

(Figure 4 and Table 1). A high protein requirement when producing eggs might reflect that synthesis of the egg-yolk protein vitalize in females is dependent on the incorporation of amino acids (Adams and Gerst, 1991; Markow *et al.*, 1999). This confirms the work of Sisodia and Singh (2012) in *D. ananassae*. They also found that flies reared in protein rich media had greater fecundity.

In the present study, altered diet effect on ovariole number was also studied in *P. straiata*. This is because both ovarioles number and fecundity are positively related. It was noticed that flies grown on protein rich diet had a significantly greater number of ovarioles than on the other two diets (Figure 5 and Table 1). This suggests that diet has significant effect on fecundity and ovariole numbers. Thus these studies in *P. straiata* suggest that altered diet had significant influence on reproductive performance.

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***Drosophila suzukii* larvae suppress *Aspergillus nidulans* growth particularly at high densities of larvae.**

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Introduction

Drosophila suzukii Matsumura is an Asian species distributed from northern Japan southwards to the semi tropics and westwards at least as far as India. It has recently been introduced by human agency to the Pacific, North and South America, and Europe (Asquith and Messing, 2012; Calabria *et al.*, 2012; Cini *et al.*, 2012; Hauser, 2011) and is now quite widely spread in these areas. Its arrival has caused great concern in the soft fruit industry, because it lays eggs in fruit that are still on the tree or bush and are not decaying.

Several moulds kill insects including *Drosophila* larvae (Courtney *et al.*, 1990; Hodge *et al.*, 1999; Rohlf *et al.*, 2005). But, because it lays in fresh fruit, the larvae of *D. suzukii* might not be able to defend themselves against the moulds that occur in fruit decaying on the ground. We, therefore, tested this ability by challenging *D. suzukii* larvae with the mould *Aspergillus nidulans*. Wild type mould of this species produces a number of compounds toxic or fatal to insects including *Drosophila* larvae. But, in addition to the wild type mould, we also challenged larvae with a transgenic strain deficient in toxins. The transgenic strain (Δ laeA) cannot express the gene LaeA that regulates secondary metabolism. Blocking LaeA expression suppresses

several secondary metabolites (Kale *et al.*, 2008; Perrin *et al.*, 2007). We expected that *D. suzukii* larvae might be able to overcome the deficient mould but succumb to the toxin producing wild type.

Methods

The *D. suzukii* larvae came from a strain derived from numerous females collected in July 2004 in suburban Tokyo. They were reared on a malt medium until 2010 and thereafter on a standard medium (Shorrock, 1971) supplemented with domestic mushrooms (*Agaricus bisporus*). Mushroom provides nutrients that are not otherwise present in standard *Drosophila* media. The mould strains were provided by Nancy P. Keller (University of Wisconsin). They were cultured on malt extract agar at 25°C, L:D 14:10, for 4-5 days. Mature conidia (asexually produced spores) were washed off with 0.9% NaCl in distilled water containing the surfactant Tween 80 (0.1%). The conidia collected were then stored at 4°C for less than a week. Before inoculating the experimental Units, the suspensions were adjusted to a titre of 1000 conidia μ^{-1} .

I put 0, 1, 2, 3, or 4 female *D. suzukii* onto 1000 mm³ *Drosophila* medium in small beakers of 2000 mm³. There were 20 replicates of each number of females. After the females had been in the beakers for 24 h, I removed the females and counted the eggs laid. After counting the eggs, I added spores of WT *A. nidulans* at the rate of 3000 per beaker to 10 replicates in each combination. I added spores of LaeA deficient *A. nidulans* at the same concentration to the other 10 replicates in each combination.

The larvae were then allowed to feed and the mould to develop until no further adult flies emerged. The beakers were monitored daily and the growth of the mould recorded. At the end of the experiment, I measured the percentage of the medium surface covered by mould. I also estimated the thickness of the mould on a scale of 1 to 5 where 1 was the highest and 5 the lowest thickness. My index of mould growth is then (percentage cover/thickness). A complete covering of thick mould thus has a growth index of 100, a slight covering of thin mould an index of $\ll 1$.

Some larvae might die during development. Therefore, the number of larvae feeding in each replicate is better represented as a combination of the number of eggs laid and the number completing development. The combination I used was (number of eggs + number of puparia/2).

I grouped the replicates for WT or deficient mould into larval number classes in order to estimate the variability of the mould growth index between tubes with similar numbers of larvae. The class "Control" contained those beakers that had not received female flies. Class 0 contained those beakers that received flies but in which no eggs were laid. Class 1 contained those with 1, 2 or 3 larvae, class 2 those in which there were 4-6 larvae, and so on up to class 6. Class 7 contained those beakers with >21 larvae. Most of these contained 22-30 larvae but there was one replicate with 39 and one with 47.

Results

The growth of mould, whether of the toxic or non-toxic genotype, was negatively related to the number of feeding larvae. The correlations were strong and nonlinear (toxic mould; Kendall T = -0.627, 2-tailed $p < 0.001$, $n = 45$; non-toxic mould; Kendall T = -0.745, 2-tailed $p < 0.001$, $n = 48$). Strong negative correlations remained even if control beakers were omitted (toxic mould; Kendall T = -0.460, 2-tailed $p < 0.001$, $n = 35$; non-toxic mould; Kendall T = 0.718, 2-tailed $p < 0.001$, $n = 38$). The number of eggs per beaker was correlated with the original number of females put into the beaker but the variability around this relationship was very large.

The mould growth index was thus also negatively related to larval number class (Figure 1). Ranked growth index differed very significantly between larval number classes ($F_{6,581}^8 = 13.896$, $p = 0.002$). There was also an apparent difference between the index for WT and deficient mould ($F_{7,182}^1 = 5.540$, $p = 0.05$), but this cannot be considered significant with these ranked data. The complete model was also significant ($F_{8,199}^1 = 25.109$, $p = 0.001$), but the interaction of mould type and larval number class was not. However, the mould growth index was higher for non-toxic moulds at low larval numbers than for toxic moulds.

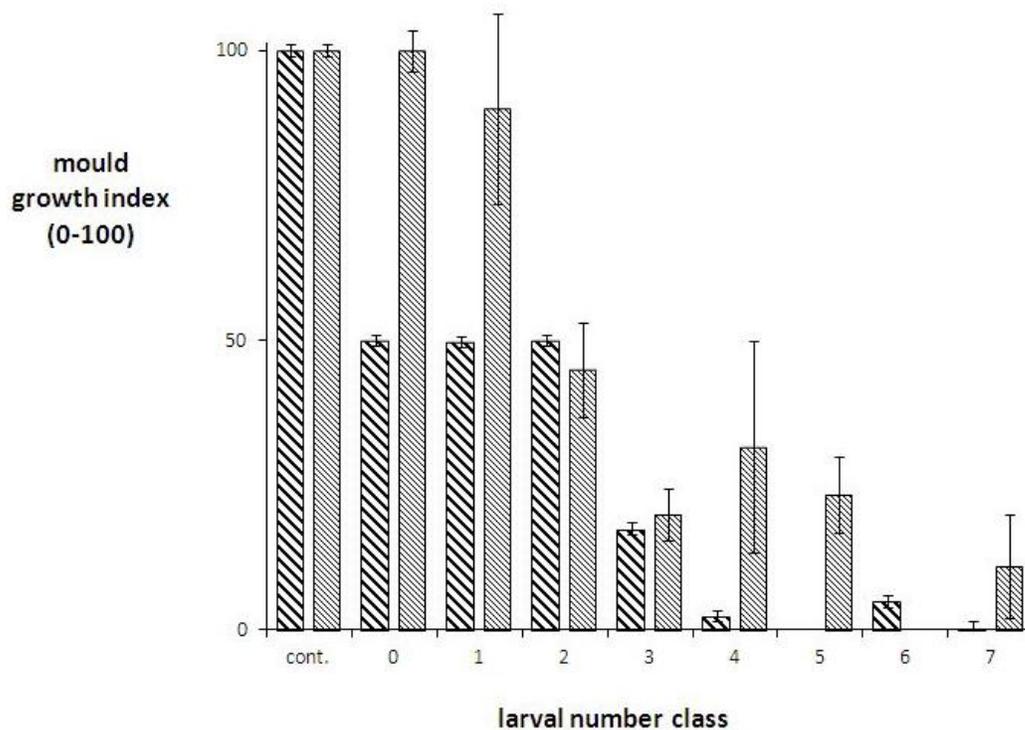


Figure 1. Relationship of median mould growth index to larval number class for toxic (heavy shading) and non-toxic (light shading) mould. Mould growth index is percentage cover divided by thickness. Thickness is a scale of 1-5 where 5 is low. Larval number class C is control (without any flies), class 0 is with flies but where no eggs were laid, class 1 contains larval numbers 1-3, class 2 4-6, and similarly up to class to 6 19-21. Class 7 contains larval numbers >21. Error bars are the standard range (*i.e.*, range/N where N is the number of replicates in that larval number class).

Discussion

Drosophila suzukii larvae are thus able to inhibit the growth of *A. nidulans*, as is *D. melanogaster* (Trieniens *et al.*, 2010). This ability of *D. suzukii* is true whether or not the mould is capable of producing toxins. The greater the larval density, the greater the inhibition (Figure 1). However, there is a particularly steep decline in mould growth between larval number class 2 and 3, *i.e.* at about 7 larvae per 1000 cubic millimetres. Larval densities of >15 largely prevent mould growth. The high growth of the non-toxic mould at low larval densities may arise from compensatory growth by this genotype which cannot increase toxin production. Any compensatory growth is overwhelmed, however, at high larval densities.

Drosophila suzukii does not, therefore, suffer great mortality from *A. nidulans* mould as long as larval densities are not very low. It is able to counteract not only toxin deficient *A. nidulans* but also the wild type that contains particularly potent mortality agents. Because *D. suzukii* can deal with these agents, it is also likely to resist other moulds, such as *Penicillium* known to be toxic against insects. The larvae of *D. suzukii* are, therefore, not prevented by moulds from developing in decaying fruit were females to lay in such substrates. Thus, decaying fruit may well be a resource that *D. suzukii* can successfully exploit for breeding. Indeed, *D. suzukii* breeds in decaying flowers and in sap streams (summarized in Wilson *et al.*, 2013) and I have reared it from decaying fruit baits laid out on the ground in both China and Japan. If this is generally so, *D. suzukii* will be even more difficult to control than is currently envisaged and decaying fruit must also be targeted in any control scheme.

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Retraction

Gürbüz, Mehmet, 18 July 2014

Mehmet Gürbüz contacted the Editors to explain that “In my earlier studies, I could not write in a good way, due to I did not know the rules of writing. Whereas, ‘... appropriating another person’s ideas or words (spoken or written) without attributing those word or ideas to their true source” according to Brown “plagiarism”. When transferring someone else’s ideas, I did not change sentences. I used sentences belonging to others, in my own articles. I have cited, but I know now that it is also plagiarism. I very much regret. For that reason, please remove my article in electronic systems.”

Gürbüz, M., 2009, The effects of exogenous estrogen and progesterone on developmental stages of *Drosophila melanogaster*. *Dros. Inf. Serv.* 92: 60-63.

Gürbüz, M., and H. Uysal 2009, Toxic effects of Patulin to some developmental stages of *Drosophila melanogaster*. *Dros. Inf. Serv.* 92: 41-43.

Gürbüz, M., and H. Uysal 2009, Effects of fumonisin B1 to developmental stages of F2 offspring of *Drosophila melanogaster*. *Dros. Inf. Serv.* 92: 78-80.

At his request, the Editors are unlinking these three citations from the online index for 2009, volume 92. But, of necessity, the articles themselves must remain in the intact published issue for 2009 and the complete online issue.