cerevisiae*					4	*Metschnikowia pulcherrima*				
		Eggs						**Eggs**						**Eggs**					
Date	Day	WASS	MEA-C	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/24	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/25	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/26	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/27	4	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0
5/28	5	0	1	0	1	0	0	0	1	3	4	0	0	0	0	0	0	0	0
5/29	6	0	0	2	2	0	0	7	1	11	19	0	0	0	0	1	1	0	0
5/30	7	0	0	0	0	0	0	0	0	5	5	0	0	1	0	0	1	0	0
5/31	8	0	1	3	4	0	0	1	1	5	7	0	0	0	0	5	5	0	0
6/01	9	0	0	2	2	0	0	0	0	14	14	0	0	6	0	10	16	0	0
6/02	10	0	2	3	5	0	0	34	4	33	71	1	0	2	1	9	12	0	0
6/03	11	0	3	5	8	0	0	13	2	4	19	0	0	1	2	10	13	0	0
6/04	12	1	1	3	5	0	0	67	0	13	80	0	0	0	0	8	8	0	0
6/05	13	0	5	9	14	0	0	67	2	11	80	0	0	11	0	33	44	0	0
6/06	14	0	7	8	15	0	0	119	5	19	143	0	0	0	1	37	38	0	0
6/07	15	0	4	6	10	0	0	111	1	4	116	0	0	0	0	9	9	0	0
6/08	16	0	11	10	21	0	0	149	0	7	156	0	0	1	1	59	61	0	0
6/09	17	0	3	6	9	0	0	123	0	8	131	0	0	0	6	39	45	0	0
6/10	18	0	0	9	9	0	0	88	0	0	88	0	0	14	1	28	43	0	0
6/11	19	0	0	8	8	0	0	124	0	8	132	0	0	0	0	19	19	0	0
6/12	20	0	0	17	17	0	0	58	0	5	63	0	0	0	1	19	20	0	0
6/13	21	0	1	7	8	0	0	82	3	14	99	0	0	0	0	43	43	0	0
6/14	22	0	3	6	9	0	0	79	0	16	95	0	0	39	1	9	49	0	0
6/15	23	0	2	7	9	0	0	155	1	3	159	0	0	2	0	21	23	0	0
6/16	24	0	2	6	8	0	0	76	0	2	78	0	0	42	0	18	60	0	0
6/17	25	0	3	12	15	0	0	121	0	4	125	0	0	27	0	26	53	0	0
6/18	26	0	5	10	15	0	0	130	0	8	138	1	0	27	11	14	52	0	0
6/19	27	0	2	19	21	0	0	126	8	7	141	0	0	45	5	26	76	0	0
6/20	28	0	3	7	10	0	0	93	0	0	93	0	0	63	0	9	72	0	0
6/21	29	0	2	8	10	0	0	135	3	13	151	0	0	54	14	2	70	0	0
6/22	30	1	1	10	12	0	0	86	0	12	98	0	1	37	11	6	54	0	0
6/23	31	0	7	7	14	0	0	124	0	2	126	0	0	60	17	22	99	0	0
6/24	32	0	1	7	8	0	0	124	0	3	127	0	0	49	18	14	81	0	0
6/25	33	1	1	2	4	0	0	172	0	2	174	0	0	81	7	20	108	0	0
6/26	34	0	0	10	10	0	0	138	0	5	143	0	0	43	11	9	63	0	0
6/27	35	0	7	13	20	0	1	152	12	11	175	0	0	39	18	2	59	0	0
6/28	36	0	0	11	11	0	0	104	0	0	104	0	1	43	17	12	72	0	0
6/29	37	0	0	16	16	1	0	147	3	0	150	0	0	75	14	4	93	0	0
6/30	38	0	0	8	8	0	0	74	0	1	75	0	0	86	11	20	107	0	0
7/01	39	0	3	8	11	0	0	135	0	1	136	0	0	50	20	11	81	0	0
7/02	40	0	11	1	12	0	0	91	0	1	92	0	0	40	19	8	67	0	0
7/03	41	0	6	10	16	0	0	130	6	0	136	0	0	59	6	3	68	0	0
7/04	42	0	1	13	14	0	0	78	0	4	82	1	0	54	28	12	94	0	0
7/05	43	1	7	8	16	0	1	107	0	1	108	0	0	87	15	8	110	0	0
7/06	44	0	5	11	16	0	0	119	2	8	129	0	0	50	1	4	55	0	0
7/07	45	0	6	3	9	0	0	107	0	1	108	0	0	38	1	3	42	0	0
7/08	46	0	1	14	15	0	0	112	1	0	113	0	0	70	0	6	76	0	1
7/09	47	0	10	7	17	0	0	92	1	3	96	0	0	87	1	1	89	0	0
7/10	48	2	2	4	8	0	0	87	0	1	88	0	2	51	0	6	57	0	0
7/11	49	0	3	7	10	0	0	76	0	3	78	0	0	95	0	5	100	0	0
7/12	50	37	2	0	39	0	1	81	0	2	83	0	0	76	1	1	78	0	0
			Remaining flies:			9	7		Remaining flies:			7	6		Remaining flies:			10	9

		4	*Candida sp.*					4	*Hanseniaspora uvarum*				
		Eggs						**Eggs**					
Date	Day	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/24	1	0	0	0	0	0	0	0	0	0	0	0	0
5/25	2	0	0	0	0	0	0	0	0	0	0	0	0
5/26	3	1	0	0	1	0	0	0	0	0	0	0	0
5/27	4	1	0	0	1	0	0	0	0	0	0	0	0
5/28	5	0	0	1	1	0	0	0	1	1	2	0	0
5/29	6	12	0	5	17	0	0	3	0	6	9	0	0
5/30	7	0	0	2	2	0	0	11	0	11	22	0	0
5/31	8	5	0	7	12	0	0	0	0	11	11	0	0
6/01	9	5	0	5	10	0	0	2	4	12	18	0	0
6/02	10	3	0	15	18	0	0	26	0	24	50	0	0
6/03	11	0	0	6	6	0	0	2	0	60	62	0	0
6/04	12	19	0	14	33	0	0	1	0	61	62	0	0
6/05	13	4	0	16	20	0	0	44	3	76	123	0	0
6/06	14	0	1	10	11	0	0	15	0	71	86	1	1
6/07	15	1	0	20	21	0	0	77	2	18	97	0	2
6/08	16	20	0	7	27	0	0	54	0	17	71	1	0
6/09	17	13	0	11	24	0	0	56	0	24	80	0	0
6/10	18	17	0	20	37	0	0	86	0	12	98	0	0
6/11	19	0	6	17	23	0	0	88	0	15	103	0	0
6/12	20	4	0	13	17	0	0	76	0	7	83	0	0
6/13	21	4	0	13	17	0	0	70	0	6	76	0	0
6/14	22	0	0	25	25	0	0	82	0	4	86	0	0
6/15	23	1	1	7	9	0	0	103	0	3	106	0	0
6/16	24	0	1	11	12	0	0	53	0	2	55	0	0
6/17	25	1	8	17	26	0	0	94	0	3	97	0	0
6/18	26	0	1	11	12	0	0	73	0	3	76	0	1
6/19	27	0	0	33	33	0	0	74	0	0	74	0	0
6/20	28	18	0	16	34	0	0	69	1	0	70	0	0
6/21	29	7	12	2	21	0	0	102	1	0	103	0	0
6/22	30	18	0	10	28	0	0	86	0	0	86	0	0
6/23	31	0	2	15	17	0	0	101	0	1	102	0	0
6/24	32	23	10	10	43	0	0	122	0	0	122	0	0
6/25	33	0	0	23	23	0	0	96	0	0	96	0	0
6/26	34	12	0	7	19	0	0	90	0	0	90	0	0
6/27	35	7	8	21	36	1	0	123	0	0	123	0	0
6/28	36	0	0	6	6	0	0	60	0	1	61	0	0
6/29	37	0	8	20	28	0	0	125	0	1	126	0	0
6/30	38	8	0	24	32	0	0	78	0	0	78	0	0
7/01	39	0	0	10	10	0	0	88	0	0	88	0	0
7/02	40	13	1	11	25	0	0	79	0	1	80	0	0
7/03	41	0	7	11	18	0	0	65	0	0	65	0	0
7/04	42	19	0	11	30	0	0	60	0	2	62	0	0
7/05	43	11	12	2	25	0	0	72	4	0	76	0	0
7/06	44	22	0	11	33	0	0	72	2	3	77	0	0
7/07	45	68	1	12	81	0	1	69	1	0	70	1	1
7/08	46	92	0	2	94	0	0	60	0	1	61	0	0
7/09	47	59	7	0	66	0	0	57	4	5	66	0	0
7/10	48	23	0	1	24	0	0	83	0	1	84	0	0
7/11	49	75	0	1	76	0	0	93	0	2	95	0	0
7/12	50	34	0	0	34	0	0	96	0	1	97	0	0
			Remaining flies:			9	9		Remaining flies:			7	5

8.5. Replication 5

		5	Control					5	*Saccharomyces cerevisiae*					5	*Metschnikowia pulcherrima*				
		Eggs						Eggs						Eggs					
Date	Day	WASS	MEA-C	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/25	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
5/26	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/27	3	0	0	0	0	0	0	0	0	6	6	0	0	0	0	0	0	1	0
5/28	4	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
5/29	5	0	0	0	0	0	0	0	1	1	2	0	0	0	0	1	1	0	0
5/30	6	0	0	0	0	0	0	2	0	0	2	0	0	2	0	0	2	0	0
5/31	7	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
6/01	8	0	0	0	0	0	0	3	0	3	6	0	0	0	2	3	5	0	0
6/02	9	0	1	2	3	0	0	9	1	4	14	0	0	0	1	2	3	0	0
6/03	10	0	0	2	2	0	0	12	8	1	21	0	0	0	0	2	2	0	0
6/04	11	0	0	3	3	0	0	1	2	20	23	0	0	7	2	8	17	0	0
6/05	12	0	1	0	1	0	0	0	3	3	6	0	1	0	2	9	11	0	0
6/06	13	0	5	0	5	0	0	0	16	3	19	0	0	0	1	9	10	0	0
6/07	14	0	1	2	3	0	0	1	10	4	15	0	0	3	12	2	17	0	0
6/08	15	1	0	4	5	0	0	0	3	4	7	0	0	0	12	6	18	0	0
6/09	16	1	1	1	3	0	0	8	2	3	13	0	0	0	2	10	12	0	0
6/10	17	0	0	0	0	0	0	20	1	2	23	0	0	0	0	3	3	0	0
6/11	18	1	2	3	6	0	0	1	3	3	7	0	0	1	14	6	21	0	0
6/12	19	0	3	1	4	0	0	8	0	7	15	0	0	16	0	8	24	0	0
6/13	20	2	1	1	4	0	0	6	3	5	14	0	0	0	24	5	29	0	0
6/14	21	0	2	2	4	0	0	2	0	3	5	0	0	0	3	9	12	0	0
6/15	22	1	4	2	7	0	0	27	8	2	37	0	0	16	15	5	36	0	0
6/16	23	1	2	1	4	0	0	14	1	5	20	0	0	37	5	5	47	0	0
6/17	24	0	5	3	8	0	0	23	9	8	40	0	0	23	25	2	50	0	0
6/18	25	0	4	0	4	0	0	33	1	2	36	0	0	45	0	2	47	0	0
6/19	26	0	2	1	3	0	0	19	4	6	29	0	0	63	0	0	63	0	0
6/20	27	1	3	4	8	0	0	32	2	1	35	0	0	50	0	1	51	0	0
6/21	28	0	12	1	13	0	0	60	15	3	78	0	0	55	2	1	58	0	0
6/22	29	0	3	0	3	0	0	36	0	2	38	0	0	68	0	1	69	0	0
6/23	30	0	9	4	13	0	0	48	27	1	76	0	0	55	1	0	56	0	0
6/24	31	1	1	3	5	0	0	47	0	1	48	0	0	90	0	1	91	0	0
6/25	32	0	1	0	1	0	0	61	30	0	91	0	0	65	0	0	65	0	0
6/26	33	4	4	1	9	0	0	46	12	0	58	0	0	59	0	0	59	0	0
6/27	34	0	9	2	11	0	0	35	37	0	72	0	0	78	1	1	80	0	0
6/28	35	0	1	1	2	0	0	35	0	3	38	0	0	70	0	1	71	0	0
6/29	36	0	1	6	7	0	0	66	34	1	101	0	0	92	0	0	92	0	0
6/30	37	0	1	4	5	0	0	77	1	1	79	0	0	74	0	0	74	0	0
7/01	38	0	6	4	10	0	0	43	27	3	73	0	0	63	6	0	69	0	0
7/02	39	0	3	2	5	0	0	66	0	1	67	0	0	67	1	0	68	0	0
7/03	40	0	6	3	9	0	0	47	22	0	69	0	0	40	0	0	40	0	0
7/04	41	3	5	4	12	1	0	77	0	0	77	0	0	83	0	1	84	0	0
7/05	42	1	9	3	13	2	2	56	2	0	58	0	1	74	2	0	76	0	0
7/06	43	2	7	2	11	1	0	59	0	1	60	0	0	46	0	0	46	0	0
7/07	44	1	2	1	4	0	0	38	7	0	45	0	0	64	1	1	66	0	0
7/08	45	0	9	2	11	1	0	39	0	2	41	0	0	62	0	0	62	0	0
7/09	46	5	12	2	19	0	0	51	3	1	55	0	0	78	2	4	84	0	0
7/10	47	2	15	1	18	0	0	66	0	0	66	0	0	72	11	0	83	0	0
7/11	48	16	16	0	32	0	0	58	3	0	62	0	0	61	3	0	64	0	0
7/12	49	17	7	0	24	0	0	74	0	0	74	0	0	88	0	0	88	0	1
7/13	50	26	8	0	34	0	0	66	0	0	66	0	0	46	10	0	56	0	0
			Remaining flies:			5	8		Remaining flies:			9	8		Remaining flies:			9	9

		5	***Candida sp.***					5	***Hanseniaspora uvarum***				
		Eggs						**Eggs**					
Date	Day	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/25	1	0	0	0	0	0	0	0	0	0	0	0	0
5/26	2	0	0	0	0	0	0	0	0	0	0	0	0
5/27	3	0	0	0	0	0	0	0	1	0	1	0	0
5/28	4	0	0	0	0	0	0	0	1	0	1	0	0
5/29	5	0	0	0	0	0	0	0	1	1	2	0	0
5/30	6	0	0	0	0	0	0	1	0	0	1	0	0
5/31	7	0	0	1	1	0	0	0	1	2	3	0	0
6/01	8	0	0	0	0	0	0	1	0	5	6	0	0
6/02	9	0	0	4	4	0	0	0	0	7	7	0	0
6/03	10	1	1	5	7	0	0	1	7	6	14	0	0
6/04	11	0	0	5	5	0	0	0	6	3	9	0	0
6/05	12	0	1	2	3	0	0	0	7	11	18	0	0
6/06	13	1	1	1	3	0	0	15	16	12	43	1	0
6/07	14	2	0	5	7	0	0	0	12	11	23	0	0
6/08	15	1	0	4	5	0	1	0	4	10	14	0	0
6/09	16	0	8	3	11	0	0	0	4	4	8	0	0
6/10	17	0	0	1	1	0	0	0	7	12	19	0	0
6/11	18	1	0	3	4	0	0	17	2	5	24	0	0
6/12	19	0	1	5	6	0	0	13	1	5	19	0	0
6/13	20	0	4	8	12	0	0	25	6	3	34	0	0
6/14	21	0	2	3	5	0	0	27	0	11	38	0	0
6/15	22	0	1	4	5	0	0	5	0	7	12	0	0
6/16	23	0	0	2	2	0	0	19	2	21	42	0	0
6/17	24	0	4	4	8	0	0	19	6	19	44	0	0
6/18	25	0	0	3	3	0	0	13	1	16	30	0	0
6/19	26	0	0	3	3	0	0	84	0	2	86	0	0
6/20	27	1	7	4	12	0	0	58	1	3	62	0	0
6/21	28	9	5	1	15	0	0	85	0	1	86	0	0
6/22	29	1	0	1	2	0	0	91	0	2	93	0	0
6/23	30	17	1	1	19	0	1	93	0	13	106	0	0
6/24	31	0	2	1	3	0	0	127	0	1	128	0	0
6/25	32	27	1	1	29	0	0	146	0	0	146	0	0
6/26	33	8	0	2	10	0	0	118	1	1	120	0	0
6/27	34	13	1	4	18	0	0	129	4	0	133	0	0
6/28	35	12	2	1	15	0	0	80	0	2	82	0	0
6/29	36	6	0	3	9	0	0	169	0	2	171	0	0
6/30	37	22	0	2	24	0	0	108	0	0	108	0	0
7/01	38	3	0	3	6	0	0	137	0	2	139	0	0
7/02	39	9	1	0	10	1	1	108	2	0	110	0	0
7/03	40	4	0	1	5	0	0	135	0	1	136	0	0
7/04	41	5	1	3	9	0	0	114	1	0	115	0	0
7/05	42	6	2	2	10	0	0	129	0	0	129	0	1
7/06	43	7	0	1	8	0	0	113	0	0	113	0	0
7/07	44	10	1	0	11	0	0	128	0	3	131	0	0
7/08	45	2	1	2	5	0	0	119	0	1	120	0	0
7/09	46	27	0	0	27	0	0	136	0	0	136	0	0
7/10	47	14	0	1	15	0	0	144	0	0	144	0	0
7/11	48	23	1	0	24	0	0	89	0	0	89	0	1
7/12	49	29	0	0	29	0	0	133	5	0	138	0	0
7/13	50	16	0	1	17	0	0	98	1	1	100	0	1
			Remaining flies:			9	7		Remaining flies:			9	7

8.6. Replication 6

		6	Control					6	*Saccharomyces cerevisiae*					6	*Metschnikowia pulcherrima*				
		Eggs						Eggs						Eggs					
Date	Day	WASS	MEA-C	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/29	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/30	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/31	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6/01	4	0	0	0	0	0	0	10	0	0	10	0	0	0	0	1	1	0	0
6/02	5	0	1	0	1	0	0	4	1	13	18	0	0	0	0	0	0	0	0
6/03	6	0	5	0	5	0	0	1	0	6	7	0	0	1	0	1	2	0	0
6/04	7	0	2	2	4	0	0	0	0	14	14	0	0	0	0	2	2	0	0
6/05	8	0	4	1	5	0	0	6	6	46	58	0	0	0	0	1	1	0	0
6/06	9	0	2	1	3	0	0	0	0	14	14	0	0	0	2	0	2	0	0
6/07	10	0	4	1	5	1	0	0	0	25	25	0	0	0	0	10	10	0	0
6/08	11	0	14	1	15	0	0	1	2	26	29	0	0	9	0	3	12	0	0
6/09	12	0	1	1	2	0	0	17	1	19	37	0	0	4	0	8	12	0	0
6/10	13	0	1	6	7	0	0	0	0	28	28	0	0	0	0	6	6	0	0
6/11	14	0	5	2	7	0	0	47	6	17	70	0	0	0	0	9	9	0	0
6/12	15	0	2	9	11	0	0	16	0	30	46	0	0	0	0	9	9	0	0
6/13	16	0	5	3	8	0	0	21	3	33	57	0	0	1	3	8	12	0	0
6/14	17	0	2	12	14	0	0	26	0	32	58	0	0	0	0	16	16	0	0
6/15	18	0	1	9	10	0	0	45	2	2	49	0	0	0	1	14	15	0	0
6/16	19	0	0	10	10	0	0	42	0	11	53	0	0	0	2	17	19	1	0
6/17	20	0	2	13	15	0	0	100	2	27	129	0	0	0	3	12	15	0	0
6/18	21	0	0	13	13	0	0	72	0	9	81	0	0	1	1	14	16	0	0
6/19	22	0	1	10	11	0	0	103	4	9	116	0	2	1	0	9	10	0	0
6/20	23	0	0	6	6	0	0	57	0	1	58	0	1	1	0	12	13	0	1
6/21	24	1	0	2	3	0	0	102	0	1	103	0	2	10	6	13	29	0	1
6/22	25	0	1	17	18	0	0	44	0	2	46	0	0	0	10	6	16	0	0
6/23	26	0	3	2	5	0	0	36	0	1	37	0	0	0	13	9	22	1	0
6/24	27	0	2	1	3	0	0	63	0	0	63	0	0	1	16	4	21	0	0
6/25	28	2	4	3	9	0	0	54	0	0	54	0	0	0	16	5	21	0	0
6/26	29	9	1	0	10	0	0	42	0	1	43	0	0	0	28	4	32	0	0
6/27	30	7	1	21	29	0	0	69	0	4	73	0	1	0	48	7	55	0	0
6/28	31	0	1	6	7	0	0	9	0	1	10	1	0	0	20	6	26	0	0
6/29	32	0	1	8	9	0	0	46	5	8	59	0	0	0	57	2	59	0	0
6/30	33	0	1	6	7	0	0	33	0	2	35	0	0	0	39	1	40	0	0
7/01	34	0	3	10	13	0	0	45	1	3	49	0	0	0	42	1	43	0	0
7/02	35	1	2	2	5	0	0	46	0	3	49	0	0	11	60	0	71	0	0
7/03	36	6	2	2	10	0	0	44	1	1	46	0	0	0	28	0	28	0	0
7/04	37	1	10	6	17	0	0	37	0	0	37	0	1	0	51	1	52	0	0
7/05	38	0	3	15	18	0	0	35	0	0	35	0	0	2	79	3	84	0	0
7/06	39	6	1	7	14	0	0	14	2	0	16	0	0	0	64	3	67	0	0
7/07	40	4	0	9	13	0	0	5	0	2	7	0	0	0	56	4	60	0	1
7/08	41	2	1	7	10	0	0	0	0	0	0	0	0	0	27	4	31	0	0
7/09	42	21	0	0	21	0	0	38	4	4	46	0	0	2	52	1	55	0	0
7/10	43	8	0	9	17	0	0	16	1	1	18	0	0	0	29	2	31	0	0
7/11	44	27	0	1	28	0	0	18	0	0	18	0	0	27	29	3	59	0	0
7/12	45	29	0	1	30	0	0	31	0	0	31	0	0	33	19	4	56	0	0
7/13	46	22	0	0	22	0	0	8	2	1	11	0	0	1	28	2	31	0	0
7/14	47	14	1	5	20	0	0	0	0	3	3	0	1	0	10	1	11	0	0
7/15	48	18	0	2	20	0	0	1	1	3	5	0	0	0	34	0	34	0	0
7/16	49	24	0	1	25	0	1	0	0	3	3	0	0	0	17	1	18	0	0
7/17	50	20	0	1	21	0	0	26	0	0	26	0	0	1	36	1	38	0	0
			Remaining flies:			9	9		Remaining flies:			9	2		Remaining flies:			8	7

		6	*Candida sp.*					6	*Hanseniaspora uvarum*				
		Eggs						Eggs					
Date	Day	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/29	1	0	0	0	0	0	0	0	0	0	0	0	0
5/30	2	0	0	0	0	0	0	0	0	0	0	0	0
5/31	3	0	0	0	0	0	0	0	0	0	0	0	0
6/01	4	0	1	0	1	0	0	0	1	2	3	0	0
6/02	5	1	1	0	2	0	0	0	10	0	10	0	0
6/03	6	1	0	1	2	0	0	0	2	7	9	0	0
6/04	7	0	0	1	1	0	0	6	4	1	11	0	0
6/05	8	0	0	1	1	0	0	0	2	10	12	0	0
6/06	9	0	0	1	1	0	0	2	4	9	15	0	0
6/07	10	18	0	3	21	0	0	0	3	22	25	0	0
6/08	11	10	0	3	13	0	0	0	1	10	11	0	0
6/09	12	12	0	9	21	0	0	38	0	13	51	0	0
6/10	13	0	0	8	8	0	0	17	1	8	26	0	1
6/11	14	13	0	8	21	0	0	11	1	23	35	0	0
6/12	15	12	0	8	20	0	0	39	1	9	49	0	0
6/13	16	14	2	5	21	0	0	24	1	15	40	0	0
6/14	17	7	0	11	18	0	0	39	2	10	51	0	0
6/15	18	4	0	8	12	0	0	63	1	30	94	0	0
6/16	19	12	0	22	34	0	0	65	0	14	79	0	0
6/17	20	0	0	8	8	0	0	75	3	18	96	0	0
6/18	21	2	1	7	10	0	0	93	2	10	105	0	0
6/19	22	7	0	19	26	0	0	87	0	0	87	0	0
6/20	23	12	0	9	21	0	0	89	1	10	100	0	0
6/21	24	4	1	10	15	0	0	124	1	2	127	0	0
6/22	25	11	0	15	26	0	0	101	0	1	102	0	0
6/23	26	8	2	7	17	0	0	161	1	0	162	0	0
6/24	27	41	0	5	46	0	0	133	0	5	138	0	0
6/25	28	17	0	7	24	0	0	134	0	5	139	0	0
6/26	29	42	0	11	53	0	0	132	0	0	132	0	0
6/27	30	42	0	10	52	0	0	133	1	2	136	0	0
6/28	31	20	0	0	20	0	0	101	0	10	111	0	0
6/29	32	44	0	7	51	0	0	168	1	6	175	0	0
6/30	33	36	0	0	36	0	0	109	1	0	110	0	0
7/01	34	41	0	1	42	0	0	120	1	1	122	0	0
7/02	35	36	1	0	37	0	0	114	0	0	114	0	0
7/03	36	44	0	1	45	0	0	139	0	1	140	0	0
7/04	37	26	0	1	27	0	0	130	2	1	133	0	0
7/05	38	45	0	0	45	0	1	136	1	9	146	1	0
7/06	39	34	0	0	34	0	0	126	0	1	127	0	0
7/07	40	32	0	0	32	0	0	155	0	2	157	0	1
7/08	41	30	0	0	30	0	0	147	1	1	149	0	0
7/09	42	41	0	1	42	0	0	128	1	1	130	0	0
7/10	43	25	0	2	27	0	0	124	0	1	125	0	0
7/11	44	54	0	2	56	0	0	142	0	0	142	0	0
7/12	45	56	0	1	57	0	0	107	1	1	109	0	0
7/13	46	30	1	1	32	0	0	145	0	1	146	1	0
7/14	47	38	0	1	39	0	0	130	3	0	133	0	0
7/15	48	29	0	1	30	0	1	157	1	5	163	0	1
7/16	49	29	0	1	30	0	0	124	1	1	126	0	0
7/17	50	11	0	0	11	0	0	142	1	2	145	0	0
			Remaining flies:			10	8		Remaining flies:			8	7

8.7. Replication 7

		7	Control					7	*Saccharomyces cerevisiae*					7	*Metschnikowia pulcherrima*				
		Eggs						Eggs						Eggs					
Date	Day	WASS	MEA-C	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/31	2	0	4	1	5	0	0	0	0	0	0	0	0	0	0	2	2	0	0
6/01	3	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
6/02	4	0	1	0	1	0	0	1	7	6	14	0	0	0	0	6	6	0	0
6/03	5	0	3	3	6	1	0	0	0	3	3	0	0	0	1	1	2	0	0
6/04	6	0	8	5	13	0	0	1	1	1	3	0	0	0	0	17	17	0	0
6/05	7	0	5	1	6	0	0	0	5	3	8	0	0	0	0	10	10	0	0
6/06	8	1	3	0	4	0	0	0	8	13	21	0	0	0	0	8	8	0	0
6/07	9	0	9	0	9	0	0	0	2	2	4	0	0	1	5	3	9	0	0
6/08	10	0	9	4	13	0	0	0	17	2	19	0	0	9	23	4	36	0	0
6/09	11	0	6	1	7	0	0	1	31	3	35	0	0	0	32	14	46	0	0
6/10	12	1	10	5	16	0	0	0	1	10	11	0	0	0	0	19	19	0	0
6/11	13	0	4	4	8	0	0	3	7	9	19	0	0	42	25	5	72	0	0
6/12	14	1	6	4	11	0	0	0	0	8	8	0	0	12	0	2	14	0	0
6/13	15	0	1	1	2	0	0	0	37	12	49	0	0	27	22	5	54	0	0
6/14	16	9	7	2	18	0	0	0	2	6	8	0	0	21	0	9	30	0	0
6/15	17	0	6	6	12	0	0	0	26	8	34	0	0	20	9	10	39	0	0
6/16	18	11	0	1	12	0	0	1	5	8	14	0	0	42	8	16	66	0	0
6/17	19	9	18	1	28	0	0	0	16	8	24	0	0	29	0	13	42	0	0
6/18	20	0	16	3	19	0	0	0	1	13	14	0	1	15	0	12	27	0	0
6/19	21	0	0	4	4	0	0	7	10	9	26	0	0	16	3	10	29	0	0
6/20	22	15	2	1	18	0	0	0	2	5	7	0	0	47	1	7	55	0	0
6/21	23	7	1	7	15	0	0	0	42	7	49	0	0	12	19	9	40	0	0
6/22	24	0	2	0	2	0	0	13	15	1	29	0	0	15	6	3	24	0	0
6/23	25	10	1	2	13	0	0	0	50	8	58	0	0	26	20	12	58	0	0
6/24	26	6	0	4	10	0	0	0	13	5	18	0	0	9	21	7	37	0	0
6/25	27	0	0	2	2	0	0	0	79	7	86	0	0	17	43	11	71	0	0
6/26	28	5	4	2	11	0	0	1	2	2	5	0	0	6	0	6	12	0	0
6/27	29	0	2	4	6	0	0	11	67	3	81	0	0	24	45	7	76	0	0
6/28	30	0	8	2	10	0	0	0	1	3	4	0	0	11	17	3	31	0	0
6/29	31	8	0	1	9	0	0	3	80	2	85	0	0	34	8	7	49	0	0
6/30	32	0	0	6	6	0	0	0	7	3	10	0	0	11	37	6	54	0	0
7/01	33	0	2	2	4	0	0	36	56	4	96	0	1	12	56	9	77	0	0
7/02	34	0	7	1	8	0	0	20	0	3	23	0	0	37	54	1	92	0	0
7/03	35	0	0	1	1	0	0	22	43	2	67	0	0	13	44	8	65	0	0
7/04	36	0	0	0	0	0	0	65	1	3	69	0	0	42	44	1	87	0	0
7/05	37	0	1	1	2	0	0	52	5	2	59	0	0	31	77	1	109	0	0
7/06	38	0	3	1	4	0	0	57	1	1	59	0	0	40	43	0	83	0	0
7/07	39	0	1	1	2	0	1	64	13	9	86	0	0	21	62	0	83	0	0
7/08	40	0	1	1	2	0	0	69	4	1	74	0	0	31	30	14	75	0	0
7/09	41	1	5	3	9	0	0	65	0	1	66	0	0	42	58	3	103	0	0
7/10	42	1	2	1	4	0	0	76	0	1	77	0	0	33	47	1	81	0	0
7/11	43	7	2	3	12	0	0	76	0	3	79	0	0	35	39	7	81	1	0
7/12	44	2	2	1	5	0	0	87	2	2	91	0	0	67	47	0	114	0	0
7/13	45	1	2	2	5	0	0	70	0	1	71	0	1	51	29	0	80	0	1
7/14	46	7	2	1	10	0	0	71	0	0	71	0	1	25	12	0	37	0	0
7/15	47	0	3	2	5	2	0	46	0	2	48	0	0	1	59	8	68	0	0
7/16	48	0	1	0	1	0	0	68	0	0	68	0	0	31	49	1	81	0	0
7/17	49	0	1	6	7	0	0	41	0	4	45	0	0	14	48	1	63	0	0
7/18	50	0	1	1	2	0	0	39	0	1	40	0	0	4	50	1	55	0	1
			Remaining flies:			7	9		Remaining flies:			10	6		Remaining flies:			9	8

		7	*Candida sp.*					7	*Hanseniaspora uvarum*				
		Eggs						**Eggs**					
Date	Day	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.	WASS	MEA-Y	PTSS	Sum	Dead M.	Dead F.
5/30	1	0	0	0	0	0	0	0	0	0	0	0	0
5/31	2	0	0	0	0	0	0	0	0	0	0	0	0
6/01	3	0	0	0	0	0	0	0	0	0	0	0	0
6/02	4	4	0	1	5	0	0	1	1	0	2	0	0
6/03	5	0	0	1	1	0	0	0	0	2	2	0	0
6/04	6	0	0	2	2	0	0	0	0	3	3	0	0
6/05	7	0	0	0	0	0	0	0	2	2	4	0	0
6/06	8	0	0	1	1	0	0	0	0	2	2	0	0
6/07	9	0	0	0	0	0	0	0	7	4	11	0	0
6/08	10	0	0	4	4	0	0	0	9	8	17	0	0
6/09	11	0	0	1	1	0	0	0	13	10	23	0	0
6/10	12	0	0	1	1	0	0	1	1	10	12	0	0
6/11	13	0	0	5	5	0	0	1	3	4	7	0	0
6/12	14	1	0	3	4	0	1	2	0	5	7	0	0
6/13	15	0	0	7	7	0	0	5	4	7	16	0	0
6/14	16	0	0	9	9	0	1	0	16	16	32	0	0
6/15	17	0	1	3	4	0	0	0	1	17	18	0	0
6/16	18	0	1	2	3	0	0	7	1	21	29	0	0
6/17	19	2	0	4	6	0	0	12	1	12	25	0	0
6/18	20	0	0	2	2	0	0	0	2	10	12	0	0
6/19	21	0	1	3	4	0	0	7	2	7	16	0	0
6/20	22	0	1	2	3	0	0	1	15	25	41	0	0
6/21	23	0	1	1	2	0	0	0	3	15	18	0	0
6/22	24	0	1	3	4	0	0	0	3	18	21	0	0
6/23	25	0	4	1	5	0	0	60	12	6	78	0	0
6/24	26	0	0	4	4	0	0	20	0	12	32	0	0
6/25	27	0	0	2	2	0	0	20	2	7	29	0	0
6/26	28	0	1	3	4	0	0	49	4	8	61	0	0
6/27	29	1	5	1	7	0	0	29	22	5	56	0	0
6/28	30	13	0	1	14	0	0	66	0	8	74	0	0
6/29	31	9	2	7	18	0	0	92	1	9	102	0	0
6/30	32	10	1	1	12	0	0	52	0	4	56	0	0
7/01	33	7	0	4	11	0	0	60	2	16	78	0	0
7/02	34	16	2	1	19	0	0	91	2	6	99	0	0
7/03	35	8	0	3	11	0	0	90	1	7	98	0	0
7/04	36	6	1	2	9	0	0	87	1	3	91	0	0
7/05	37	10	0	1	11	0	0	93	1	3	97	0	0
7/06	38	0	0	3	3	0	0	91	6	1	98	0	1
7/07	39	12	1	2	15	0	0	100	9	3	112	0	0
7/08	40	17	0	2	19	0	0	117	1	5	123	0	0
7/09	41	29	0	1	30	0	0	88	1	4	93	0	0
7/10	42	36	2	1	39	0	0	82	1	1	84	0	0
7/11	43	49	0	0	49	0	0	94	3	2	99	0	0
7/12	44	80	0	0	80	0	0	131	0	1	132	0	0
7/13	45	38	0	1	39	0	0	129	0	0	129	0	0
7/14	46	15	0	0	15	0	0	128	1	4	133	0	0
7/15	47	35	0	1	36	0	0	97	0	4	101	0	0
7/16	48	10	0	0	10	0	0	129	2	1	132	0	0
7/17	49	35	0	0	35	0	0	123	1	3	127	0	0
7/18	50	29	0	1	30	0	0	75	2	3	80	0	0
			Remaining flies:			10	8		Remaining flies:			9	9