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Fruit phosphorus concentration does not affect oviposition preference in the invasive pest *Drosophila suzukii*

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Abstract

1. The broad variation in host use among polyphagous insects is well documented but still poorly understood. In numerous pest insects, the proximate mechanisms responsible for variation in oviposition preference among host plants remain to be elucidated *Drosophila suzukii* is an invasive crop pest that attacks a wide range of host fruits. Females prefer ovipositing on particular fruit media (blackberry, cherry, blackcurrant) that are rich in phosphorus. As phosphorus is known to be involved in female reproduction in insects and Drosophila, phosphorus concentration could drive oviposition preference in *D. suzukii*.

2. To test the effect of phosphorus on oviposition, we measured the egg-laying preferences of *D. suzukii* in a choice environment containing 12 media with different concentrations of phosphoric acid (the main molecule that contains phosphorus within fruits).

3. In our assay, *D. suzukii* female did not prefer ovipositing in media with high phosphorus concentrations. As an experimental control, we verified the previous result of a higher female preference for media made of fruits rich in phosphorus.

4. Two alternative hypotheses could explain our results. A molecule containing phosphorus, but other than phosphoric acid, could drive the preference for phosphorus-rich fruits. Alternatively, one or more molecules that do not contain phosphorus could be responsible for the preference for these fruits. Studying the proximate mechanisms driving host use will ultimately help improve the management of any crop pest.

Keywords

spotted-wing drosophila; host choice; chemical ecology; insect behavior; oviposition site selection

Introduction

Elucidating the mechanisms that drive patterns of host use by polyphagous insect pests is an important goal in ecological entomology. Oviposition preference, one of the main traits that determines host use in polyphagous insects, varies widely among host fruits and among varieties within a given host fruit (Singer, 1986). Both the physical (skin thickness, fruit shape and color) and chemical (nutritional quality, presence of toxins) properties of fruits are thought to affect female oviposition preference (Renwick, 1989).

Drosophila suzukii is an insect pest, native to Asia, which invaded both Europe and North America in 2008 (Fraimout *et al.*, 2017). This extremely polyphagous pest can infest fruit in at least 19 different plant families (Kenis *et al.*, 2016). In particular, it infests soft fruits (strawberry, raspberry, blueberry, mulberry), stone fruits (cherry) and grape resulting in important economic losses for farmers (for example $US 36.1 million in revenue loss between 2009 and 2011 for Californian raspberry producers; Farnsworth *et al.*, 2017). Current control methods rely heavily on the use of broad-spectrum insecticides (Schetelig *et al.*, 2018). However, as the timing of infestation coincides with ripening, pesticide use is restricted to protect consumers from exposure to chemical residues (Haviland & Beers, 2012). Given the known negative impacts of insecticides on human health and on the environment (Bourguet & Guillemaud, 2016), alternative pest management strategies are deeply needed. Notably, the development of push-pull strategies (e.g., trap-plants) requires a good understanding of the factors driving oviposition preference (Alkema *et al.*, 2019).

The roles of certain physical (Lee *et al.*, 2011; Burrack *et al.*, 2013) and chemical characteristics (Olazcuaga *et al.*, 2019) in driving oviposition preference of *D. suzukii* are well known. For instance, oviposition preference for a given fruit increases as pH increases and as the force necessary to penetrate the skin decreases (Kinjo *et al.*, 2013; Lee *et al.*, 2015; Baena *et al.*, 2022). The ratio of protein to carbohydrates (P:C ratio) also affects female oviposition preference (Lee, 2015) with females preferring to lay eggs in media rich in carbohydrates relative to proteins (Silva-Soares *et al.*, 2017; Young *et al.*, 2018). The effects

of other chemical compounds on oviposition preference have been little investigated (Olazcuaga *et al.*, 2019). Olazcuaga *et al.*(2019) found that females preferred to lay eggs in media made from fruits rich in phosphorus (specifically, blackberry, cherry and blackcurrant) and furthermore that this preference was positively correlated with the concentration in phosphorus within those fruits in a choice environment. Given this correlation, and because phosphorus is important for female reproduction in insects (Markow *et al.*, 1999), including some Drosophila species (King & Wilson, 1955; Markow *et al.*, 1999), we hypothesize that females *D. suzukii* could seek phosphorus-rich fruits for feeding and potentially for oviposition (Markow *et al.*, 1999). The aim of this study is to test whether oviposition preference of *Drosophila suzukii* depends on the concentration of phosphorus.

Material and methods

Fly population and maintenance

To minimize phenotypic variation, we used an inbred line of *D. suzukii* (WT3 2.0; Paris *et al.*, 2020). Individuals were maintained in vials with standard laboratory fly food (GF, Backhaus *et al.*, 1984) for several discrete generations (15 days of development, 6 days of adult maturation and 24h to lay eggs; growth chamber at 21°C (+/- 2°C), 65% relative humidity and with a 16:8 (L:D) light cycle). We performed oviposition preference assays under the same environmental conditions.

Media with increased concentrations of phosphorus for the oviposition preference assay

To test whether the oviposition preference of *D. suzukii* depends on phosphorus concentration, we supplemented a minimum fly food medium with phosphorus. More precisely, we created the minimum medium by adding 1% (wt/vol) of agar, 6% of inactive malted brewer's yeast, 1.5% of yeast extract, and an antimicrobial solution (consisting of 6

ml of 99% propionic acid, 10 ml of 96% ethanol and 0.1% of methylparaben sodium salt) to 1000 ml of sterile deionized water. Then, to vary the concentration of phosphorus in our minimum medium, we used monopotassium phosphate (KH_2PO_4). Because KH_2PO_4 dissolves in water into potassium hydroxide (KOH) and phosphoric acid (the main source of phosphorus in the 12 fruits used in Olazcuaga *et al.*, 2019), it alters the pH. Thus we also adjusted the pH of each medium to 4 (i.e., the pH of fruit media used in Olazcuaga et al. 2019 and in the present assay) using hydrogen chloride (HCl).

To maximize the ability to detect an effect of phosphorus concentration on oviposition preference, we used a phosphorus concentration ranging from 0 to 1.65 g/L (corresponding to a concentration of KH_2PO_4 ranging from 0 to 7.25 g/L), several times larger than the range of phosphorus concentration of the 12 fruit media used in Olazcuaga *et al.*, 2019 (0 to 0.30 g/L).

Oviposition preference assay

Following Olazcuaga *et al.*, 2019, oviposition preference was measured in oviposition arenas (choice assay) with 12 compartments. We randomly distributed each of 12 different phosphorus concentrations across the 12 compartments of each oviposition arena. In each arena, we placed 20 flies aged 6 days (held 6 days after emergence as adults on standard medium). After 24h, we counted the number of eggs laid in each compartment. We replicated this assay 129 times. As a control, we performed the same protocol using 12 different fruit media in each arena to replicate the positive correlation between oviposition preference and the phosphorus concentration of fruit media found in Olazcuaga *et al.*, 2019. To estimate the number of replicate arenas for this control, we performed a power analysis with simulations based on the subsampling of the original data (see Sup. Mat.). We found that an assay with 6 control arenas provided enough power to detect a significant effect of phosphorus on oviposition preference in more than 99% of the simulations. Hence, we used

20 control arenas for the control assay. The position of 129 test arenas and 20 control arenas was randomized within the growth chamber.

Statistical analyses

To investigate whether oviposition preference depends on phosphorus concentration, we tested for the effect of phosphorus concentration on the number of eggs laid in each compartment using a generalized mixed linear model (Poisson distribution for count data). The model included phosphorus concentration as a fixed effect (continuous variable). To control for potential correlations in the number of eggs among compartments across and within arenas, we added arena identity and the position of the compartment within the arena as random effects (with 129 and 12 levels respectively). To account for overdispersion, we added an observation-level random effect (1548 levels). We tested for the effect of phosphorus concentration using a Likelihood Ratio Test (LRT; Bates *et al.*, 2015). To quantify the proportion of variance in oviposition preference explained by phosphorus concentration, we computed the likelihood-based coefficient of determination using the *rr2* package (Ives, 2019). As a control, we separately performed the same analysis on the data from the 20 control arenas using the phosphorus concentration of the 12 fruit media estimated in Olazcuaga *et al.*, 2019.

Results

Testing for a causal effect of phosphorus on oviposition preference

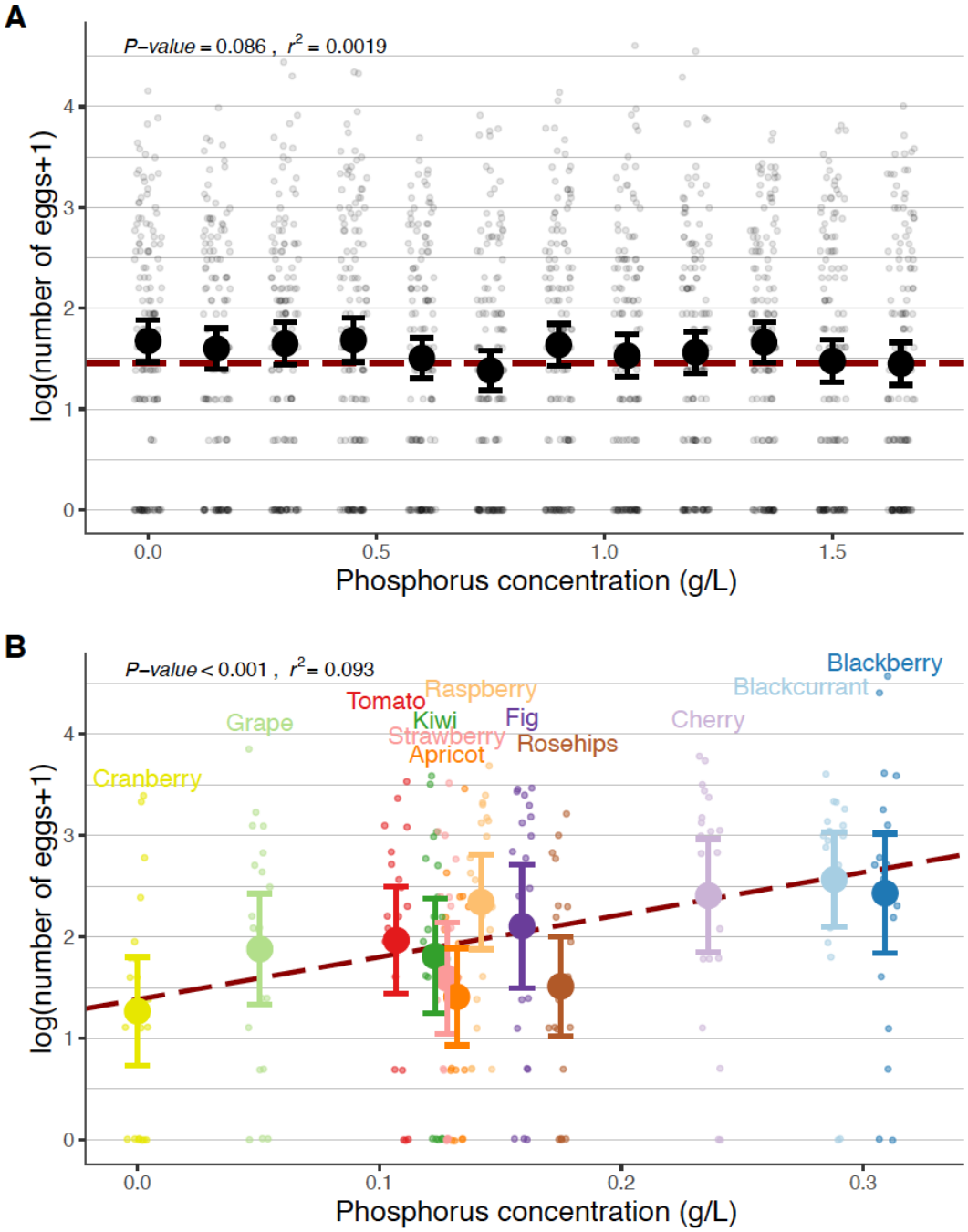

Fig. 1: Relationship between female oviposition preference and 12 different phosphorus concentrations (A) supplemented in standard medium or (B) in 12 different artificial fruit media. For each phosphorus concentration or fruit, large dark circles represent the mean with 95% CI error bars, small light circles represent the observed data. The dotted red line represents the number of eggs predicted by the best model. The *P*-value and coefficient of determination of the effect of phosphorus concentration on oviposition preference is given above each panel.

The number of eggs laid in each compartment did not increase with the phosphorus concentration (LRT $\chi2$ = 2.94, df = 1, *P*-value = 0.09; Fig. 1A). In contrast, the number of eggs laid in each compartment increased with the phosphorus concentration of the 12 fruit media in control arenas (LRT $\chi2$ = 23.53, df = 1, *P*-value < 0.001; Fig. 1B).

Discussion

We used an inbred line of *D. suzukii* to test whether phosphorus concentration affects oviposition preference in this invasive pest. Our results confirm previous findings of a greater oviposition preference for fruit media with the highest concentration in phosphorus. However, females did not prefer to lay eggs on minimum media with high phosphorus concentrations.

Identifying molecules potentially involved in oviposition preference

Three hypotheses may explain why we found a preference for fruit media with high phosphorus concentrations, but not for minimum media with high phosphorus concentrations. First, other molecules containing phosphorus than phosphoric acid might drive oviposition preference in *D. suzukii*. Even if phosphorus may be present in fruits within other molecules, this hypothesis seems unlikely because the concentration of these other molecules is likely much smaller than the concentration of phosphoric acid in fruits (50–80% of phosphorus stored in grains and seeds is in phosphoric acid; Lott *et al.*, 2009). Second, oviposition preference could be driven by one or more molecules that contain no phosphorus, but whose concentration may be correlated with the concentration of phosphorus across the 12 fruits studied. Some volatile compounds, such as linalool, are known to be involved in the oviposition choice of *D. suzukii* (Baena *et al.*, 2022). However, whether these compounds correlate with the concentration in phosphorus remain to be determined. Finally, this correlation may arise due to different individual compositions of the fruits that are by chance associated with phosphorus concentration.

Deciphering mechanisms that may influence oviposition preference

Two proximate mechanisms could explain the oviposition preference for specific fruits. First, females might be attracted by olfactory compounds which are honest cues of the quality of the fruit substrate (olfaction-driven preference). In this scenario, oviposition preference would be an active choice made by females for oviposition (i.e., associated with a non-random flight targeted towards the best fruit substrate for either feed or egg development). Second, females might not be attracted by olfactory compounds, but may spend more time on fruits that are more nutritious for them (gustation-driven preference). If females randomly lay eggs when they feed, they might eventually lay more eggs on fruits they feed on the most. An experiment comparing oviposition preferences when flies have access to such volatile compounds (e.g., choice environment with the odor of the fruit, as in this study) versus without (e.g., choice environment over-ventilated or where odors are blocked by a single compound) would allow us to dissociate these two mechanisms and thus help to better identify the factors driving host preference in *D. suzukii*. Finally, the detection of specific volatile compounds could help increase the trapping efficiency of this pest and improve the prediction of potential damages (Burack et al 2015).

Conclusion

By addressing an important and long-standing ecological question regarding the factors driving oviposition preference in a crop pest, our study proposes a straightforward methodology to identify chemical compounds that may determine oviposition preference in an insect pest. This methodology can be used to test for the effects of other chemical compounds on the oviposition of insect pests.

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Author contributions

Conceptualization: L.O., N.O.R., R.G.

Experimental design and data acquisition: L.O. and J-L.C with inputs of R.G. and N.O.R.

Statistical analyses and writing: L.O. and N.O.R with inputs of R.G. and J-L.C.

Data availability statement

The data and R scripts for our analyses are available at:

https://github.com/olazlaure/PhosphorusPreference2022

They will be archived on Figshare upon acceptance of the manuscript.

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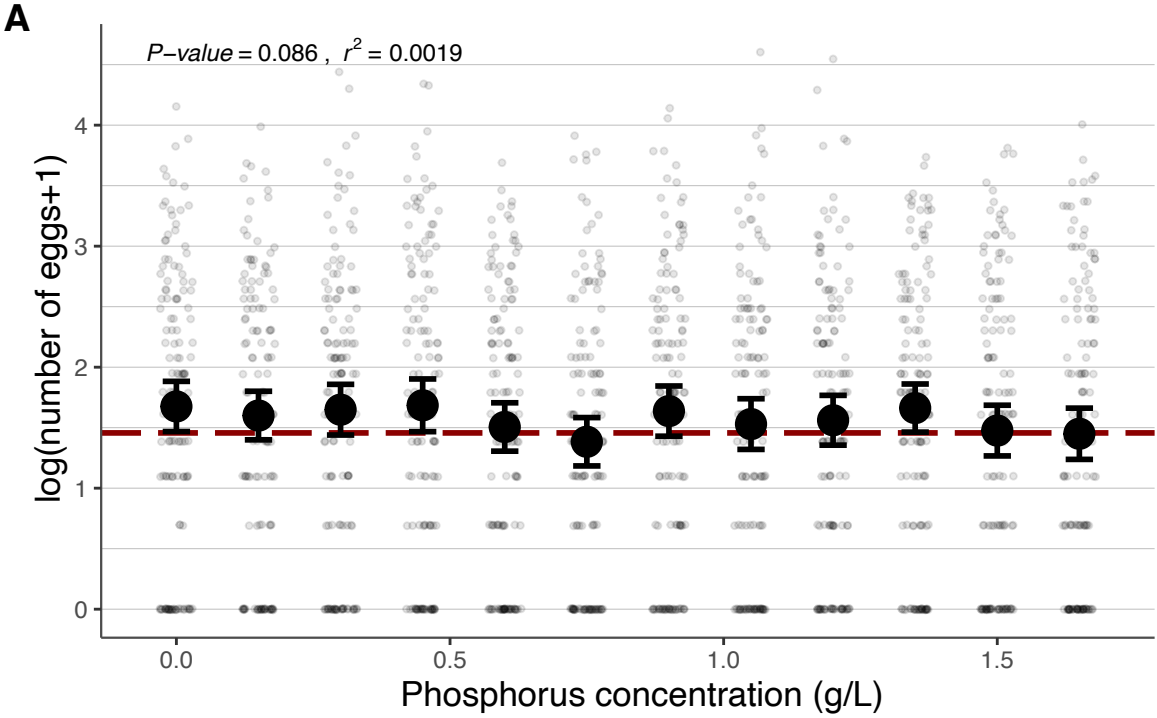
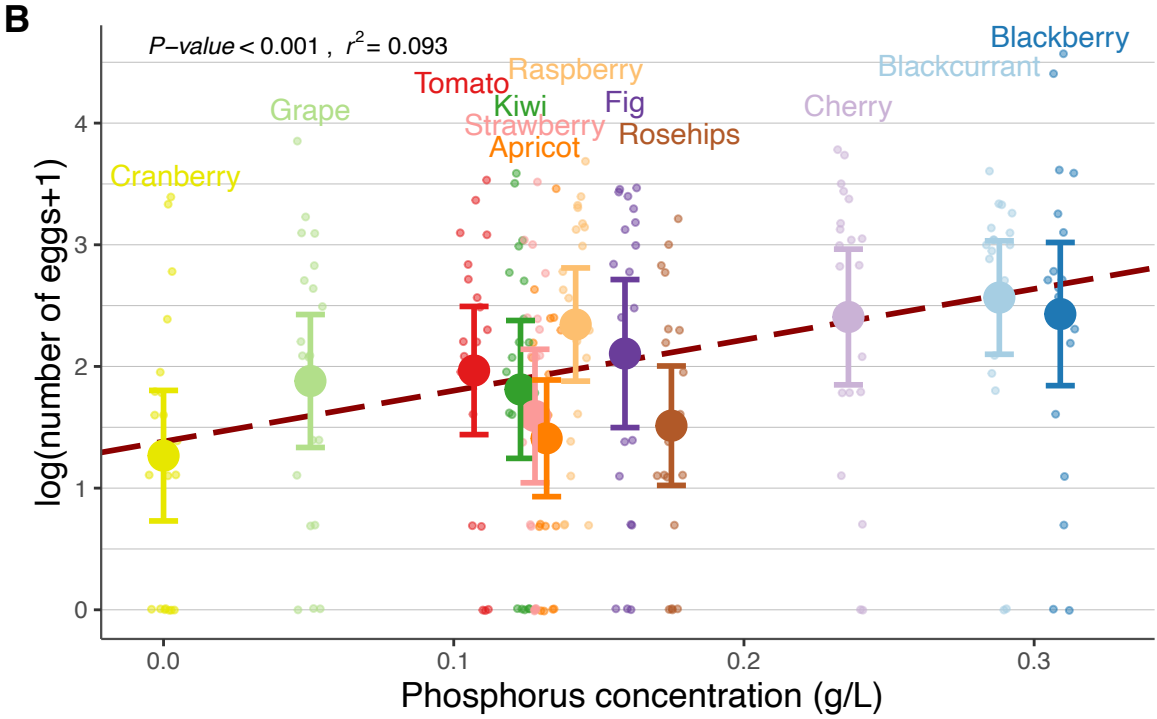

A
P–value = 0.086 , r2 = 0.0019
log(number of eggs+1)
0
1
2
3
4
0.0
0.5
1.0
1.5
Phosphorus concentration (g/L)


B
P–value < 0.001 , r2 = 0.093
Blackberry
Blackcurrant
Raspberry
Tomato
Grape
Kiwi
Fig
Cherry
Strawberry
Rosehips
Apricot
Cranberry
log(number of eggs+1)
0
1
2
3
4
0.0
0.1
0.2
0.3
Phosphorus concentration (g/L)