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Virulence and Impact of Brazilian Strains of *Puccinia psidii* on Hawaiian ʻŌhiʻa (*Metrosideros polymorpha*)[1]

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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 Hawaiʻi, but this strain has caused only mild levels of damage to the state's predominant native forest tree, ʻōhiʻa (*Metrosideros polymorpha*). Multiple other strains of *Puccinia psidii* have been identified from Brazil and characterized via extensive sampling and microsatellite analyses. Potential effects of other Brazilian *P. psidii* strains on Hawaiʻi's ʻōhiʻa were investigated with two inoculation experiments conducted in Brazil. The first, a split-plot experiment, was conducted to determine pathological impact of five Brazilian *P. psidii* strains on ʻōhiʻa seedlings and to assess variation in susceptibility of seedlings from six different open-pollinated ʻōhiʻa parent trees to each *P. psidii* strain. The second experiment was conducted to determine influence of the rust disease on growth and survival of ʻōhiʻa seedlings. Three of the five *P. psidii* strains were highly virulent on most of the inoculated ʻōhiʻa seedlings (93%–100% infection rates), and none of the ʻōhiʻa 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 ʻōhiʻa seedlings at 6 months postinfection. These results have immediate implications for designing Hawaiʻi's quarantine barriers.

In 2005, the rust pathogen *Puccinia psidii* was first reported on an ʻōhiʻa seedling (*Metrosideros polymorpha*) growing in a greenhouse on the Hawaiian island of Oʻahu (Killgore and Heu 2007). Subsequently, this rust was reported on all major islands of Hawaiʻi (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 Hawaiʻi at Mānoa, Honolulu, pers. comm.) out of the approximately 200 myrtaceous species occurring in Hawaiʻi.

In Hawaiʻi, *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 Hawaiʻi. Since the introduction of *P. psidii*, rose apple has experienced heavy defoliation and widespread mortality following infection. *Puccinia psidii* has also had a devastating impact on *Eugenia koolauensis*, one of Hawaiʻi's endangered endemic species. It can also severely affect *Rhodomyrtus tomentosa*, an invasive shrub. However, the impact of *P. psidii*

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infection on most of the other myrtaceous hosts, including ʻōhiʻa, has been less severe to date.

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

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

Although *P. psidii* has only lightly impacted Hawaiʻi's natural ʻōhiʻa to date, major concerns remain about potential future impacts of this rust pathogen on the ecologically important ʻōhiʻa trees. Specifically, research by Zhong et al. (2008, 2011) indicated that only one genotype of *P. psidii* had been introduced to Hawaiʻi. Thus, plant health experts are concerned about the inadvertent introduction of other rust-pathogen strains that might be more aggressive on ʻōhiʻa (Loope and Uchida 2012). Of further concern, the variation in rust susceptibility among ʻōhiʻa 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 various genetic relationships and often different host associations. The studies reported here were designed to (1) assess variability in pathogenicity of several Brazilian *P. psidii* strains on seedlings representing diverse ʻōhiʻa families in Hawaiʻi, and (2) estimate the impact of severe *P. psidii* infections on growth and survival of ʻōhiʻa seedlings.

MATERIALS AND METHODS

Pathogenicity of Different Strains of Puccinia psidii *on Different Families of ʻŌhiʻa*

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

To bulk up urediniospore numbers for inoculation tests, single-uredinial isolates of each strain were separately inoculated as a spore suspension (2×10^4 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°C until required for assay.

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

The seed collections were processed in accordance with the requisite phytosanitary procedures and shipped to Brazil. The ʻōhiʻa

TABLE 1

Host Species and Locations in Brazil from Which Each of the Five *Puccinia psidii* Strains Were Isolated

Strain No.	Host Tree Species from Which Strain Was Isolated	Location Coordinates, Elevation (masl) and Brazilian State
1	*Eucalyptus grandis*	S22.5986, W58.8003, 550 masl, São Paulo
2	*E.urophylla* × *E. grandis* hybrid	S18.4865, W39.9849, 78 masl, Bahia
3	*Myrciaria cauliflora* (jabuticaba)	S20.6554, W42.8367, 675 masl, Minas Gerais
4	*Psidium guajava* (guava)	S20.4097, W43.0507, 587 masl, Minas Gerais
5	*Psidium guineense* (Brazilian guava)	S15.5903, W39.2892, 120 masl, Bahia

TABLE 2

Locations of the Six ʻŌhiʻa (*Metrosideros polymorpha*) Trees on the Island of Hawaiʻi from Which Seeds Were Collected for These Experiments

Family No.	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	1,177
3	*glabberima*	N19.68644266000, W155.29324703900, Saddle Road	1,293
4	*glaberrima*	N19.68624485870, W155.29317747700, Saddle Road	1,284
5	*polymorpha*	N19.67743892930, W155.31851862500, Saddle Road	1,452
6	*polymorpha*	N19.68629912190, W155.29175752700, Saddle Road	1,232

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 earlier, and the split plots in each block consisted of each of the six different ʻōhiʻa families. Each split plot contained three seedlings from one of the six ʻōhiʻa families. Three completely randomized replications (blocks) of this design were implemented.

The inoculum for each strain was prepared as an aqueous suspension of 2×10^4 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 hr 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 postinoculation. The two most infected leaves on each plant were categorized for disease development according to Junghans et al. (2003*b*): 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 it received a score of S1, S2, or S3. A total of 83 to 86 ʻōhiʻa 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 (Plate I).

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 analysis of variance (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 ʻōhiʻa 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-ʻōhiʻa 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 = .05) were used for testing the contrasts. The SAS v.9.3 MIXED procedure (SAS Institute 2010) was used to estimate the means and test the comparisons. Using this approach, statistically valid inferences could be made about each of the following: (1) differences among disease severity caused by *P. psidii* strains; (2) differences in disease severity among ʻōhiʻa families; and (3) differences in disease severity caused by different *P. psidii* strains on different ʻōhiʻa families.

Impact of Severe Infection by P. psidii *on Growth and Survival of ʻŌhiʻa Seedlings*

To evaluate the impact of severe rust disease on height growth and mortality rates of ʻōhiʻa seedlings, 32 10-month-old seedlings from each of three OP families of ʻōhiʻa (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 earlier. The other 16 seedlings of each family served as control plants, which were not inoculated with *P. psidii* but were sprayed with 1 ml/liter of the fungicide trifloxystrobin + tebuconazole (Nativo, Bayer). Additional sprays of Nativo were applied to the noninoculated, 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 ʻŌhiʻa*

The results from the inoculation test with the five *P. psidii* strains and six ʻōhiʻa families indicated that three of the strains used [Strain 1 (UVF2 derived from *Eucalyptus grandis*), Strain 2 (EUBA1 derived from *E. urophylla* × *E. grandis* hybrid), and Strain 3 (derived from *Myrciaria cauliflora*] were highly virulent on all six of the ʻōhiʻa families, but Strain 4 (derived from *Psidium guajava*) and Strain 5 (derived from *P. araça*) displayed lower virulence across the ʻōhiʻa families. This result was evident from the levels of "resistant seedlings" based on the criteria of Junghans et al. (2003*b*); seedlings inoculated with Strains 1, 2, and 3

TABLE 3

Resistance Levels of ʻŌhiʻa Seedling Families in Response to Inoculation by Five Different Brazilian Strains of *Puccinia psidii*

Puccinia psidii Strain No.	Percentage of Resistant ʻŌhiʻa Seedlings[a]
1 (UVF2; derived from *E. grandis*)	0
2 (EUBA1; from the *E. urophylla* × *E. grandis* hybrid)	7
3 (derived from *M. cauliflora*)	0
4 (derived from *P. guajava*)	61
5 (derived from *P. guineense*)	84

[a] The method of Junghans et al. (2003*b*) was used to evaluate the resistance of each seedling to *P. psidii*: 83 to 86 ʻōhiʻa seedlings were tested for each *P. psidii* strain.

TABLE 4

Mean Percentage of Leaf Coverage by Uredinia after Inoculation with Five Different *Puccinia psidii* Strains on Six Different ʻŌhiʻa (*Metrosideros polymorpha*) Families at 20 Days Postinoculation

P. psidii Strain	No. of ʻŌhiʻa Families	Mean[a] Leaf Damage (%)	SE of Mean Leaf Damage (%)
Strain 1 (UFV2)	6	18.1a	2.0
Strain 2 (EUBA1)	6	14.4a	1.5
Strain 3	6	15.3a	1.4
Strain 4	6	4.1b	1.6
Strain 5	4	3.0b	1.8

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

showed only 0%, 7%, and 0% resistance, respectively, whereas the seedlings inoculated with Strains 4 and 5 showed 61% and 84% resistance, respectively (Table 3).

Variation in susceptibility of ʻōhiʻa 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 ʻōhiʻa families for each strain (Table 5).

Impact of Severe Infection by P. psidii *on Growth and Survival of ʻŌhiʻa 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 postinoculation (Table 6). *T*-tests showed that this difference was statistically significant at the .0001 level of probability for Families 2 and 4 and at the .005 level of probability for Family 3. Analyses also indicate that the inoculated seedlings had a 27% greater chance of mortality over this

TABLE 5

Mean Percentage Leaf Coverage with Uredinia of Leaves of Six Different ʻŌhiʻa (*Metrosideros polymorpha*) Families in Response to Different *Puccinia psidii* Strains at 20 Days Postinoculation

Family	No. and Name of *P. psidii* Strains Used	Mean Leaf Damage (%)	SE 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 Area Under the Plant Growth Curve [AUPGC]) and Survival of Three Families of ʻŌhiʻa (*Metrosideros polymorpha*) Seedlings at 180 Days Postinoculation

ʻŌhiʻa Family	Growth of Noninoculated Seedlings AUPGC	Survival (%)	Growth of Inoculated Seedlings AUPGC	Survival (%)
2	354	69	79	44
3	762	56	245	19
4	530	50	199	31
Averages	549	58.3	174	31.3

Note: T-tests for growth showed significance at the .001, .05, and .0001 levels for families 2, 3, and 4, respectively.

same period, compared with noninoculated, control seedlings.

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 into Hawaiʻi.

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 ʻōhiʻa (*Metrosideros polymorpha*) families tested. In contrast, Strains 4 and 5, derived from *P. guajava* and *P. guineense*, respectively, were much less virulent on these same ʻōhiʻa families.

These results clearly demonstrate that some *P. psidii* strains/genotypes, which are not yet present in Hawaiʻi, could potentially cause devastating disease to native ʻōhiʻa in Hawaiʻi should these strains be introduced. Furthermore, this test did not include all currently known strains of *P. psidii*, including those from the mainland United States, Central America, South America, the Caribbean, Australia, and elsewhere. Because *P. psidii* strains that are not present in Hawaiʻi appear to pose a threat to native ʻōhiʻa in Hawaiʻi, proactive measures are critical to avoid the introduction of new *P. psidii* strains into Hawaiʻi. 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 Hawai'i), exist that could pose a major threat to a given plant resource (A. Tschanz, Animal and Plant Health Inspection Service [APHIS], pers. comm.). The results of this study (and others) should be considered by the appropriate regulatory authorities in the Hawaiian Department of Agriculture (HDOA) and in APHIS when developing appropriate protection and quarantine regulations to prevent the introduction of new *P. psidii* strains to Hawai'i via incoming myrtaceous plant materials.

The finding that all *P. psidii* strains are not equally virulent on 'ōhi'a 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 multiple *P. psidii* strains indeed 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 'ōhi'a may reflect coevolution 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 Hawai'i (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 ha 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 'ōhi'a in Japan (Kawanishi 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 'ōhi'a 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 'ōhi'a families from more geographically diverse provenances. Increased understanding of natural variation in resistance/susceptibility of diverse 'ōhi'a families to diverse *P. psidii* strains would be useful for predicting 'ōhi'a population dynamics should different *P. psidii* strains become established in Hawaiian forests.

Also, if a large amount of natural variation in rust resistance is found, this genetic variation could be exploited by planting 'ōhi'a 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 (Junghans et al. 2003*a*, Alfenas et al. 2009) and could serve as a valuable model in Hawai'i for increasing resistance of 'ōhi'a and other native species to *P. psidii*. However, the economic, ecological, and logistical constraints on wide-scale replanting of 'ōhi'a forests in Hawai'i 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 Hawai'i at Mānoa, Honolulu, pers. comm.). In some of the split plots, as much as 33% of the leaf surface of 'ōhi'a 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 'ōhi'a seedlings.

CONCLUSIONS

These studies conclusively demonstrate the existence of *P. psidii* strains, not yet in Hawai'i, that can be highly virulent on 'ōhi'a from Hawai'i. 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 'ōhi'a forests of Hawai'i. 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 Hawai'i, our results indicate that other strains of this rust pathogen pose a potential additional threat to the 'ōhi'a forests of Hawai'i 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 toward articulating effective biosecurity policies.

These studies show that three of the five *P. psidii* strains studied from Brazil are highly virulent on 'ōhi'a seedlings under experimental conditions. Although this experiment was conducted explicitly to explore the risks that other strains of *P. psidii* might pose to the 'ōhi'a of Hawai'i, the Myrtaceae family contains over 4,000 species and, indeed, is one of the largest tree families in the world. Other states or nations with extensive myrtaceous populations may wish to draw insight from these studies or conduct their own pathogenicity trials with diverse *P. psidii* strains.

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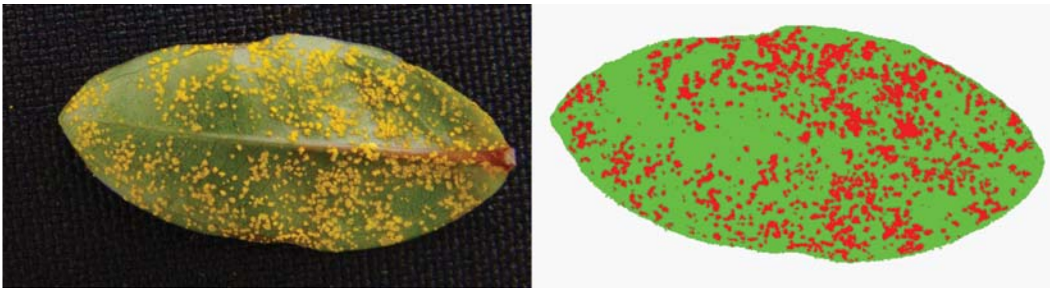

PLATE 1. Photo of an ʻōhiʻa leaf infected by *Puccinia psidii (left)* and the image of this same leaf after transformation by the QUANT Image Processing Software (Vale et al. 2003) *(right)*. In this example, 26.65% of the area of the leaf was colonized and this was recorded as the disease severity for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