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Two Pests Overlap: *Drosophila suzukii* (Diptera: Drosophilidae) Use of Fruit Exposed to *Halyomorpha halys* (Hemiptera: Pentatomidae)

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Abstract

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) and brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), are global economic pests that may co-occur on small fruits. We investigated whether fruit recently exposed to *H. halys* affected subsequent host use by *D. suzukii*. Laboratory no-choice and choice tests presented *D. suzukii* with *H. halys*-fed and unfed raspberries and blueberries immediately or 3 d after *H. halys* feeding. Resulting *D. suzukii* eggs, or larvae and pupae, were counted. The number of *D. suzukii* immatures among fed and unfed fruit was not significantly different in lab studies. There was no relationship between the intensity of *H. halys* feeding, as estimated by the number of stylet sheaths, and *D. suzukii* oviposition on blueberry. Lastly, field studies compared *D. suzukii* infestation between *H. halys*-fed and unfed raspberries. Raspberries were previously exposed to *H. halys* for 3 d or simultaneously exposed to both pests for 7 d. Natural infestation by *D. suzukii* in the field was similar among raspberries previously or simultaneously exposed to *H. halys* compared to control fruit.

Key words: blueberry, competition, invasive, raspberry, resource overlap

The invasive *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) and brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), are economic pests of worldwide concern (Rice et al. 2014, Asplen et al. 2015). Raspberries are highly susceptible to *D. suzukii*, and blueberries are moderately susceptible (Lee et al. 2011, Burrack et al. 2013). If left unmanaged, the small fruit industry could lose an estimated US$247 million annually in the Pacific United States (Bolda et al. 2010) and 13% of revenue in northeastern Italy (De Ros et al. 2015) from *D. suzukii*. The impact of *H. halys* on small fruits is a growing concern. Nymphal and adult *H. halys* have been observed feeding on raspberries from mid-July to September (Basnet et al. 2014). Confinement of *H. halys* on blueberry clusters led to eventual necrosis, discoloration, reduced berry weight, and reduced °Brix levels on some cultivars (Wiman et al. 2015). Blueberries injured by *H. halys* had lower °Brix and higher phenolic levels, and conspecific nymphs fed more on uninjured than injured berries in laboratory choice-tests (Zhou et al. 2016). Also, *H. halys* produce odorous defensive compounds. When *H. halys* contaminate wine-grapes they release trans-2-decenal, which can taint wine (Mohekar et al. 2017).

Given that both *H. halys* and *D. suzukii* attack small fruits, the question arises whether prior feeding by *H. halys* could result in an increase, decrease, or no effect on *D. suzukii* infestation rates. Feeding by other hemipterans can affect plant quality, which in turn affects the performance of other herbivores (Mattson et al. 1989; Denno et al. 1995, 2000). Because *H. halys* can taint and reduce fruit quality, this could affect the ability of *D. suzukii* to locate the fruit or may deter *D. suzukii* from utilizing the fruit for oviposition. Under this scenario, co-occurrence of *D. suzukii* and *H. halys* may synergistically increase crop damage because *D. suzukii* avoids fruits fed on by *H. halys*. Thus, the total sum of damaged fruits is greater with selective avoidance than random feeding events. If *D. suzukii* randomly infests fruit regardless of prior *H. halys* exposure, then crop damage will be intermediate. If *D. suzukii* prefers *H. halys*-fed fruit, total damage will be the lowest of the three scenarios.

Our objectives were to: 1) determine whether *D. suzukii* oviposits in recently *H. halys*-fed raspberries or blueberries differently from unfed berries in laboratory and field trials; 2) determine if the intensity of *H. halys* feeding affects *D. suzukii* oviposition; and 3) determine *D. suzukii*'s response to berries 3 d after feeding by *H. halys*, in case feeding alters fruit quality over time.

Table 1. Experimental details, mean, and statistical outcomes comparing the number of *D. suzukii* in fruit exposed to *H. halys* or not

Assay	Fruit in arena	n^a	*D. suzukii* stage	Avg *D. s.* ± SE/berry		df	*t* or *F*	*P*	Trial dates
				Fed	Unfed				
Laboratory									
Raspberry No-choice	5 fed or unfed	14	Larva–pupa	17.9 ± 1.9	16.6 ± 2.0	1,24	0.33	0.57	21, 26 Aug., 17 Sept. 2014
Choice	5 fed + 5 unfed	14	Larva–pupa	20.4 ± 1.7	26.6 ± 2.5	1,24	4.0	0.056	27 Aug., 3, 9 Sept. 2014
Blueberry No-choice	10 fed or unfed	15	Egg	5.1 ± 0.36	4.8 ± 0.37	1,26	0.28	0.60	24, 25 Feb., 3 Mar. 2015
Choice	5 fed + 5 unfed	30	Egg	3.5 ± 0.29	3.3 ± 0.31	1,56	0.02	0.90	17, 18, 19 Feb. 2015
Choice delay	5 fed + 5 unfed	28	Egg	5.6 ± 0.41	5.0 ± 0.36	1,52	0.74	0.39	23, 26 Feb., 3 Mar. 2015
Field									
Raspberry Choice 1—prior exposure	5–12 fed +5–12 unfed	20	Larva	27.2 ± 2.6	30.3 ± 4.3	19	0.76	0.46	11–15 July 2016
Choice 2—simultaneous exposure	5–12 fed +5–12 unfed	16	Larva	3.1 ± 0.8	4.3 ± 1.2	15	1.15	0.27	11–18 July 2016

[a] Number of replicates per fed and unfed treatment.

Materials and Methods

Laboratory Study

Drosophila suzukii were from a colony described in Woltz et al. (2015). *Halyomorpha halys* adults were collected by beat sheet from ornamental plants in the Willamette Valley of Oregon. Raspberries were from an unsprayed mixed raspberry cultivar field at the USDA/Lewis Brown research farm in Corvallis, OR. Green raspberries were enclosed in mesh bags to prevent infestation from either pest in the field. Upon ripening, raspberries were randomly selected from different plants, and mixed prior to trials. Organic blueberries, likely imported from the southern hemisphere, were purchased from a store. To create treatments of *H. halys*-fed and unfed fruit, half of the fruit were placed inside 28- by 28- by 28-cm plastic mesh cages containing ~50 *H. halys* adults for 24 h. Adults were observed feeding on the fruit. For the unfed treatment, the fruit were placed in identical cages without *H. halys* for 24 h.

Raspberry—No-Choice and Choice Trials

Fruit were transferred to arenas with *D. suzukii*. Each arena contained four 14–17-d-old mated female *D. suzukii* in a 23- by 23- by 25-cm plastic cage with a water wick. The number of fruits exposed, replicates, and trial dates are in Table 1. After 24 h of exposure to *D. suzukii*, the raspberries were stored in 120-ml plastic cups covered with mesh netting for 7 d at 24 °C. Raspberries were dissected and the number of *D. suzukii* larvae and pupae were counted. Larval and pupal counts were used to estimate oviposition because of the difficulty of counting eggs on raspberries.

Blueberry—No-Choice and Choice Trials

Fed and unfed fruit were transferred to arenas with *D. suzukii* for 24 h as described earlier. In "delay" choice trials, *H. halys*-fed and unfed blueberries were held at 24 °C for 72 h before being exposed to *D. suzukii*. After exposure, blueberries were tracked individually for the number of eggs laid by *D. suzukii*, the number of *H. halys* stylet sheaths, and °Brix (sugar content). To count stylet sheaths, all berries including controls were soaked for 15 min in a mixture of 1 g acid fuchsin, 1 ml glacial acetic acid, and 100 ml dH_20 (Anonymous 2017) to stain stylet sheaths bright pink for viewing under magnification. Lastly, berries were rinsed and stored frozen until each berry was macerated to obtain °Brix readings (Hanna Instruments, Woonsocket, RI). Prior acid fuchsin soak was not expected to alter °Brix readings. In a protocol comparison, fed or punctured blueberries soaked in acid, intact berries soaked in acid, and intact nonsoaked berries had similar °Brix levels of 13.2 ± 0.25, 13.7 ± 0.22, and 13.6 ± 0.24, respectively ($F_{2,111} = 1.2$, $P = 0.31$, $n = 36–39$).

Field Study

For both trials, green raspberry fruit at the USDA research field were "prebagged" with mesh bags prior to experimentation to prevent infestation. Each raspberry plant had one to three bagged pairs of adjacent fruit clusters.

Trial 1

Prebagged clusters were re-opened; overly ripe berries and any excess of 12 berries per cluster were removed. One cluster from a pair received four *H. halys* adults and was re-bagged, and the other cluster was re-bagged without *H. halys*. During trials 1 and 2, three bags were also set up simultaneously as feeding controls with *H. halys* to ensure that *H. halys* were feeding. After 3 d, all bags were removed. Fruit from the feeding controls were collected to count stylet sheaths in the lab; this destructive sampling could not be done on fruit used in the experiment. Bag removal initiated the access period for *D. suzukii*, where naturally occurring flies could oviposit on fruit previously caged with *H. halys*. After 4 d of access by *D. suzukii*, all fruit clusters were taken back to the lab and stored at 24 °C for 3 d such that any eggs and early instar larvae could develop into second or third instars for easier identification. Fruit were crushed and live larvae floated out in a mixture of 3.9 liter of water and 237 ml salt.

Trial 2

Prebagged clusters were re-opened and prepared as in trial 1. Then *H. halys* were caged on fruit for 7 d using a 4-mm grid mesh bag that allowed entry of *D. suzukii*. Thus, there was simultaneous exposure to *H. halys* and access by *D. suzukii* for 7 d. Afterwards, fruit clusters and feeding controls were processed as in trial 1.

Statistical Analyses

Laboratory no-choice, choice, and choice delay trials of both fruits were analyzed separately. For raspberry trials, the total number of *D. suzukii* larvae and pupae developing from *H. halys*-fed and unfed fruit were compared with treatment as a fixed effect and trial date as a random effect. For blueberry trials, the number of *D. suzukii* eggs laid was compared with treatment as a fixed effect and trial date as a random effect. Among *H. halys*-fed fruit, separate linear regressions tested the effect of the number of *H. halys* stylet sheaths

Table 2. Regression analyses for blueberries fed upon by *H. halys* with the number of stylet sheaths as the independent variable

Assay	Dependent variable	df	*F*	*P*
No-choice	Eggs laid by *D. suzukii*	1,148	3.59	0.060
	°Brix of berry	1,148	0.045	0.832
Choice	Eggs	1,148	1.12	0.293
	°Brix	1,148	2.09	0.151
Choice delay	Eggs	1,137	0.61	0.437
	°Brix	1,137	0.34	0.559

on either eggs laid or °Brix. For the field studies, a paired *t*-test compared the abundance of *D. suzukii* larvae between the *H. halys* and control pairs. The number of *D. suzukii* larvae per cluster was divided by the ending number of fruit per cluster because this varied. After both field trials were found nonsignificant, retrospective power analyses examined whether having more samples would result in significance. Analyses were done in JMP 12.0 (SAS 2015).

Results and Discussion

Raspberry—Lab

In no-choice trials, there were no differences in the total number of *D. suzukii* larvae and pupae among *H. halys*-fed and unfed raspberries (Table 1). In choice trials, there were marginally fewer *D. suzukii* larvae and pupae developing in *H. halys*-fed compared to unfed raspberries. This could suggest that oviposition of *D. suzukii* was lowered on fed raspberries assuming that the number of *D. suzukii* larvae and pupae correlates with eggs laid. We did not examine whether prior *H. halys* feeding affects *D. suzukii* survival, and the result could reflect lowered survival of immature *D. suzukii* on fed raspberries.

Blueberry—Lab

Halyomorpha halys fed on blueberries within 24 h. An average of 5.92 ± 0.54, 5.79 ± 0.49, and 4.76 ± 0.35 stylet sheaths per berry were found on exposed blueberries from the no-choice, choice, and choice "delay" trials, respectively. Unexposed blueberries had no stylet sheaths. The number of *D. suzukii* eggs laid in *H. halys*-fed and unfed blueberries did not differ in the no-choice, choice, and choice "delay" trials (Table 1).

Regression analyses showed no significant relationships between eggs laid on a berry or °Brix level of berry with respect to the number of *H. halys* stylet sheaths on the same berry (Table 2). Trials had 140+ samples and a range of 0–29 sheaths per berry. Thus, the oviposition behavior of *D. suzukii* did not appear to be influenced by the intensity of *H. halys* feeding that occurred within 1 or 3 d. The °Brix of fruit did not vary with intensity of *H. halys* feeding, whereas Wiman et al. (2015) and Zhou et al. (2016) found a ∼12–39% reduction in °Brix among *H. halys*-fed blueberries compared to unfed controls. Our study exposed *H. halys* to harvested blueberries for 24 h, whereas Wiman et al. (2015) and Zhou et al. (2016) exposed *H. halys* to a blueberry cluster on the plant for 1 wk and 24–72 h, respectively.

Raspberry—Field

Fruit caged with *H. halys* in the feeding controls had an average of 0.85 ± 0.25 and 2.6 ± 0.38 stylet sheaths per berry after 3 or 7 d of exposure, respectively. Both raspberries caged with *H. halys* for 3 d prior or simultaneously exposed to *H. halys* for 7 d had similar infestation rates from *D. suzukii* as unfed raspberries in the field (Table 1). The number of *D. suzukii* larvae was 10% and 29% numerically lower in the *H. halys*-fed fruit in trials 1 and 2, respectively. Retrospective power analyses revealed that the probability that additional data may show that the treatments are different is 0.088 in trial 1 and 0.135 in trial 2. There were 25+ *D. suzukii* larvae per fruit in trial 1 compared to 3+ larvae per fruit in trial 2. The difference may have been due to fruit clusters being completely accessible to *D. suzukii* in trial 1, whereas *D. suzukii* had to pass through a 4-mm-diameter hole to oviposit on fruit in trial 2.

In summary, our lab and field studies with blueberry and raspberry suggest that *D. suzukii* used fruit fed upon by *H. halys* within the previous 7 d similarly to unfed fruit. Detailed studies with harvested blueberry showed no preference in oviposition rates based on *H. halys* feeding intensity that occurred 1 or 3 d previously. Additional studies could determine if prolonged feeding or earlier feeding by *H. halys* a few weeks beforehand might affect *D. suzukii*. However, the potential for *H. halys* to have this effect depends on its natural propensity to feed on the same fruit for an extended period or feed on unripe fruit in the field. In the lab, *H. halys* prefer to feed on fruit not previously fed upon by conspecifics (Zhou et al. 2016). Currently, *H. halys*'s preference for unripe small fruits is unknown under unconfined conditions. Wild *D. suzukii* mainly oviposit on ripe blueberry and raspberry in the field (Lee et al. 2015).

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