Obtaining Biocontrol agents against Polyphagous Shot Hole Borer from SE-Asia.

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Abstract:

Goal of the work was to improve obtain natural enemies for the Polyphagous and Kuroshio shot hole borers in California

Natural enemies are present in the native range of the beetles Taiwan.

Cooperation was obtained from local scientists to try to establish lab populations of natural enemies: parasitoids and nematodes.

In general, it is difficult to establish laboratory cultures of these natural enemies, because of the hidden lifestyle of the beetles and consequently the parasitization could not be observed.

Cooperators were not able to establish beetle parasitoid populations in the lab, no successful offspring was obtained from the wasps exposed to beetle containing logs of wood.

As a result we decided to try to establish cultures ourselves from field collected infested avocado wood in Taiwan.

Two trips were made were we collected large numbers of avocado branches from near Tainan in Taiwan and shipped them to our quarantine facility at UCR.

The January trip resulted in 169 parasitoids (5 species) emerging and May trip in 112 parasitoids (5 species)

Long-term rearing at UCR was not successful from the January trip one generation of offspring was obtained, while the May trip resulted in up to 3 generations of offspring before the cultures died out.

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Introduction/Background:

The species morphologically identified as *Euwallacea fornicatus* has been present in Southern California for at least a decade (Eskalen et al 2013). It is unknown how this species arrived in California but transport in wooden packing material seems to be an obvious possibility. For the earliest detection in 2003 until 2012 little was known about the distribution of this beetle. Historically *Euwallacea fornicatus* was associated with problems in tea in Sri Lanka (Walgama, 2012), where the density of these beetles could reach 1,500,000 beetles per acre. While *E. fornicatus* in Asia was a big economic problem in tea crops, other tree species were also attacked and Danthana (Danthana, 1973) reported that up to 90 species of hosts were known.

Once we became involved in the research on these beetles it soon became clear that the beetle that had invaded the Los Angeles area was genetically different from the "Tea Shot Hole Borer", to distinguish it from that beetle we named it the "Polyphagous Shot Hole Borer" to indicate that this beetle attacks many different host plant species (Eskalen et al 2013). In 2014 a second invasion of a species morphologically identified as *E. fornicatus* was detected in El Cajon, CA and upon genetic fingerprinting it became clear that this again was a different cryptic species close to *E. fornicatus*. This species was found in its native range in Taiwan and Okinawa and was named Kuroshio Shot Hole Borer (KSHB) (Rugman-Jones and Stouthamer, 2016). Based on our phylogeographic work we now know that the PSHB occurs in Asia in Northern Taiwan, Southern China, Vietnam, Taiwan and Okinawa, while the KSHB occurs only in Taiwan and Okinawa (Stouthamer et al., 2017) . The DNA sequences of the PSHB would indicate that the invasion found in California originated from either southern China or from Vietnam. The KSHB sequences are identical to sequences found in beetles from Taiwan.

These SHB species carry with them a specific combination of fungi that they use to culture for food, inside the trees (O'Donnell et al., 2015, Eskalen et al., 2012, Freeman et al., 2013). Each generation females leaving their natal gallery will leave with a set of fungi in special organs inside their heads that they will use to seed their own galleries. The mode of reproduction of these SHB is similar to bees and ants, in that inseminated females can regulate the access of sperm to their eggs, and in allowing a sperm to fertilize an egg, the resulting offspring will become a female, while eggs that remain unfertilized develop into males. Thus the mother can control the sex ratio of her offspring. She does this in a very precise manner and produces mainly daughters and only a few sons. In addition to this unusual sex ratio, the siblings also mate with each other, this so called sibmating is common among ambrosia beetles. No negative effect of sibmating are known in these species. The result of this sibmating is that female beetles that emerge from galleries once they are adult will already have mated with their brother, and have stored his sperm in a special organ called spermatheca. Once such females have created their own galleries they will be able to produce both sons and daughters.

This specific lifecycle –spending most of their time inside the tree, cultivating their fungi, where both the mother and her offspring exclusively feed on these fungi, leaves few opportunities to control these insects with insecticides/fungicides. Research both here in California and in Israel (Mendel et al., 2012) has not yet resulted in an effective control strategy. Control of these beetles is hampered by the fact that they spend most of their life inside their galleries inside trees. Thus reaching them with pesticides is difficult once they are inside the tree. During their dispersal phase they spend a short time outside their native tree, either to walk on the bark surface to find another spot to construct a new gallery or to fly away and find another suitable host.

In countries in south-east Asia where the beetle is native the beetles appear to cause little damage to trees. We spent considerable time in Vietnam and in Taiwan to look for damage on street trees, and we found very few street trees attacked, this in contrast with the situation here in California. For instance, in California weeping willows are attacked heavily while in Taiwan we could not find any evidence of beetle attack on this species. Similarly, in Vietnam no evidence was found of attack on street trees. In Vietnam, beetle attacks were common in plantations of acacia. In Taiwan, it was also difficult to find beetles attacked by our shot hole borers. The highest infestations we could find were in avocado orchards, but even there the infestation rate was much lower than what we find in our avocado orchards. Because Taiwan is in the native range of both the Kuroshio and the Polyphagous shot hole borer we concentrated on trying to collect parasitoids from there. At least five species of parasitoids were discovered in Taiwan, and most of these are new to science. Several techniques were tried to establish laboratory colonies of these wasps and we were successful in maintaining them for up to 4 generations in the laboratory before we did not have an additional generation.

Objectives: the main objectives of the project.

1. Determine which natural enemies attack the shot hole borer species in their native range
2. Culture these parasitoids so that non-target studies, which are required for before parasitoids can be release in biological control programs

Material and Methods:

Foreign exploration

Taiwan:

We have visited Taiwan in 2016 at the end of March beginning of April to set up an agreement with the Taiwan institute of forestry research for the collection of natural enemies. During this trip we visited several natural areas where the densities of SHB was extremely low, the only locality where a number of SHB could be found was in avocado groves. We have set up a system for our cooperator to first attract beetles to cut bolts hung in trees for the attraction of beetles to the bolts. Once the beetles have established in the bolts they will become vulnerable to their natural enemies and by recollecting the bolts some 6-8 weeks after deployment they can be kept in the laboratory for their natural enemies to emerge. At the same time cultures of beetles are maintained in the lab that then will form the hosts of the potential parasitoids thus collected. During our trip we set up bolts and discussed with our cooperator how to go about doing this collection.

Taiwan January 2017

January 10-22, 2017

We visited Taiwan from January 10-22, 2017 and collected from avocado groves near the city of Tainan. In this effort, we were accompanied by our contact from the Taiwan Forestry Research Institute. We collected from Avocado trees, short pieces of avocado wood infested containing beetle holes, in the hope that beetles were still present in the wood and that some of them would be parasitized. The pruning that took place in these avocado orchards often left short branches attached to the tree, and these stumps were often attacked by shot hole borers (see figure1). These stumps were cut off the trees and collected for shipment to the UCR quarantine under permit (USDA-APHIS Permit P526P-16-03142).

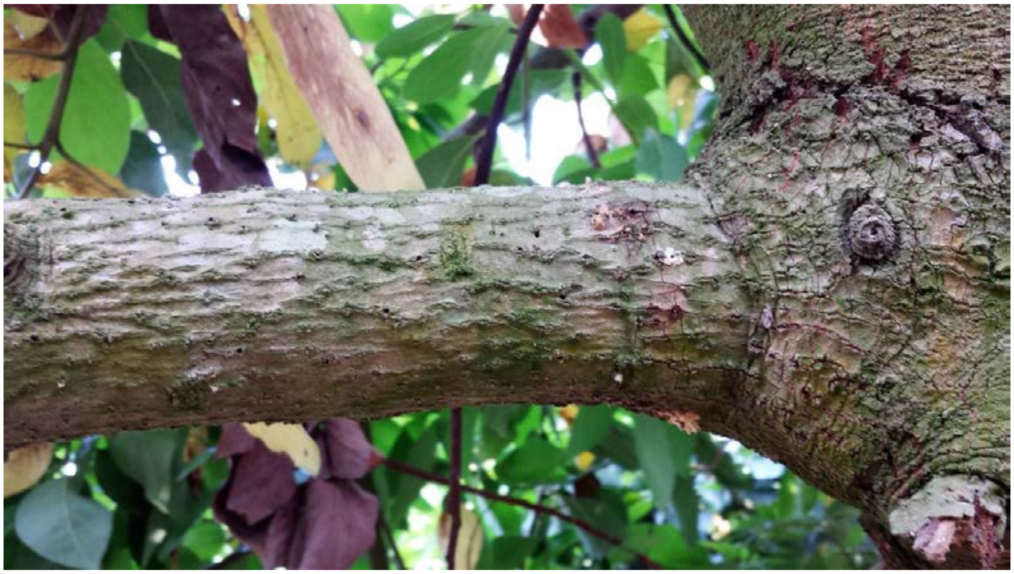

Figure 1: Branch of avocado tree with shot hole borer holes in it.

On the day that the stumps were collected (Figure 2), we dipped the cut parts of the stumps in molten wax in order to slow down the drying out of these stumps. When stumps dry out the beetles residing in them often die.

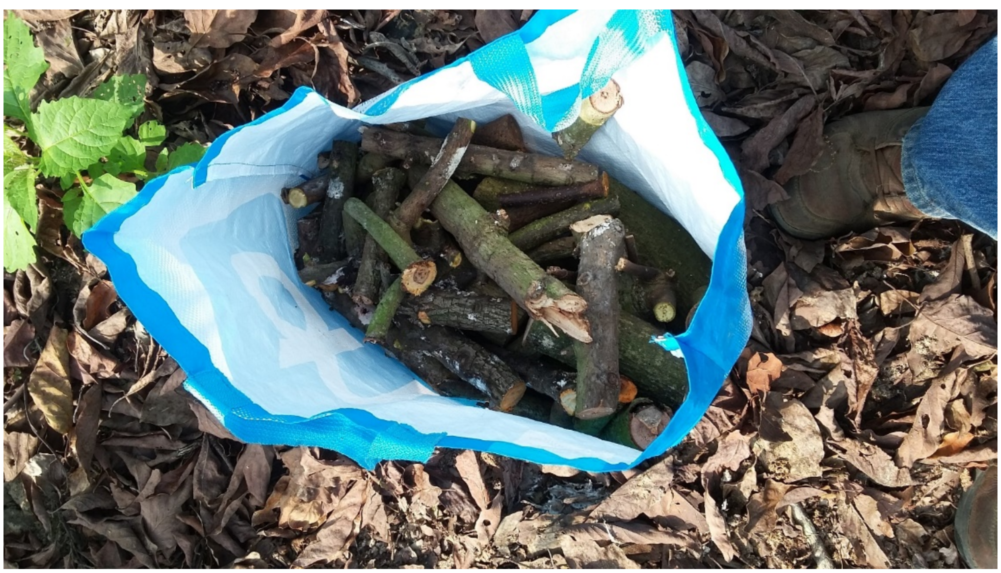

Figure 2. Stumps collected from one of the avocado orchards.

Several methods had to be developed for shipping the material, since these were pieces of wood which still had quite a bit of humidity in the wood and the fear was that during the transport fungal growth would cover the branches and potentially kill the content of the branches. We therefore decided that we needed to ship the material in such a way that the contents would be able to exchange water and air with the environment. To accomplish this, while also containing insects that might emerge during the transport, we came up with the following approach. In Taiwan, boxes with a cloth exterior are sold for storing clothes under vacuum. Glued to the interior bottom of the box is a large zip lock bag with an air valve that can be used to suck a vacuum (Fig 3). These boxes were modified by cutting a hole in the top of the zip lock bag, and covering this hole with a layer of cloth mesh fine enough to keep the insects inside the bag, and covered both on the inside and on the outside with a layer of metal mesh to further inhibit egression by insects.

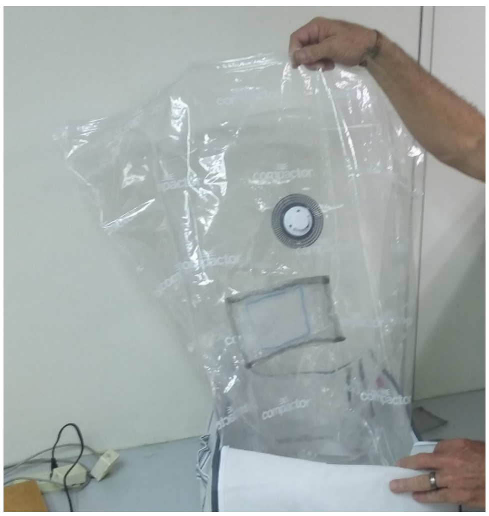

Figure 3. Ziplock bag attached to cloth box, equipped with ventilation hole.

Next the wood was first packed in ziplock vacuum bags fitted with the same hole and hole coverings. Per box two ziplock bags with wood could be fitted in the box-ziplock bag. To assure ventilation of the bottom zip lock bag with wood, a single hole –covered as described above- was cut in the top of the bag. For the top ziplock bag with wood, two holes were cut and covered as described above. When the two ziplock bags with wood were placed in the ziplock bag of the box, all the holes lined up so that ventilation was possible from the bottom bag, to the top bag, to box ziplock bag.

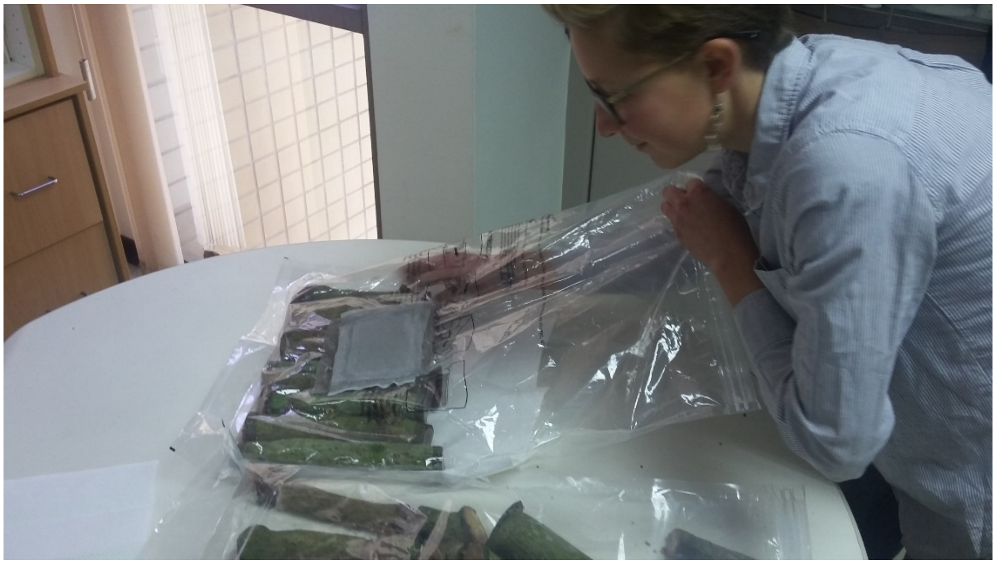

Figure 4. Ziplock bag, equipped with ventilation hole, is made ready to be shipped. This bag will be placed inside the ziplock bag shown in Fig 3.

The cloth box (Fig 5) was then placed inside a sturdy cardboard box and filled with styrofoam peanuts, followed by closing the box with tape.

Figure 5. Cloth box containing ziplock bags with wood samples.

The boxes were shipped on Tuesday afternoon (Taiwan time) and arrived at a post office close to the LAX Plant Inspection Station by Friday morning. Unfortunately, the post office did not deliver them on Friday, but an effort to deliver them was made on Saturday, when the inspection station was closed. On Monday mid-day the three boxes were delivered to the inspection station and after inspection FedEx was asked to pick them up and deliver them to our Quarantine facility. Unfortunately, they did not pick them up on Monday, so on Tuesday morning I called the USDA permit unit to see if I would be able to change the permit conditions in such a way that I could pick them up from the Inspection station. With the help of Bob Tichenor (USDA-PPQ) this was arranged and on Tuesday afternoon I delivered the boxes to the UCR quarantine facility.

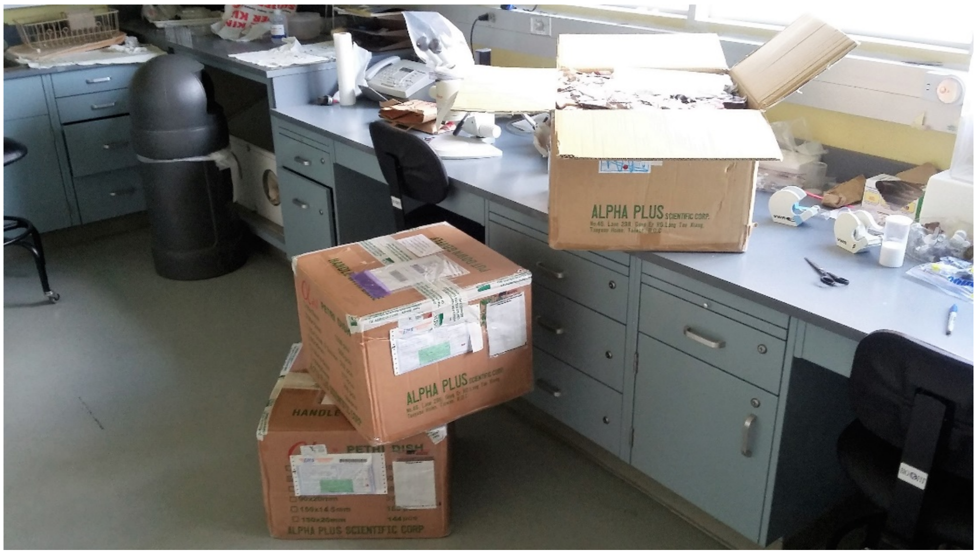

Figure 6. Cardboard boxes arrived in good shape in UCR quarantine.

Overall the material arrived in very good shape (Fig 6) with little fungal growth and the material was subsequently transferred to bug dorms, the next day the individual stumps were transferred to clear plastic containers with ventilated lids to avoid condensation.

Insects have been emerging from these containers including a large number of different parasitoid species and individuals. A complete accounting of the emergence and their identity will be given in the next report, early on in the emergence several bethylid wasps emerged, later a substantial number of braconid wasps emerged. These were identified as belonging to the genus Cosmophorus, a genus known to parasitize bark and ambrosia beetles. The wasps in this genus are characterized by the possession of large mandibles that they use both to clear the opening to the beetle gallery from debris as well as for grabbing the beetles while ovipositing in them. In addition, several other families of parasitoids emerged from the stumps: Encyrtidae, Pteromalidae and Eulophidae. For each of these batches of parasitoids separate cages were initiated containing castor bean logs infested with Polyphagous Shot Hole Borer. Even after one month in our quarantine facility wasps are still emerging from the stumps, thus it appears that some of the parasitoids have a long life cycle. No F1 wasps have yet emerged from the logs that we have provided them, which again may indicate a long lifecycle.

The collection of wood made in January consisted of about 36 kg of avocado stumps infested with shot hole borers. This collection of wood was maintained in the UCR quarantine and checked daily for emergence of insects. The number of parasitoids and beetles that emerged were tallied daily.

We have learned a lot from this trip and are about to embark on a second collection trip May 15-22nd, to collect more wood. This wood will again be used to establish both parasitoid natural enemies and also nematodes. We have streamlined our infested wood availability and have developed better cages for emergence of the parasitoids. Part of the emerged beetles will be used to obtain nematodes. For this we have consulted with nematode specialists at UCR.

Finally, several of the emerged beetles were identified in order to make sure that we indeed were dealing with the *Euwallace fornicatus* species complex, and to our surprise we collected all three of the species known to occur in Taiwan from the avocado wood. The majority of the material was the PSHB, next several specimens of the TSHB and only two individuals of the KSHB were found.

Taiwan May 2017

In May 2017 we again visited the area near Tainan where most of the avocado groves are located. We collected material for one week and again shipped the material back to UCR for emergence.

<u>Rearing methods</u>

<u>January Trip</u>

The wasps that had emerged were placed in bug dorms with logs infested with PSHB that were produced in our insectary. We wanted to make sure that all possible beetle stages were present in these logs so that the proper stage was available for the wasps to parasitize. The logs we used for producing the offspring was castor bean, since that is the host where the beetles develop the best and the fastest in our rearing system. The Braconid wasps were observed to go into the holes in the logs produced by the beetles.

<u>May Trip</u>

Based on our experience from the January rearing effort we changed several aspects of our rearing syste. First we changed the emergence cages in which the wood collected in Taiwan was kept. We used plastic containers (Fig. 7) instead of bug dorms to allow the beetles and wasps to emerge. In the containers we made honey available for the emerging wasps to feed on. In addition we raised the relative humidity in the rooms was increase to 70% to more closely resemble the conditions in Taiwan. Wasps that emerged were allowed to mate with males of their own species if they were available and introduced in similar containers and supplied with a log containing beetle colonies. Some of these logs containing beetles were collected in the field, while in other case we used logs from our mass rearing.

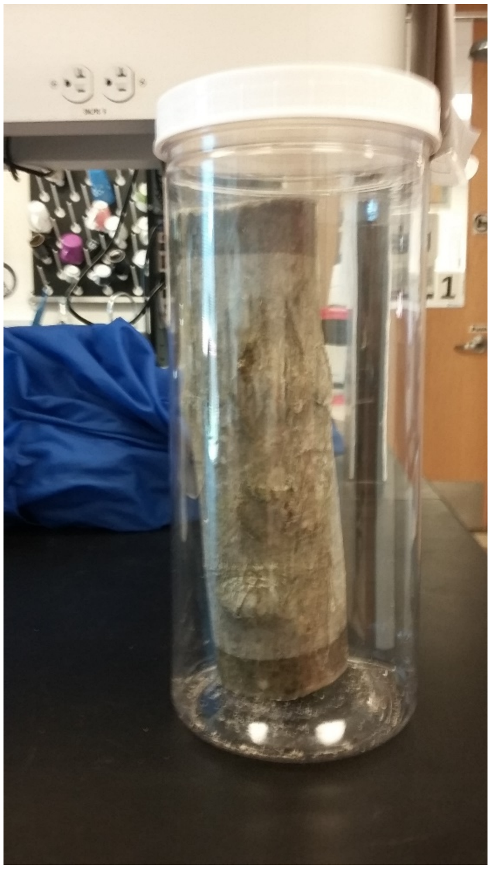

Figure 7. Containers used for emergence and rearing Shot Hole Borer Parasitoids

Results

Taiwan January 2017

Total emergence of parasitoids

Table 1 Family identity of the parasitoids emerged from the wood collected in January 2017

Bethylidae	6F
Braconidae	4F,3M
Synuatophorus	46F, 46M
Encyrtidae	34
Megaspilidae	4
Pteromalidae	26

The identity of the parasitoids was determined using both morphology and molecular identification. The results showed that the Eulophids belonged to the genus Phymastichus, the Bethylids belonged to the genus Cephalonomia, and the Braconidae- to the genus Sinuatophorus. Wasps of the Braconids produced an F1 generation consisting of 41 females and 76 males. Unfortunately, the F1 generation did not produce any offspring.

Taiwan May 2017

Table 2 Family identity of parasitoids emerged from the wood collected in May 2017

Bethylidae	50
Braconidae	13F,16M
Eulophidae	26
Eupelmidae	4
Eurytomidae	2

Table 3. Successive generations produced by the wasps that emerged from wood collected in May 2017

	P	F1	f2	f3
Braconidae	13F,16M	9f,11m	2f, 15m	2f,1m
Bethylidae	50	9		
Eulophidae	26	11f,5m	4f,5m	3f,2m

Discussion

From our collections and observations in Taiwan, it is obvious that the beetles there are being kept at a much lower level than here in California. We assume that natural enemies there play and important role in controlling the beetles. Collections in Taiwan have shown that several different species of parasitoids can be collected from beetle-infested wood. Three families of parasitoids were the most common in our collections. The species that we collected were all three new to science and their specific name, will require species descriptions by specialists in the field. From our observations in our rearing efforts, it appears that all three species emerge from adult beetles. However, the hidden lifestyle of the beetles, makes it difficult to observe what is going on in the parasitization process, and consequently it is not known which beetle stages are parasitized. Since we were not able to get large and growing populations in rearing, it makes it difficult to study their life history in more detail. This inability thus far to mass rear the parasitoids in the laboratory also makes it impossible to do extensive non target testing using these wasps. It is vital for the potential biocontrol of the beetles in California that we develop an efficient rearing system. We have made progress for the first collection that was imported in January where we were only able to rear one generation of a single species before the population crashed, to the collection in May where we were capable of rearing at least one generation for the bethylid, and several generations for the eulophids and braconids. We expect to make another trip to Taiwan early next year, which will hopefully result in our ability to have a stable production of one or more of these wasp species.

Conclusions

Parasitoids are most likely keeping the Kuroship and Polyphagous shot hole borer populations at a low level in Taiwan. Several species of parasitoid are present there that can play a role in the control of the beetles. Despite our efforts to mass-produce these parasitoids in California we have been unsuccessful so far in rearing more than just a handful of generations of these parasitoids. We will need to improve our rearing techniques in order to progress to the ultimate goal of releasing these natural enemies for the biological control of these beetles.

Summary

- At least three species of parasitoids parasitize shot hole borers in Taiwan
- We were able to rear them in the laboratory for 1-4 generations
- We were not able to establish permanent lab populations

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