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Ambrosia Beetle Diversity, Vector Potential and Response to Solarization Treatments Associated with Sassafras Trees Killed by Laurel Wilt

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Abstract

Laurel wilt is a plant disease caused by the fungus *Harringtonia lauricola* and vectored by the invasive red bay ambrosia beetle (*Xyleborus glabaratus*). Laurel wilt affects trees in the Lauraceae family and has caused extensive mortality of redbay (*Persea borbonia*) forests in the southeastern US. The disease is currently spreading north on sassafras (*Sassafras albidum*), which is also in the Lauraceae family. Other species of ambrosia beetles colonize dead host trees and are able to carry the *H. lauricola* fungus. My study investigated the presence of ambrosia beetles and *H. lauricola* in sassafras trees in an area recently infected with laurel wilt. I also evaluated the use of solarization (covering infested material with plastic sheeting to kill insects and pathogens with heat), as a potential method for sanitizing infested sassafras. Emerging ambrosia beetles were collected from felled sassafras trees killed by laurel wilt. The diversity of beetle communities found within sassafras trees was compared to that captured in funnel traps, and live beetles that emerged from sassafras logs were tested for *H. lauricola*. To evaluate solarization, infested material was wrapped in plastic sheeting and left in the sun. After one month, beetle emergence from solarized logs was compared to emergence from controls. We did not recover any redbay ambrosia beetles in funnel traps or within felled trees. The overall ambrosia beetle community in sassafras logs was more diverse than in funnel traps, but seasonality is likely a key factor in this finding. The pathogen *H. lauricola* was recovered from several ambrosia beetle species, including species not previously documented as carrying the fungus. Solarization reduced beetle emergence, but not significantly. Improvements on methodology are discussed as a way to further evaluate solarization as a viable method for sanitization.

1. Introduction

Laurel wilt is an infectious plant disease affecting trees in the Lauraceae family, and is caused by the fungus *Harringtonia lauricola*, a symbiont of the invasive red bay ambrosia beetle (RAB, *Xyleborus glabaratus*). RAB and other ambrosia beetles colonize dead or weakened trees and live within the sapwood, feeding on fungi transported in specialized mouthparts. In the native range of the RAB in Asia, this process usually does not occur in live trees. In laurels of North America, however, the RAB attacks live, healthy trees. Once a tree is infected, *H. lauricola* spreads in the vascular system, and the tree's immune response leads to restriction of water flow, causing the leaf wilting and death within weeks (NCFS 2022).

Laurel wilt has been most destructive in the southeastern US where it affects redbay (*Persea borbonia*) and swampbay (*Persea palustris*) forests. These forests exhibit a high density of host trees in a climate similar to Southeast Asia, where RAB originates (Fraedrich et al. 2008). Laurel wilt also affects avocado (*Persea americana*), but the damage is not as severe as in other *Persea* spp. (Ploetz et al. 2017, Olatinwo et al. 2021). Laurel wilt has killed hundreds of millions of trees in twelve states in the southeastern US (Fig 1, Olatinwo et al. 2021, Ward and Riggins 2023). Furthermore, it is spreading to northern areas without redbay, swampbay, or avocado because it is able to colonize sassafras trees (*Sassafras albidum*, Fig. 2, Ward and Riggins 2023).

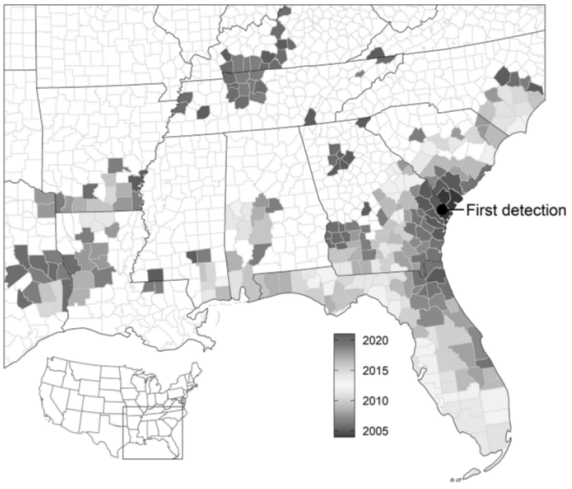

Figure 1. Counties documented with laurel wilt (Olatinwo et. al 2021)

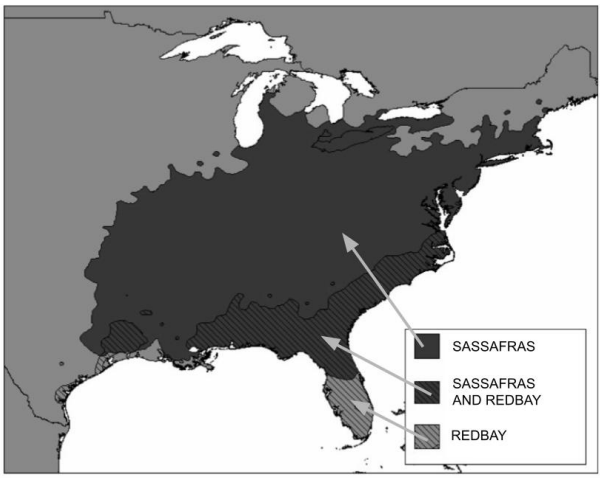

Figure 2. Range of sassafras and redbay trees (Ward and Riggins 2023)

Sassafras is an early successional tree that has been described as having limited commercial and ecological value (Sullivan 1993). Its commercial usage is mostly in furniture and safrole oil which serves as a scented additive to a variety of products. Ecologically, sassafras twigs are important to deer browsing. Several species of bird also consume the fruits, although in low quantities (Sullivan 1993, Olatinwo et. al 2021).

Cold tolerance tests suggest that RAB could survive in > 90% of the geographic area where sassafras is found. However, an average temperature increase of 1.5° C by 2050 would lead to 99% of the range of sassafras not experiencing winter temperatures cold enough to kill RAB (Formby et al. 2017). Chemical treatments of laurel wilt on redbay and avocado have shown limited success outside of greenhouse experiments (Olwatinwo et al 2021) so management has focused on monitoring and prevention through public awareness. An additional factor in management is the abundance of other species of ambrosia beetles, which can vector *H. lauricola* in redbay and avocado (Carillo et al. 2014). While these species do not normally carry *H. lauricola* as their fungal symbiont, they are able to obtain and spread the fungus after being in contact with trees infected with laurel wilt. This is worrisome, not only because it demonstrates greater vector potential but because it suggests the retention of dead trees in the environment may play a role in spreading laurel wilt.

One of the major causes of the noncontiguous spread of laurel wilt is the movement of infested firewood (Olatinwo et al. 2021), as *H. lauricola* has been recovered in standing dead redbay trees up to 14 months after infection (Spence et al. 2013). These findings highlight the need for monitoring and testing the presence of ambrosia beetles and the *H. lauricola* fungus on trees that have already been killed by laurel wilt, as vector potential may still exist. Furthermore, dead and infested material needs proper treatment or sanitization to limit the spread. Effective methods of sanitizing infested material remain limited. Wood chipping has shown success but can be cumbersome and costly. Solarization is a method of sanitization by covering infested material in plastic sheeting. In Southern California, solarization has yielded significant mortality of ambrosia beetles in boxelder (*Acer negundo*) and live oak (*Quercus virginiana*, Jones and Paine 2015) and applying this methodology to ambrosia beetles in sassafras may help limit the spread of laurel wilt in the southeastern US.

Much of the research regarding laurel wilt has been performed in avocado and redbay but not replicated in sassafras, a species crucial to managing the continued spread. Our study objectives were to: (i) Determine if funnel trap catches are representative of ambrosia beetle diversity found in sassafras trees, (ii) Evaluate the presence of the laurel wilt pathogen on ambrosia beetles emerging from sassafras, and (iii) Evaluate solarization as a potential method of sanitizing trees infected by laurel wilt.

2. Materials and Methods

2.1 Ambrosia Beetle Comparison

Five sassafras trees infected with laurel wilt were felled on 1 September 2022 at Panther Creek State Park in Hamblen County, Tennessee. This field site was selected as it has shown recent detections of laurel wilt in 2019 and RAB was detected in low levels in August 2022 (Mayfield, unpublished data, USFS 2022) Browning foliage, signs of RAB bore holes, and discolored xylem were used as symptoms to recognize trees with laurel wilt. Trees were cut down via chainsaw and divided into four 1-m long sections. Each 1-m section was cut into three bolts ranging in length from 27 to 33 cm.

The five trees produced a total of 60 bolts. Bolts from each section were randomly assigned to one of three treatment categories: no treatment (control), solarization treatment, and field control.

Two funnel traps (Fig. 3) were placed 10 m from each of the five sassafras stumps and left from 15 September to 27 October 2022. Traps were baited with a 50% alpha-copaene bubble lure (Synergy Semiochemicals Corp. Lot # 210816), and collection cups were filled with a propylene-glycol solution as a preservative. Beetles were collected from funnel traps three times, at two-week intervals.

2.2 Recovery of *H. lauricola* fungus

Live subsamples of emerged ambrosia beetles in all three treatments were shipped overnight to a pathology lab (USFS SRS, Athens GA) where DNA detection was used to identify *H. lauricola* on the beetle samples. In this process, each beetle sample was surface-disinfected in 20 µl of 70% ethanol for 15 s, rinsed with sterile distilled water, and blotted dry on a clean filter paper. Individual beetles were transferred into a 1.5 ml microcentrifuge tube containing 100 µl sterile distilled water and macerated into a mixture with a sterile plastic rod. Fungal DNA was extracted from the mixture using the DNeasy Qiagen Plant Mini Kit, according to the manufacture protocol. Extracted DNA for each sample was used as a template in polymerase chain reaction (PCR). The presence of *H. lauricola* in DNA samples was confirmed by positive PCR amplification of the large subunit ribosomal RNA gene region using species-specific primers (Olatinwo, et al. 2021).

2.3 Solarization of Sassafras Bolts

In the field, sassafras bolts assigned to solarization and field control treatments were stacked in ten groups of four bolts. Five groups (solarized) were covered in translucent sheet plastic and left outdoors in direct sunlight for five weeks (1 September to 6 October 2022). The other five groups (field control) were left without plastic sheeting for the same time period. Bolts of sassafras used to monitor for emergence were kept in a climate-controlled room in insect rearing buckets. Adult ambrosia beetles that emerged into collection vessels were identified to species and tallied. Beetles in the ‘no treatment’ bolts were collected from 1 September 2022 to 21 January 2023. Beetles from solarized and field control bolts were collected from 6 October 2022 to 21 January 2023.

Figure 3: Example of a funnel trap (WITASEK 2023)

Figure 4. Stacks of solarized and field control sassafras bolts in our field site collected from sassafras (felled 9/1/2022)

The species composition of beetles collected in funnel traps was compared with that of beetles emerging from sassafras trees using species abundance, richness, and Simpson's Diversity Index (1-D). To further evaluate differences in the solarization treatment, a mixed model ANOVA was used to test the null hypothesis that emerged beetles per square centimeter of wood did not differ by treatment. Solarization treatment was considered a main effect while tree number was modeled as a random effect. Analyses were run using the Fit Model procedure in JMP® 14.0.0 (SAS Institute Inc., 2018) using a Standard Least Squares approach. *F*-tests for fixed effects were considered significant when $P < 0.05$ and means separation was performed using Student's t-test.

3. Results

3.1 Ambrosia Beetle Comparison

No redbay ambrosia beetles were found in sassafras bolts or funnel traps. A total of 1635 beetles emerged from sassafras bolts and 261 beetles were collected in funnel traps. Beetles in sassafras bolts showed higher Simpson's Index of diversity (0.69) than those collected from funnel traps (0.50). Beetle species richness in sassafras bolts was

25% greater than in funnel traps (Table 1). Three species (*Xylosandrus crassiusculus*, *Xyleborus affinis*, and *Euwallacea validus/interjectus*) made up 88% of beetles in sassafras bolts while two species (*Xylosandrus crassiusculus* and *Xyleborinus saxesenii*) made up > 90% of beetles in funnel traps. Funnel traps documented two species not seen in sassafras bolts, but lacked three species that were present in sassafras bolts. *Xyleborus affinis* made up a third of emerged beetles in sassafras bolts but < 1% beetles in funnel traps. *Xyleborinus saxesenii* made up 27% of funnel trap catches but < 5% of emerged beetles. The number of beetles caught in funnel traps dropped significantly after the first collection (Fig 5).

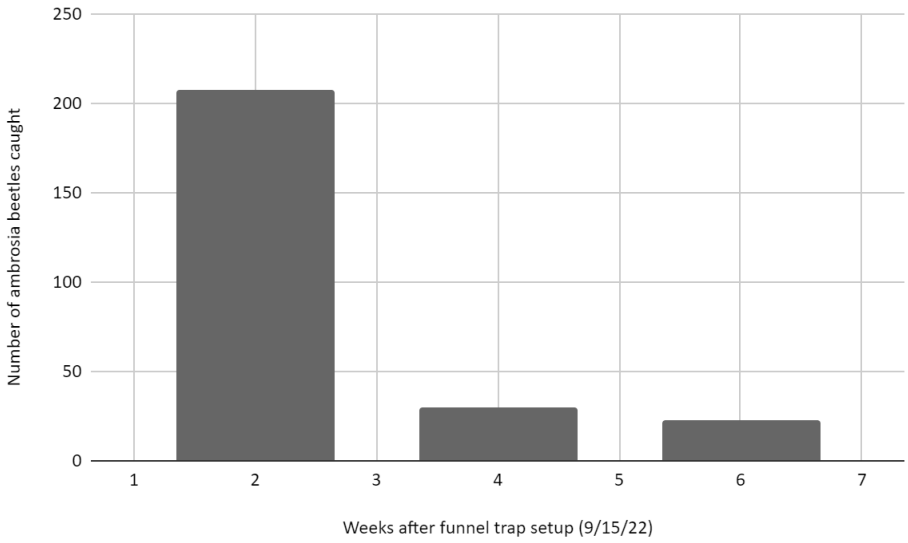

Figure 5. Ambrosia beetles from funnel traps placed 9/15/22, Hamblen County, TN

Table 1. Comparison of emerged ambrosia beetle composition from sassafras bolts (felled 9/1/22) and beetles in funnel traps (9/22-10/22), Hamblen County, TN.

	Sassafras Bolts		Funnel Traps	
Species	Number	Percent	Number	Percent
Xylosandrus crassiusculus	614	37.6	170	65.1
Xyleborus affinis	536	32.8	1	0.4
Euwallacea validus/interjectus	291	17.8	-	-
Xyleborinus saxesenii	69	4.2	71	27.2
Dryoxylon onoharaense	44	2.7	4	1.5
Monarthrum mali	22	1.3	7	2.7
Xylosandrus germanus	20	1.2	1	0.4
Monarthrum fasciatum	15	0.9	-	-
Xyleborus ferrugineus	14	0.9	4	1.5
Euplatypus compositus	7	0.4	-	-
Pityopthorus sp.	2	0.1	-	-
Cyclorhipidion bodoanum	1	0.1	-	-
Hylocuris rudis	-	-	2	0.8
Cnestus mutilatus	-	-	1	0.4
Total	1635	100.0	261	100.0
Species Richness	12		9	
Simpson's Index of Diversity (1-D)	0.69		0.50	

3.2 Recovery of *H. lauricola* fungus

A total of 72 live ambrosia beetles across nine species that emerged from sassafras bolts were tested for *H. lauricola*. Thirty percent of beetles across six species tested positive for carrying the fungus (Table 2). The three most common beetle species averaged 28% positive for *H. lauricola*. Three species that did not test positive had a sample size of less than five individuals. Three species tested positive that had not been previously known to carry *H. lauricola* (*E. validus/interjectus*, *Monarthrum fasciatum,* and *Dryoxylon onoharaense*).

Table 2. Recovery of laurel wilt pathogen, *Harringonia lauricola*, from ambrosia beetles emerged from sassafras bolts (felled 9/1/22) Hamblen County, TN.

Species	No. specimens evaluated	% positive for *H. lauricola*
Xyleborus affinis	29	41
Xylosandrus crassiusculus	17	24
Xyleborinus saxesenii	10	20
*Euwallacea validus/interjectus**	6	17
*Monarthrum fasciatum**	3	33
*Dryoxylon onoharaense**	2	100
Xyleborus ferrugineus	1	0
Xylosandrus germanus	3	0
Monarthrum mali	1	0
Total	72	31
* Not previously reported in literature in association with *H. lauricola*		

3.3 Solarization of Sassafras Bolts

Sassafras bolts that were solarized for five weeks showed lower beetle emergence than bolts left in the field for five weeks without plastic sheeting (field control) and bolts immediately placed in emergence buckets indoors (no treatment). The difference in emergence between the ‘no treatment’ group and the ‘solarized’ and ‘field control’ groups was significant (P=0.004). Differences in beetle emergence between the ‘solarized’ and the ‘field control’ group were not statistically significant (P=0.265, Fig 6). Because there was a significant difference between the no treatment group and the other two groups left in the field to test solarization, we reject the null hypothesis that emerged beetles per square centimeter did not differ by treatment. However, without significant differences between the solarized and field control group, we cannot conclude that solarization was an effective form of sanitation.

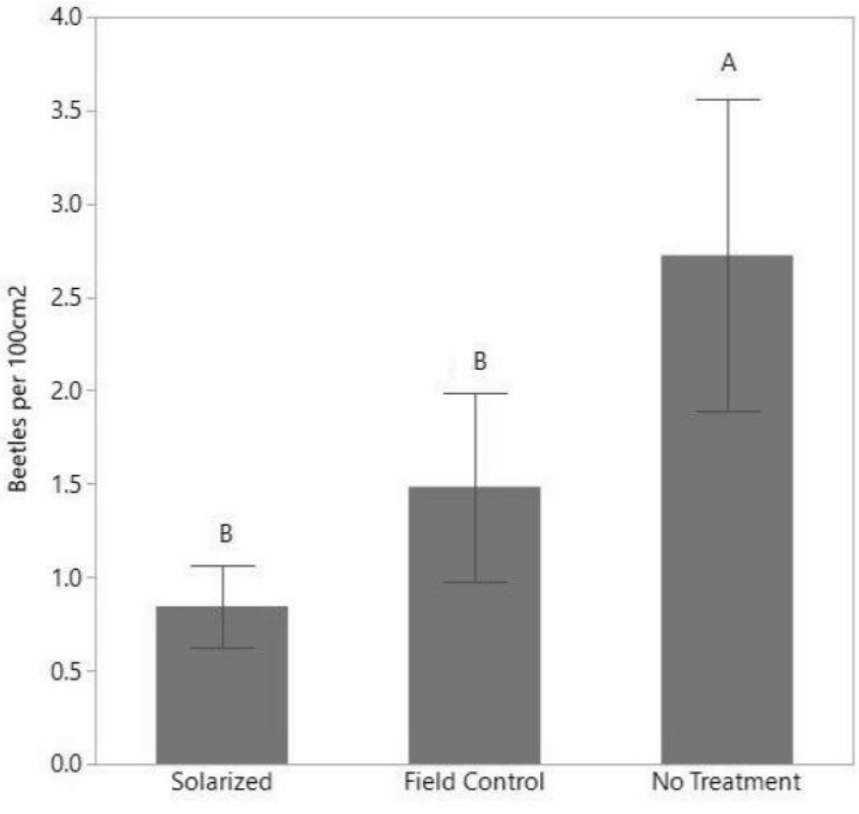

Figure 6. Mean ambrosia beetle density across three treatments in sassafras trees affected with laurel wilt (felled 9/1/22) Hamblen County, TN. Bars with the same letters do not differ significantly ($P > 0.05$).

4. Discussion

4.1 Ambrosia Beetle Comparison

Monitoring ambrosia beetle populations and quantifying RAB presence involves comparing funnel trap catches in the field to beetle communities emerging from infested material. In our study, we expected little RAB presence in infected sassafras bolts, as RAB selects healthy hosts while other species of ambrosia beetle colonize trees after initial RAB invasion and infection of laurel wilt (Huges et al. 2015). As such, funnel traps were an important method for quantifying RAB presence. We did not find any RAB in alpha-copaene baited funnel traps set out from 15 September to 27 October 2022 or in infected sassafras trees felled on 1 September 2022. Dates are critical in this result. Research into seasonality of RAB by Crane et. al (2011) shows peak RAB funnel patterns occur from June to October in Florida. Cooler temperatures at our field site would indicate that traps set in September were likely at the tail end of RAB seasonality.

In our funnel traps, 75% of catches occurred in the first two weeks with a sharp dropoff in subsequent weeks. Funnel traps baited with alpha-copaene have been shown to be the most effective form of RAB capture, however, their use in early stages of an epidemic is shown to be limited as these traps do not pull beetles from a large area and finding RAB has been shown to be difficult in areas without severe laurel wilt presence (Hanula et al. 2016) Emerged beetles from felled sassafras seemed to be a better indicator of ambrosia beetle populations, as those counts yielded higher species richness and diversity than we found in funnel traps. Our results for no presence of RAB must be regarded with caution as this experiment did not take place during peak ambrosia beetle seasonality.

4.2 Recovery of *H. lauricola* Fungus

The presence of *H. lauricola* has been documented in several species of ambrosia beetle recovered from redbay and avocado (Carillo et al. 2013, Ploetz et al. 2017, Cruz et al. 2021). We recovered *H. lauricola* from six out of nine beetle species in sassafras. Ploetz et al. (2017) reported that *H. lauricola* was recovered from 34% of beetles in redbay but only 6% in avocado. In our study, we recovered *H. lauricola* from 31% of our beetles, suggesting mechanisms of *H. lauricola* transfer from RAB to other ambrosia beetle species may be similar in redbay and sassafras.

We recovered *H. lauricola* from three beetle species that had not been previously documented as carrying the *H. lauricola* fungus: *E. validus/interjectus*, *M. fasciatum, and D. onoharaense* (Carillo et al. 2013, Ploetz et al. 2017, Cruz et al. 2021). These three species made up < 2% of beetles captured from funnel traps, highlighting the importance of monitoring infested material in addition to funnel traps for an accurate assessment of *H. lauricola* presence. Although the fungus was documented, it is not confirmed that these beetle species are able to vector laurel wilt into healthy sassafras trees. Carillo et. al (2014) determined that it is possible for other ambrosia beetle species, including four found in our experiment (*Xyleborus affinis*, *Xyleborus ferrugineus Xyleborinus saxesenii*, and *Xylosandrus crassiusculus*), to act as vectors to healthy redbay trees. Carillo et al. (2014) performed this experiment in a greenhouse under no-choice conditions that only allowed ambrosia beetles to colonize healthy trees. Ambrosia beetles other than RAB do not select healthy trees in field conditions. Therefore, we cannot conclude that ambrosia beetles carrying *H. lauricola* are acting as independent vectors or that the infested material they colonize is contributing to the spread of laurel wilt on sassafras. Regardless, the presence of the fungus on local ambrosia beetle species heightens the need for public awareness in regards to the movement of infested material, and the need for better sanitation methods.

4.3 Solarization of Sassafras Bolts

In our solarization experiment, beetle emergence was lower in the solarized group after five weeks, although this difference was not significant. Again, seasonality is an important factor in this result. Bolts were solarized on 1 September 2022, after

temperatures had begun to fall. Temperature data loggers showed a peak temperature of 38° C in solarized groups. A review by Jones and Paine (2015) of solarization treatments in bark beetles documents that temperatures need to reach >45° C to significantly reduce beetle survival. Negron et al. (2001) highlights the importance of seasonality and limiting bolt stacking, suggesting a single layer of bolts would yield highest temperatures. Our temperature logs were placed in between layers of bolts. Since our experiment took place during a season when temperatures did not get high enough to solarize properly, we cannot draw conclusions from our results.

5. Summary and Conclusion

This study documented differences in the ambrosia beetle communities collected in funnel traps in the field and those emerging from sassafras trees killed by laurel wilt. We found that funnel traps did not fully represent the species of ambrosia beetle that were carrying the *H. lauricola* fungus. Ambrosia beetles within sassafras trees killed by laurel wilt were carrying the *H. lauricola* fungus, including three species that had not previously been documented as doing so. The potential for these species to act as vectors of laurel wilt remains unknown. Solarization of infested sassafras did not yield definitive results. Ambrosia beetle populations and their response to solarization are heavily influenced by seasonality, so replication during summer months would yield more representative results. Our research has highlighted the need for continuous monitoring of *H. lauricola* and local ambrosia communities in sassafras to help manage the spread of laurel wilt. Limiting the spread involves identifying laurel wilt in its early stages of infestation and sanitizing infected wood in an efficient way in order to reduce vector populations.

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