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Repellency of mustard (*Brassica juncea*) and arugula (*Eruca sativa*) plants, and plant oils against the sweetpotato whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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ABSTRACT

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an economic complex of at least 36 cryptic species, comprising a highly polyphagous and serious pest of vegetable, fiber and ornamental crops. Sustainable alternative measures such as cultural controls can be effective in integrated pest management of *Bemisia*, but have received relatively little research effort. "Push – pull" strategies are a form of cultural control based on behavioral manipulation of insect pests and their natural enemies. Pests are repelled from a protected resource ("push" component) and simultaneously attracted to a trap crop ("pull" component) where they are subsequently removed, preferably through biological control or other appropriate means. In this study, we conducted laboratory studies using an olfactometer or odor-detecting equipment to determine the effect of volatiles from whole plants and plant oils to repel the sweetpotato whitefly. In addition to volatiles, we tested responses to colors known to attract whitefly adults. Finally, we monitored whitefly behavior using a video recorder and behavioral analysis software in response to repellent oils. Results indicated significant movement of whitefly adults towards attractants, such as cucumber plants and the color green, and movement away from mustard plants (Caliente and giant red mustard varieties). Repellency was seen to odor of arugula and mustard plants. In addition, the highest total distances traversed and the rate of insect movement were recorded in hot pepper wax, mustard oil and the cucumber controls. Time allocation analysis indicates attraction towards the cucumber control and hot pepper wax, as well as aversion to garlic oil and mustard oil. The clean air control elicited no directional movement. These studies have indicated mustard and mustard oil for use as "push" components in "push-pull" management. Present results suggest that garlic oil can be an effective "push" candidate. Finally, we suggest modified olfactometers and the use of behavioral analysis software as alternative solutions to traditional Y-tube olfactometer studies.

Additional index words: olfactometer, garlic oil, mustard oil, hot pepper wax, giant red mustard, insect behavior

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a complex of at least 36 putative cryptic species, comprising a highly polyphagous and serious worldwide economic pest of vegetable, fiber and ornamental crops worldwide. This species has been listed in the world's 100 worst invasive species (Barbosa et al. 2014). Reliable estimates of global economic loss are difficult to obtain because of the extensive geographical areas and diverse crops, as well as differing monetary systems (Oliveira et al. 2001). The effectiveness of chemical control is mitigated by the development of resistance to insecticides such as neonicotinoids (Castle et al. 2013) and organophosphates and pyrethroids (Gauthier et al. 2014). Sustainable alternative measures such as cultural controls can be effective in integrated pest management of *Bemisia*, but have received relatively little research effort (Hilje et al. 2001).

"Push – pull" strategies are a form of cultural control based on behavioral manipulation of insect pests and their natural enemies. Pests are repelled from a protected resource ("push" component) and simultaneously attracted to a trap crop ("pull" component) where they are subsequently removed, preferably through biological control (Cook et al. 2007) or other appropriate means (Shelton and Badenes-Perez 2006). Several "push-pull" strategies were reviewed by Cook et al. 2007. Successful application of push-pull agriculture was developed in Africa for subsistence farmers against stemborers of maize and sorghum. Push crops were non-host repellent intercrops, primarily molasses grass, silverleaf or green desmodium. Pull crops were Napier or Sudan grass (Cook et al. 2007). Effective intercrop combinations reduced pest infestation and increased efficiency of natural enemies. Despite the potential of push-pull as a tool in integrated pest management, adoption has been limited since the time the term was coined (by Pyke et al. 1987), with only a handful of programs progressing to commercial use (Cook et al. 2007).

Insect attraction or repellency is due to visual or chemical cues (Cook et al. 2007). The action of volatiles is often measured directly by insect movement through olfactometer studies or indirectly in measurements such as oviposition rates. Legaspi and Simmons (2012) evaluated four commercial oils as repellents or oviposition deterrents against *Bemisia tabaci* (= *B. argentifolii* (Bellows and Perring) through choice and no-choice potted plant experiments. Mustard oil was the most promising push component, compared to garlic, hot pepper wax and horticultural petroleum oils. Y-tube olfactometer experiments tested whitefly orientation responses to volatiles from six plants: tomato, tobacco, cabbage, cotton, cucumber and celery (Li et al. 2014). Four plant extracts induced strong attraction responses, but not those from celery. Bemisia tabaci showed strong attraction to (E)-2-hexenal, 3-hexen-1-ol and mixtures of these compounds, which were identified as potential push components for B. tabaci (Li et al. 2014). In field experiments in Sudan, push – pull tactics showed promise against B. tabaci on the common bean (*Phaseolus vulgaris*) (Oji and Mohamed 2005). Coriander (*Coriandrum sativum*) served as the push crop and *Dolichos lablab* served as the trap crop.

Visual stimuli may affect crop attractiveness to insects (Mound 1962), but may be less studied in the context of push-pull strategies. Traps have been designed to attract adult whiteflies which are drawn to the color yellow, without reliance on sticky materials or bait (Chu and Henneberry 1998). In field studies conducted in the southwestern United States, China and India, nine trap base colors were compared for attractiveness to *B. argentifolii*: white, rum, red, yellow, lime green, spring green, woodland green (dark green), true blue, and black. Trap colors most attractive to the whitefly were lime green, yellow and spring green (Chu et al. 2000).

Both visual and olfactory cues are used by whiteflies in host selection. We used a variety of olfactometers to measure response of *Bemisia tabaci* to potentially repellent volatiles when in the presence of attractive color and cucumber odor. We used a video recorder and behavioral analysis software to monitor whitefly behavior.

Here we used a modified Y-tube olfactometer to measure responses of *Bemisia tabaci* adults to volatiles from putative repellent and attractive plants, as well as to repellent oils. In addition to volatiles, we tested responses to colors known to attract whitefly adults. Finally, we monitored whitefly behavior using a video recorder and behavioral analysis software in response to repellent oils.

MATERIALS AND METHODS

Insects and Plants. B-biotype sweetpotato whitefly (*B. tabaci*) were reared on potted collard plants (*Brassica oleracea*) kept in a greenhouse. Newly emerged female whiteflies were removed from the underside of the leaf surface and kept in a small plastic holding cage (17 cm height 19 cm wide x 17 cm long) on the day of testing. Pots were 18 cm (diam) x 19 cm (height) and filled with Berger BM6 (Berger Peat Moss, Quebec City, Canada) potting medium. All plants were fertilized once a week using Miracle Grow All Purpose Plant Food (24-8-16). Plants used for olfactometer tests were planted in small plastic pots (11.5 cm x 11.5 cm diam.) (Dillen Products, Middlefield, OH). Plants tested included arugula (Eruca sativa cv. "Nemat"), mustard (*Brassica juncea* cv. "Caliente 19"), and Giant Red Mustard (Brassica juncea). Cucumber plants (*Cucumis sativus* var. "Poinsett 76") were used to provide host odor to test with repellent oils. Plants were tested for repellency to whiteflies when they were approximately two months old and had six leaves.

Olfactometer Experiment 1. A modified olfactometer was constructed because of difficulties in studying repellency using traditional Y-tube olfactometers (Legaspi et al. 2011). Thus individual whiteflies were tested in a straight long clear acrylic tube (75 cm long, 3 cm diam.) (Tap Plastics, Oakland, CA). An 11 cm air mixing chamber was created in this tube by gluing in an acrylic disk which had a 9 mm aperture and created air flow down the center of the tube. In order to introduce test whiteflies, an opening was made in the device at 45 cm and the top of a micro centrifuge tube was glued into place to create a closable door. The end of the tube was covered with a removable piece of mesh fabric. Air flowed into the device from a pressurized air cylinder and passed through activated carbon filters, through two flow meters, and into two glass holding chambers (41 cm height, 16 cm diam.) in which plants were placed. Air flowed out of these chambers into the olfactometer at a rate of 0.25 L / min. (Analytical Research Systems, Gainesville, FL). The olfactometer was placed upon black poster board on a laboratory bench to provide a contrasting background in relation to the whiteflies. This arena was surrounded on three sides by 36 cm high walls made from white foam core poster board. Experiments were conducted under lighting from two fluorescent bulbs on the laboratory ceiling directly above the arena. Temperature was maintained at ~25 ˚C. Small potted test plants were placed in one of two test chambers after the top of the pots were covered with aluminum foil to reduce odors coming from the potting soil. In trials with odors, we placed a cucumber plant in one chamber to provide an attractive upwind odor source. Leaves of each plant tested were lacerated as previously described (Legaspi et al. 2011). In addition to testing whitefly attraction to plant odors, we observed movement to colors by placing yellow or green discs inside the olfactometer. Spectral reflectance curves for each colored disc are given in Fig. 1. The different treatments were as follows: a) cucumber plant odor alone (C), b) green disc alone (G), c) cucumber plant odor and green disc (C + G), d) yellow disc alone (Y), , e) cucumber plant odor and yellow disc (C + Y), f) cucumber odor, green disc and Nemat arugula plant odor (C + G + N), g) cucumber plant odor, green disc and giant red mustard plant odor (C + G + M), h) cucumber plant odor, green disc and Caliente mustard plant odor (C + G + Ca) and i) control (no plant odor and no color).

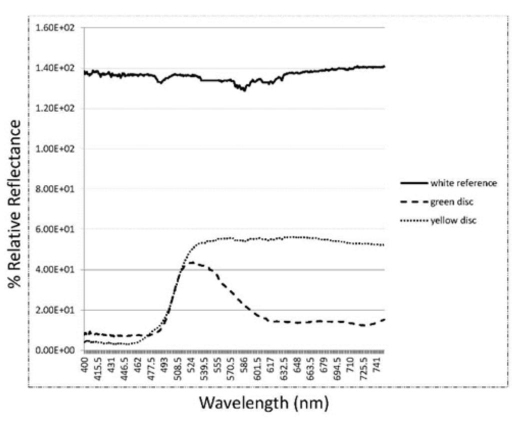

Fig. 1. Spectral reflectance curves of green and yellow colors used in olfactometer tests.

At the start of each 10 minute trial, one female whitefly was removed from the holding cage and gently placed through the olfactometer door using an aspirator attached to a black straw equipped with a plastic mesh screen. The positive (upwind) or negative (downwind) position of the whitefly was recorded at the end of each trial. Trials were ended before 10 minutes if the insects traveled more than 20 cm in either positive or negative direction. The olfactometer was cleaned with soapy water between uses with different treatments.

Olfactometer Experiment 2. Behavioral data was collected using EthoVision® (Version 7) tracking software (Noldus Information Technology Inc., Leesburg, VA). We constructed a chamber from plexiglass (3 cm high, 3 cm wide, 28 cm long) (Tap Plastics, Oakland, CA) in order to film whitefly behavior. A plexiglass wall with a 9 mm aperture was placed 10 cm into the device to direct airflow down the center of the chamber. The end of the chamber was covered with a removable piece of mesh fabric. A door was made by gluing a micro centrifuge tube into a hole on top of the chamber in the center of the filming arena (9 cm) (Fig. 2). These trials were filmed with a Sony DCR-SR68 Handycam (Sony Corp, Tokyo) mounted on a tripod 48 cm above the arena. The air source, filtration and flow meters attached to this arena were the same as in Experiment 1, except that only one chamber was used to hold plants. Air flowed at a rate of 0.25 LPM. Small cucumber plants (25-30 cm) were used as an attractive odor source and air flowed over these plants in treatments both with and without potentially repellent oils. Repellent oils were applied to filter paper (9 cm diam.)

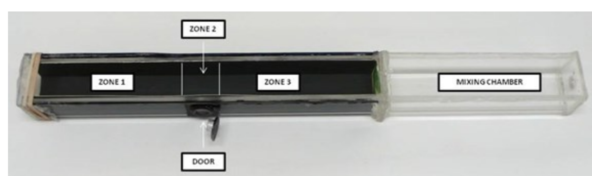

Fig. 2. Modified olfactometer used in Experiment 2 showing the mixing chamber, microcentrifuge tube that served as a closable door, and organza cloth screen. Zone 1 was located farthest from the odor source and was in the last 8 cm of the chamber; Zone 2 was located in the 2 cm around the door of the chamber; and Zone 3 was located near the odor source in the first 8 cm of the chamber.

inside a plastic petri dish (9 cm diam, 1.3 cm height) placed inside the chamber with the plant. Treatments included hot pepper wax (Pepper), mustard oil (Mustard), and garlic oil (Garlic). Oil was applied at label recommended rates – garlic oil (11%), hot pepper wax oil (3%), and mustard oil (3%) (Legaspi and

Simmons 2012). The controls were cucumber plants alone (Control) without the repellent oil and an empty chamber with no plant odor or plant oil odor (Clean Air).

At the start of each trial female whiteflies were placed inside the chamber door and their behavior was filmed for 10 minutes. Trials were ended if the insect traveled all the way to either end (upwind or downwind) of the olfactometer. Films were edited using Power Director Pro. X and Y coordinates from whitefly movement throughout the trials were obtained with Ethovision allowing movement tracks to be calculated. From these tracks calculations were made for length of trial, position at the end of trial, positive movement, negative movement, mean speed (cm/s), and distance (cm).

Movement was measured in three zones in the chamber: Zone 1 was located the farthest from the odor source and was in the last 8 cm of the chamber, Zone 2 was located in the 2 cm around the door of the chamber, Zone 3 was located near the odor source in the first 8 cm of the chamber (Fig. 2).

Statistical analysis. In experiment 1, One-way Analysis of Variance (ANOVA) tests were performed on distance traveled by the whitefly adult as affected by host plant and color. In Experiment 2, the effects of oil treatments on distance traveled and whitefly speed were analyzed using 1-way ANOVA. Each treatment and control were analyzed separately for the effects on total duration spent in each of the 3 experimental zones. Statistical analyzes were performed using SAS Software (SAS Institute Inc., Cary NC). Means were separated by LSD tests at $P = 0.05$.

RESULTS

Experiment 1. Whiteflies exposed to the color green both with and without cucumber odor, and whiteflies exposed to cucumber odor alone traveled significantly further upwind than whiteflies exposed to no color or odor stimuli ($P < .05$) (Table 1). Whiteflies exposed to yellow in this test did not travel up wind significantly more than insects exposed to air alone. When the odor of nemat arugula or giant red mustard was added to the stimuli of green color and cucumber, whiteflies showed a reduction of movement. When the odor from caliente mustard was added, whiteflies moved in a negative direction ($F = 3.48$; df = 8, 183; $R^2 = 0.13$; $P < 0.001$).

Experiment 2. Total distance traversed by test insects was greater in the pepper, mustard oil treatments and cucumber control, than in the garlic and clean air treatments (Fig. 3) (F = 6.5; df = 4, 74; R2 = 0.26; P < 0.001). Here, insect traversal rate was measured directly.

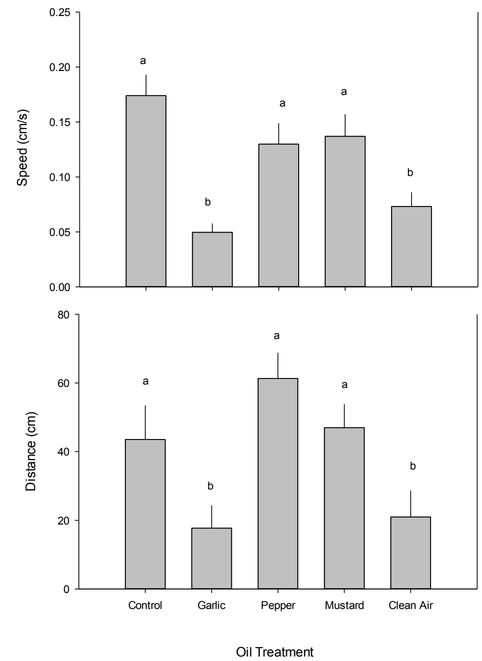

Fig. 3. Mean distance (cm) and mean speed (cm/s) of *Bemisia tabaci* under the following oil treatments: Cucumber plant odor (control), Garlic oil (garlic), hot pepper wax oil (pepper), mustard oil (mustard) and no plant odor (clean air). Different letters indicate that means were significantly different (One-way ANOVA; LSD test, $P = 0.05$).

As in distance traversed, insect speed was higher in the cucumber control, mustard and pepper treatments, than in the garlic and clean air (Fig. 3) (F = 8.5; df = 4, 78; R2 = 0.32; $P < 0.001$). Treatments that resulted in greatest distance traversed also showed highest rate of

Table 1. Experiment 1 – Mean distance (cm ± SE) traveled by *Bemisia tabaci* in a modified olfactometer under different disk color and plant odor treatments.

N	Disk color	Plant odor	Distance (cm ± SE)
22	green	cucumber	11.64 ± 1.96 a
20	green	none	11.25 ± 2.37 ab
20	none	cucumber	8.25 ± 3.53 ab
20	yellow	cucumber	3.9 ± 3.21 bcd
20	yellow	none	2.5 ± 2.31 cd
20	none	none	2.15 ± 2.28 cd
20	green	Cucumber + Nemat mustard	0.45 ± 3.27 d
30	green	Cucumber + GRM mustard	0.017 ± 2.16 d
20	green	Cucumber + Caliente mustard	-1.6 ± 2.66 d

N = number of replicates; GRM = giant red mustard; Different letters within the distance column indicate that means were significantly different (LSD test, Proc GLM, SAS, P = 0.05).

Table 2. Experiment 2 - Total durations (sec ± SE) in 3 different zones of a modified olfactometer under the different treatments: Garlic oil (Garlic), Hot pepper wax oil (Pepper), Mustard oil (Mustard). Controls were the following: Cucumber plant odor (Control) and no plant odor (Clean air). Zone 1 was located farthest from the odor source and was in the last 8 cm of the chamber; Zone 2 was located in the 2 cm around the door of the chamber; and Zone 3 was located near the odor source in the first 8 cm of the chamber.

Treatment	Zone 1	Zone 2	Zone 3	Statistics
Control (n=9)	175.0 ± 37.4b	51.2 ± 18.2b	318.8 ± 34.3 a	F = 18.5; df = 2, 24; R^2 = 0.61; P < 0.001
Garlic (n=20)	302.8 ± 49.2a	97.5 ± 24.9b	160.0 ± 47.9b	F = 6.2; df = 2, 57; R^2 = 0.18; P < 0.01
Pepper (n=16)	205.1 ± 38.2ab	108.1 ± 25.8b	265.0 ± 40.0a	F = 5.1; df = 2, 45; R^2 = 0.18; P < 0.05
Mustard (n=19)	308.4 ± 54.3a	103.1 ± 32.0b	180.6 ± 40.9b	F = 5.7; df = 2, 54; R^2 = 0.17; P < 0.01
Clean air (n=15)	171.5 ± 54.5a	218.5 ± 48.8a	149.1 ± 53.7a	F = 0.5; df = 2, 42; R^2 = 0.02; P = 0.64

n = number of replicates; For each zone, total duration was analyzed by one-way ANOVA to compare between treatments and controls; Different letters within each column indicate that means were significantly different (LSD test, SAS, P = 0.05).

movement.

Time allocations in the three experimental zones are summarized in Table 2. Test whiteflies in the cucumber control and hot pepper wax spent significantly more time in Zone 3, closest to the odor source, suggesting attraction to the odor. Insects spent more time in Zone 1, farthest from the odor source, in the garlic and mustard treatments, suggesting repellency. Finally, whiteflies were equally distributed across all three zones in the clean air control, indicating no directional movement.

DISCUSSION

Oil extracts can be important pest repellents or oviposition deterrents against *Bemisia tabaci* (Zhang et al. 2004, Al-mazra'awi and Ateyyat 2009, Yang et al. 2010). Cottonseed oil repelled sweetpotato whitefly adults for up to 9 days when applied to cotton, squash, lettuce, and carrot seedlings (Butler et al. 1989). In greenhouse and laboratory tests, bifenthrin and Sunspray oil repelled *B. tabaci* adults for up to 7 and 5 days, respectively, followed by the insecticidal soap “M-Pede” and extract of *Nicotiana gossei*. A Garlic Barrier treatment was not significantly different from the water control (Liu and Stansly 1995). Bioassays for repellency and oviposition deterrence in essential oils from the leaves of several plants indicated that extract from *Plectranthus neochilus* Schltr.(“lobster flower”) was most effective against *B. tabaci* in tomato (Baldin et al. 2013).

Host location in whiteflies is largely dependent on visual cues, but olfactory stimuli are also important (Bleeker et al. 2009). Whiteflies are weak fliers, but are also capable of direct, active flight (Byrne 1999). Therefore, olfactometer experiments can be useful in assessing the potential of candidate repellent oils, plant extracts or whole plant in designing “push-pull” tactics against whiteflies. Legaspi et al. (2011) demonstrated possible repellent effects of mustard (*Brassica juncea* (L.) Czern) volatiles to whiteflies through olfactometer studies. Planting mustard as a companion crop with collards (*Brassica oleracea* L. var. acephala) in outdoor experiments resulted in reduced whitefly landings, but not in reduced oviposition on the collards. Y-tube olfactometer experiments showed some attraction of *B. tabaci* to cucumber extract, as well as repellency to asparagus lettuce (Zhao et al. 2014). Using a vertical olfactometer, Zhang et al. (2004) found that increasing concentrations of ginger oil resulted in increased repellency over a distance of 1 – 2 mm and at concentrations >0.5%.

Olfactometer experiments using whole mustard plants showed evidence of repellency against adult whitefly females, as well as reduced landings in collards intercropped with mustard (Legaspi et al. 2011). However, the reduced landings did not translate into lower oviposition on the collards (Legaspi et al. 2011). In a separate greenhouse study on collard and mustard planted together on the same pots, the proximity of the mustard seemed to reduce egg counts on the adjacent collard plant, and field counts of whitefly eggs were lower on mustard compared to collards and other field crops (Legaspi 2010). However, the use of mustard crop transects did not appear to reduce whitefly oviposition in nearby crops. These previous studies suggest that volatiles from whole mustard plants have a weak repellent effect on the whiteflies which often does not result in measurable reduction in oviposition on an economic crop, thereby indicating the need for complementary tactics, such as commercial extracts.

In these experiments, a key distinction must be made between distance measurements. In Experiment 1, distance was measured away from the odor source during the test duration, whereas in Experiment 2, total distance traversed was measured, regardless of directional movement towards or away from the odor source. Instead, total time near the source or away from it were measured using the Zone designations. The hot pepper wax and mustard oil treatments, together with the cucumber control elicited greatest movement in the test insects. Movement was directional towards the odor source in the hot pepper wax treatment and cucumber control, and away from it in the mustard oil treatment. The garlic oil treatment and clean air control showed lowest rates of insect movement and total distance traversed, but differed in that movement in the garlic oil treatment was directional away from the odor source, whereas the clean air control resulted in non-directional movement.

In summary, Experiment 1 showed measurable movement of whitefly adults towards attractants, such as cucumber plants and the color green, and movement away from mustard (Caliente and Giant red mustard varieties) and arugula (Nemat var.) plants. Experiment 2 showed highest total distances traversed and rate of insect movement in hot pepper wax, mustard oil and the cucumber controls. Time allocation analysis indicates attraction towards the cucumber control and hot pepper wax, as well as aversion to garlic oil and mustard oil. The clean air control elicited no directional movement. Findings reported here are in general agreement with previous greenhouse studies indicating repellency in mustard plants (Legaspi 2010) and mustard and garlic oils (Legaspi and Simmons 2012), and provided stronger evidence of repellency than earlier experiments using traditional Y-tube olfactometers (Legaspi et al. 2011). These studies indicated mustard and mustard oil for use as "push" components in "push-pull" management. Present results suggest garlic oil may also be a promising candidate. These results are similar to those conducted on *B. tabaci* using choice and no-choice experiments testing mustard, garlic, and hot pepper wax vs. water alone (Legaspi and Simmons 2012).

Traditional Y-tube olfactometer studies have proven very useful in measuring attractive responses of insects to volatiles. However, the use of olfactometers to measure repellent responses is problematic (Legaspi et al. 2011). Previous olfactometer studies that measured repellency to volatiles used different approaches. Zhang et al. (2004) used a vertical olfactometer, taking advantage of the natural tendency of whiteflies to move upwards due to positive phototaxis. Zhao et al. (2014) used a standard Y-tube olfactometer, but measured repellency in differential movements towards the volatile treatments in the two olfactometer branches. Here, we suggest modifications to the Y-tube olfactometer and the use of behavioral analysis software as alternative solutions.

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