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# Virulence and impact of Brazilian strains of *Puccinia psidii* on Hawaiian ‘ohia (*Metrosideros polymorpha*)

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**Abstract**

A single strain of *Puccinia psidii*, the causal agent of rust disease on Myrtaceae, was recently reported on multiple myrtaceous hosts in Hawaii, but this strain has caused only mild levels of damage to the state’s predominant native forest tree, ‘ohia (*Metrosideros polymorpha*). Multiple other strains of *Puccinia psidii* have been identified from Brazil and characterized via extensive sampling and microsatellite analyses. The potential effects of other Brazilian *P. psidii* strains on Hawaii’s ‘ohia were investigated with two inoculation experiments conducted in Brazil. The first, a split-plot experiment, was conducted to determine the pathological impact of five Brazilian *P. psidii* strains on ‘ohia seedlings and to assess variation in susceptibility among six different ‘ohia families to each *P. psidii* strain. The second experiment was conducted to determine the influence of the rust disease on growth and survival of ‘ohia seedlings. Three of the five *P. psidii* strains were highly virulent on most of the inoculated ‘ohia seedlings (93-100% infection rates), and none of the ‘ohia families used in this test showed significant resistance. The other two strains tested were much less virulent. Infection by the highly virulent strains of *P. psidii* resulted, on average, in a 69% reduction in height growth and 27% increase in mortality of ‘ohia seedlings at 6 months post infection. These results have immediate implications for designing Hawaii’s quarantine barriers.

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## Introduction

In 2005, the rust pathogen *Puccinia psidii* was first reported on an ‘ohia seedling (*Metrosideros polymorpha*) growing in a greenhouse in the Hawaiian Island of Oahu (Killgore and Heu 2007). Subsequently, this rust was reported on all major islands of Hawaii (Uchida et al. 2006, Anderson 2012). As of February 2013, this rust had been found to infect young, unsclerified, foliage of 33 myrtaceous tree and shrub species (Janice Uchida, University of Hawaii, Manoa, pers. com.) out of the approximately 200 myrtaceous species occurring in Hawaii.

In Hawaii, *P. psidii* infection has had variable impacts among myrtaceous species (Anderson 2012). The most striking impact of this rust disease has been on rose apple (*Syzygium jambos*), an invasive tree that had previously colonized tens of thousands of hectares in Hawaii. Since the introduction of *P. psidii*, rose apple has experienced heavy defoliation and wide-spread mortality following infection. *P. psidii* has also had a devastating impact on *Eugenia koolauensis*, one of Hawaii’s endangered endemic species. It can also severely affect *Rhodomyrtus tomentosa*, an invasive shrub. However, the impact of *P. psidii* infection on most of the other myrtaceous hosts, including ‘ohia, has been less severe to date.

It is especially fortunate for Hawaii that *P. psidii* has had only light impact on ‘ohia, because the ‘ohia tree is the most wide-spread tree species in Hawaii; it constitutes approximately 80 percent of all forest trees in Hawaii’s native rainforests (Loope and LaRosa 2008) and occupies about 400,000 hectares (1,000,000 acres) in Hawaii (Petteys et al. 1975). For these reasons, ‘ohia is the most critical species for protecting Hawaii’s watersheds and native ecosystems that provide habitat for much of the state’s wildlife (Loope and Uchida 2012). ‘ohia is a highly variable species in terms of its physical appearance (height, form, leaf morphology, flowers etc. and as suggested by the specific epithet “polymorpha”); it is estimated to have arrived to the Hawaiian Islands about 3.9 million years ago and has subsequently evolved on diverse site types found across the Hawaiian Islands (Percy et al. 2008).

In the natural environment in Hawaii, ‘ohia has been only lightly infected by *P. psidii,* with infections found on young leaves of a few ‘ohia plants scattered around Hawaii (Anderson 2012). *P. psidii*-caused mortality of ‘ohia has occurred on rare occasion, but then only when conditions were extremely conducive for disease development. One instance of *P. psidii*-caused mortality occurred at a commercial nursery on Oahu where potted ‘ohia seedlings received mist irrigation and were situated downwind from nearby stands of *P. psidii*-infected rose apple that produced abundant urediniospores (assessment made by P. Cannon in 2007). This situation was alleviated by removing the infected rose apple trees and applying appropriate fungicides to subsequent batches of ‘ohia seedlings.

Although *P. psidii* has only lightly impacted Hawaii’s natural ‘ohia, to date, major concerns remain about potential future impacts of this rust pathogen on the ecologically important ‘ohia trees. Specifically, research by Zhong et al. (2008, 2011) indicated that only one genotype of *P. psidii* had been introduced to Hawaii. Thus, plant health experts are concerned about the inadvertent introduction of other rust-pathogen strains that might be more aggressive on ‘ohia (Loope and Uchida 2012). Of further concern, the variation in rust susceptibility among ‘ohia sources has not been well characterized, so it remains unknown which host populations are more susceptible to *P. psidii*.

To assess genotypic diversity of *P. psidii* in Brazil, extensive sampling was conducted from diverse myrtaceous species across widely ranging geographic locations, and *P. psidii* genotypes were determined by microsatellite analysis (Graça et al. 2011). This analysis indicated the presence of multiple *P. psidii* genotypes in Brazil, which reflected varying genetic relationships and often different host associations. The present studies were designed to 1) assess variability in pathogenicity of several Brazilian *P. psidii* strains on seedlings representing diverse ‘ohia families from Hawaii, and, 2) estimate the impact of severe *P. psidii* infections on growth and survival of ‘ohia seedlings.

Materials and Methods:

Pathogenicity of different strains of *Puccinia psidii* on different families of ‘ohia.

For tests on the pathogenicity of different *P. psidii* strains on ‘ohia, inoculations were conducted with five Brazilian strains (Table 1), which represented wide genotypic diversity and diverse host associations.

Table 1 goes approximately here

To bulk up urediniospore numbers for inoculation tests, single-uredinial isolates of each strain were separately inoculated as a spore suspension (2 x $10^4$ urediniospores $mL^{-1}$) to leaves on host plants of the same species from which the strains were originally derived. After 20 days in the inoculation chamber, newly formed urediniospores were collected and stored at -80$^o$ C until required for assay.

To test differences in susceptibility among ‘ohia families, at least 300 ‘ohia (*Metrosideros polymorpha*) seeds were collected from each of six different open-pollinated (OP) ‘ohia trees growing at diverse locations on the island of Hawaii (Table 2).

Table 2 goes approximately here.

The seed collections were processed in accordance with the requisite phytosanitary procedures and shipped to Brazil. The ‘ohia seeds were germinated in the greenhouse of the Universidade Federal de Viçosa, and established as potted seedlings.

A split-plot inoculation experiment was conducted in which the five main plots in each block consisted of the five *P. psidii* strains described above, and the split plots in each block consisted of each of the six different ‘ohia families. Each split plot contained three seedlings from one of the six ‘ohia families. Three completely randomized replications (blocks) of this design were implemented.

The inoculum for each strain was prepared as an aqueous suspension of $2\times10^4$ urediniospores $mL^{-1}$ according to the methods developed by Ruiz et al. (1989). For inoculations, this spore suspension was spray-inoculated onto abaxial and adaxial leaf surfaces of all leaves to the point of runoff for all seedlings in the plot. To verify that the urediniospores used were viable, two rose apple cuttings were utilized as susceptible controls in each block. After inoculation, plants were kept in a mist chamber at 25±2°C for 24 h in the dark, and subsequently transferred to a growth chamber at 22±2°C with a 12-hr light cycle (Ruiz et al. 1989); each set of seedlings that had been inoculated with a different *P. psidii* strain was physically separated from sets of seedlings that had received other *P. psidii* strains.

Rust resistance/susceptibility was evaluated for each inoculated plant at 20 days post-inoculation. The two most infected leaves on each plant were categorized for disease development according to Jungans et al. (2003): S0 = immunity or hypersensitive reaction; S1 = punctiform pustules <0.8 mm in diameter; S2 = medium pustules from 0.8 to 1.6 mm in diameter; and S3 = large pustules >1.6 mm in diameter and, in some cases, where pustules developed on the leaf petioles and young branchlets. The term “fleck” was also used to characterize the immune reaction that was occasionally observed. Flecking occurs when localized leaf cells become chlorotic, but a small black spot, which would be indicative of a hypersensitive reaction, does not develop. A seedling was considered resistant if it had been scored S0 or if it exhibited flecking; a seedling was susceptible if received a score of S1, S2 or S3. A total of 83 to 86 ‘ohia seedlings were evaluated for resistance to each *P. psidii* strain.

QUANT Image Processing Software (Vale et al. 2003) was used to evaluate the percentage of leaf area covered by rust, based on digitized images of infected leaves (Figure 1). Figure 1 goes approximately here.

The split-plot experiment resulted in an unbalanced randomized block design due to some seedling mortality (for disparate reasons) that resulted in the loss of all seedlings of family 12 that had been inoculated with strain 5 in Blocks II and III and all seedlings of family 8 that had been inoculated with strain 5 in Block II. For this reason, the usual ANOVA approach was modified, and a different mixed general linear model (McCullagh and Nelder 1991) was used for the statistical analysis. The combination of the inoculum strain and ‘ohia family (IS-F) was viewed as a categorical fixed effect (with 5*6-2=28 levels), with block (B) and the interaction of the block by *P. psidii* strain-’ohia family combination (B*IS-F) as random effects. To examine the effects of interest, and compare the strain virulence, contrasts were used from the combination IS-F. The maximum likelihood ratio test and the Bonferroni’s adjustment for multiple pairwise comparisons (for an experiment-wise alpha =0.05) were used for testing the contrasts. The SAS v.9.3 MIXED procedure (SAS Institute Inc. 2002-2010, Cary, NC, USA) was used to estimate the means and test the comparisons. Using this approach, statistically valid inferences could be made about each of the following: A) Differences among disease severity caused by *P. psidii* strains; B) Differences in disease severity among ‘ohia families; and C) Differences in disease severity caused by different *P. psidii* strains on different ‘ohia families.

Impact of severe infection by *P. psidii* on growth and survival of ‘ohia seedlings.

To evaluate the impact of severe rust disease on height growth and mortality rates of ‘ohia seedlings, 32 10-month-old seedlings from each of three OP families of ‘ohia (Families 2, 3 and 4) were used. Sixteen of these 32 seedlings for each family were inoculated with Strain 1

(UVF2) of *P. psidii* in accordance with the same methodology described above. The other 16 seedlings of each family served as control plants, which were not inoculated with *P. psidii*, but were sprayed with 1 mL/L of the fungicide trifloxystrobin + tebuconazole (Nativo®, Bayer). Additional sprays of Nativo® were applied to the non-inoculated, control seedlings every 15 days. This experiment was conducted in a completely randomized design in greenhouse conditions. Lateral branches were pruned during the subsequent growth period. Height growth in millimeters and survival were recorded for all 96 seedlings at 30-day intervals over the next 180 days. At the end of the assessment period, the Area Under the Plant Growth Curve (AUPGC) was calculated for all seedlings using the method of Shaner and Finney (1977).

## Results

### Pathogenicity of different strains of *Puccinia psidii* on different families of 'ohia.

The results from the inoculation test with the five *P. psidii* strains and six 'ohia families indicated that three of the strains used– Strain 1 (UVF2 derived from *Eucalyptus grandis*), Strain 2 (EUBA1 derived from *E. urophylla* x *E. grandis* hybrid), and Strain 3 (derived from *Myrciaria cauliflora*) – were highly virulent on all six of the 'ohia families, while Strain 4 (derived from *Psidium guajava*) and Strain 5 (derived from *P. araca*) displayed lower virulence across the 'ohia families. This result was evident from the levels of "resistant seedlings" based on the criteria of  Junghans et al. (2003b); seedlings inoculated with strains 1, 2, and 3 showed only 0%, 7% and 0% resistance, respectively, whereas the seedlings inoculated with stains 4 and 5 showed 61% and 84% resistance, respectively (Table 3).

Table 3 goes approximately here.

Variation in susceptibility of 'ohia to the different *P. psidii* strains is also apparent when considering the percentage of leaves covered by uredia (Table 4). In the statistical comparison of calculated P-values with Bonferroni alpha values (Table 4), no statistically significant differences were found among the disease severities caused by *P. psidii* Strain 1, Strain 2, and Strain 3, and no significant differences were observed in disease severity caused by Strain 4 and Strain 5. However, these Bonferroni values also showed large and statistically significant differences between the disease severity caused by the highly virulent group (Strains 1, 2, and 3) and the lower virulence group (Strains 4 and 5).No statistically significant differences in disease severity were observed among the six different ‘ohia families for each strain (Table 5).

Table 4 goes approximately here.

Table 5 goes approximately here.

Impact of severe infection by *P. psidii* on growth and survival of ‘ohia seedlings.

Surviving seedlings inoculated with *P. psidii* Strain 1 (UFV2) had, on average, only 31% of the height growth (Area Under the Plant Growth Curve) of uninfected seedlings from the same families at 6 months post-inoculation (Table 6). T-tests showed that this difference was statistically significant at the 0.0001 level of probability for Families 2 and 4 and at the 0.005 level of probability for Family 3. Analyses also indicate that the inoculated seedlings had a 27% greater chance of mortality over this same period, compared to non-inoculated, control seedlings.

Table 6 goes approximately here.

Discussion

Conducting these two tests in Viçosa, Minas Gerais, Brazil, conferred two major advantages: 1) The Plant Pathology team at the Universidade Federal de Viçosa had extensive experience with pathogenicity tests with *P. psidii*, and 2) it was possible to examine a diverse range of *P. psidii* strains without risking the introduction of new strains.

The salient results from these experiments show that three *P. psidii* strains used in this test (Strains 1, 2, and 3) were highly virulent on seedlings representing all six of the ‘ohia (*Metrosideros polymorpha*) families tested. In contrast, Strains 4 and 5, derived from *P. guajava* and *P. araça*, respectively, were much less virulent on these same ‘ohia families.

These results clearly demonstrate that some *P. psidii* strains/genotypes, which are not yet present in Hawaii, could potentially cause devastating disease to native ‘ohia in Hawaii should these strains be introduced. Furthermore, this test did not include all presently known strains of *P. psidii*, including those from the mainland USA, Central America, South America, the Caribbean, Australia and elsewhere. Because *P. psidii* strains that are not present in Hawaii appear to pose a threat to native ‘ohia in Hawaii, proactive measures are critical to avoid the introduction of new *P. psidii* strains into Hawaii. This is especially noteworthy because plant protection and quarantine regulations are generally applied only at the species level for a plant pathogen. Thus, special regulatory measures are needed to regulate *P. psidii* at a strain level. Development of special regulatory measures are typically based on a Pest Risk Analysis in which solid scientific evidence is presented that other strains, not yet present in a country (or state in the case of Hawaii), exist that could pose a significant threat to a given plant resource (A. Tschanz, Animal and Plant Health Inspection Service (APHIS), personal communication). The results of this study (and others) should be considered by the appropriate regulatory authorities in the Hawaiian Department of Agriculture (HDOA) and in the Animal and Plant Health Inspection Service (APHIS) when developing appropriate protection and quarantine regulations to prevent the introduction of new *P. psidii* strains to Hawaii via incoming myrtaceous plant materials.

The finding that all *P. psidii* strains are not equally virulent on ‘ohia is interesting but not unexpected. Several previous reports based on cross-inoculation studies have demonstrated marked differences in the ability of *P. psidii* isolates to infect diverse myrtaceous species

(MacLachlan 1938, Marlatt and Kimbrough 1980, Ferreira 1981, De Castro et al. 1983, Coutinho and Figueiredo 1984, Coelho et al. 2001, Rayachhetry *et al*. 2001, Aparecido et al. 2003). Thus, a primary conclusion from this study is that indeed multiple *P. psidii* strains pose a threat to other hosts in other locations, but not all strains threaten all myrtaceous host species in all geographic locations. The reason for this variation in virulence remains unknown, but the low virulence of *P. psidii* Strains 4 and 5 on ‘ohia may reflect co-evolution of some *P. psidii* strains on specific host species, which has resulted in rust strains with different impacts on the different myrtaceous species.

Furthermore, to date, only one genotype of *P. psidii* has been found in Hawaii (Zhong et al. 2008, Kadooka 2010, Graça et al. 2011). To avoid the introduction of the Hawaiian strain of *P. psidii* into Brazil, which has over 5,000,000 hectares of eucalyptus plantations and many other myrtaceous species, the Hawaiian strain of this fungus could not be used in these experiments in Brazil. Plans to conduct virulence tests in a controlled biological containment facility that compare Hawaiian and Brazilian *P. psidii* genotypes are currently under consideration.

Recently, *P. psidii* isolates have been collected on different myrtaceous hosts from Uruguay, Paraguay, Costa Rica, Puerto Rico, Florida, Mexico, and Australia (Carnegie et al. 2010), and, on rooted cuttings of ‘ohia in Japan (Kawanishhi et al. 2009). Continued genetic characterization and virulence tests are needed to determine the invasive threats posed by these other sources of *P. psidii*.

Although not statistically significant, data show trends that indicate potential variation in resistance/susceptibility among ‘ohia families inoculated with the same strain of *P. psidii*. Perhaps more family-level variation in resistance/susceptibility to different *P. psidii* strains could be detected by testing ‘ohia families from more geographically diverse provenances. Increased understanding of natural variation in resistance/susceptibility of diverse ‘ohia families to diverse

*P. psidii* strains would be useful for predicting ‘ohia population dynamics should different *P. psidii* strains become established in Hawaiian forests.

Also, if significant natural variation in rust resistance is found, this genetic variation could be exploited by planting ‘ohia sources that have been selected or deliberately bred for increased resistance to known *P. psidii* strains. The Brazilian programs to develop *Eucalyptus* species, hybrids, and clones that are resistant to *P. psidii* have been especially successful (Alfenas et al. 2009), and could serve as a valuable model in Hawaii for increasing resistance of ‘ohia and other native species to *P. psidii*. However, the economic, ecological, and logistical constraints on wide-scale replanting of ‘ohia forests in Hawaii are sizeable.

The use of leaf photos and QUANT Image Processing Software (Vale et al. 2003) to measure infected-leaf-area was very effective for obtaining a quantitative measure of infection level. This approach provided precise quantitative data (Tables 4 and 5) that were conducive to robust statistical analyses. However, it should be noted that this measurement could greatly underestimate the amount of leaf mesophyll that is infected by *P. psidii*. Anatomical examinations show that *P. psidii* mycelia can occupy a leaf area in the mesophyll that is two to three times larger than the area occupied by the uredia on the leaf surface (Janice Uchida University of Hawaii, Manoa, pers. com.[*]). In some of the split plots, as much as 33% of the leaf surface of ‘ohia plants was occupied by uredia; this could translate to between 66% and 99% infection of the leaf mesophyll by *P. psidii* mycelia. Furthermore, the growth and mortality impact study clearly demonstrated that under highly conducive conditions, *P. psidii* infection can have a tremendously deleterious impact on the health and survival of ‘ohia seedlings.

## Conclusions

These studies conclusively demonstrate the existence of *P. psidii* strains – not yet in Hawaii – that can be highly virulent on ‘ohia from Hawaii. Because these tests were conducted under conditions very conducive to infection (high inoculum levels, high air moisture content and ideal temperature), it is not possible to predict precisely the epidemiological behavior and ecological ramifications of these same strains should they be introduced into the natural ‘ohia forests of Hawaii. Nevertheless, it seems especially prudent to avoid this threat by any practical means.

Despite the fact that one strain of *P. psidii* already exists in Hawaii, our results indicate that other strains of this rust pathogen pose a potential additional threat to the ‘ohia forests of Hawaii should they become introduced. This information lends support to improving state and federal restrictions to reduce the risk of future introductions of additional strains of *P. psidii*. Recognition of the variability in virulence of different strains of *P. psidii* should be useful towards articulating effective biosecurity policies.

These studies show that three out of the five *P. psidii* strains studied from Brazil are highly virulent on ‘ohia seedlings under experimental conditions. Although this experiment was conducted explicitly to explore the risks that other strains of *P. psidii* might pose to the ‘ohia of Hawaii, the Myrtaceae family contains over 4000 species and, indeed, is one of the largest tree families in the world. Other states or nations with significant myrtaceous populations may wish to draw insight from these studies or conduct their own pathogenicity trials with diverse *P. psidii* strains.

Acknowledgements

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Table 1. Host species and locations in Brazil from which each of the five *Puccinia psidii* strains were isolated.

| Strain Number | Host Tree Species From Which Strain Was Isolated | Location coordinates, elevation(masl) and Brazilian State |
|---|---|---|
| 1 | *Eucalyptus grandis* | S22.5986 W58.8003, 550 masl, Sao Paulo |
| 2 | *E.urophylla x E. grandis* hybrid | S18.4865 W39.9849, 78 masl, Bahia |
| 3 | *Myrciaria cauliflora* (jaboticaba) | S20.6554 W42.8367, 675 masl, Minas Gerais |
| 4 | *Psidium guajava* (guava) | S20.4097 W43.0507, 587 masl, Minas Gerais |
| 5 | *Psidium araça* (Brazilian guava) | S15.5903 W39.2892, 120 masl, Bahia |

Table 2. The locations of the six ‘ohia (*Metrosideros polymorpha*) trees on the Island of Hawaii from which seed were collected for these experiments.

| Family Number | Variety of *M. polymorpha* | Location coordinates and landmarks | Elevation masl |
|---|---|---|---|
| 1 | *incana* | N19.47887757870, W154.83296994800, Kapoho Town | 25 |
| 2 | *glaberrima* | N19.42388426190, W155.23639821300, Volcano Town | 1177 |
| 3 | *glabberima* | N 19.68644266000, W155.29324703900, Saddle Road | 1293 |
| 4 | *glaberrima* | N 19.68624485870, W155.29317747700, Saddle Road | 1284 |
| 5 | *polymorpha* | N 19.67743892930, W155.31851862500, Saddle Road | 1452 |
| 6 | *polymorpha* | N 19.68629912190, W155.29175752700, Saddle Road | 1232 |

Table 3. Resistance levels of ‘ohia seedling families in response to inoculation by five different Brazilian strains of *Puccinia psidii*.

| *Puccinia psidii* strain number | Percentage of Resistant ‘ohia Seedlings * |
|---|---|
| 1 (UVF2; derived from *E. grandis*) | 0 |
| 2 (EUBA1; from the *E. urophylla* x *E. grandis* hybrid) | 7 |
| 3 (derived from *M. cauliflora*) | 0 |
| 4 (derived from *P. guajava*) | 61 |
| 5 (derived from *P. araca*) | 84 |

*The method of Jungans et al. (2003) was used to evaluate the resistance of each seedling to *P. psidii*: 83 to 86 ‘ohia seedlings were tested for each *P. psidii* strain.

Table 4. Mean percentage of leaf coverage by uredinia following inoculation with five different *Puccinia psidii* strains on six different ‘ohia (*Metrosideros polymorpha*) families at 20 days post-inoculation.

| *P. psidii* Strain | No. of ‘ohia families | Mean Leaf Damage % | Std. error of Mean Leaf Damage % |
|---|---|---|---|
| Strain 1 (UFV2) | 6 | 18.1 a[1] | 2.0 |
| Strain 2 (EUBA1) | 6 | 14.4 a | 1.5 |
| Strain 3 | 6 | 15.3 a | 1.4 |
| Strain 4 | 6 | 4.1 b | 1.6 |
| Strain 5 | 4 | 3.0 b | 1.8 |

[1]Means followed by the same letter are not significantly different from each other.

Table 5. Mean percent-leaf-coverage with uredinia of leaves of six different ‘ohia (*Metrosideros polymorpha*) families in response to different *Puccinia psidii* strains at 20 days post-inoculation.

| Family | Numbers and names of *P. psidii* strains used | Mean Leaf Damage % | Std. error Leaf Damage % |
|---|---|---|---|
| 1 | 5 (Strains 1, 2, 3, 4, 5) | 11.5 | 4.3 |
| 5 | 5 (Strains 1, 2, 3, 4, 5) | 9.7 | 3.5 |
| 7 | 5 (Strains 1, 2, 3, 4, 5) | 10 | 3.2 |
| 8 | 4 (Strains 1, 2, 3, 4) | 10.3 | 3.4 |
| 11 | 5 (Strains 1, 2, 3, 4, 5) | 11.9 | 1.7 |
| 12 | 4 (Strains 1, 2, 3, 4) | 16.6 | 4.2 |

Table 6. Impact of severe disease caused by *Puccinia psidii* (Strain 1; UFV2) on growth (calculated by measuring the Area Under the Plant Growth Curve; AUPGC) and survival of three families of ‘ohia (*Metrosideros polymorpha*) seedlings at 180 days post-inoculation. T-tests for growth showed significance at the 0.001, 0.05, and 0.0001 levels for families 2, 3 and 4, respectively.

| ‘ohia | Growth of Non-inoculated Seedlings | | Growth of Inoculated Seedlings | |
|---|---|---|---|---|
| Family | AUPGC | Survival % | AUPGC | Survival % |
| 2 | 354 | 69 | 79 | 44 |
| 3 | 762 | 56 | 245 | 19 |
| 4 | 530 | 50 | 199 | 31 |
| Averages | 549 | 58.3 | 174 | 31.3 |

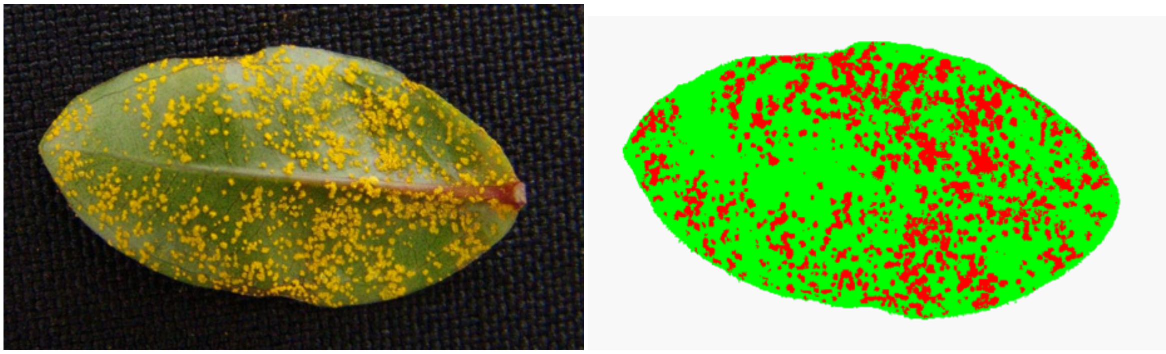

Figure 1. Photo of an ‘ohia leaf infected by *Puccinia psidii* and the image of this same leaf after transformation by the QUANT Image Processing Software (Vale et al. 2003). In this example, 26.65% of the area of the leaf was colonized and this was recorded as the disease severity for this leaf. This leaf is 30 mm in length, actual size.

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