Viet Nam exploration Feb 9th- March 2nd, 2014

Foreign exploration for the Polyphagous Shot Hole Borer and associated fungi in Viet Nam (Feb 9th- March 2nd, 2014)

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Introduction

The polyphagous shot hole borer (*Euwallacea fornicatus*) is an invasive species in California that has been present at least since 2003. It is an ambrosia beetle that lives in close symbiosis with several fungi on which both the adults and the offspring feed. The pest status of this insect and associated fungi was not realized until in 2012 a backyard avocado tree in South Gate was found to be infected by both beetle and its fungi by Dr. A. Eskalen (Department of Plant Pathology and Microbiology, UCR). Using DNA finger prints Dr. R. Stouthamer's lab at UCR determined that the PSHB invading California shared a DNA finger print with the invasion that was taking place in Israel where this beetle/fungus combination caused severe damage to avocado. In addition the DNA finger printing showed that the beetle in California and Israel differed from known *E. fornicatus* populations found in Hawaii, Florida, Sri Lanka, southern Thailand, Indonesia and Australia. The difference in DNA sequences between the California invasion and the beetles found in the aforementioned locations was such that it would be highly unlikely that they were the same species. The only collections that we had that showed larger similarity but not identity with our beetle came from the northern part of Thailand. Subsequently, additional samples showed that our beetle also occurred in Okinawa (Japan) but again these beetles were not identical to ours. It was not until we received a large sample from Viet Nam that we found beetles having DNA fingerprints identical to ours, in addition to beetles with slight variants of the DNA finger print were found in Viet Nam was well, indicating that Viet Nam most likely belong to the native area of this beetle. Native areas of insects will generally contain the largest amount of genetic variation in both beetle and its symbionts, but more importantly from our point of view, will also contain the most diverse natural enemy complex. Our cooperator in Viet Nam had already found several species of flies that he assumed were predaceous on the beetle.

In Viet Nam we were taken around by our cooperator Dr. Thu and people from his department of the Forest Protection Research Center of the Vietnamese Academy of Forest Sciences. Our trip in Viet Nam consisted of going out to different localities where the PSHB had been found by Dr. Thu and his team consisting mainly of Acacia and Cinnamon plantations, and upon our request areas where avocado was grown commercially. Our mode of action on these field trips would be to find PSHB infested trees, cut parts out of the tree, take them to the lab and dissect the log there to 1. Collect all insects and mites found in the galleries, and 2. Make fungal preparations from the galleries to determine the fungal composition and to collect potential antagonists to the harmful fungi that the beetle carries. Taking the logs apart meant cutting them first into smaller sections of about 4 inches lengths and followed by cutting those sections into smaller slices. This allowed us to look into the galleries of the beetles and to remove any insect or mite specimens that were present there.

Travel schedule:

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10-Feb	hanoi	arrived	activity
11-Feb	yen bai	Left for Yen Bai (N of Hanoi)in the morning	Visited Acacia and Cinnamon plantations, collected wood, also collected castor bean and tea for dissection
12-Feb	hanoi	Arrived back in Hanoi late at night	Vissited Acacia and Cinnamon plantations collected wood for dissection in Hanoi
13-Feb	hanoi	Lab work Hanoi	Dissected logs, collected insects and initiated fungal and bacterial colonies
14-Feb	ngoc lac	Left from Ngoc Lac (S of Hanoi) in the morning	Here the main intention was to look for castor beans, collected many logs
15-Feb	hanoi	Arrived back in Hanoi late at night	Again searched for Castor beans and went to a single Acacia plantation to collect logs. On the way back to Hanoi stopped to collect more castor bean
16-Feb	hanoi	Day off	
17-Feb	hanoi	Lab Work Hanoi	Dissected logs, collected insects and initiated fungal and bacterial colonies
18-Feb	hanoi	Lab Work Hanoi	Dissected logs, collected insects and initiated fungal and bacterial colonies
19-Feb	hanoi	Lab Work Hanoi	Dissected logs, collected insects and initiated fungal and bacterial colonies
20-Feb	tuy hoa	Left for Tuy Hoa (middle of Viet Nam on coast)	
21-Feb	tuy hoa	Field work Tuy Hoa	First day of field work consisted mainly of visiting Acacia plantations, and several avocado trees on a farm
22-Feb	tuy hoa	Field Work Tuy Hoa	Visited acacia plantations and a relatively young avocado grove,
23-Feb	tuy hoa	Day off	
24-Feb	hanoi	Left for Hanoi	
25-Feb	hanoi	Lab work Hanoi	Dissected logs, collected insects and initiated fungal and bacterial colonies
26-Feb	hanoi	Lab work Hanoi	Dissected logs, collected insects and initiated fungal and bacterial colonies
27-Feb	hanoi	Lab work Hanoi	Dissected logs, collected insects and checked fungal and bacterial colonies
28-Feb	hanoi	Lab work Hanoi	Dissected logs, collected insects and checked fungal and bacterial colonies, prepared insect specimen and fungal specimens preserved in ethanol for shipment home
1-Mar	leave	Left Hanoi for Korea	
2-Mar		Left Korea for LAX	

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Results:

We have collected around 450 samples of insects from the logs, the insects included many beetles, predaceous thrips, several species of mites, predaceous beetle larvae and fly larvae. In addition we received material collected by the cooperators of the potential natural enemies, 2-3 species of flies. We are in the process of identifying these species to determine if indeed they are predators. No evidence was found for nematode infection of the beetles but in several cases beetles were found in the galleries that had been attacked by fungal entomopathogens. These beetles were passed on to Akif for further isolation of the fungi and several cultures of the entomopathogens are maintained in Hanoi by our cooperator. Akif will identify these fungi from the ethanol preserved material he brought back. In total Akif made 142 fungal isolates from Acacia, castor bean, tea and avocado, 47 of them are *Fusarium euwallaceae*, 22 of them are *Graphium* sp. and seven of them are entomopathogenic fungus probably *Beauveria bassiana* and the rest of them are in the process of identification.

In one location near Tuy Hoa we found beetles in avocado as well. We visited two locations with avocado, one consisting of a new large plantation of avocado and, and the second location was a mixed farm near Tuy Hoa. In the latter location we found avocadoes that were attacked by an ambrosia beetle. Beetles and fungi were collected and once they are identified we will know if the beetle species is the same as the one invading CA.

The pattern of PSHB infestation that we found in Viet Nam was somewhat surprising. According to our host Dr. Thu, acacia had been grown in Viet Nam for about 12 years and only in the last 4 years or so the beetle had become a major pest in the acacia plantations. Even now when we went to the area around Tuy Hoa we could find acacia plantations there that were heavily infested yet some 5 km away there were plantations free of the beetle, similarly we found several castor bean plants in the region where we could not find any beetle. Also in Hanoi itself and in some of the smaller cities we visited we did not find much evidence for beetle infestation in street trees this in contrast to the situation here in southern California. In Vietnam the beetle appeared to be mostly a pest in plantations of tree species, such as acacia and cinnamon. There are several potential hypotheses that may explain the pattern found in Vietnam:

1. The beetle is invasive there as well and is not a native species there. Our genetic evidence is such that we believe the beetle is native in Vietnam and this refutes this hypothesis
2. The beetle is native but the fungal pathogen that is causing infections in the acacia trees is a new fungal form that may have invaded from up north (S- China) and therefore we would expect the infestation to be earlier and more severe closer to China than in parts further away. Although the infestation was relatively heavy up north and could explain the pattern found, infestations further south were also heavy.
3. The presence of a large number of host trees such as offered by the acacia plantations resulted in an adaptation by the fungal pathogens to the local host tree and this allows for a more rapid growth of the beetle population. It is not a single adaptation to the host plant but each local population of the beetle and fungus adapts to the abundant host and causes a similar infestation pressure.

We can test the hypothesis 2 and 3 by doing reciprocal transplants of beetles grown on one tree species on another, for instance beetles from castor bean on other castor beans and on box elder, and similarly box elder beetles on either box elder or on castor bean. In these experiments we would determine the offspring production of the beetles and the expectation would be that beetles would produce more offspring on the host on which they grew up than on the reciprocal host.

In southern California the beetle appears to be doing well on a range of host plants why this is the case versus what happens in Viet Nam is not clear. Potentially in Viet Nam there are other biotic factors that keep the beetle population limited to only those hosts occurring in plantations where the beetles have become well adapted to the particular host species, while here in California because of the lack of these biotic factors (such as for instance predators or entomopathogens) the beetles are able to invade additional hosts.

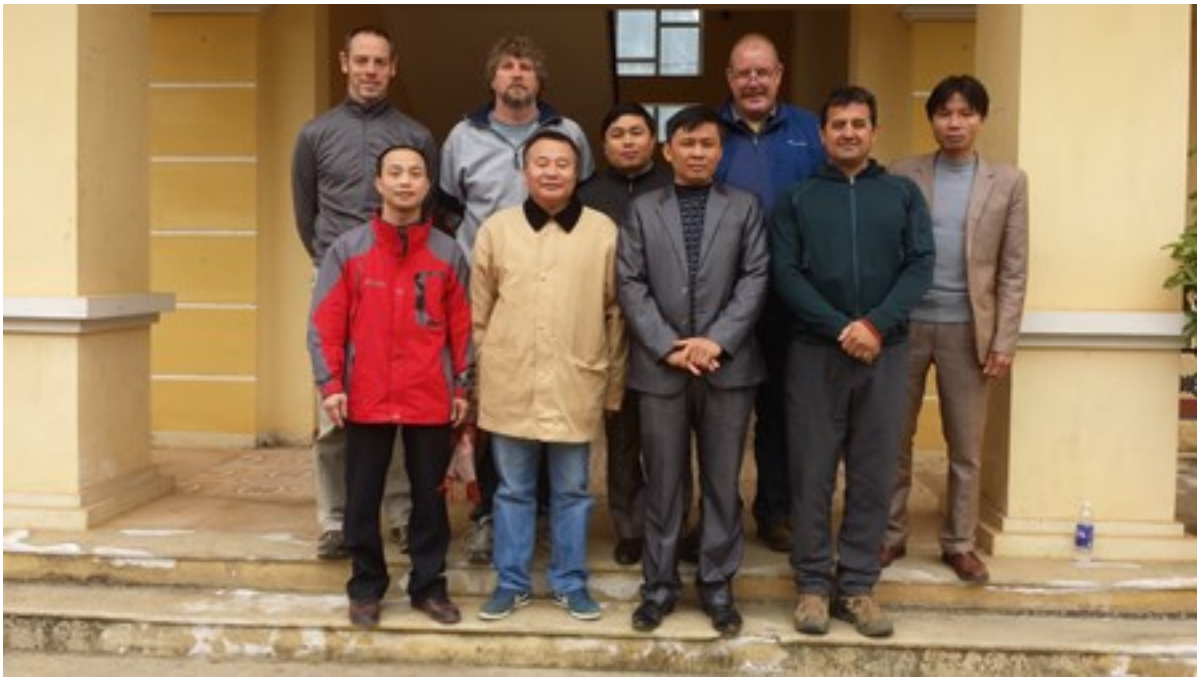

Figure 1. Photo taken during our visit to Agricultural school in Ngoc Lac. Back row (L toR) Paul Rugman-Jones (Entomology, UCR), Tim Thibault (Huntington Library and Gardens), School teacher, Richard Stouthamer (Entomology, UCR0, School teacher. Front row Dr. Quan (Vietnamese Academy of Forest Sciences), Dr. Thu (Vietnamese Academy of Forest Sciences), School teacher, Akif Eskalen (UCR, Plant Pathology and Microbiology)

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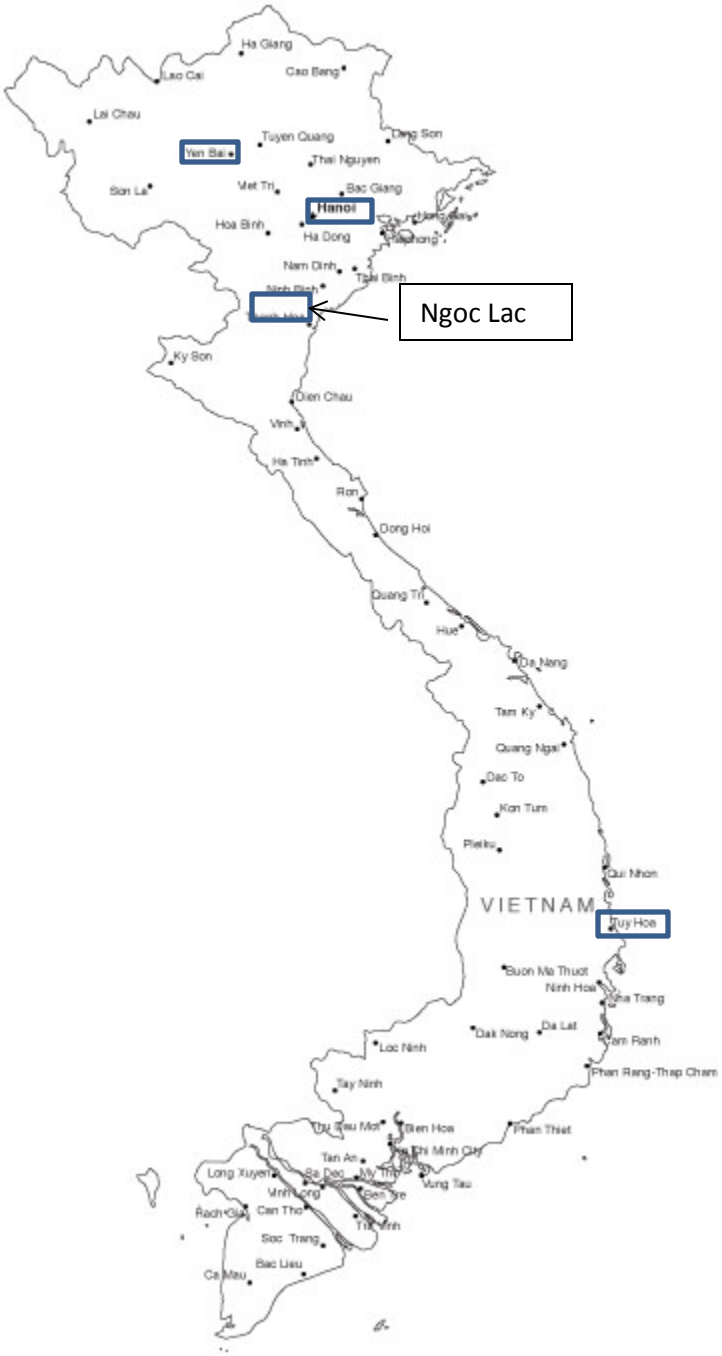

Figure 2. Locations visited during trip.

Figure 3. Acacia forest Yen Bai

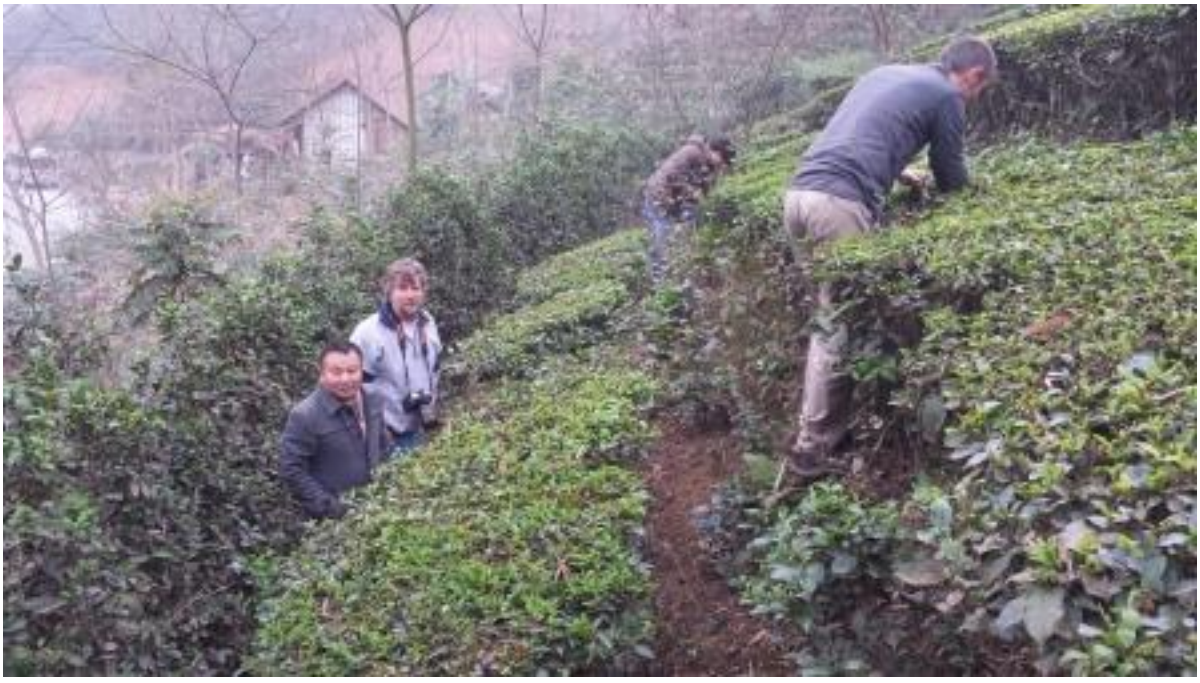

Figure 4. Collecting infested wood in a tea plantation in Yen Bai

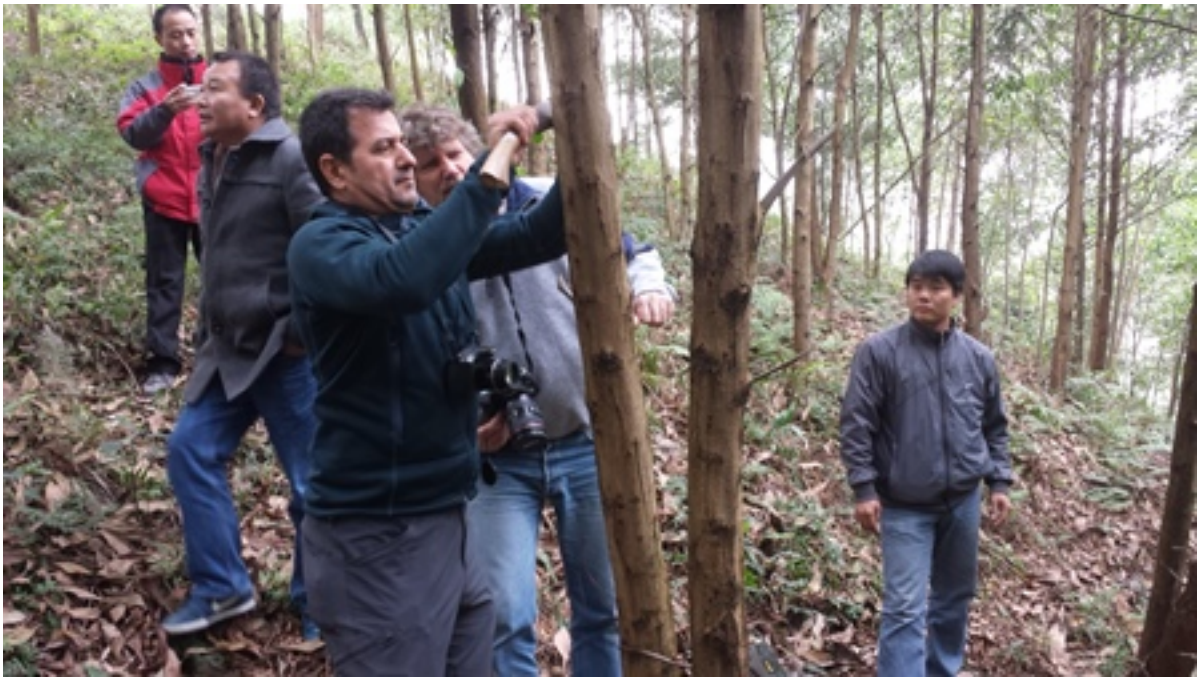

Figure 5. Collecting fungal samples from acacia in Yen Bai

Figure 6. Cross section of an acacia tree infested with a shot hole borer, galleries and fungal discoloration are visible.

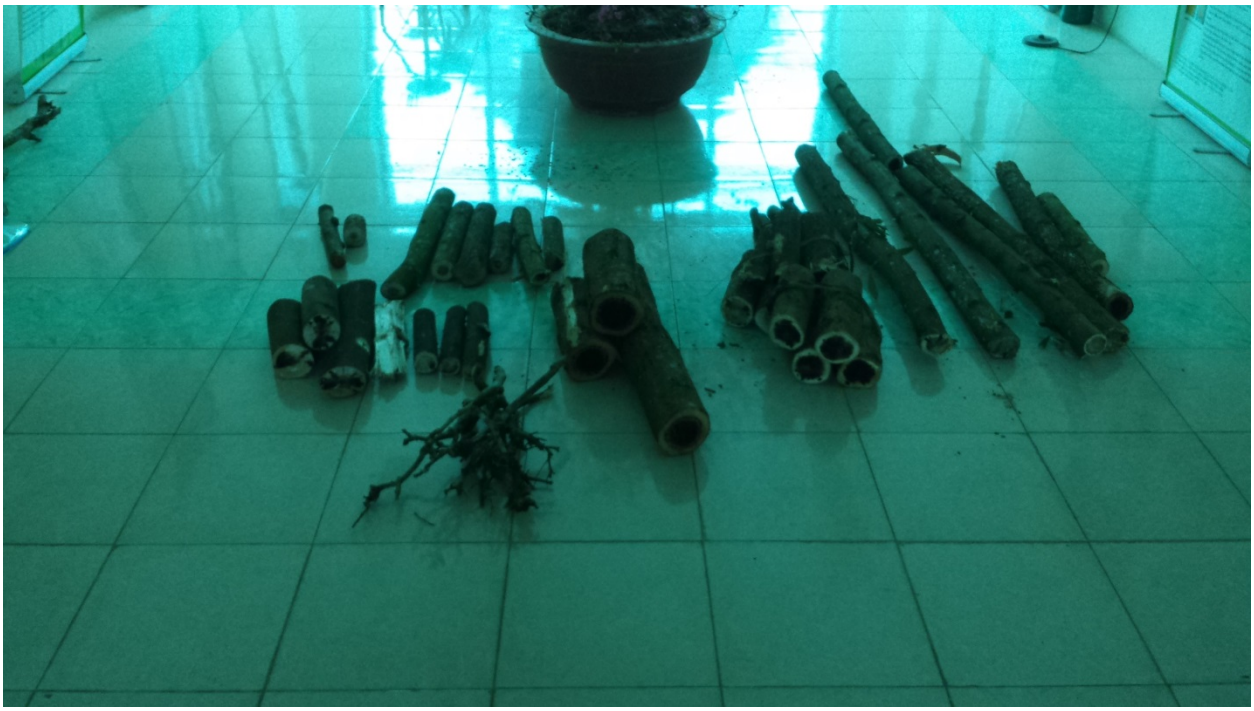

Figure 7. Logs collected from Yen Bai, including tea, acacia, cinnamon and castor bean.

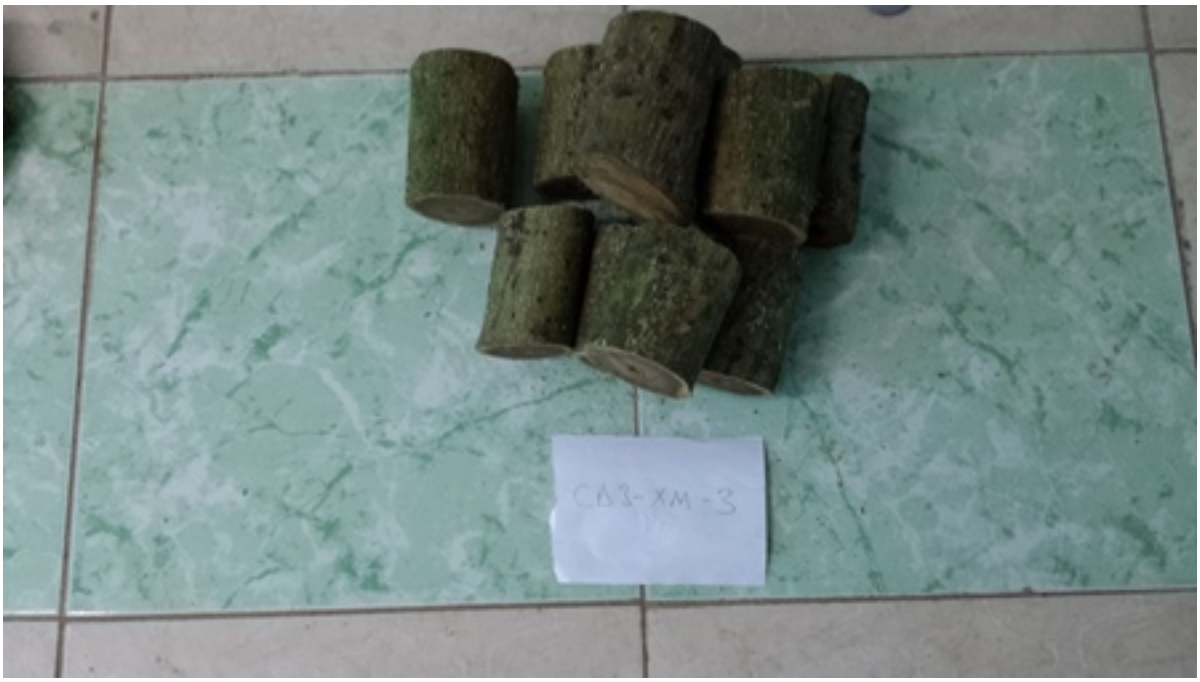

Figure 8. Each log was cut in smaller pieces (~4 inches long)

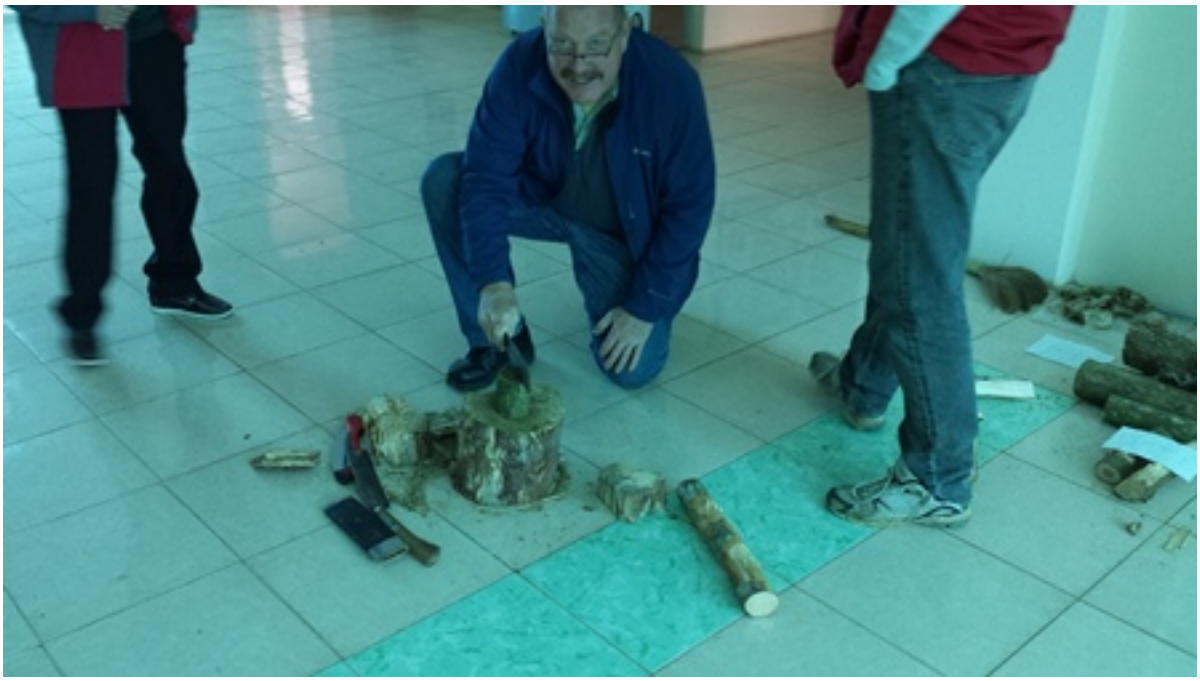

Figure 9. Smaller pieces were cut into slices using a cleaver.

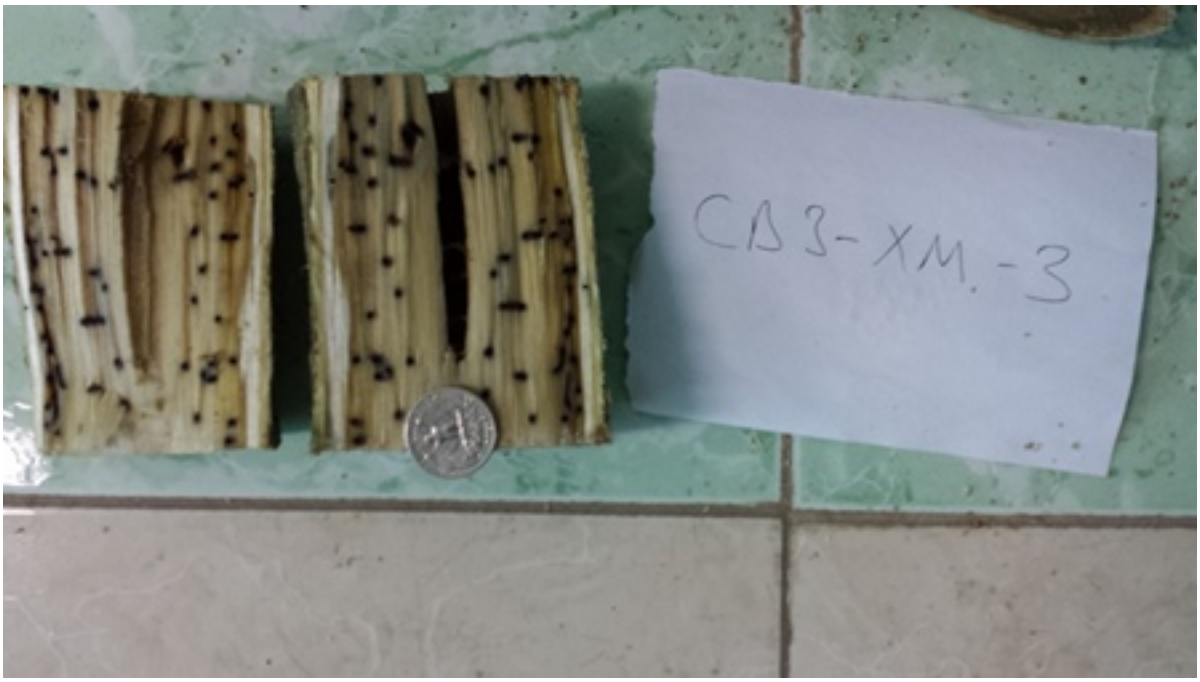

Figure 10. Castor bean smaple cut in two, showing beetle galleries

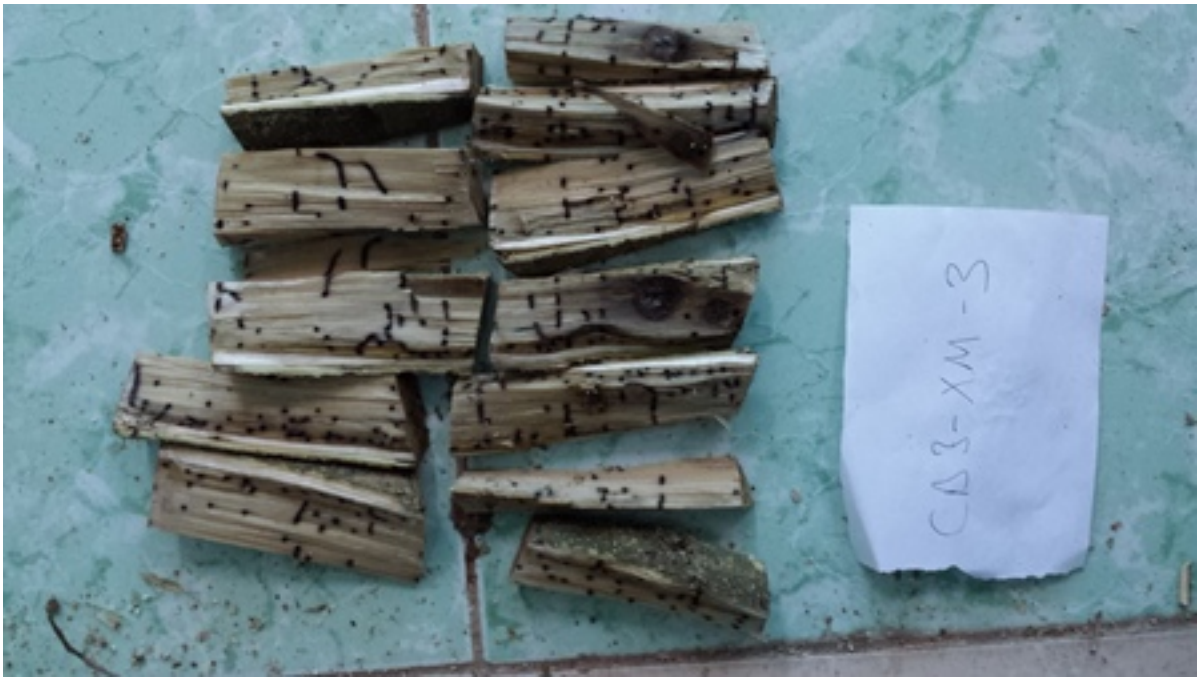

Figure 11. Castor bean sample cut in smaller slices to be examined under microscope for insect and fungal extraction

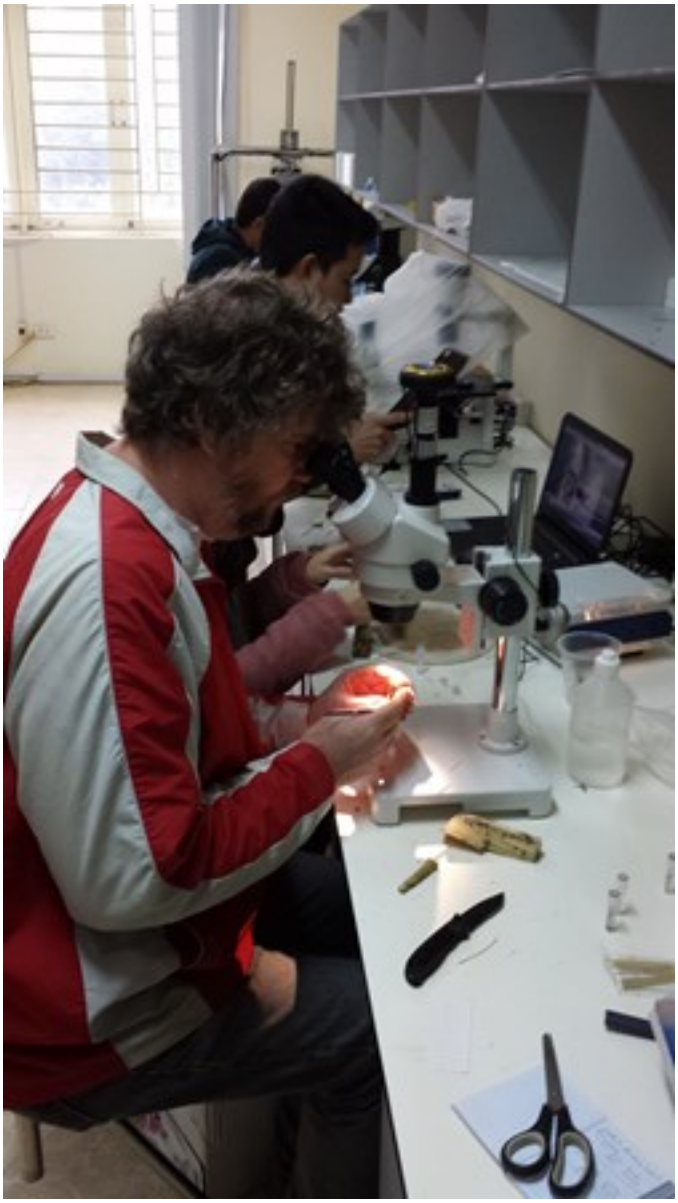

Figure 12. Examination of the slices under microscope for insect and fungal extraction.