Behavioral Response of *Halyomorpha halys* (Hemiptera: Pentatomidae) and Its Egg Parasitoid *Trissolcus japonicus* (Hymenoptera: Scelionidae) to Host Plant Odors

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Insects use a range of cues to help them interact with each other and their host plants. Among these cues, olfaction plays a major role in host selection. The present study investigated the behavioral response of the brown marmorated stink bug, *Halyomorpha halys* (Stål), and its egg parasitoid, *Trissolcus japonicus* (Ashmead), to host plant-related odors. We used *H. halys* nymphs since their response to host odors is relatively unknown. In a Y-tube, we first evaluated the behavioral response of *H. halys* nymphs to whole-fruit odors of apple (*Malus domestica* (Borkh.)) and peach (*Prunus persica* (L.) Batsch). Subsequently, we tested the behavioral response of *H. halys* and *T. japonicus* to 18 selected synthetic volatiles previously identified from *H. halys* and its common host plants. In the greenhouse, we further tested *H. halys* attraction to the most promising of these volatiles individually and as blends. In single-choice tests, *H. halys* nymphs preferred odors from apple and peach over the control (no odor). In dual-choice tests, *H. halys* did not show any preference between apple and peach odors. Among the 18 volatiles tested, *H. halys* nymphs were attracted to ethyl salicylate (ES), undecane (UN), and ethyl acetate (EA) compared to the control. In the greenhouse, *H. halys* nymphs were similarly attracted to blends of 1:1 ratio of ES and EA but not to single compounds. Also in the Y-tube, female *T. japonicus* preferred the arm that had ES, β-caryophyllene, and decanal and a blend of these three compounds at a 1:1:1 ratio. *Trissolcus japonicus* was more attracted to the control arm than to the arm containing tridecane or α-pinene. These results indicate the potential of developing *H. halys* and *T. japonicus* attractants or repellents based on host plant volatiles and suggest possible adaptive responses of this pest and its egg parasitoid to similar host plant odors.

**Keywords:** brown marmorated stink bug, Y-tube olfactometer, invasive species, host plant volatiles, tritrophic interaction
INTRODUCTION

Research in insect chemical ecology has led to the discovery of effective attractants for use in insect pest monitoring. Most insects, particularly herbivores, have evolved to depend on plants for survival (Ehrlich and Raven, 1964). Insects use plants to obtain resources, like food and oviposition sites, which they locate through chemical cues commonly referred to as volatile organic compounds (VOCs) produced by intact [host plant volatiles (HPVs)] or mechanically and/or herbivore-damaged plants [herbivore-induced plant volatiles (HIPVs)] (Choh et al., 2013; Xu and Turlings, 2018). Although plants are ubiquitous, their availability and quality changes because of selection pressure (Bruce et al., 2005), and production of volatiles varies in space and time (Holopainen and Blande, 2013; Conchou et al., 2017, 2019), which means insects have to develop efficient and effective sensory organs to discriminate between host and non-host odors.

Several VOCs have been identified from different plant parts (Knudsen et al., 1993), with each of the compounds or their combinations reported to perform different biological roles during an insect's trophic interactions with plants (Paré and Tumlinson, 2002; Reddy and Guerrero, 2004). In many situations, phytophagous insects in particular respond to individual VOCs described as signature compounds or blends (Szendrei and Rodriguez-Saona, 2010; Rodriguez-Saona et al., 2012) that assist in locating plant species on which they can feed and reproduce. Moreover, insects respond to VOCs when they synergize with visual and olfactory cues, such as color, shape, and pheromones, which are used in monitoring traps particularly in moths (Yang et al., 2004; Varela et al., 2011; Sans et al., 2016; Rodriguez-Saona et al., 2020). This has provided opportunities where VOCs are used as stand-alone compounds or synergized with other cues to monitor many destructive agricultural and forest pests and improve pest management by increasing accuracy of detection (Knight and Light, 2001; Light and Knight, 2005).

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a key invasive pest responsible for causing severe feeding damage to major fruit, vegetable, and field crops in North America and Europe (Leskey et al., 2012; Rice et al., 2014; Leskey and Nielsen, 2018). *Halyomorpha halys* is a polyphagous insect, and its behavioral ecology, feeding habits, and movement within the landscape are driven by a number of factors. Notably among them are host switching due to decreasing host quality, strong requirement for diet mixing to satisfy its developmental requirements (Nielsen and Hamilton, 2009; Acebes-Doria et al., 2016; Dingha and Jackai, 2017), and VOCs produced by host plant species during attractive stages (Blauuw et al., 2019). Among these factors, HPVs inform host selection when the insect is in the active accessible space for host choice. Yet, little is known on the behavioral response of *H. halys* nymphs to host plant odors.

Like most insects, *H. halys* selection of host plants is dependent on the chemical profile released by the plants. However, polyphagous insects accept a wide range of host plants with distinct odor profiles. Thus, selection of candidate compounds is more challenging with generalist than specialist insects (Braasch et al., 2012; Kaplan, 2012).

Additionally, there is strong evidence that entomophagous insects including parasitoids also use VOCs associated with herbivory and egg deposition to locate their host and thus may respond to synthetic VOCs associated with these behaviors (Turlings and Tumlinson, 1992; Van Loon et al., 2000; Dudareva et al., 2006; Tentelier and Fauvergue, 2007; Ngumbi et al., 2009; Pashalidou et al., 2015). This has been demonstrated in some *Trissolcus* species (Colazza et al., 2004). However, limited studies have been done on the behavioral responses of *H. halys*’ coevolved egg parasitoid, *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae), although *T. japonicus* does positively respond to host karomones on exposed leaf surfaces (Boyle et al., 2020).

Our primary aim was to investigate the behavioral response of *H. halys* nymphs and female *T. japonicus* parasitoids to HPVs that may serve as attractants for food and oviposition sites, respectively, and for improving the monitoring of both species. Specifically, as adult and nymphs share the same host plants, we tried to isolate host plant cues that nymphs might use during host searching behaviors. As a non-reproductive stage, nymphs are only seeking nutritional resources, which may be signaled by VOCs. This study follows up on Blauuw et al. (2019) findings that *H. halys* nymphs chose host plants depending on host phenology and characterized VOCs that are present during attraction. Apple and peach fruits were chosen because they are common host plants of *H. halys* (Akotsen-Mensah et al., 2018), and *H. halys* survive well and damage peach and apple based on laboratory feeding studies (Acebes-Doria et al., 2016; Dingha and Jackai, 2017). Whole fruits were used because *H. halys* attack mainly fruiting parts, and plant parts are commonly used as experimental methods to identify novel plant compounds for herbivore insects (Knolhoff and Heckel, 2014).

Further, we hypothesized that *T. japonicus*, during its own host searching behaviors, will respond positively to similar plant volatiles as its primary host, *H. halys*. Our specific objectives were to: (1) evaluate the behavioral response of *H. halys* nymphs to whole-fruit odors of apple [*Malus domestica* (Borkh.)] and peach [*Prunus persica* (L.) Batsch] in laboratory (Y-tube) assays; (2) test the behavioral response of *H. halys* and *T. japonicus* to 18 selected, previously identified synthetic fruit volatiles in laboratory (Y-tube) assays; and (3) test *H. halys* attraction to the most promising of these volatiles individually and as blends in a greenhouse.

MATERIALS AND METHODS

Test Insects

*Halyomorpha halys* second instars used for the study came from eggs sourced from the New Jersey Department of Agriculture (NJDA, Trenton, NJ, United States), and a colony at Rutgers Agricultural Research and Extension Center (RAREC; Bridgeton, NJ, United States). Newly hatched nymphs were fed *ad libitum* with pesticide-free raw sunflower seeds, green beans, carrot, and water provided in a cotton wick and were kept in Petri dishes until ready for use.
**Trissolcus japonicus** females came from the laboratory colony maintained at RAREC using protocols developed by Talamas et al. (2015) and Zhang et al. (2017). Briefly, fresh *H. halys* eggs obtained from the NJDA were exposed to the parasitoids for 24 h. Upon emergence, the parasitoids were sexed, and females were transferred to plastic containers with a feeding station (honey solution). Females aged between 2 and 7 days were used in the experiments.

Insect colonies were maintained in an incubator at 25 ± 1°C, 65–70% relative humidity, and 16:8 h (L:D) photoperiod.

**Y-Tube Olfactometer Bioassays**

A Y-tube olfactometer (Sigma Scientific LLC, Micanopy, FL, United States) was used to determine the behavioral response of second instar *H. halys* starved for 3 h and adult (female) *T. japonicus*. The Y-tube was placed in a particle board box in a darkroom illuminated with a 20-W red LED light and maintained at approximately 25°C. The olfactometer was similar to that described by Blackmer et al. (2004). Briefly, the apparatus consisted of a Y-tube glass (40-mm diameter × 36-cm length) with the two arms angled at 50° through which laboratory-grade air (Airgas Company, Vineland, NJ, United States) was drawn through and the airflow was controlled by an inline flowmeter (Gilmont Instr., Barnant Co., Barrington, IL, United States) set at 5 L/min to deliver 2.5 L/min to the olfactometer arm. The arm in which the treatments were placed was randomized in all experiments, and the Y-tube was rinsed in acetone and air dried between replicates.

Whole undamaged apple (*M. domestica*) and peach (*P. persica*) fruit were tested in two customized 4.5-L stainless steel stock pots (Figure 1; Experiment 1 below). Each pot had two openings through which air could flow in and out of a glass chamber (odor source). Volatile sources (apple vs. peach) were placed in their designated olfactometer arm for 2 min prior to release of test insects to ensure the stabilization of the diffusion of the test material. Synthetic compounds (2- and 0.5-μl aliquots of synthetic VOCs for *H. halys* and *T. japonicus*, respectively) were pipetted onto 5-cm² filter paper (Whatman®, Thermo Fisher Scientific, Waltham, MA, United States), and the filter paper was replaced after testing 10 insects per compound. In each experiment, a single insect was introduced into the base of the Y-tube and allowed to walk to either arm at the Y-junction, and the time taken to make a choice was recorded. The insects were continuously observed until they made a choice. Individuals that did not make a choice after 12 min were recorded as not responding and removed from the analysis. Thirty (30) insects were tested for each compound, with each insect representing a replicate. Pots and other apparatus were cleaned with 5% bleach and 95% ethanol between replicates.

**Halyomorpha halys** Response to Synthetic HPVs

**Experiment 2**

In experiment 2, second instar *H. halys* behavioral responses to volatiles identified in headspace collections from pepper (*Capsicum frutescens* Mill.), soybean (*Glycine max* L.), sweet corn (*Zea mays* L.), Swiss chard (*Beta vulgaris* L.) (Blauw et al., 2019), peach (*P. persica*), and apple (*M. domestica*) (Akotsen-Mensah et al., unpublished), and those reported in the literature (Horvat and Chapman, 1990; Horvat et al., 1990; Knudsen et al., 1993; Casado et al., 2006; Rout et al., 2012; Izumi et al., 2015) were tested. In all, 18 synthetic compounds consisting of five alkanes, four terpenes, four esters, four aldehydes, an alcohol, and an acid (Table 1) and their blends (Table 2) were purchased from commercial sources (Sigma Aldrich, St. Louis, MO, United States) and tested in the Y-tube olfactometer. Compounds that significantly attracted nymphs in the olfactometer were tested as binary and ternary blends of equal ratios to investigate the existence of possible synergisms. Blends consisted of two (binary) compounds in a ratio of 1:1 and three (ternary) compounds in ratios of 1:1:1. Because of potential of compounds reacting when mixed, blends used in the Y-tube were released from separate filter papers.

**Experiment 3**

In experiment 3, nymphal response to promising compounds from experiment 2 were tested in a mesh cage under greenhouse conditions. Six mesh cages were used for each trial (93.0 cm × 47.5 cm × 47.5 cm, BugDorm-44590DH, Megaview Science Co., Ltd., Taiwan) and separated by at least 0.5 m. The cages were placed in a greenhouse aligned lengthwise from north to south, and the test compounds and control (hexane) were randomly assigned to either end of the cage. The test compounds (treatments) consisted of four single compounds, namely, (1) ethyl salicylate (ES), (2) ethyl acetate (EA), (3) nonanal (NO), and (4) tridecane (TR) (Table 1), and seven blends, namely, (5) blend 1, (6) blend 2, (7) blend 3, (8) blend 4, (9) blend 5, (10) blend 6, and (11) blend 7 (Table 2). Each blend was prepared by putting 2 μl each in 2 ml of hexane and tested individually. Individual compounds or blends were dispensed by transferring 1.0-μl aliquots into release devices consisting of a 1.5-ml microcentrifuge vial (VWR International, Radnor, PA, United States) with a cotton string (~2.5 cm long) threaded through a hole drilled (0.125 mm) in the lid. The release device was suspended on top of a customized trap.
FIGURE 1 | (A) Customized olfactometer, showing the source of stimuli via airflow through each of the two arms. Air was pumped from a gas cylinder through the stainless container to the two-way part of the olfactometer. The flow rate of the air was checked regularly, ensuring that it was 100 ml/min through each arm (200 ml/min into the central portion of the olfactometer). (B) Greenhouse setup consisted of a customized black pyramid trap placed in a bugdorm and nymphs released from the center of the two treatments. The pyramid trap is 15 cm tall and 10 cm wide at the base. These were placed in a 90 × 45 × 45 BugDorm tent. The release mechanism consisted of a 1.5-ml microcentrifuge vial with a cotton string (∼2.5 cm long) threaded through a hole drilled (0.125 mm) through the lid and placed on top of the pyramid trap.

(Figure 1B) to collect insects attracted to the compounds. For each compound, 10 late second instar H. halys, starved for 3 h, were released from Petri dishes (100 mm × 15 mm; VWR International, Radnor, PA, United States) placed in the center of each cage. After 24 h, the location of the nymphs was recorded. A nymph was recorded as making a “choice” if it was on a baited trap or on the cage directly above it (e.g., a circle of a given diameter directly aligned above the treatments; defined as a “choice-zone”). Cages were soaked in 10% bleach for 2 h and washed thoroughly with tap water before each trial.

Trissolcus japonicus Response to Synthetic HPVs

Experiment 4
To measure the behavioral response of T. japonicus to volatiles identified as attractive to H. halys (based on experiments 2 and
Blends of compounds were made from single compounds which T. japonicus showed greater than 50% response were made into a blend (blend 1) and tested in a Y-tube. Also, those which showed less than 50% response were made into a blend (blend 2) and also tested. The blends (Table 2) used were (1) blend 1 (ES + BC + DE), (2) blend 2 (AP + TR + Lim + Lin), and (3) blend 3 (blend 2 + ES). To maintain the integrity of the compounds and avoid potential interaction of compounds, blends were not mixed, but each was put separately on a filter paper. The loading rate tested for each compound was 0.5 μl.

**Statistical Analyses**

For all tests involving choice between two treatments, data were analyzed using Chi-square contingency analyses. A Chi-square goodness-of-fit test based on the assumption that there would be 50:50 chance of insect responding to both treatments was used to test for significance at $p = 0.05$. For the greenhouse experiment, a Student’s $t$-test was used to determine difference between the treatments. All analyses were done using JMP®, Version 15 (SAS Institute Inc., Cary, NC, United States).

**RESULTS**

_Halyomorpha halys_ Response to Apple and Peach Fruit Volatiles

_Halyomorpha halys_ nymphs were significantly attracted to apples of the variety Golden Delicious (20.0%) ($\chi^2 = 16.28, p < 0.001$), but not to those of the variety Ginger Gold ($\chi^2 = 1.68, p = 0.302$).
Peaches of the Encore variety attracted significantly more *H. halys* nymphs, while those of the variety Harrow Beauty did not exhibit significant attraction between the odors of apples (var. Golden Delicious: $\chi^2 = 0.28$, $p = 0.599$) and peach (var. Encore: $\chi^2 = 3.28$, $p = 0.0673$), although nymphs tended to orient more to the arm containing apples.

**Halyomorpha halys** Response to Synthetic HPVs

Among the 18 synthetic compounds evaluated in the Y-tube, ES ($\chi^2 = 8.53$, $p = 0.004$), undecane (UN) ($\chi^2 = 8.53$, $p = 0.004$), and EA ($\chi^2 = 6.08$, $p = 0.016$) were significantly attractive to the nymphs (blank) (Figure 2). In contrast, nymphs demonstrated repellant behavior to BC ($\chi^2 = 8.53$, $p = 0.004$), octanal ($\chi^2 = 5.83$, $p = 0.016$), DE ($\chi^2 = 4.67$, $p = 0.031$), and octane ($\chi^2 = 4.67$, $p = 0.031$) (Figure 2). When ES and EA were blended (blend 1) and compared to other blends including those modeled from volatiles emitted from pepper, soybean, sweet corn, and Swiss chard at $2\times$ (blend 6) and $5\times$ (blend 7) concentrations (Blaauw et al., 2019), blend 1 ($\chi^2 = 4.52$, $p = 0.0336$) and blend 2 (ES and Lin) ($\chi^2 = 9.5$, $p = 0.002$) significantly attracted the nymphs (Figure 3), while blends 6 and 7 showed no attraction compared with control. The combined blends of ES and EA were not more attractive than individual compounds, which suggests there was no synergistic effect between ES and EA and other blends too.

**Trissolcus japonicus** Response to Synthetic HPVs

*Trissolcus japonicus* females were attracted to ES, BC, and DE but not to AP and TR (Figure 5). To test whether there could be a synergistic effect of VOCs that showed significant attraction and those that did not, only blend 1 (ES + BC + DE) showed significant attraction ($\chi^2 = 4.9$, $p = 0.026$); however, the attraction was not greater than the individual compounds. Also, when ES was added to compounds that did not show attraction (blend 2), ES did not improve the attraction of this blend. This indicates that ES alone may be attractive, but its effect may be suppressed when combined with other non-attractive compounds.

**DISCUSSION**

The primary aim of this study was to investigate the behavioral responses of *H. halys* nymphs and females of the *H. halys* primary predator, *Trissolcus japonicus*. The results showed that *H. halys* nymphs and females were more attracted to synthetic compounds that mimic pheromones and plant volatiles, particularly ES, BC, and DE. This suggests that these compounds could be used as lures to attract *H. halys* for biological control strategies. However, the lack of synergistic effect between these compounds indicates that their attractiveness may be additive rather than synergistic.

In the greenhouse, response of *H. halys* nymphs to the four individual synthetic VOCs tested (ES, EA, NO, and TR) was not significant compared to the control (Figure 4). However, of the six blends tested, *H. halys* showed significant positive response to blend 1 (ES + EA) ($t = 8.93$, $p = 0.014$) but were repelled by blend 6 ($t = 7.27$, $p = 0.022$) (Figure 4).

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parasitoid *T. japonicus* to HPVs, with the goal of identifying attractants for improving the monitoring of both species. The results showed that *H. halys* nymphs are attracted to volatiles from whole apple and peach fruit and to some synthetic VOCs and their blends. When offered matured Golden Delicious apple or matured Encore peach, nymphs showed attraction to volatiles emitted by both fruit compared to control (only air). However, when given a choice between these fruits, *H. halys* nymphs responded similarly to both odors. This is in broad agreement with host discrimination by insects using plant...
volatiles (Bruce et al., 2005; Webster et al., 2010; Bruce and Pickett, 2011; Pickett et al., 2012) and shows a clear preference of *H. halys* nymphs for fruit volatiles in the absence of other choices but a lack of discrimination when two attractive volatile blends are present simultaneously. Although *H. halys* survival on peaches is higher than on apples (Acebes-Doria et al., 2016), this may be due to host plant quality or nutrition during fruit development, whereas we evaluated mature fruits. As a generalist insect, *H. halys* specializes on maturing fruits in landscapes with highly heterogeneous and ephemeral resources (Martinson et al., 2015; Blaauw et al., 2019); thus, we expected mature fruits to be more attractive. Peach is a common early season host for *H. halys* (Leskey and Nielsen, 2018), and further studies including fruits across different phenological growth stages (i.e., early maturation to mature) are needed to correlate emissions of fruit volatiles with *H. halys* nymphal arrival to hosts. Although *H. halys* nymphs did not seem to discriminate between the odors of mature apple and peach VOCs, differences in the fruit quality, which is a combination of total number, identity, and concentration of VOCs emitted by different cultivars of apples (Dixon and Hewett, 2000) and peaches (Akotsen-Mensah et al., unpublished), could explain the observed differences in attractiveness between peach and apple varieties.

With respect to the synthetic VOCs, the results revealed that *H. halys* nymphs showed attraction to ES, UN, and EA. These compounds are commonly found in some host plants of *H. halys*, and thus, it is not surprising to observe this positive response of the nymphs toward them. The strongest attraction was found for ES, which is emitted in high amounts from several fruits including apples (e.g., var. "Granny Smith" and "Jonagold"), blueberries, plum, passion fruit, feijoa fruit, raspberry, tomato, among others (Knudsen et al., 1993), many of which are preferred host of *H. halys*. Also, EA is a volatile found in nature and emitted by many plants (Knudsen et al., 2006).

Although, *H. halys* did not respond to the majority of VOCs, individually and as blends, tested in this study, we cannot conclusively establish that they were not attractive for several reasons. First, it is possible that the dose and blend ratio (Bruce et al., 2005; Tasin et al., 2006a,b; Najar-Rodriguez et al., 2010; Bruce and Pickett, 2011) and release rates of some of the tested compounds and blends were not optimized to trigger a behavioral response. Another reason may be because most Pentatomid receptor neurons are not strongly tuned toward specific plant odor compounds (Dickens et al., 1993) and, thus, the variations in the response of *H. halys* to these compounds. The combined blends of ES and EA were not more attractive than the individual compounds, which suggests that there was no synergistic effect between ES and EA and other blends too. Morrison et al. (2018) tested blends of ubiquitous compounds found in apple and peach headspace volatiles and green leaf volatile compounds commonly produced by most plants, but found little evidence for synthetic compounds retaining *H. halys* in apple and peach fields. They also found no evidence for synthetic host plant blends synergizing with the *H. halys* aggregation pheromone. They, however, noted that the compounds used were not optimized for *H. halys* in either emission rate, concentration, or deployment strategy, which provides further opportunity to investigate the responses of *H. halys* to other potential HPVs.

For some of the VOCs tested, the nymphs selected the clean air. This indicates that some HPVs may repel *H. halys* and that no single compound may fully account for the response to fruit volatiles. Thus, host location in *H. halys* is likely not mediated by a single compound but instead by a combination of attractive (and possibly also repellant) compounds
in the blend, which makes the polyphagous *H. halys* attracted to multiple hosts. This also means some of these VOCs could be potential repellents and could have a place in a push–pull strategy in managing *H. halys*.

Our results support our hypothesis that the coevolved parasitoid *T. japonicus* is attracted to similar VOCs as its primary host *H. halys*. The HPV ES elicited positive behavioral responses by both species. This response to the same VOCs across trophic levels has likely been selected to increase the likelihood of *T. japonicus* locating host resources (i.e., *H. halys* eggs) across a diverse landscape (Aldrich et al., 1995; Tillman et al., 2010). Further studies should evaluate if there is an increase in host finding (i.e., *H. halys* eggs) by *T. japonicus* in the presence of attractive VOCs as well as their potential use as a monitoring lure for both species.

In conclusion, we have demonstrated convergence across trophic levels by a polyphagous species and its coevolved natural enemy to similar HPVs. We found that *H. halys* nymphs are attracted to odors from peach and apple fruits and that one common host-associated ester, ES, was attractive to *H. halys* nymphs and *T. japonicus* females. While we evaluated nymphs, adult and nymphal stages of *H. halys* share host plants and are expected to respond to the same cues. Cues shared between *H. halys* and its parasitoid suggest that *T. japonicus* utilizes HPVs to locate egg masses for oviposition. Given that plants emit a complex mixture of VOCs, belonging to the same or to other chemical groups, other VOCs not tested here could also be involved. However, the VOCs tested in our study are all found in host plants of *H. halys*, and thus, its response to them is highly plausible. If ES and EA, and their blends, are used in conjunction with the aggregation pheromone to improve *H. halys* trapping.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

BB, CA-M, AN, and CR-S conceived the idea, planned the experiments, and analyzed and interpreted the data. CA-M performed both the greenhouse and Y-tube experiments. All authors contributed to the article and approved the submitted version.

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