

# SUCCESSION IN GALLS ON SYZYGIUM MALACCENSE AND THEIR IMPACT ON LEAF AGING

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*Abstract.* Plant-insect symbiosis and succession are important components of tropical ecosystems for understanding natural history and biodiversity. There are not many studies of gall succession and gall life cycles in tropical environments, even though it is an important component of tropical ecology. A number of gall specimens on *Syzygium malaccense* were followed over time, sampled for insect communities, and tested for areas of fungal coverage to determine if they underwent successional development and caused leaf decay outside the gall boundaries. Different gall stages were classified over this time period and were found to contain different communities. Desiccation and decay were found to cause gall color change, and the decay was limited to the gall boundaries avoiding the *S. malaccense* leaf. *Syzygium malaccense* galls were thus found to model succession, and also present a unique symbiotic system amongst a diverse set of tropical organisms.

*Key words:* *Syzygium malaccense*, *Megatrioza vitiensis*, *Mo'orea*, *Succession*, *Symbiosis*, *Tortricid*, *Penicillium? sp*

## INTRODUCTION

Understanding biotic interactions in tropical ecosystems is an important way of adding to our current knowledge of natural history. Plant-insect symbioses provide interesting models for such interactions, especially co-evolution. Co-evolution is a type of symbiosis in which the change in one organism includes a change in its symbiont (Raman 1997). In many known co-evolutionary relationships, insects often parasitize and exploit their host plants for nutrients or shelter at a cost to the host. By reducing the overall health of their hosts, such insects reduce plant productivity and detrimentally impact plants harvested for commercial use (Mello 2002). However, most co-evolved relationships require the insect to monitor its parasitism and refrain from killing too many plant cells (Felt et al 1940). Limiting plant cell death thus enables the plant host to grow and reproduce so that the insect parasite and its progeny may continue to survive as well. Certain plant-insect symbioses also create opportunities for secondary infestations and infections in the plant (Paul et. all 2000). This succession of multiple organisms follows a specific pattern of change over time and

parallels concepts of secondary succession or the directional change in an occupied community over time (Roskam 1997). Oftentimes, the presence of one organism facilitates another organisms' ability to colonize the area (Campbell et al 2005) which increases biodiversity and complexity in the system.

Insect-driven gall formation models co-evolution, as insects form specific relationships with plants for food, oviposition sites, and habitation (Panda and Khush 1995). Galls are abnormal outgrowths of plant tissue that can form on the leaf, stem, root, or flower of a plant (Fay, Hartnett, and Knapp 1996) and redirect nutrients from the plant's natural growth to form highly organized, fleshy outgrowths of tissue. (Felt et al 1940). Gall formers range from bacteria, viruses, and fungi, to insects and vary in host and host tissues' specificity (Darlington and Hirons 1968). Insect-produced galls develop in a series of stages. According to Felt (1940), adult or instar stimulus induces gall formation before the gall tissue envelopes the instar. He found that the instar resides within the gall until ready to emerge as an adult. He also found that in the instance of leaf galls, the gall either remains on the leaf and erupts to release

the insect or falls to the ground for insect emergence. While some galls drop off after their gall-former matures, others remain on the plant for lengthy periods of time and provide the opportunity for secondary insect and fungal infections (Roskam 1997). Such re-infestations or infections may redirect additional nutrients from the plant and further decrease its overall health; however, little research has been done on this phenomenon.

One group of gall forming insects is the Psylloidea family, Triozidae, commonly known as psyllids or jumping plant lice. Psyllids induce gall formation by sucking sap from the underside of a leaf, triggering pit formation around the nymph (Downer 1991). Hodkinson stated that in closed leaf galls, the nymph instar resides opposite to the site of entry, at the bottom of the pit, protected from environmental factors, particularly desiccation, drying out (Ananthakrishnan 1984). Gall forming psyllids are extremely host specific and have very low reproductive success when ovipositing on plants other than their specific host (Van Klinken 2000). Biological limitations on psyllid reproduction are also affected by synchrony with the host plant (Hodkinson 2010; Raman 2005). Insect life cycles that are dependent on a host plant tend to be seasonally based to coincide with the emergence of new buds, leaves, or fruit, especially in temperate zones (Tauber 1981). However, insects found in the tropics tend to be multivoltine because of smaller variations in seasonality and year-around growth of the host plant (Raman 2005). Psyllids preferentially oviposit on younger leaves with more nutrients for gall formation and their life cycles often coincide with new leaf outgrowths (Luft & Paine 1997). Thus, tropical gall forming psyllids in the tropics experience a multivoltine life cycle synchronous to year-round leaf flushes.

*Syzygium malaccense* is a cultivated fruit-bearing tree in the tropics and ranges throughout the Pacific. It is thought to originate from either the Indo-Malayan region or Southeast Asia but its true origin is unknown (Whistler 2006). Introduced to the Society Islands by Polynesian voyagers, it was traditionally used in their sacred temples,

maraes, but is now found growing wild in forested areas (White 2011). However, *S. malaccense* was also cultivated as a crop and medicinal plant in private gardens (Whistler 2009). Kirkaldy discovered *Megatrioza vitiensis*, a gall-forming psyllid, was commonly found in association with *Syzygium malaccense* in the Society Islands of French Polynesia (Ananthakrishnan 1984). *Megatrioza vitiensis* creates galls on the leaves of *S. malaccense* that remain on the leaves of the plant for long periods of time after the psyllid emerges. The relationship between *M. vitiensis* and *S. malaccense* must be an old symbiosis as ancient Polynesians would ground up the gall, *M. vitiensis* nymph, and *S. malaccense* leaf together to create traditional medicine (Whistler 2006; Hinano Murphy, personal communication, September 26, 2012). Because of its importance as a crop, sacred plant, and medicinal plant, *S. malaccense* was preferentially planted in personal gardens and maraes and can be found in abundance below the Belvedere in Mo'orea, French Polynesia (Hinano Murphy, personal communication, September 26, 2012). Although the formation of galls on *S. malaccense* is commonly known by Polynesians, little is known about the gall life cycle and what happens to the galls after *M. vitiensis* emerges from the galls.

The goals of this study were to determine if there were differences between insect communities in the galls on *S. malaccense* over time and to establish if there is definitive succession of the galls themselves. Additionally, this study examined the cause of gall color change and determined whether later gall stages affect leaf decay beyond the gall. It was hypothesized that after the gall former leaves the gall, other organisms then colonize the micro-compartment for food and shelter because the gall remains on the leaf. It is also hypothesized that gall color changes as the gall desiccates and decays over time and that secondary colonization of the gall would cause leaf decay outside the gall boundary.

## METHODS

### *Study system*

There were four organisms involved in this study system: *Syzygium malaccense*, *Megatrioza vitiensis*, moth larvae from family Tortricidae moth larva, and white, fungal fruiting bodies from *Penicillium? sp?*. *Syzygium malaccense* is a medium sized, tropical tree that produces, bright cerise, bottle brush flowers and fleshy, oval shaped apples. The leaves are opposite, simple, oblong shaped and are often covered in insect galls (Whistler 2006) which vary from shades of green to brown (Appendix A). *Megatrioza vitiensis* is a psyllid found in the tropics associated with *S. malaccense*. According to Kirkaldy, as a nymph, *M. vitiensis* create galls on the leaves of *S. malaccense* (Ananthakrishnan 1984). The Tortricid moth larva was found inside the *S. malaccense* galls after the *M. vitiensis* nymph had emerged as an adult (Appendix A). The white *Penicillium? sp* fungus was found on the Tortricid moth larva frass, feces, as clusters of white, fruiting bodies (Appendix A).

#### Study sites

Three study sites were chosen below the Belvedere where *S. malaccense* was preferentially planted around maraes by the Polynesians at GPS points at -17.53613468, -149.82833947, -17.53494575, -149.82675901, and -17.53425835, -149.828837445 (Figure 1). Collections, observations, and experiments were conducted between 25 September 2012 and 14 November 2012. The three study sites were chosen by rolling a die at two different forks in the Three Pines Trail to determine which stand to sample from based on the number rolled and stand encountered (e.g. rolled 3 meant the third stand I found would be a site). I sampled from large, cluttered stands of *Syzygium malaccense* found along or slightly off the Three Pines Trail.

Site 1 was located to the left of the first fork in the Three Pines Trail with a cluster of about thirty trees, ten meters in any direction

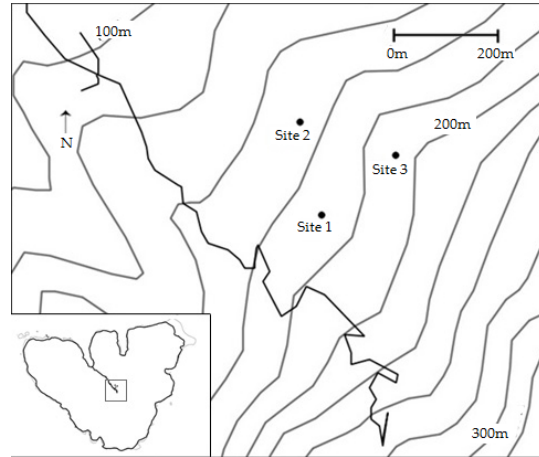


FIG. 1. Map of Mo'orea with locations of Study Sites 1, 2, and 3

from the GPS point. Site 2 was located to the right of the first fork and left of the second fork in the Three Pines Trail with a cluster of about forty trees, ten meters in any direction from the GPS point. Site 3 was located to the right of the first fork and right of the second fork in the Three Pines Trail with a cluster of about twenty-five trees, ten meters in any direction from the GPS point.

#### Field observations: gall successional stages

Leaves with different gall color combinations and sizes were marked and recorded from 08 October 2012 to 15 November 2012. No more than one leaf was marked per tree and no more than ten galls were tracked per leaf. Over one hundred galls were tracked over time. To create a chronological timeline of the gall life cycle, pictures were taken of the top and bottom of each leaf three times a week on two or three day intervals. Pictures were taken with a color wheel and a Nikon Aw100 so changes in size and color could be standardized and analyzed in ImageJ. Data from these images was compiled and used to create Appendix B, Figure 2, and Figure 4.

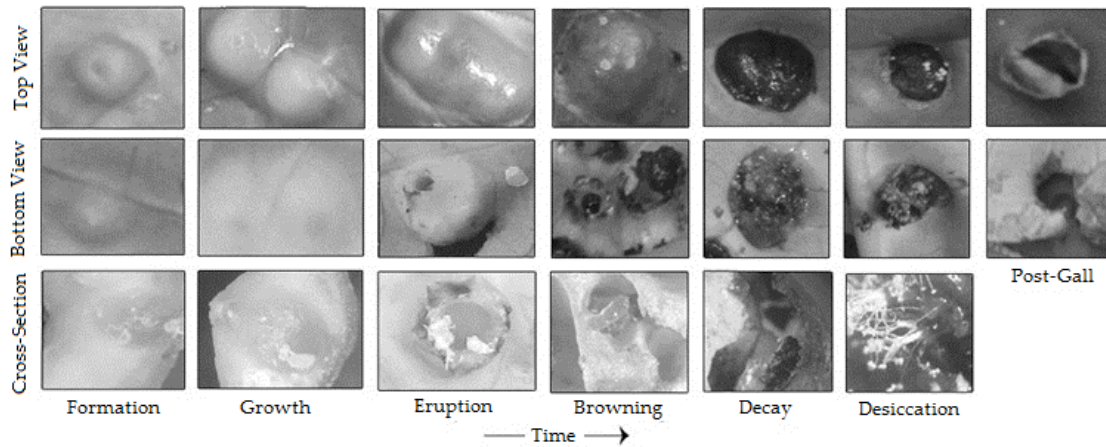


FIG. 2. Photos of galls representing different gall stages over time from left to right. Top Row: Top view of gall stages over time. Middle Row: Bottom view of gall stages over time. Bottom Row: Cross-section of gall stages over time.

Galls were grouped into different classifications based on several characteristics in Appendix B. Galls were sorted into the following stages: Formation, Growth, Eruption, Browning, Decay, Desiccation, and Post-gall. Gall stages were determined by the following characteristics: percent-coverage of brown on the gall's top and bottom surface area, gall size diameter, gall eruption, presence of *M. vitiensis* nymph, Tortricid larva, wet Tortricid larva frass, dry Tortricid larva frass, white *Penicillium? sp* fungus, and if the gall was hollow or gone. A hollow gall was defined as a gall that only had an external layer of plant tissue along the top and bottom of the gall without any fleshy, green or brown gall tissue or moisture inside. Images from each defined gall stage were selected to portray the gall life cycle in Figure 2. A top and bottom view as well as a cross section picture demonstrate the progression of a gall's life over time and its different stages.

#### *Field observations: species composition*

Leaves with various stages of gall formation were randomly sampled across all sites and trees. No more than two leaves were taken from any tree. Approximately thirty galls of each stage were observed for exterior characteristics and classified according to Appendix B before dissection. Galls were cut open vertically with the underside of the leaf

facing up. The presence of a *M. vitiensis* nymph, Tortricid larva, wet Tortricid larva frass, dry Tortricid larva frass, and white *Penicillium? sp* fungus was recorded for each gall and totaled as a percentage in Figure 3. Pictures of the cross-sectioned gall were taken and used to portray the gall life cycle in Figure 2 as well.

Information from Appendix B, Figure 2, and Figure 3 were combined with information from galls tracked over time. Figure 4 modeled the gall life cycle and characteristics of each stage over time as depicted by over one hundred galls tracked over time. Days were grouped into the different gall stages listed in Appendix B and all gall progressions were aligned to the date of their gall eruption. Five characteristics were tracked, compiled, and averaged over time from gall successional stage pictures to show average percentages of characteristics present. Gall eruption for each gall was tracked and recorded at each time point. The percentage of galls erupted at each time point was then calculated and marked on the graph. The presence of an *M. vitiensis* nymph and a Tortricid larva were also noted at each time point and averaged across all the galls to determine what percentage of galls contained an individual characteristic at each stage. The presence of the *M. vitiensis* nymph when the gall was closed was confirmed when the gall erupted and the presence of the Tortricid larva was measured by the presence

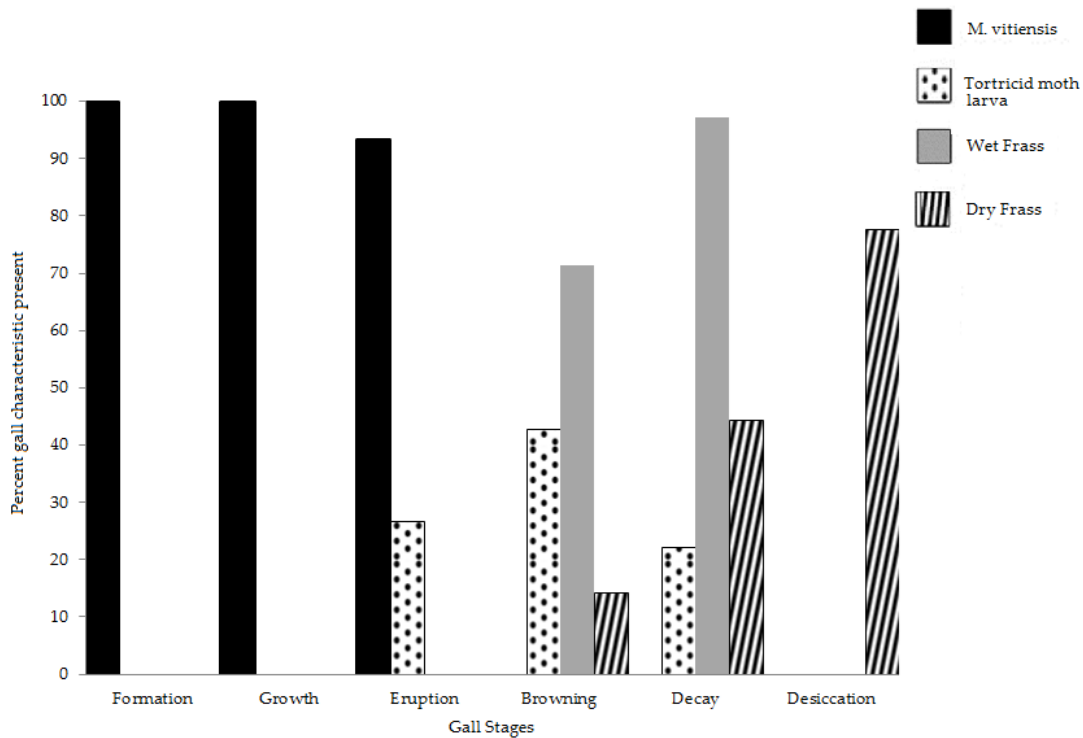


FIG. 3. Percent of gall characteristics present in gall stages over time. A sample of galls was taken for each stage and cross-sectioned. The characteristic found inside were tallied for each stage and visualized as percent presence in the stage.

of Tortricid larva frass on the underside of the gall.

Finally, brown coverage and fungal coverage were recorded by measuring the amount of brown or fungal coverage on the underside of each gall to obtain a percentage. The percent-coverage of brown or fungus was calculated for each gall in a time point. Then the percent-coverage of brown or fungus was totaled and averaged for one time point to get an average percent-coverage of brown or fungus for that time point.

*Field experiments: desiccation and decay rate*

Two treatments of gall manipulation were performed to monitor rates of percent-coverage of brown and the percent-coverage of *Penicillium? sp* fungus on galls after herbivory from 29 October 2012 to 15 November 2012. Percent-coverage of browning on the gall indicated what percentage of the gall tissue on the underside of the gall that was dried out or desiccated. Meanwhile percent-coverage of fungus

indicated the percent of the gall tissue that was decaying or decayed by the *Penicillium? sp* fungus. For each treatment, two or three similarly sized healthy leaves with new or newly erupted, fleshy green galls were chosen at each site from different trees. Five galls on the underside of each leaf were cut so that a 0.25cm<sup>2</sup> area was removed that was no more than 0.05cm deep to emulate insect herbivory. There were a total of twenty-five galls tested per treatment. For the cut treatment, a drop of distilled water was placed on the cut gall tissue as a methods control. For the spore treatment, a drop of spore solution was placed on the cut gall tissue to inoculate the tissue with fungal spores. A total of 0.5 grams of brown insect frass with white *Penicillium? sp* fungus was collected and mixed into 1 ml of distilled water to make the spore solution (Choi 1999). Pictures of the top and bottom of each leaf were taken three times a week every two or three days to observe percent-coverage of brown, percent-coverage of fungus, and other changes in the wounds over time. Pictures were taken with a color wheel and a

Nikon Aw100 so changes in size and color could be standardized and analyzed in ImageJ].

#### *Statistical methods*

All statistical tests were performed in JMP. A regression line was fitted to the Desiccation and Decay experiments testing for percent-coverage of brown coverage and percent-coverage of fungus. Regression lines were compared between the cut and spore treatment in the percent-coverage of brown and Figure 5 compares the cut and spore treatment regression lines in the percent-coverage of fungus test. The R<sup>2</sup> value was listed to show how well the line fit to the data and p-value was listed to show if there was a significant correlation in the data.

## RESULTS

#### *Field observations*

Six distinct stages of the gall life cycle were observed and recorded (Appendix B). In the first stage of the gall life cycle, Formation, the gall was a small, yellow-green bump on the leaf that was less than 5mm in diameter. The gall was comprised mostly of fleshy plant tissue with a small, hollowed out pit with a *M. vitiensis* nymph inside. It was completely closed with a slight, raised pucker on the underside of the gall. The gall tissue swelled upward and transformed into a brighter green color in the Growth phase as the psyllid nymph and gall pit were enlarged as well. The gall continued to enlarge until the Eruption stage in which the underside of the gall split and spread out to make an opening in the gall. At this point, the psyllid emerged from the gall. Occasionally, a Tortricid moth larva would enter a gall between the transition stages of Eruption and Browning. The entire gall would then turn brown during the browning stage with the bottom of the gall along the edges opening from the Eruption stage browning first. This initial browning occurred as the plant tissue oxidized and began to dry out. After the gall turned completely brown, white fungal fruiting bodies would appear on the underside of the

gall signaling the Decay stage. The white fruiting bodies grew throughout the entire gall and would grow on both the bottom and top of the gall. The gall dried out and desiccated in the final Desiccation stage and was often times completely devoid of tissue with the exception of the exterior tissue covering. The remaining gall tissue pulled away from the surrounding leaf tissue in the final stage and eventually only a hole marked where the gall resided.

There were distinct differences in the communities and characteristics of galls overtime. The psyllid, *M. vitiensis*, remained in the gall in the Formation, Growth, and part of the Eruption stage. After *M. vitiensis* left the *S. malaccense* erupted gall, two other organisms colonized the gall: a Tortricid moth larva and white *Penicillium? sp* fungus. The Tortricid moth larva was found in some but not all of the galls after the Eruption stage. It colonized the gall after Eruption and ate its way into the fleshy, gall tissue to create tunnels for it to reside in. Sometimes the larva would be present in the gall tissue with *M. vitiensis* still inside the erupted gall but was not found prior to the Eruption stage (Figure 2). Moth larvae were observed to crawl along the leaf to move between galls for shelter and tunneled into adjacent galls as well. The moth larva was found inside the gall tissue throughout the Eruption, Browning, and Decay stages but was not found in the Desiccation stage (Figure 3). While not all galls contained moth larva, many galls contained piles of orange-brown, sticky frass, which clung to the underside of the gall (Figure 2). This frass was produced by Tortricid moth larvae and provided a surface for white *Penicillium? sp* fungal fruiting bodies to grow. While the white fruiting bodies were primarily found on wet and dry larva frass, it was also observed to reside on wet, brown, soggy gall tissue as well. The complete transition of the gall from green, photosynthetic tissue to brown tissue, and fungal growth signaled the Decay stage. As the gall tissue dried up overtime in the Desiccation stage, the white fungal bodies also desiccated but remained until the gall tissue fell off (Figure 3).

There were several important trends seen in Figure 4, the gall life cycle and succession.

The percentage of galls with psyllids - *M. vitiensis* - inside decreased dramatically as the percentage of galls erupted increased showing an inverse relationship. Psyllid nymphs were always found in the gall before the gall erupted while Tortricid moth larvae were found after the gall erupted. The percentage of larva present and percent brown increased overtime before plateauing at 39% and 100% respectively (Figure 4). Fungus grew after the underside of the gall had 100% coverage of brown and increased steadily for eighteen days before leveling off at an average of 60% coverage of fungus across all the galls sampled. The Desiccation stage of the gall life cycle was not reached in Figure 4. However, in Figure 2, pictures of galls in the Desiccation stage were recorded, as well as holes left on the *S. malaccense* leaf where galls dropped off, Post-gall.

#### Field experiments

Percent-coverage of brown on the underside of galls in both the cut and spore treatment progressed at similar rates. The cut treatment regression line had a slope of 13.01

with a y-intercept of 16.26. The p-value was <0.001 with a R2 value of 0.665. The spore treatment regression line had a slope of 12.961 with a y-intercept of 21.848. The p-value was <0.001 and the R2 value was 0.642. The two treatments had very similar slopes with only a 0.049 difference. Percent-coverage of fungus also had similar slopes. The cut treatment regression line had a slope of .71 with a y-intercept of -3.711. The p-value was <0.001 with a R2 value of 0.153. The spore treatment regression line had a slope of 0.052 with a y-intercept of -.27. The p-value was <0.001 and the R2 value was 0.113. The regression lines of both test and both treatments were significant indicating a positive correlation between time and the increase of browning and fungal coverage. The slopes of between treatments in both tests were too similar to determine clear differences in rates of browning and fungal growth. Thus, based on the slopes, there was no distinct difference in the percent-coverage of brown or percent-coverage of fungus in either treatment.

#### DISCUSSION

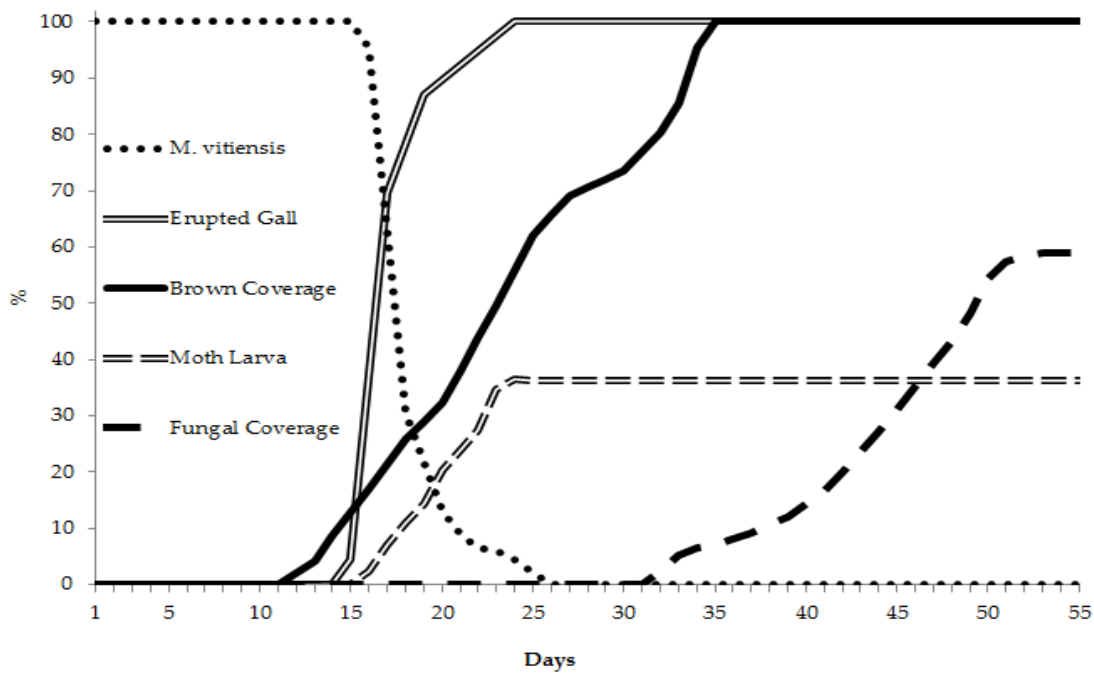


FIG. 4. Gall life cycle and succession percent characteristics present over time. A set of galls was followed over time and observed for the presence of five characteristics.

The results of this research show succession in the *S. malaccense* gall as different communities occupy the gall over time. As shown in Figure 3, the psyllid nymph occupies the gall first in the gall life cycle and is followed by the Tortricid moth larva and white *Penicillium? sp* fungus. While the presence of the psyllid nymph is required for the formation and the development of the gall, the presence of the moth larva was not required for the following stages of the gall life cycle. It also seemed that the presence of the white *Penicillium? sp* fungus was not necessary for the progression of the gall life cycle. However, the presence of the white *Penicillium? sp* fungus was only denoted by the occurrence of the visible, white, fungal fruiting bodies and not the hidden fungal mycelium. The extent of the fungal mycelium in the gall and leaf tissue of *S. malaccense* was not observed and may have been present in gall tissue without visible fruiting bodies. Additionally, while it seemed that the white *Penicillium? sp* fungus produced fruiting bodies on wet, brown gall tissue as well as the moth larva frass in Figure 2, the moth larva frass could have been hidden by the growth of the fungal fruiting bodies. The moth larva frass could have also dried out and been concealed by the dried, brown gall tissue. As this white *Penicillium? sp* fungus was only identified to genus, it would be interesting to note if it is a native or introduced fungus and whether it is specialized or not. If *Penicillium? sp* is native to this system, it would suggest that its role in the gall system is highly co-evolved. This would be additionally supported if the fungus was also a specialist to this system.

While the presence of an individual moth larva was not found in every gall, one moth larva could have a large impact on a single *S. malaccense* leaf in multiple galls. A single moth larva could tunnel between the gall tissue of

several adjacent, touching galls and then crawl across the leaf to another group of galls. This movement between galls was a source of error in Figure 4, as the existence of a moth larva in the gall was determined by the presence of its frass. The moth larva defecates on the underside of the gall, the inside of the gall pit, and in the tunnels of the gall tissue which greatly increased the surface area available for the fungus to produce fruiting bodies. In Figure 4, there seems to be a trend between the increased browning of the galls and the increased presence of the moth larvae. This could be because moth larvae continuously fed on gall tissue, which increased the surface area of exposed plant tissue and thus oxidation and browning of the gall. This is the second recorded observation of this Tortricid moth and exemplifies the increase of biodiversity associated with and possibly resultant from succession. It is currently an undescribed species and was also found by Peter Oboyski on the *S. malaccense* flower below the Belvedere in Mo'orea during the Biocode Project (Peter Oboyski, personal communication, October 9, 2012). These moth larvae seem to be generalist feeders that take advantage of edible plant tissue on the *S. malaccense* tree. Strangely enough, moth larvae do not seem to eat the tissue on the *S. malaccense* leaves, and only ate the gall tissue in the observations of this study. It would be interesting to note whether this Tortricid moth is an endemic species to the island that colonized *S. malaccense* trees by chance or was an introduced or if it is an invasive species that naturalized on this plant. Regardless, the presence of this undescribed species in a known symbiosis supports the concept that plant-insect symbioses create opportunities for secondary colonizers as described by Paul et al (2000) and increases the overall biodiversity of the system.



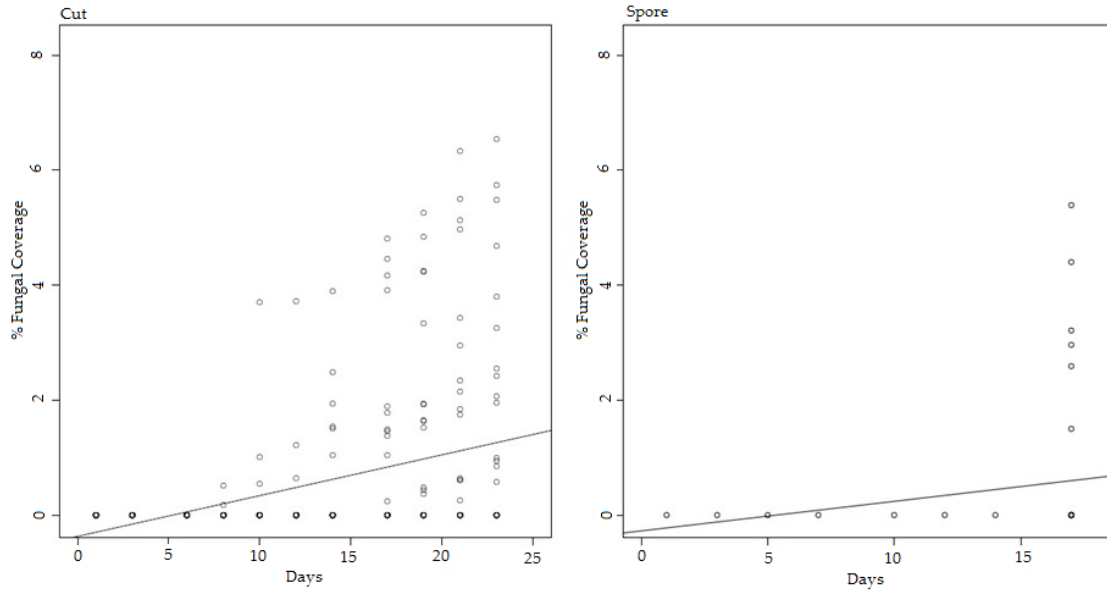


FIG. 5. Percent-coverage of fungus over time in cut treatment (left) and spore treatment (right) Fitted with a regression line: cut treatment p-value <.001,  $R^2=0.153$  and spore treatment p-value <.001,  $R^2=.113$ .

This study also aimed to examine the causes of gall color change and whether the presence of the gall induces leaf decay outside the gall boundaries. Oxidation caused by plant wounding and browning caused by plant tissue drying out was found to be responsible for the color change in galls. While colonization by the Tortricid moth larvae was not directly responsible for the gall color change, it did increase the rate of color change from green to brown. The moth larvae increased the rate of oxidation, browning, and desiccation through herbivory. It also provided additional nutrients for the white fungus to grow on its larva frass. Galls that did not experience herbivory had less browning and remained green for longer periods of time than those that did. As shown by the desiccation and decay experiments, the rate of browning between the two treatments was very similar regardless of the presence of fungal spores, signifying that the presence of fungal spores did not affect browning.

While browning was limited to where wounds on the leaf and gall were made, decay as indicated by the presence of fungal fruiting bodies was confined to the gall area as well. In Figure 5, both the cut and spore treatment had similar regression slopes however, there were more galls with some *Penicillium? sp*

fungal growth seen on the cut treatment than on the spore treatment. This was unexpected as the cut treatment that was not inoculated with fungal spores and had more fungal fruiting bodies than the spore treatment that was inoculated with fungal spores. These findings suggest that in order for *Penicillium? sp* fruiting bodies to grow, the fungus needs another component for growth aside from an herbivory wound. One reason why the spore treatment might not have had as many fungal fruiting bodies was that the spore solution may have been at too low of a spore concentration to properly inoculate the gall. The more likely reason for the difference between the two treatments, however, was the presence of Tortricid larvae in some of the cut treatment galls and not in the spore treatment. As most *Penicillium? sp* fruiting bodies were observed on insect frass in the field, the *Penicillium? sp* fungus might require the presence and maybe nutrients from the frass to grow. A pilot study was unsuccessfully conducted to track browning and fungal growth with the presences of a transplanted moth larva. The stress of removing the moth larva from its original gall to a new gall caused the moth larva to pupate within a day and remain outside of the gall. The white fruiting bodies were not found outside the gall

boundaries on the leaf tissue which correlated with where the moth larva frass was found. Further studies should be done to examine the mycelium of the white *Penicillium? sp* fungus and to determine how extensive its network is on the *S. malaccense* leaf.

The observations of the successional stages and shifts as well as the experimental studies on the spread of desiccation and decay provide new information about the life cycles of the community observed, and the symbioses between plants and insects, and also confirm past models of succession. The life cycle information of *M. vitiensis* matches the outlined life cycle information of its family, Trioziidae established by Downer (1991) and Hodkinson (Ananthakrishnan 1984). The life cycle information of galls on *S. malaccense* up until gall eruption correlates to Hodkinson's findings as well (Ananthakrishnan 1984). Information about the gall after eruption, however, has not been observed before, and stands alone as new information about the gall life cycle. This new information about the *M. vitiensis* and *S. malaccense* gall life cycles demonstrates co-evolution as these organisms change due to their influence on each other (Raman 1997).

This study was the first to record all organisms found in the later stages of the gall life cycle and the presence of the Tortricid moth larva and white *Penicillium? sp* fungus in *S. malaccense* galls. The colonization of the gall after *M. vitiensis* leaves the gall demonstrates the directional shift in the gall community and indicates succession as defined by Roskam (1997). The psyllid constructs an environment - the gall - to live in, which also creates gall tissue for the moth larvae to consume and reside in. In turn, the moth larvae defecate around and in the gall, providing an ideal surface for the white fungus to grow fruiting bodies on. Thus, the Tortricid moth larvae and the white fungus benefit from living on the gall as the moth larvae receives nutrients and shelter and the white fungus receives nutrients and is able to produce spores for propagation. *Syzygium malaccense* does not benefit from this symbiosis, and actually experiences reduced productivity, as stated by Mello (2002). This parasitic symbiosis does not kill too many *S. malaccense* cells though, and

supports Felt's findings in other co-evolved relationships (Felt et al 1940).

Though a majority of the gall's life cycle has been elucidated, there are several gaps in its life cycle that should be observed and further researched. While galls were clustered into several stages according to different characteristics, the actual age of each gall was not known. This would be important to observe in order to compile a more exact timeline of the gall life cycle. By rearing an adult and allowing it to oviposit on a *S. malaccense* leaf, time points could then be recorded from when the eggs were laid, when the leaf tissue began swelling to form the gall, and when the nymphs hatched. This additional information would enable a finer partitioning of the currently delineated formation stage of the gall life cycle and give a more comprehensive view of the relationship between *M. vitiensis* and *S. malaccense*. The mechanism of gall eruption would also be an interesting component of the gall life cycle to observe. This study would reveal whether *M. vitiensis* evolved a trigger to stimulate the eruption of the *S. malaccense* gall when ready to molt into an adult, or if the gall erupted at a critical mass and the psyllid adjusted its growth to match the timing of gall eruption.

Additional studies to observe the secondary organisms that occupy the gall would also add complexity to this plant-insect relationship. In order to determine the host range of the white *Penicillium? sp* fungus, galls and larva frass with and without the white *Penicillium? sp* fruiting bodies should be dissected and observed for mycelium. This would help determine the *Penicillium? sp* fungus' specificity and could provide further information on whether decay, or the growth of this fungus, was limited to the gall boundaries and moth larva frass, or grew throughout the entire leaf. Finally, a study about the Tortricid moth larvae's life cycle would add insight to how the *S. malaccense* tree is utilized in this moth's life cycle and how it influences the gall life cycle. As this is only the second time this Tortricid moth has been recorded, very little is known about its life cycle and if it is endemic to Mo'orea or French Polynesia.

There are many more fragments of this study system and other plant-insect interactions to be observed and explained. While this study looked at one facet of symbiosis, co-evolution, and succession found in this tropical ecosystem, a plethora of other relationships reside, condensed in this diverse system. Understanding connections between organisms also enables scientists to recognize and conserve biodiversity. With every new study, the complexities of the tropical ecosystem build to expand old concepts and create new theories. A comprehensive knowledge of these tropical ecosystems and their combined interactions add important knowledge to appreciating biodiversity by ecological succession and understanding the natural history of the world.

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## APPENDIX A

*Syzygium malaccense*



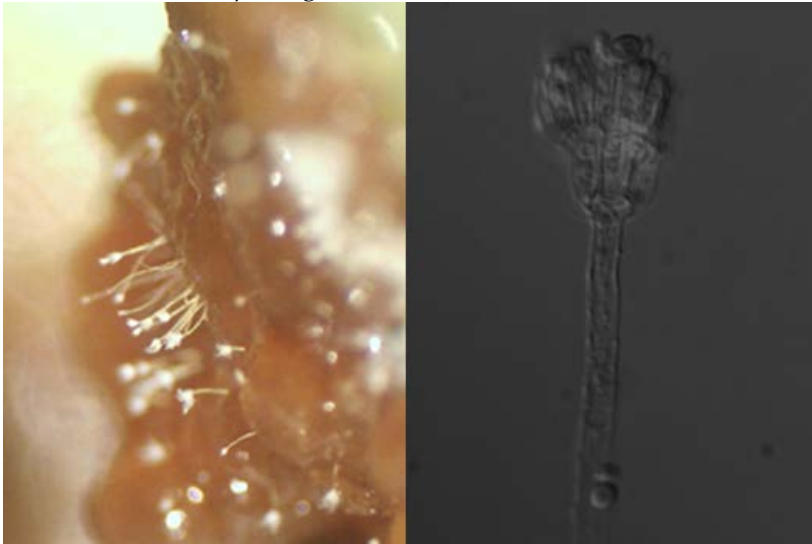
*Syzygium malaccense*, commonly known as *Ahi'a* in Tahitian, is a medium sized, tropical tree that can grow up to twelve meters in the wild (Whistler 2006). Whistler observed that *S. malaccense* has seasonal growth, primarily flowering in the spring, but can flower up to three times a year in tropical environments. He observed that bright cerise, bottle brush flowers grew two to three times a year, and produced fleshy, oval shaped apples that deteriorated rapidly after ripening. The leaves are opposite, simple, oblong shaped and are often covered in insect galls (Whistler 2006), which vary from shades of green to brown. These trees prefer humid valleys but can grow at elevations up to 600 meters above sea level. While not an invasive species, it naturalizes in areas where introduced to, and normally lives for several decades. (Whistler, 2006).

*Megatrioza vitiensis*



*Megatrioza vitiensis* was found throughout the Pacific on *Syzygium malaccense* and other *Syzygium* species by Kirkaldy (Ananthakrishnan 1984). It is a member of family Triozidae and has a flattened nymph instar stage that resides at the base of the gall's interior pit. They form closed, green, pit galls on leaves that preferentially cluster along the leaf's midrib with one nymph per gall (Mulherin, 2010). The *M. vitiensis* nymph resides inside the gall between two to three weeks before the gall erupts and then emerges. The nymph crawls out of the gall onto the underside of the leaf where the nymph exoskeleton split in half and the fully-formed, winged, adult emerges. Hodkinson found that after the adults oviposit on the leaf, psyllid nymphs hatch from their eggs and feed on nutrients from the phloem, triggering gall formation (Ananthakrishnan 1984).

White *Penicillium?* sp Fungus



The white *Penicillium?* sp fungus was found on *Syzygium malaccense* galls, mostly on Tortricid moth larva frass. It was identified down to the genus *Penicillium?* sp by Todd Osmundson of the University of California Berkeley.

Tortricid moth



Tortricid moth larvae were found on the *Syzygium malaccense* flowers (Peter Oboyski, personal communications 09 October 2012) and galls and then reared into adults. It is currently identified as a Tortricid moth, species 8 (Oboyski 2010).

## Appendix B

Stage	% Brown Top	% Brown Bottom	Size (mm)	Open/Close	Psyllid	Moth Larva	Wet Larva Frass	Dry Larva Frass	% Fungus	Hollow	Notes
Formation	0	0	0-5	Close	A	N	N	N	N	N	Newest galls observed were at least one day old
Growth	0-5	0-10	5-8	Close	A	N	N	N	N	N	Slight browning can occur where the gall opening is
Eruption	0-10	0-20	5-9	Open	S	S	S	N	N	N	Erupted galls form a "star" shape opening
Browning	0-25	5-100	5-9	Open	N	S	S	N	S	N	Has a significant amount of fleshy green gall tissue
Decay	25-100	75-100	5-9	Open	N	S	S	S	S	N	Has little to no green gall tissue left
Desiccation	75-100	75-100	4-9	Open	N	N	N	S	S	A	Gall shrinks and a hole starts to form
Post-Gall	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	Hole in the leaf where the gall tissue use to be

Key. A-Always, S- Sometimes, N-Never