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Field evaluation of eight attractant traps for *Bactrocera minax* (Diptera: Tephritidae) in a navel orange orchard in China

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Abstract

The Chinese citrus fly, *Bactrocera minax* (Enderlein) (Diptera: Tephritidae), is the most destructive pest in many citrus orchards of south central China. Methyl eugenol and cuelure, 2 potent male lures that are effective for capturing related species, are believed not to attract this species. Limited knowledge is available about the effectiveness of attractant traps for this pest. A field trial was carried out to determine the efficacy of 8 attractant traps to *B. minax*. The study was conducted during the adult occurrence season in an orchard of navel orange, *Citrus sinensis* (L.) Osbeck (Rutaceae), in Zhijiang County, Hunan Province, China, in 2016. To link the efficacy of these attractant traps with the pest population, fruit infestations in the orchard also were investigated. As expected, this study confirmed that methyl eugenol and cuelure were not attractive to *B. minax*. Green-colored sticky spheres trapped significantly more flies and males than methyl eugenol and cuelure-baited traps. On average, ammonium acetate + putrescine trapped more females than males, but it was not statistically better than any of the other tested attractant traps. Fruit infestation rates by *B. minax* ranged from 0.7 to 11.1% in the replicates. Judging by the results of trapping and actual field infestation, it appears that trap effectiveness of the 8 attractant traps was low. A more potent attractant trap is needed, especially for early detection of the pest.

Key Words: Chinese citrus fly; citrus; lure; trapping

Resumen

La mosca china de los cítricos, *Bactrocera minax* (Enderlein) (Diptera: Tephritidae), es la plaga más destructiva en muchos huertos de cítricos del sur de China central. Se cree que el metil eugenol y la cuelure, dos señuelos potentes para los machos son efectivos para capturar especies relacionadas,pero se cree que no atraen a esta especie. Existe conocimiento limitado sobre la efectividad de las trampas atrayentes para esta plaga. Se realizó una prueba de campo para determinar la eficacia de 8 trampas atrayentes para *B. minax*. El estudio se realizó durante la temporada cuando los adultos estran presentes en un huerto de naranja navel, *Citrus sinensis* (L.) Osbeck (Rutaceae), en el condado de Zhijiang, Provincia de Hunan, China, en el 2016. Para vincular la eficacia de estas trampas atrayentes con la población de la plaga, también se investigaron las infestaciones del fruto en el huerto. Como se esperaba, este estudio confirmó que el metil eugenol y la cuelure no fueron atractivos para *B. minax*. Las esferas pegajosas de color verde atraparon significativamente más moscas y machos que el metil eugenol y las trampas con cebo de cuelure. En promedio, el acetato de amonio + putrescina atrapó más hembras que machos, pero no fue estadísticamente mejor que cualquiera de las otras trampas atrayentes probadas. Las tasas de infestación de frutos por *B. minax* varían entre el 0.7 y 11.1% en las réplicas. A juzgar por los resultados de las trampas y las infestaciones en el campo, parece que la eficacia de las trampas de las 8 trampas atrayentes fue baja. Se necesita una trampa atrayente más potente, especialmente para la detección temprana de la plaga.

Palabras Clave: mosca china de los cítricos; cítrico; señuelo; captura

The Chinese citrus fly, *Bactrocera minax* (Enderlein) (Diptera: Tephritidae), is one of the most economically important citrus pests in south central China, Bhutan, and northwest India, causing up to 100% fruit loss. The pest is a significant threat to world citrus production (Zhang 1989; Wang & Luo 1995; Dorji et al. 2006; Dong et al. 2013).This univoltine pest damages *Citrus* plants only (Zhang 1989; White & Wang 1992; Wang & Luo 1995; Allwood et al. 1999). Adults usually occur from late Apr or early May until Aug, depending on local temperatures (Wang & Luo 1995; Dorji et al. 2006; Zhou et al. 2012). Sexually mature males and females mate and oviposit on the fruits multiple times (Lu et al. 2007; Zhang et al. 2007; Dong et al. 2014). Generally, fruits that are 11 mm in diameter or larger are susceptible to oviposition (van Schoubroeck 1999; Liu et al. 2014). The period of susceptibility lasts for about 1 mo, from mid-Jun to mid-Jul (Dorji et al. 2006). The infested

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fruits have noticeable oviposition punctures (Zhang 1989; Zhang et al. 2007). Larvae feed inside fruits until completing the third instar, then emerge from fruit and pupate in the soil until Apr or May of the next yr (Wang & Luo 1995; Dorji et al. 2006; Dong et al. 2013).

Methyl eugenol and cuelure, 2 potent male attractants for many *Bactrocera* species, were reported to not be attractive to *B. minax* (White & Elson-Harris 1992). A few other attractant traps, such as sugar-vinegar-wine mixture (Wang & Luo 1995), green-yellow colored sticky spheres (Drew et al. 2006; Chen et al. 2017), and hydrolyzed protein (Zhou et al. 2012; Mahat et al. 2016), were claimed to be attractive to *B. minax*. In addition, studies were conducted to explore the sex pheromone (Xiao et al. 2013), and citrus fruit volatiles (Liu & Zhou 2016) as attractants for the species.

The performance of currently available lures and traps used in China for *B. minax* are generally poor and inconsistent. This contributes to the widespread and long-lasting outbreaks of this pest in China. The overall goal of this study was to evaluate the efficacy of 8 commonly used attractant-based traps for *B. minax* under field condition. To better understand the effectiveness of these attractant traps, fruit infestation at the test site also was investigated at harvest.

Materials and Methods

FIELD SITE

Experiments were conducted in an orchard of 180 ha, 12-yr-old navel orange, *Citrus sinensis* (L.) Osbeck (Rutaceae), from Apr to Sep 2016 in Zhijiang County, Hunan Province, China (27.2377°N, 109.3761°E). The plant density in the orchard was about 900 trees per ha, with trees averaging about 2.5 m in height with a canopy diameter of about 2 m. The orchard was surrounded by shrubs and other trees. No management practices for *B. minax* or other pest control were carried out in the orchard during the experimental period.

ATTRACTANT TRAPS AND TRAPPING

Eight attractants were used in the study: (1) sugar mixture: a homemade sugar solution with vinegar and wine mixture (10 g sugar, 5 g ethylic acid, and 3 mL alcohol in 100 mL water); (2) torula yeast: 3 pellets per 300 mL water (Chemtica Internacional S.A., Santa Rosa, Costa Rica); (3) Great bait: Great® fruit fly bait, a protein-based bait, 1:3 (vol:vol) solution at the recommended application rate (Hubei Great® Biotech Co. Ltd, Wuhan, China); (4) sticky sphere: a green-colored sticky sphere, 7 cm diam, green polystyrene sphere, with 1 mm thickness transparent sticky glue on the surface (Nongjie Technology Development Co. Ltd, Changsha, Hunan, China); (5) methyl eugenol: a solid sustained-release preparation (Chemtica Internacional S.A., Costa Rica); (6) cuelure: a solid sustained-release preparation (Chemtica Internacional S.A., Costa Rica); (7) 2-component: a 2-component fruit fly bait with a solid sustained-release preparation of ammonium acetate + putrescine (Scentry Biologicals, Inc., Billings, Montana, USA); and (8) 3-component: a 3-component fruit fly bait with a solid sustained-release preparation of ammonium acetate + putrescine + tri-methylamine (Scentry Biologicals, Inc., Billings, Montana, USA).

Three types of food-based lures: sugar mixture, torula yeast, and Great bait, were baited with 300 mL aqueous solution in McPhail traps. One mL of 80% dichlorvos EC (Tianjin Agrevo Pesticide Technology Co. Ltd, Tianjin, China) was diluted in the aqueous solution as a toxicant. Sticky spheres were hung with wire inside tree canopies. Four types of olfactory lures, (methyl eugenol, cuelure, 2-component, and 3-component) were placed inside Steiner traps. To prevent the captured flies from escaping, a dichlorvos sustained-release strip (2 cm in length) (Plato Industries Ltd., Houston, Texas, USA) was placed in each Steiner trap as toxicant. All traps were attached to the tree canopy at about 1.5 m above the soil.

The trapping work was carried out from early Apr to late Jul 2016. A randomized complete block design was used to assign blocks. Blocks were separated at least 200 m from each other. Each block was regarded as a replicate. A total of 5 replicates, 40 attractant traps (5 × 8) were deployed in the trial. Traps in a replicate were located randomly in a 50 m separation from each other. The food-based lures were replaced every 2 wk, sticky sphere traps were replaced each wk, and the olfactory lures were replaced every 4 wk. Table 1 summarizes information on the attractant traps used in this study. All attractant traps were checked weekly. Captured flies were collected for species identification in the lab. The attractant traps were moved to new locations within the block at the weekly check. The experiment was terminated when no flies were captured for 2 consecutive wk.

FRUIT INFESTATION

Oviposition punctures of *B. minax* on the fruits were investigated in the same orchard in early Sep. The oviposition punctures were examined based on the method described by Zhang et al. (2007). The fruits with 1 or more oviposition punctures were regarded as infested fruits. Thirty-six trees per replicate, and 27 fruits per tree were randomly sampled using random numbers. The first and last 3 rows of each replicate were discarded to avoid the influence of edge effect.

STATISTICAL ANALYSES

Due to the large number of zeros, trapping data did not conform to the assumptions of analysis of variance (ANOVA). Instead, the nonparametric Friedman test, was used for analysis of the differences in the numbers of trapped males, females, and the total number of flies. Post hoc pairwise comparisons between each pair of attractant traps were conducted with *P* values adjusted using Bonferroni's correction to avoid increases of type I error due to multiple testing. Adjusted *P* values of pairwise comparisons less than 0.05 were considered statistically significant. The nonparametric Wilcoxon test was used to analyze the difference in the number of trapped males and females for each attractant trap, with *P* values less than 0.05 considered significant.

Fruit infestation rate (%) per tree was calculated using the formula: [(number of infested fruits) / (number of fruits selected)] × 100%. Because there were a number of zeros in infestation rate, the data did not conform to ANOVA assumptions. Differences in infestation rate among replicates were tested using nonparametric Kruskal-Wallis one-way ANOVA. Post hoc pairwise comparisons between each pair of replicates were conducted with *P* values adjusted using Bonferroni's

Table 1. Attractant traps used in this study.

Attractant traps	Lure type	Lure form	Lure placement	Lure refresh interval (wk)
Sugar mixture	Food-based	Liquid	McPhail	2
Torula yeast	Food-based	Liquid	McPhail	2
	Food-based	Liquid	McPhail	2
Sticky sphere	Visual cue or device	Spherical	N/A[a]	1
Methyl eugenol	Olfactory	Solid	Steiner	4
Cuelure	Olfactory	Solid	Steiner	4
2-component	Olfactory	Solid	Steiner	4
3-component	Olfactory	Solid	Steiner	4

[a]Not applicable.

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correction to avoid increases of type I error due to multiple testing. Adjusted *P* values of pairwise comparisons less than 0.05 were considered statistically significant.

In addition, the correlation between infestation rates and attractiveness of each attractant trap was analyzed with the Spearman rank correlation test. All statistical analyses were performed using IBM SPSS Statistics version 22.0 software (IBM Corp., Armonk, New York, USA).

Results

TRAPPING

The numbers of flies captured during the trapping periods are presented in Figure 1. Both methyl eugenol and cuelure trapped zero *B. minax* during the entire trapping period, while other attractant traps captured the flies from 20 May to 1 Jul. Two peak captures by sticky spheres occurred on 17 and 24 Jun, with total captures of 17 and 16 flies, respectively (Fig. 1A), and male captures of 15 flies on each date (Fig. 1B).

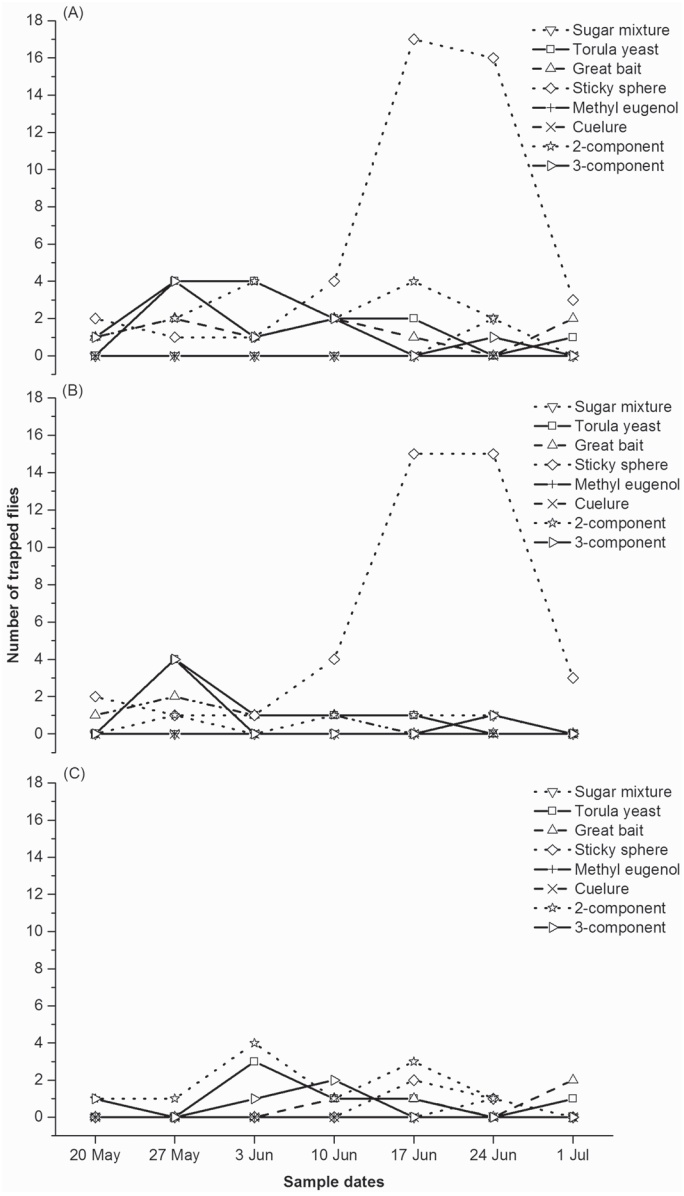


Fig. 1. Weekly trapping data for *Bactrocera minax* using 8 attractant traps in a navel orange orchard in Zhijiang County, Hunan Province, China, in 2016. Data represent the total number of captured flies from 5 replicates for each attractant trap on each sampling date. (A) total (male plus female), (B) male, and (C) female.

There were significant differences among the attractant traps in terms of the number of total captured flies (Friedman test: $\chi^2 = 24.263$; df = 7; $n = 5$; $P = 0.001$; Fig. 2), total captured males (Friedman test: $\chi^2 = 22.471$; df = 7; $n = 5$; $P = 0.002$; Fig. 2), and total captured females (Friedman test: $\chi^2 = 19.923$; df = 7; $n = 5$; $P = 0.006$; Fig. 2). Pairwise comparisons revealed that sticky spheres trapped significantly more total flies than methyl eugenol and cuelure did (adjusted $P = 0.035$ in each case; Fig. 2), as well as total male flies (adjusted $P = 0.028$ in each case; Fig. 2). Pairwise comparisons suggested that the numbers of females captured were not statistically different among the attractant traps (adjusted $P > 0.05$ in each case; Fig. 2).

Within a given attractant trap, significantly more males than females were trapped by sticky spheres (Wilcoxon test: $Z = -2.023$; $P = 0.043$; $n = 5$; Fig. 2). On the other hand, significantly more females than males were trapped by the 2-component trap (Wilcoxon test: $Z = -2.070$; $P = 0.038$; $n = 5$; Fig. 2). The remainder of the attractant traps did not show statistically significant differences in captures between male and female ($P > 0.05$ in each case; Fig. 2).

INFESTATION RATE AND CORRELATIONS WITH TRAPPING

Mean fruit infestation rates were 11.1, 0.7, 10.1, 1.5, and 6.4% in replicate 1, 2, 3, 4, and 5, respectively. The infestation rates indicated pest pressure in the field. The rates were significantly different among the replicates (Kruskal-Wallis ANOVA: $H = 53.86$; df = 4; $n = 180$; $P < 0.001$; Fig. 3). Pairwise comparisons indicated that the fruit infestation rates in replicates 1, 3, and 5 were significantly higher than those of replicates 2 and 4 (adjusted $P < 0.05$ in each case; Fig. 3). In addition, both of the numbers of total flies and males ensnared in sticky sphere were significantly correlated to infestation rates ($n = 5$; $r_s = 1.000$; $P < 0.001$; 2-tailed). For the remainder of the attractant traps, the Spearman's correlation coefficient (r_s) were very low, not significant ($P > 0.05$; 2-tailed), indicating there is no correlation between the infestation rates and performances of the traps.

Discussion

Other studies have been conducted to evaluate the effectiveness of various attractant traps to *B. minax* (Dorji et al. 2006; Drew et al. 2006; Zhou et al. 2012; Mahat et al. 2016; Chen et al. 2017). This study has substantial differences from previous studies. Firstly, previous studies focused primarily on 1 type of attractant trap, either food-based lures (e.g., Dorji et al. 2006; Zhou et al. 2012; Mahat et al. 2016), or visual cue traps (e.g., Drew et al. 2006; Chen et al. 2017). This study is the first that included almost all commercially available tephritid fruit fly attractant traps. Secondly, based on the fruit infestations in the field during this study, the fly populations were not high. Previous trials likely were conducted in orchards where abundance of *B. minax* was greater than in this study. This is especially true in the study of Zhou et al. (2012), in which both a protein bait and sugar mixture had a peak weekly capture of more than100 flies. Thus, this study is different than most of other studies, but better reflects what citrus growers might expect to see in a well-managed field.

The result of this study revealed that all food-based lures, especially sugar mixture (which is widely used in China for trapping *B. minax*) performed poorly in the field. This result was different from some pre-

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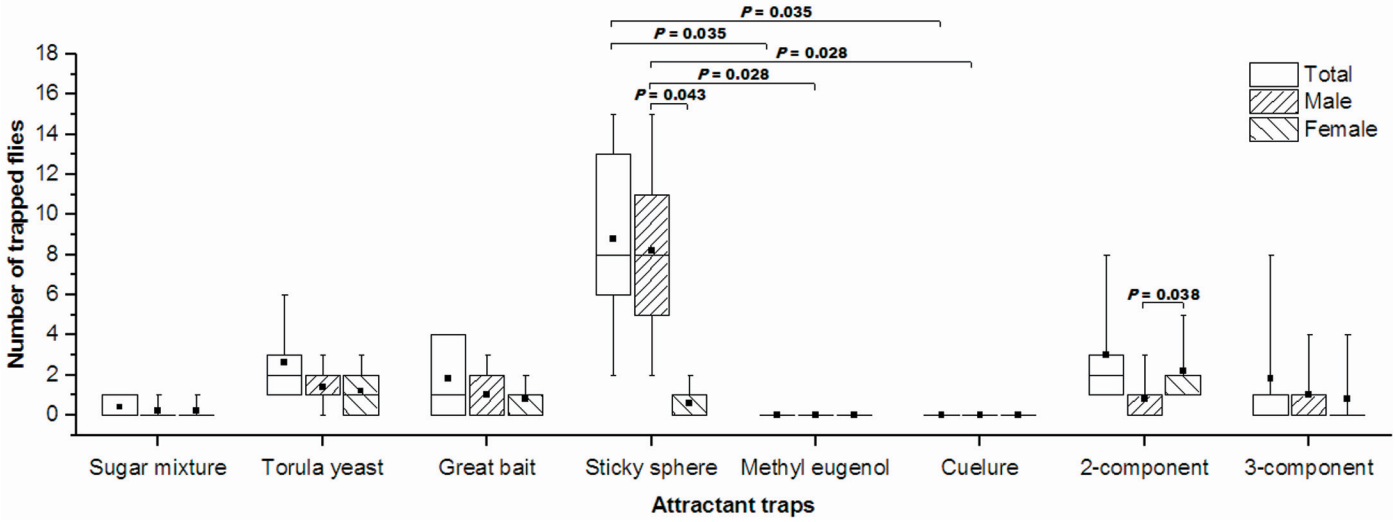


Fig. 2. Total numbers of *Bactrocera minax* trapped by each attractant trap in a navel orange orchard in Zhijiang County, Hunan Province, China, in 2016. Data are represented as box plots, with the mean (square symbols), the median (central lines), the 25th and 75th percentiles (top and bottom of boxes), and the 5th and 95th percentiles (cap of lower and upper whiskers). The Friedman test and post hoc pairwise comparisons of all attractant traps for the number of males, females, and total number of flies were analyzed. Adjusted *P* values less than 0.05 of pairwise comparisons are shown. Differences between numbers of male and females captured by an attractant trap were analyzed using the Wilcoxon test. All the *P* values less than 0.05 are shown.

vious studies (e.g., Wang & Luo 1995; Zhou et al. 2012). Because most food-based lures are made locally, the quality as well as the mix of the ingredients could be substantially different.

Drew et al. (2006) suggested that the visual responses of *B. minax* to spheres is most likely a host fruit-seeking response, and probably reflects a genetically based propensity for these flies to be attracted to the shape of their common *Citrus* host species, because *B. minax* is thought to have evolved with the plant genus *Citrus*. We could not assess the trapping effectiveness of sticky spheres in the study by Drew et al. (2006), because the abundance of *B. minax* adults was unknown in their study. In our study, the visual cue trap, the sticky sphere, demonstrated attractiveness to *B. minax*. Although the sticky sphere did not display a statistically significant advantage over other tested attractants, except for methyl eugenol and cuelure, on average the visual traps caught more flies than other attractant traps. The variance among replicates is likely masking the significance of the traps.

This study confirmed that the 2 *Bactrocera* male para-pheromones, methyl eugenol and cuelure, showed no attractiveness to the fly (White & Elson-Harris, 1992). At present, use of olfactory cue for long-distance orientation of *B. minax* has not been well demonstrated (Liu & Zhou 2016). However, females of this fly appear to accept certain olfactory cues for their egg-laying, because oviposition preference is observed to relate to emission of some chemical compounds such asnonanal, citral, limonene, and linalool from host fruits (Liu & Zhou 2016). Although more females than males were trapped by the 2-component trap, there were no statistically significant differences between 2-component and other attractant traps in terms of overall attractiveness to the fly.

As stated previously, *B. minax* is a very destructive citrus pest. Invasion of this pest into other major citrus production areas, either through trade or human movement, can result in significant disruption to local citrus industries. Early detection is among the critical measures in preventing the invasion. An effective lure or trapping technique is necessary for early detection of invaders. This study suggests that currently available attractant traps being used in China and elsewhere may not be effective enough for early detection, and more effective attractant traps need to be discovered and developed.

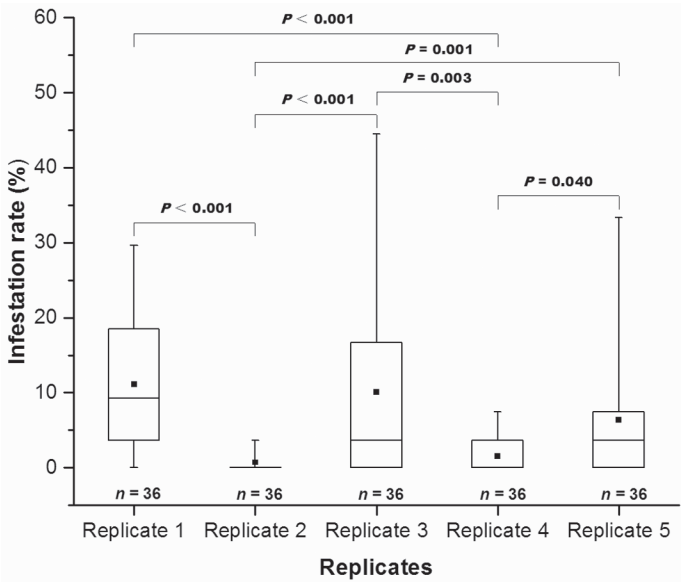


Fig. 3. Fruit infestation rates by *Bactrocera minax* in a navel orange orchard in Zhijiang County, Hunan Province, China, in 2016. Data were available from 36 trees (sample size, *n* = 36) from each replicate. Data are represented as box plots, with the mean (square symbols), the median (central lines), the 25th and 75th percentiles (top and bottom of boxes), and the 5th and 95th percentiles (cap of lower and upper whiskers). Kruskal-Wallis ANOVA and post hoc pairwise comparisons of all replicates used for analysis. Adjusted *P* values less than 0.05 of pairwise comparisons are shown.

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