Determining if There are Lines of Guava Rust (*Puccinia psidii*) That Could Seriously Impact Ohia (*Metrosideros polymorpha*), in Hawaii

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Introduction

The rust, *Puccinia psidii*, was first found on the leaves, stems and fruit of guava in Brazil in 1894 (Winter, 1984). As a result, it was first called guava rust. It has subsequently been identified in other countries of the western hemisphere including Paraguay in 1884, Uruguay in 1989, Puerto Rico in 1913, Colombia in 1913, Cuba in 1926, Jamaica in 1936, Florida in 1979, Mexico in 1981, El Salvador in 1987 (Simpson *et al*, 2006). It has also been found in California in 2004. This progression of finds suggests that this rust fungus has been moving to new locations, especially in a northerly and westerly direction from its point of origin.

In 2005, *P. psidii* showed up in Oahu, Hawai'i (Killgore and Heu, 2005; Uchida *et al*, 2006). Within two years after arriving on Oahu, this rust was well-distributed on every other Hawaiian Island, as well. One hypothesis is that the Hawaiian strain came from California. However, this rust is very scarce in California (CFPC, 2008). It will probably take molecular genetic analyses of appropriate samples to determine the actual route this rust took to get to Hawaii.

Worldwide, *Puccinia psidii* has already shown a capacity to attack plants in 20 genera in the family Myrtaceae (Simpson *et al*, 2006). Eucalyptus is one genus in the family Myrtaceae that is of great interest internationally. In one test in Brazil, with 39 species of *Eucalyptus* and *Corymbia*, 37 of the species tested were found to be susceptible to this fungus (Alfenas and Zauza, unpbl. data, reported in Glen *et al*, 2007).

To date, the strain of the rust that has made it to Hawaii has not been found on any eucalypts, but is has killed a very high proportion of one already-endangered native Myrtaceae (*Eugenia koolauensis*). An exotic-invasive bush species, *Syzyzium jambos* (the Indian Rose Apple) has also been very heavily impacted. Previously, *S. jambos* had colonized many tens of thousands of hectares of Hawaii mainly in the form of one to five hectare clumps of very tall bushes. The rust only affects the current year's flushes of foliage of the rose apple, but it can annihilate this foliage completely and after about three years of loosing new foliage, the rose apple simply dies.

The Impact on Ohia

The strain of *P. psidii* that has made it to Hawaii can also attack ohia (*Metrosideros polymorpha*) and this is undoubtedly the item of greatest concern in Hawaii. Ohia trees comprise about 85% of all native forests in Hawaii. If this rust could kill extensive amounts of Hawaii's ohia forests, the impact would be hugely deleterious to the biodiversity, watersheds, natural beauty and many other attributes that these forests provide.

So far, the impact of the rust has not been very heavy on existing ohia trees found across Hawaii, even where there are heavily-infected clumps of rose apple right nearby. In arboretum settings it is apparent that some ohia varieties are much more susceptible to this fungus than are others. But even with the most vulnerable varieties, only the most juvenile leaves become infected.

Perhaps the most troubling demonstration of the possible impact of this fungus is in nurseries where ohia are being raised and where there happens to be

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In: Adams, J. comp. 2010. Proceedings of the 57th Western International Forest Disease Work Conference; 2009 July 20-24; Durango, CO. Forest Health Technology Enterprise Team Ft Collins, CO
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nearby, heavily-infected, rose apple clumps that are providing a heavy spore load. Under these circumstances a majority of ohia seedlings show sign of infection and some die. Fortunately, in the natural forests, although there are some ohia seedlings that have been infected by this rust, the proportion of affected seedlings found to date has been very low.

From a purely pragmatic point of view, therefore, the impact of this particular rust strain might be considered a nuisance in Hawaii, but a tolerable one.

Figure 1. *Puccinia psidii* pustules on the leaves of a heavily attacked Rose apple (*Shyzyzium jambos*) stem (Photo courtesy of J.B. Friday).

The possibility of more aggressive strain of *P. psidii* coming to Hawaii

Although P. *psidii* is already widespread in Hawaii, the genetic marker analyses that have been performed on about 60 different samples of this fungus, collected from all over Hawai'i, suggests that all of the sources have the same genetic composition.

For this reason, the Hawaiian authorities are very concerned that a more aggressive strain of *Puccinia psidii* will find its way to Hawaii and cause considerably more damage to local myrtaceae, and especially the ohia trees.

Towards preventing this from happening, the Hawaiian authorities set up an interim measure to prevent the entrance of any myrtaceous plant or plant parts from coming into the state. This interim law was in effect from March 2006 until March of 2007 and was then automatically voided. The law has been replaced with an inspection routine. Currently all shipments of myrtaceous species coming into Hawaii are examined by Agricultural Inspectors at the ports of entry. If no sign of **P. psidii** can be found on the inspected plants, then they are allowed to pass on to their intended destinations.

There are a few limitations in the logic of using either a total blockage approach of Myrtaceae into Hawaii or of using just an inspection at entry approach. One of the major shortcomings in both is that it is unknown whether there might actually be a more aggressive strain of guava rust.

The Test

Towards resolving this question of whether there might be strains of *Puccinia psidii* that could be much more pathogenic on ohia, an appropriate test is being initiated. It involves the following steps:
About 300 viable seeds were collected from each of 24 distinct families of ohia from many diverse locations in Hawaii; these seed have been shipped to Vicosa, Brazil where they are being grown as seedlings to about one year of age.

Approximately 300 single-pustule isolations have been made of *Puccinia psidii* in Brazil and neighboring countries and these have been (or are being) bulked up to produce sufficient quantities of each strain so that the DNA of each strain can be extracted and analyzed for their respective genetic markers.

Once all of these genetic analyses are completed, the phylogeny of this rust will be established and this information, coupled with any information on the apparent aggressiveness of a strain, will be used to select 8 distinct strains for use in pathogenicity tests (note: one of the strains that will be used will be one that matches up with the strain already in Hawai'i; this will take some special attention but will not be covered exhaustively here). The pathogenicity test will consist of an 8 x 24 factorial test with six

blocks. In each block of this test, 8 strains of this rust will be inoculated onto 15 seedlings of each of the 24 different ohia families.

The degree to which the rust has developed on all 11,520 ohia plants in this test will be evaluated at three weeks and six weeks following inoculation and the appropriate statistical tests will be performed.

The data and the conclusions of this study, whatever they might be, will be presented to the authorities in Hawaii and should provide an accurate estimation of whether there could be a more aggressive strain of guava rust for the ohia tree.

Based on the above, it is hoped and expected that the authorities in Hawaii will have a much more accurate concept of the risk that an influx of additional strains of *P. psidii* might present to their islands. Armed with this information they will be able to make better-informed decisions regarding regulations to place on Myrtaceous species coming into Hawaii.

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