USDA FOREST SERVICE
INTERNATIONAL ACTIVITY REPORT

SUBMITTED TO: USDA Forest Service, Forest Health Protection, International Activities Team

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ACTIVITY TITLE: Developing an agreement for the University of Vicosa, in Brazil, to study guava rust for the State of Hawaii.

ACTIVITY SUMMARY:

This report has several sections. Each is semi-autonomous. The reader should study this summary and then choose the section(s) of interest to them. The first section describes a trip made by the author to Brazil from July 12-20th. Travel costs for this trip were covered by the International Activities Team. This trip had the purpose of reviewing forest pathology facilities and personnel at the Federal University of Vicosa, Brazil, and of setting up a working agreement with their lead forest pathologist, Dr. Acelino Couto Alfenas. The collaboration from Dr. Alfenas' group is needed to make a critical set of studies on the genetic diversity of guava rust (*Puccinia psidii*) and for determining the capacity of various strains of this rust for attacking varieties of ohia (*Meterosideros polymorpha*). These tests are crucial for the State of Hawaii because ohia comprises about 85% of the trees in the native forests of Hawaii. Currently Hawaii has an interim measure in place blocking the entrance of all myrtaceous species into that state. These studies will develop the information needed to determine if it is important to make that measure permanent. This section of this report runs from pages 2-13.

The second section of this report sets the terms of the working agreement between the US Forest Service and the Federal University of Vicosa, Brazil. It discusses the strategy for sampling different rust isolates in Brazil and lays out the experimental design for pathogenicity tests in which selected strains of this rust will be artificially inoculated onto each of 24 different ohia families. It also provides a budget for the project that has been agreed on both by the author, representing the US Forest Service, and by Dr. Acelino Couto Alfenas, representing the university. This section runs from pages 14-17.

The last section of this report addresses a rather amazing list of additional complementary activities that are serendipitous spin-offs of the above-mentioned project. Some of these will enable Hawaiian pathologists and resource managers to deal more effectively with this disease. Some will strengthen the forest pathology team at the University Federal de Vicosa, Brazil, and some will benefit the greater international forest pathology community that is very concerned with the movement and impact of rust. This section runs from pages 17-28 and includes conclusions and references.

Section 1. Overview of the trip

Reason for the Trip:

One strain of guava rust (*Puccinia psidii*) has already found its way to Hawaii. It was first reported in 2005 (Killgore and Heu, 2005; Uchida *et al*, 2006). Six months later, this fungus had already become disseminated throughout the each of the Hawaiian Islands. Isolates were collected from many different locations in Hawaii and the DNA of these, along with the DNA of isolates from Brazil and Florida were analyzed for genetic markers. These analyses indicated that all of the Hawaiian isolates were the same and that there were some very different strains of this rust in other parts of the world (Zhong, 2007).

Figure 1. Orange pustules of the guava rust (*Puccinia psidii*) shown here on apple leaves

The strain of the rust that is already in Hawaii has already shown that it is very aggressive on rose apple (*Syzygium jambos*) and moderately aggressive on *Melaleuca spp*, but that it is only mildly pathogenic on the ohia tree (*Metrosideros polymorpha*). The authorities (USGS, USDA-APHIS, Hawaii Department of Agiculture, Hawaii Department of Land and Natural Resources, The Nature Consevancy, and the US Forest Service) in Hawaii are all very concerned that a more aggressive strain of this rust could establish itself in Hawaii and do a lot more damage to the ohia. This could be devastating as ohia comprises 85% of the natural forests of this state.

On the chance that there might be a more aggressive strain of the rust, the Hawaiian authorities have set up an “Interim Measure” which has the purpose of blocking the importation of any Myrtaceous species into Hawaii. There are over 4,600 species of Myrtaceaous plants world-wide and Hawaiian’s regularly import some of these (eg certain species of Eucalyptus) mainly for floral arrangements.

Figure 2. Distribution of *Puccinia psidii* in Latin America (from Booth *et al*, 2000)

This brings us to the purpose of this trip. It is basically to go to Brazil, which is considered the center of origin of this rust, and make arrangements with a local research institution to learn a great deal more about the variation of the strains of guava rust that might be found in that country and, in particular, to find out if some of these strains might be more pathogenic on various families of ohia.

An obvious first question, when wanting to find out more about the rust in Brazil, was to figure out who, in Brazil, already knows a lot about this fungus.

A review of the literature shows that there are over a dozen pathologists that have done some work with the guava rust in Brazil over the past 30 years. One forest pathologist who has especially distinguished himself for having done a great deal of research on this rust is Dr. Acelino Couto Alfenas. He runs a rather large plant pathology laboratory at the Universidad Federal de Vicosa (located in Vicosa, Brazil) and he, along

with his fellow professors, post–docs, graduate students and even undergraduate students, has been working on this rust disease for over 15 years.

A lot of Dr. Alfenas' clients are the big pulp and paper companies of Brazil since a majority of these companies grow eucalyptus species, and since many eucalyptus species, as Dr. Alfenas and associates have found out, are susceptible to this rust to one degree or another. Many genetic studies have been conducted to determine the variation in resistance to this rust between both hybrid and pure lines of the main eucalyptus species used in Brazil and the results show that there is a high degree of variation and that this is a highly heritable trait that can be captured easily through clonal forestry.

Figure 3. Eucalyptus plantations in Brazil are of clonal origin and are capable of very fast growth. Here Veracel plantations are being harvested after about six years of growth. There are 5,000,000 hectares (12,000,000 acres) of fast-growing eucalypt plantations in Brazil. The fact that the guava rust can seriously affect the growth of many eucalypts has really gotten the attention of all the major pulp companies in the country.

One of the biggest rust studies that Acelino has undertaken was a study supported with nearly $600,000 (Aus) of Australian and South African financial support. The objective of this study was to test 58 different myrtaceous species for their susceptibility to the rust. To be able to conduct this test, Acelino needed to work with an international contingent of pathologists and geneticists to set up the pathogenicity tests and he needed to set up a number of inoculation and incubation chambers (and much more) there in

Vicosa. He did all of this extremely well and some key research was published. In this study, it was determined that 32 out of 39 species of *Eucalyptus* and *Corymbia* species tested were susceptible (Glen *et al*, 2007).

In other studies carried out by Acelino and colleagues, they have determined that the disease is much more intense when there is extended periods of wet weather, and also, since Brazil is such a vast country, that favorable months for rust vary for different parts of the country.

They have also found that some tree species are affected much more intensively than others and that, in general native myrtaceae are less vulnerable than exotic myrtaceae. There are no species, however, that are more vulnerable to a rapid and heavy build-up of this rust than *Syzygium jambos*, or "jambeiro" as it is referred to locally.

Although Acelino Alfenas and colleague had clearly demonstrated that they were capable of conducting first class pathology tests when just one strain of the *Puccinia psidii* rust was being used, the experiments that Hawaii needs to have done involve the use of at least eight different strains of this rust and these tests would need to be done on a very slow-growing Hawaiian species (ohia) that is very uncommon in Brazil. There are many other nuances of these tests that also need to be fully understood by the Brazilian pathology team if these tests are to provide the Hawaiian authorities with the information needed to make fully-informed decisions on whether or not to continue with the quarantine of Myrtaceous plants.

The reader might ask, "why do these tests in Brazil?" The answer to this question is straightforward. This guava rust has its center of origin in Brazil. It is highly inadvisable, as well as illegal, to ship rust to countries where it does not already exist. Therefore, if meaningful tests were to be done with a broad range of rust inoculum, Brazil would be the logical country where the different rust sources should be sought and where the pathogenicity tests should be conducted.

For all of these reasons it was very important for the author to go to Vicosa, learn the capacities of Acelino's plant pathology lab and then go through every step of the entire set of experiments that need to be conducted to make sure that they understand why each step of the experiment has to be executed in a specific way and to determine who will be the person(s) responsible for conducting each step.

Again, the purpose of this set of experiments is to get the information needed by Hawaiian authorities to determine what risk might be incurred if additional myrtaceous species (the carriers of this particular rust) are allowed into the state.

The Trip

In this section a brief overview of the trip is offered. Some specific comments about meetings held during this visit and some of the plant pathology facilities and some of the plant pathology personnel are given in subsequent sessions.

The author flew to Rio de Janeiro on June 12, 2008 and then traveled by car, provided by Acelino, to the University Federal of Vicosa in Vicosa, Brazil,

During the next two days we visited each of Acelino Alfenas' three Plant Pathology Laboratories, reviewed the lab equipment in each, and talked with the technicians that were doing the work in these labs.

Figure 4. Dr. Acelino Couto Alfenas, Raphael Alfenas and Thayta in one of three the well-equipped phytopathology labs that Acelino manages.

Then we toured the nearby plant pathology screenhouses, green houses, inoculation chambers and incubation facilities that are right there on the campus. Acelino and colleagues have built in many customized features in order to undertake the pathological studies that they manage.

Figure 5. *Eucalyptus grandis* demonstrating both a high and low degree of susceptibility to the standard strain of the guava rust (*Puccinia psidii*). Photo was taken inside one of the many greenhouses and screenhouse managed for forest pathology studies at the University Federal de Vicosa. Lucio Guimaraes holds the resistant plant while Acelino Alfenas shows the susceptible.

Subsequently, we traveled about two km. to a three- hectare plant pathology nursery which is used to conduct larger-scale forest pathology tests. We also visited the even larger general forest nursery. This is where the rust studies were conducted on the 58 myrtaceous species in collaboration with the Australians and South African forest pathologists. At both of these nurseries there are special features that have been built to facilitate specific pathology studies. Acelino provided a step by step account of each activity that takes place when conducting each kind of pathogenicity test.

Subsequently we traveled about 10 km to the University Forest. This forest is a mix of natural forest fragments and an arboretum-like arrangement of many native tree species. Some of these trees were myrtaceous species suspected of having rust. Acelino demonstrated his approach to quickly finding the rust and for collecting and documenting samples.

Subsequent to these activities at the University, Rodrigo Graca Neves, Cristina Aun and the author made a three-day trip traveling 3,200 km to the eucalypt plantations of Veracel Celulose S. A. in Bahia State (and back). At this time of year it was necessary to travel to Bahia because Bahia was still in the late stages of the rainy season and it would be much easier to find the rust there. Veracel's Forest Pathologist, Daniela Andrade Neves met us on arrival and took us on an extensive tour to five locations where she had previously found the rust. At each location we collected the rust for subsequent molecular analysis and for possible use in the pathogenicity tests.

One of the highlights of this field trip was finding the rust on some eucalypt clones that had, until very recently, always tested as resistant. All we could do was

speculate on what might be happening. Had the rust evolved, through mutation or genetic recombination, so that it could take on this resistant clone?

Another highlight was finding the rust on Araceau, a native myrtaceae that, until very recently, had never shown any indication of being affected by the rust. This has prompted more speculation that the rust might be evolving, or that the inoculum loads have become much heavier in recent years.

In both of these cases, single pustule samples were collected in Ependorph vials and placed in an ice chest. On return to Vicosa, of course, these vials were immediately placed in the -80° C refrigerator until they can be used either for the bulking up of the DNA for molecular genetics testing or for the creation of large loads of pure-strain-inoculum for the pathogenicity tests.

Nine other rust samples were collected during this visit to Veracel Celulose.

Figure 6. Daniela Andrade Neves, Forest Pathologist for Veracel Celulose S.A., points to a pustule of *Puccinia psidii* that has managed to develop on this eucalyptus clone that was, until very recently, considered to be resistant to this rust. Veracel, as well as every other major pulp company in Brazil, maintains a very active breeding program partly so that it can continue to develop clones that are resistant to the new strains of rust that may be evolving. This photo was shot in one of about 180, tenth-hectare plots that Veracel has installed to determine the impact of the *Puccinia psidii* on the growth and yield of its eight top clones (and two susceptible checks). This test has a Randomized Complete Block design with 6 replications per location installed at 3 locations, each with distinctly different climates.

Early Saturday morning (July 19th), after having returned from Bahia the night before, the author spent three hours debriefing with Dr. Acelino Alfenas. The main topic covered were all of the tasks that needed to be carried out to bring the ohia rust test to completion. Following is a list of all of the tasks along with the names of the people who will be responsible for conducting these tasks.

TASKS	Person (people) responsible	Expected Date of Completion
Getting Inoculum For the Tests		
Collecting single pustules from at least 200 different sources in Brazil	Rodrigo Graca Neves	Oct. 2008
Bulking up DNA from each isolate and collecting the DNA	Rodrigo Graca Neves	Dec. 2008
Shipping DNA for each isolate to the US for genetic marker analysis	Rodrigo Graca Neves	Dec. 2008
Running the genetic markers	Mee-Sook Kim	Feb. 2009
Developing the phylogenetic tree	Mee-Sook Kim	Mar-09
Picking the isolates to be used as inoculum	Acelino Alfenas, Phil Cannon, Mee-Sook Kim and Rodrigo Graca Neves.	Apr-09
Bulking up the inoculum for the selected isolates	Cristina Aun	Oct. 2009
Getting the Ohia Seedlings Ready for Test		
Determining the ohia seed sources to collect	Rob Hauff, Elizabeth Stacy	Done
Overseeing the seed collection	Rob Hauff	Done
Getting the seed extracted, running pre-germination tests, getting phytosanitary papers, and mailing seed to Brazil	Rob Hauff and Hawaii APHIS staff	Done
Getting import permit into Brazil	Acelino Alfenas	Done
Picking up seed in Brazil	Rodrigo Graca Neves	Sep-08
Establishing seedlings in Brazil germinate, prick out, label	Cristina Aun	Oct-08
Growing seedling for first year	Alex	Oct-09
Running the Guava Rust Test for Hawaii		
Developing the pathogenicity test	Phil Cannon, Richard Sniezko	Done
Developing a budget for the test	Acelino Alfenas, Phil Cannon	Done
Arranging to transfer the funds to the Funarbe Foundation of the Federal University of Vicosa	Val Mazainis, Michelle Zweede, Glaucia Verissima, Elizabeth Pacheco Fontes, Julie Lydick, Acelino Alfenas, Phil Cannon	Done
Teaching the design of this test in Vicosa, Brazil	Phil Cannon	Done
Arranging seedlings for inoculation with the pre-designated rust strain	Cristina Aun	Oct-09
Conducting the inoculations	Cristina Aun	Oct-09
Tending seedings during the		

incubation period	Cristina Aun	Nov-09
Evaluating the level of disease development in each seedling.	Cristina Aun, Acelino Alfenas	Dec. 2009
Writing up the results of the experiment		
Analyzing the data	Cristina Aun, Acelino Alfenas Phil Cannon, Rich Sniezko, Janice Uchida	Jan-10
Writing white papers, publications theses	Cristina Aun, Acelino Alfenas Phil Cannon, Rich Sniezko, Janice Uchida, Rodrigo Neves, Cristina Aun	Mar-10
Using the results of these experiments		
To make new risk assessments on guava rust in Hawaii	Janice Uchida, Anne Marie La Rosa Lloyd Loope	Apr-10
To make new quarantine decisions in Hawaii	DOFAW, HDLNR, USDA-APHIS and other Hawaiian natural resource agencies.	May-10
Use results for other purposes	The world's forest pathology community especially in the Pacific Rim	2010 & onwards

The author then returned to the US arriving in the afternoon of June 20. One hundred and five hours were spent traveling during this nine-day trip. Another 23 hours were spent in meetings, giving presentations, seeing facilities, meeting key personnel and collecting rust isolates. Another 12 hours were spent eating some of the best food on the planet, always in good company.

Key Meetings

1) **Meeting with the lead rust scientists at the University Federal de Vicosa, Monday 10:00- 12:30 AM, July 14th.**

   a) Acelino Alfenas opened the meeting with a very full discourse of the history of rust in Brazil and of the involvement of himself and several members of his lab on getting a better understanding of this rust.

   b) Rodrigo Neves discussed the main goal of his PhD thesis which is already underway. Currently he is collecting isolates of over 200 different sources of the rust. All samples are single-pustule isolates. He will store each of these isolates (at -80°C), and then, when it is time to collect their DNA, he will inoculate their spores into individual jambeiro (rose apple = *Syzygium jambos*) plants which they will quickly infect. After the fungus has "bulked up" and produced a copious new rash of pustules, Rodrigo will harvest these and then isolate the DNA from this essentially pure sample. Rodrigo will be studying the molecular variation of the DNA from the 200 plus collections that he is making as a major component of his thesis. He

will be doing some of this work in Brazil (at UFV) and some of it at Washington State Universisty and the Rocky Mountain Research Laboratory in Moscow, Idaho. For much more information on Rodrigo, please see the last section of this report titled "Complementary or Spinoff Activities".

c) Lucio Guimaraes described his study of the variation in genetic susceptibility of *Eucalyptus grandis* and its hybrids to this rust fungus. This is a crux study for Brazil because this country has 5,000,000 ha of eucalyptus (about 12,000,000 acres) and much of this is growing at about 50 m3/ha/yr, which is extremely fast. Most of these eucalypt plantations are composed of clones (because select clones have the top growth rates and because these clones have wood with exceptional pulping properties). However, many of the top clones that are being used are susceptible, at least to some degree and under certain environmental conditions, to guava rust. For many years to come, forest pathologists and tree breeders will have a big job continuing to produce eucalypt clones that are sufficiently resistant to this disease.

d) Alexandro Alonso Alves described the research that he is doing (along with Acelino, Dario Grattapaglia and Cosme Dario Cruz) on the molecular genetics of rust resistance within the eucalypts. One salient result, so far, is that one Quantitative Trait Loci (QTL) explains more than 80% of the phenotypic variation. This will be a huge boon to the breeders who will now be able to screen DNA of candidate trees for the presence or absence of this QTL.

e) The author spent one hour on the white board going over the experimental design to be used when the UFV personnel will conduct the pathogenicity tests of this fungus on ohia. In this test, eight different strains of Brazilian rust will be inoculated onto 24 selected families of ohia.

Meeting Number 2, 2:00-3:30 PM, July 15th

The author and Acelino Alfenas met with Elizabeth Pacheco Batista Fontes and Glaucia Verissimo of FUNARBE Foundation to make sure that the funds for this study could be smoothly transferred from the US Forest Service to Acelino's lab at the Federal University of Vicosa.

Meeting Number 3, 5:00 - 6:30 PM, July 15th

The author gave an evening lecture to the University on Guava Rust. The title of this presentation was. "How is the guava rust disease being dealt with in Hawaii". The presentation lasted about 75 minutes. There were good questions from the audience. About 20 people in attended.

Meeting Number 4, 7:00 – 10:00 AM, July 19th

This is a three-hour, one-on-one, close-out meeting between Acelino Alfenas and the author to go over every detail of the working agreement between the USFS and the UFV. The salient result of this meeting, the task list, was covered earlier in this report.

The Physical Facilities of the Acelino Alfenas Plant Pathology Laboratory Complex

The indoor space available for the Alfenas plant pathology group consists of two labs in one building and a third lab in another. During this visit, it seemed like that largest of these labs was in perpetual motion with so many typical plant pathology lab activities that the author nick-named this particular lab the formiguero, "the ant-mound", because of its very busy nature. At any given time it had about eight lab technicians in it processing pathology samples that had been collected from all over the country. Indeed, some of these samples came from eucalypt plantations from as far as three thousand kilometers away, and even neighboring countries. Many of the samples were a part of some genetics-resistance studies for the eucalyptus rust and for other fungal and bacterial diseases. Many of the technicians were not only analyzing the incoming specimens but were also actively involved with traveling to the sites to collect the samples.

Outside the labs there is a long hallway. This is lined with oversized refrigerators and freezers (maybe ten of these in total). We opened one of these (listed as storage at -80° C) and found that it was stuffed with racks of tiny Ependorph vials each containing a collected rust sample. Acelino mentioned that he badly needed another of these -80°C freezers as these provide the conditions where he can store rust indefinitely. (pathologists reading this document will remember that rusts are obligate parasites, you cannot store them on synthetic media). Other than another freezer, Acelino he did not mention other laboratory equipment needed to have his lab operating at top condition.

Attached with these laboratories there are several meeting rooms and lecture halls, all very nicely appointed with all the requisite audio visual apparatus to facilitate easy exchanges of ideas.

Two blocks away from Acelino's laboratories, we found the plant pathology green houses and one building filled with custom-designed chambers used for the inoculation and incubation of several of the major kinds of diseases that afflict eucalypts in Brazil. All of these green houses are in good shape and are very nicely equipped.

With respect to conducting pathogenicity tests with guava rust Acelino and his colleagues have the following facilities:

1) the fridges for storing rust isolates.
2) An especially "clean" green house for producing thousands of uninfected *Syzyzium jambos* "jambeiro" plants.

3) A couple of (1m x 3m x 1.5 m) inoculation chambers (these have the insides painted very black to preclude the entrance of any light. They are also outfitted with thermal controls to keep the temperature in these chambers at 22°C and with a misting system to keep the humidity at 99%. (Note: through many trials, Acelino and associates have determined that 8 hours of continuous darkness, a temperature of 22°C and 99% humidity provide optimal conditions for ensuring that rust spores, sprayed onto the leaf surface at the time of inoculation, will have the best chance for infecting susceptible host plants).
4) A bank of 16 incubation chambers, each measuring about 1m by 0.5 m by 2.0 m, in which inoculated plants can be held to promote disease development under ideal conditions (note: environmental conditions here are 22° C, 99% humidity, but only partial shading)
5) Many hundreds of meters of raised benches to hold the inoculated plants after the incubation period. These facilities are under a shade cloth, and have a complete misting system. Inoculated seedlings reside on these benches for 3 to 5 weeks before being scored for their respective levels of rust symptoms..

Key Personnel of the Acelino Alfenas Laboratory

During this visit, the author was introduced to about 30 different people connected with Acelino's program.

Clearly the most important person of all is Marcia Brandao who is Acelino's Executive Assistant. Acelino, himself is a very cordial and spontaneous person which means that Marcia has the challenge of ensuring that Acelino meets all of his assumed obligations and, in general, of running the lab in his absence. She performs this job with phenomenal proficiency and with an outstanding spirit, to boot.

Lucio Guimaraes is the aforementioned graduate student that is working on the genetic variability and heritability of Eucalyptus rust resistance. *Eucalyptus grandis*, *E urophylla* and their hybrids are the genotypes most commonly under scrutiny.

Raphael Alfenas (Acelino's son) is doing his thesis on the genetic variation in resistance of Eucalyptus to *Cylindrocladium spp*., another fungus that is causing a serious foliar disease in Brazil.

Rodrigo Graca Neves has assumed the position of head technician for Acelino's work on the eucalyptus rust for the past couple of years. He replaced Edival Valverde Zauza, Acelinos previous rust technician and researcher extraodinaire, who received his Masters and then went to Suzano Florestal to head up their research program.

Currently Rodrigo has just finished writing up his PhD thesis proposal to work on genetic variation of the eucalyptus rust. As will be seen ahead, and in some detail, Rodrigo will be a very key person in many of the activities associated with the ohia rust

study. (See Complimentary and Spinoff Activities), but his thesis activities will make it impossible to continue with all of his current lab responsibilities.

In order to replace Rodrigo in his day to day rust work at the Lab, Acelino has hired Cristina Pierroti Aun. Cristina is only a senior undergraduate student at this point, but Acelino has already determined that she has an exceptional mind and a great interest in plant pathology. She is already managing the collection of rust isolates for Vicosa. Cristina will be the key person carrying out the ohia inoculation studies, as well.

Figure 7. Cristina Pierroti Aun demonstrates the isolation of a single pustule of *Puccinia psidii* rust into an Epindorph vial. In the vial this rust sample can be stored indefinitely at -80° C.

Thayta, Marcelo, Patricia, Vitor and Vanessa are several more technicians that work in Acelinos' labs. Julianna is on loan to Acelino's lab but is actually working more for Dario Grateppaglio's program. Dario is a world re-known molecular geneticist who became famous about 15 years ago when he, working at North Carolina State University, in Ron Sederoff's Laboratory, unraveled the entire molecular sequence of the DNA of *Eucalyptus grandis*. This was either the first or the second tree species in the world to have its entire DNA sequenced. Dario has been in Brazil since that time handling a number of molecular questions for his country. He and Acelino have had several collaborative projects.

Leandro is another one of Dario's lab associates that is currently working with Acelino.

Alex is a young buck that is just moving into the position of managing the plant pathology section in the main forestry nursery for Acelino. This is quite a nice complex. Much of the construction of this nursery was financed by Australia a few years ago when they had Acelino's team conduct their big rust studies with the 58 different myrtaceous species.

Sabastian is Alex's father and, until very recently, was the manager of the impressive school forest for the University of Vicosa. He took us to the forest and knew the history of most of the studies that were being conducted there. He also helped us find some of the host trees for the rust.

Murilo Zurbini is a professor and head of the graduate program for the Plant Pathology program. One of the projects that he has been spearheading is a massive exchange program between several programs from UFV with graduate schools in several departments at North Carolina State Univerisity.

Eduardo Mizubutil is a professor at UFV specializing in population genetics of Solanaceous plant pathogens. Eduardo is a great friend of Tobin Peever at WSU and, during my brief stay in Brazil, was able to contact him and put the finishing touches for Rodrigo's "sandwich program". Eduardo has also been Murilo's colleague on visits to NC State University.

Sergio Brommenorhenkel and Alexandro Alonso Alves (mentioned in Meeting 2d, above) are molecular geneticists at UFV. They will be collaborating with Rodrigo in doing some of the sequencing of the guava rust DNA which will, in turn, be a part of his thesis.

Daniella Andrade Neves is a graduate of Acelino's program and is now heading up pathology research for Veracel, one of the largest pulp and paper companies on the planet. During this trip, we would drive 3,200 km to Bahia to see some of the research she is doing on guava rust and make some very interesting and unique rust collections.

Finally, Elizabeth Pacheco Batista Fontes ("Bette") and Glaucia Verissimo are Director and Grants Administrator for FUNARBE, respectively. (Elizabeth did her PhD in virology at NC State and is very well respected as a scientist as well). FUNARBE is the Foundation that was set up by UFV to help administer grant funds that are coming to the University. Michele Zweede, of the USFS International Programs will be working with them as she will be helping us to get some money to support the UFV on a couple of key rust studies.

Section 2. Terms of Agreement for the Pathogenicity Tests.

The terms of agreement for the working agreement with the University to study guava rust on ohia will be laid out shortly. However, a little history will help appreciate the substantial collaborative effort that has already gone into setting this up.

In early 2007, Lloyd Loope (USGS, Maui), Anne-Marie La Rosa (USFS, Hawaii) and several leaders in the Hawaiian Department of Agriculture expressed concern about other races of guava rust coming to the Hawaiian Islands and being exceptionally destructive on Myrtaceous species, especially on the pervasive ohia trees, that perform so many important functions on these islands.

The author, cognizant that the disease had originated in Brazil and a colleague of a few Brazilian Forest Pathologists, agreed to try to contact some of these pathologists and see if they might be willing to help pursue the Hawaiian's questions.

Dr. Acelino Alfenas gave the strongest response from Brazil and further correspondence indicated that he was exceptional well-suited to collaborate on these studies, providing, of course, that there was adequate funding to cover costs.

The author developed the design for the experiment. A budget was worked out by Dr. Alfenas.

Val Mazainus (USFS International Programs) suggested that Michelle Zweede might be able to facilitate the transfer of funds to Dr. Alfenas' Laboratory. Dr. Alfenas, indicated that the FUNARBE Foundation, of the Federal University of Vicosa, could receive these funds on his end.

Rob Hauff worked with Elizabeth Stacy, to design a sampling technique to ensure the collection of the seed of a widely-representative set of the ohia trees in Hawaii. Subsequently he supervised the seed-collection process from start to finish and obtained the phytosanitary permits so that the seed could be sent to Acelino, in Brazil.

Acelino, in the meantime, obtained the import permit for these ohia seed working through the authorities in Brazil.

The author ran through the experimental design with Rich Sneizko (of DORENA), and Rich made one extremely important improvement to the design (without changing the size of the experiment).

The experiment is novel so it was decided that it would be wise if the author could travel to Brazil, meet with the crew that would be conducting the experiment, and go over the details so there was no chance for not getting the Hawaiian's questions answered.

Fortunately, the IAT agreed to finance the costs of this trip

WORKING AGREEMENT TO STUDY GUAVA RUST BETWEEN THE UNIVERSITY OF VICOSA IN BRAZIL AND THE US FOREST SERVICE
2nd Draft (includes changes to budget to reflect the latest exchange rate)

SCOPE-

This agreement will help facilitate and finance studies, to be conducted by the Federal University of Vicosa, Brazil, which will lead to a far better understanding of the pathogenicity of several different races of guava rust on a wide range of ohia germplasm. The information generated from this study will be used by the State of Hawaii to determine how important it is to keep new races of guava rust out of their state.

BACKGROUND-

About 80% of the native forest vegetation in Hawaii is comprised of ohia trees (genus Metrosideros spp., family Myrtaceae). There is known to be a great degree of variation in this species. There are several other species of Myrtaceae in Hawaii as well. Some of these are indigenous, some are endangered, some are exotics and some are invasives.

In 2005, the guava rust fungus, *Puccinia psidii*, was detected in Hawaii for the first time. Within six months it had spread to all of the islands. Molecular analysis of the DNA indicates that there is only one race in Hawaii so far. This particular race of the rust has killed a couple of invasive and indigenous Myrtaceous species, but it is not especially damaging on mature ohia trees and it is also not aggressive on eucalypt species planted there either. On the other hand, it is known that in Brazil there are some races of this rust that can cause a significant amount of damage on many eucalypt species. This, and other evidence, leads one to suspect that the race of this rust that made it to Hawaii is not especially pathogenic and that more pathogenic races may exist that have not yet found their way to Hawaii.

In fact, officials in Hawaii are so concerned that a more pathogenic race will make it to their shores that they have created an interim rule which bans (and provides measures to ensure the ban) the importation of any Myrtaceous plant parts into their state.

OBJECTIVES-

Based on the information above, the objectives of this project are set up studies which will determine if there are, indeed, other races of this particular rust, *Puccinia psidii* and to determine if some of these races could be more pathogenic on ohia.

METHODOLOGY-

Two types of studies will be conducted under this agreement. The first will have the objective of determining if there are different races of this fungus in Brazil. The

second will be to determine if some of the different races of this fungus have markedly different levels of pathogenicity over a wide range of ohia germplasm.

Study Number 1

A collection will be made of rust from about 40 distinctly different collection points from within the range of guava rust in eastern South America (note collection points will vary by geographic location, and host plant). Single spore isolates will be used to bulk up pure sources of the DNA from each of these isolates. The DNA will be extracted in Brazil and sent to the US (Rocky Mountain Forest Research Station) where it will be analyzed using molecular genetic techniques. Distinct molecular variants will be documented. Eight distinct variants will be selected for the second part of this study.

Study Number 2

Ohia seed from 28 distinct families have been collected in Hawaii. These seedlots will be sent to Brazil. In a nursery at the Federal University of Vicosa at least 350 quality seedlings will be produced for each of these families. After the seedlings are about one year old, they will be set out in nine experiments. Within each of these nine experiments the layout will consist of four blocks with an eight-tree row plot for each of the 28 families. For eight of these experiments one of the eight rust variants, selected during study number 1, will be used to inoculate all seedlings in that particular experiment when these seedlings are about one year old. Seedlings in the ninth experiment will serve as a control and will not be inoculated.

Between two and four weeks later, all seedlings in all nine experiments, will be evaluated for the amount of rust sign and symptoms they are showing (according to standards established by Dr. Arcelino Alfenas) and they will also be evaluated for their symptoms, expression of growth loss, etc.

The resultant data will then be documented and, on the basis of this documentation, the existence of a more pathogenic race of *Puccinia psidii* on ohia will then be either supported or denied.

SPECIFIC PRODUCTS EXPECTED

1) Several publications describing the above-mentioned experiments and their results (with coauthors coming from the University of Vicosa, the University of Hawaii Manoa, the Department of Forestry and Wildlife-Hawaii, and the US Forest Service)

2) At least one PhD thesis (at the University of Vicosa)

And, 3) Most importantly, all the information that Hawaii needs to decide whether to continue their interim ban on the importation of Myrtaceous species into Hawaii.

BUDGET

Activity	**Estimated Cost (US $)**
Reception of Ohia Seed in Brazil	750
Cost of Growing Ohia Seedlings in Nursery	6,000
Cost of Collecting inoculum (by biotype/strain)	3,750
Cost of doing the Inoculations	3,000
Cost of Analyzing the Inoculations (at two time periods)	4,500
Cost of Analyzing Data	5,200
Cost of Authoring (or co-authoring a report)	6,000
Overhead for Universidade Federal de Vicosa	6,000
Wire Transfer Service (Meti)	1,800
Total	37,000

Arrangements

Funds for this Project are to be deposited with the Funarbe Foundation in Vicosa, Brazil. This money will be transferred from Region 5 to the International Program of the US Forest Service and then down to the Funarbe Foundation using the Services of Meti

These Funds are to be distributed as follows.

$15,000 immediately following the acceptance of this agreement by all parties.

$15,000 after approximately six months, following the preparation and approval (by all parties) of a progress report.

And $7,000 dollars at the completion of the project as evidenced by at least two of the products listed in the section titled “Specific Products Expected”.

Section 3. Complementary or Spinoff Activities.

During this trip to Brazil, the author stayed focused on the task setting up the studies that are needed to provide Hawaii with the answer that it needs to the question, are there other strains of the rust, *Puccinia psidii*, that pose a significant risk to the health of ohia (*Metrosideros polymorpha*) in Hawaii.

Never-the-less, as a result of going through the effort to set these studies up, there is a rather amazing list of additional complementary activities that are serendipitous spin-offs. The last section of this report briefly describes 23 of these. Some of these activities will enable Hawaiian pathologists and resource managers to deal more effectively with this rust disease. Some will strengthen the forest pathology team at the University Federal de Vicosa, Brazil, and some will benefit the greater international forest pathology community that is very concerned with the movement and impact of this rust.

Metrosideros polymorpha **(ohia) seed import permit into Brazil.**

While the author was visiting in Brazil, the import to permit ohia seed finally arrived from Brazil's Department of Defense and Plant Inspection. Acelino had been working with the authorities in Brasilia to obtain this permit for over two months. We went over every detail on the permit to ensure that there will be no (or as few as possible) hassles when the seed is sent from Hawaii to Brazil. The author has subsequently translated this two-page document to English and passed this information onto Rob Hauff who has been taking charge of getting the seed collected in Hawaii and of sending the seed to Dr. Alfenas.

The development of a sampling system to get the ideal assortment of ohia seed.

Rob Hauff has collaborated with Dr. Elizabeth Stacy, a botanist at the University of Hawaii (Hilo Campus) to figure out what assortment of ohia seed should be collected to represent the variation in Hawaii's ohia seed. Twenty-four ohia families were targeted for collection.

Pre-germination tests for the different ohia seedlots.

Rob Hauff arranged for pre-germination tests to be run on each of the ohia seed lots that have been collected. Most of the seed sources had between 6 and 60 % germination rates.

Phytosanitary certificates for the ohia seed.

Rob Hauff arranged for each of the ohia seed lots to be examined and for APHIS-Hawaii to grant the phytosanitary certificates for this seed.

Transfer of information on how to grow ohia to the Brazilian nurseries.

Brazilian's are phenomenally good at growing plants, especially people like Alex who is managing the plant pathology section of the forestry nursery. Never-the-less, ohia have very small seed, which often means that conditions for optimal germination and early-growth can be fairly exacting. Rob Hauff is rounding up information on how to grow ohia from a few Hawaiian nurseries that have already demonstrated a capability of growing this species well.

Support of the rust research capacity in Brazil.

Brazil already has a very active guava rust research program. To date, much of it has focused on how this rust affects eucalyptus clones in Brazil and of finding resistance to this rust through breeding. As a result of this current set of studies, very significant advances are anticipated in terms of understanding the range in variability of *Puccinia psidii* both in terms of the variability of its DNA and in terms of the pathogenicity of its different strains. Significant advances will also be made on the evolutionary development of the fungus and the path of its geo-distribution. At the end of this project, Brazilian forest pathologists and molecular geneticists will have done all of the work or will, at least, have participated very strongly in every one of these discoveries. Therefore, by conducting the rust studies specified in this working agreement, the key players in Acelino's lab will gain far more experience in doing research on this rust and in the field of molecular genetics and will, in turn, be ever more capable and in even more demand as researchers, professors, speakers, co-authors, consultants and collaborators.

Re-affirmation of some strong forest pathology ties that have existed in the past between the Vicosa lab and US, and US Forest Service, Forest Pathologists.

Many Brazilian Forest Pathologists obtained their masters and PhDs in US universities or Canada. Furthermore, several forest pathologists from the US have spent many years working in Brazil. Among these are Chuck Hodges and Tom Harrington. These pathologists have, invariably, worked closely with pathologists and institutions, such as Dr. Acelino Couto Alfenas and the University of Vicosa, to carry this work out. Not surprisingly, one of the consequences of these interactions is that Brazilian pathologists are now very competent and essentially self-sufficient. Never-the-less, it is a characteristic of forests pathologists, world-wide, that they do their best work when they have an opportunity to meet with other pathologists and share ideas and techniques. As this study develops there will be opportunities for plenty of interaction between many forest pathologists in the US (in Hawaii, Puerto Rico, Idaho, California) and the UFV in Vicosa, Brazil.

Rodrigo Neves "sandwich" program at Washington State University

Rodrigo Graca Neves is a graduate of the Federal University of Vicosa (UFV) that has been working as Acelino's lead technician on the rust problem in Brazil. He is

also in the graduate school program and will be doing his PhD thesis on the molecular genetics of the different *Puccinia psidii* rust races in Brazil and neighboring countries.

One of the optional features of the UFV graduate school program is that it offers a short, overseas, sabbatical-like experience with another major university which is re-known internationally for leading the world in that field of research. This experience is affectionately referred to as a "sandwich program". Typically sandwich programs last for about six months and come during the middle of the thesis work.

Some months ago, Acelino indicated that Rodrigo was looking for a university in the US that might be appropriate for this kind of a sandwich program. In talking with Mee-Sook Kim and Ned Klopfenstein, Forest Pathologists at the Rocky Mountain Research Station, we were able to figure out that Washington State University might provide an excellent "sandwich" opportunity. They have the world re-known faculty in the field of molecular rust research, albeit mainly in the field of grass rust research.

The very close proximity of Washington State University with the Rocky Mountain Research Station (about 12 miles) is another big plus for this particular sandwich arrangement. This will make it easy for Rodrigo to spend time with Mee Sook Kim and Ned Klopfenstein. This will be especially important since Mee Sook will be coordinating the activities oriented around the use of genetic markers to study genetic diversity of guava rust and to help identify the eight rust strains to be used in the ohia family rust experiments in Brazil (see ahead).

Figure 8. The ever-affable and PhD-aspiring Rodrigo Graca Neves holds the branch of a *Eucalyptus grandis* sustaining a moderately heavy attack of guava rust (*Puccinia psidii*). In Brazil the disease is known as el ferrugem do eucalipto.

The idea of having Rodrigo do his sandwich program at WSU got a special boost from Dr. Eduardo Mizubutil (UFV molecular plant pathologist of solanaceous crops). He happens to be a very close friend of Tobin Peever, one of the lead rust researchers at WSU.

Connecting International Forestry of the USFS and the FUNARBE Foundation

When making contract arrangements with an entity outside the United States, one might anticipate some difficulties in transferring funds. Very fortunately, the International Programs section of the USDA Forest Service has had experience sending money to Brazil on previous contracts and the FUNARBE foundation has had experience receiving funds from outside granting institutions on behalf of the University Federal de Vicosa. Some pre-arrangements were necessary. Val Mazainis, Director of the International Programs asked Michelle Zweede to inform the author about what information would be necessary to transfer the funds to the UFV and Dr. Acelino Alfenas informed the author that FUNARBE would be the institution best placed to receive and administer these funds on behalf of the university. All that remained was for Dr. Alfenas and the author to agree on the terms of the contract and then to write up a contract.

Rust Collections in Puerto Rico, Jamaica, Dominican Republic, Florida and California

The collections of the rust by Rodrigo and colleagues in Brazil and Uruguay is leading the way in the effort to understand the variation of this rust, but the intention of an even broader study is to also get DNA samples from several different *Puccinia psidii* isolates from trees in Puerto Rico, the Dominican Republic, Jamaica, Florida, and California, as well. Jose Negron has already collected at least one isolate of this fungus from Puerto Rico and taken it back to Mee-Sook's Lab. Dr. Deborah Jean Lodge has indicated that she knows where more isolates could be collected in the Dominican Republic, Jamaica and Puerto Rico. And Pat Nolan and Paul Zambino can provide isolates from the rust in California. At the very least, Mee Sook Kim will analyze the DNA of these strains using the genetic marker protocol developed by Shaobin Zhong. Rodrigo Graca Neves has also indicated interest in obtaining some of these strains for his thesis project.

Permits to import rust and the DNA of rust into the United States

Dr. Ned Klopfenstein has obtained APHIS permits to import the spores of this rust into the US for the purposes of extracting the DNA. Mee Sook Kim can isolate the DNA from these samples once they have arrived in the US. Alternatively, if the DNA is extracted in another country, that extracted DNA can also be sent to Mee Sook and does not require a permit.

Mee Sook Kim to supervise the exploration of the variation in the DNA of different *Puccinia psidii* rust strains using genetic markers.

(An important note: this activity will be done in communication with Shaobin Zhong and with the possibility to train Rodrigo Graca Neves in this process**.**

By 2006, Shaobin Zhong, while working at the University of Hawaii, had developed set of genetic markers that are effective for making determinations between different races of the *Puccinia psidii* rust (Zhong, 2007). Unfortunately, Shaobin took another job and can no longer work on this rust. Fortunately, Mee Sook Kim (working at the Rocky Mountain Research Station) is in a position where she can help with these analyses and she has already used Shaobin's markers in previous exercises.

Over the next few months, it is expected that Mee Sook will receive the DNA of the rust from each of the following sources:

Brazil and Uruguay about 250 isolates (75 are already collected)

Puerto Rico and Jamaica about 5 isolates (one isolate is already collected)

Florida about 10 isolates

California about 6 isolates

Hawaii about 70 isolates (60 are already collected).

Once Mee Sook has the isolated DNA of a given set of rust samples, she will send these DNA extracts to the University of Wisconsin's Biotech Center for the assessment of their genetic markers (as per Shaobin's protocols).

Following the DNA analyses of all of these different sources, Mee Sook will proceed to developing a phylogenetic tree. As noted earlier, a rust strains position on the phylogenetic tree will also be one important factor considered when choosing the strains of this fungus to be used in the pathogenicity tests in Brazil.

She, along with Ned Klopfenstein, John Hanna, and Bryce Richardson will also develop theories on the evolution and spread of this fungus.

It should be noted that this work (to the greatest degree possible) will also be coordinated with the sandwich program of Rodrigo Graca Neves at WSU so that he can come over and spend two or three weeks with Mee Sook while she in engaged in these analyses.

Preliminary selection of sources to be used for this pathogenicity test

Of course, one of the main reasons for using the genetic markers and developing the phylogenetic tree is to help pick the eight strains of *Puccinia psidii* that will be used as inoculum for this set of pathogenicity tests in Brazil. An effort will be made to pick eight very distinct strains for these tests. Other factors having a bearing on the selection of these strains will be the host plant, diversity of geographic location and demonstrations of extraordinary pathogenicity (for example, the new rust strain found growing on the eucalypt clone that had previously been found to be resistant to the rust strain routinely used for evaluating resistance).

Pathogenicity Tests with the California strains of Guava Rust

Tentative plans have been made to do a pathogenicity test with ohia families and the California rust strains as well. These tests will have the same general experimental design as the one laid out for Brazil, and will involve the same ohia families. The major difference is that the test will be conducted in California with the California strains of this rust. Dr. Pat Nolan, Plant Pathologist for the County of San Diego, has agreed to conduct the tests. Dr. Paul Zambino, who has a lot of experience with rust research (and is now stationed with the USFS in San Bernadino), will be assisting her with these tests.

Pathogenicity Tests with the Puerto Rican, Jamaican and Florida strains of Guava Rust

Very early queries have also been made to learn more about the rust in Puerto Rico, Jamaica and Florida and to see if there might be someone in a situation where they could perform pathogenicity tests with their strains on the same ohia families that are being used in Brazil. There are possibilities. The author is reluctant to push these tests right now, however, as the rust strains from Brazil are likely to be much more diverse and therefore more important to use in these initial tests.

Complete Sequencing of the *Puccinia psidii* grant via Alexandro's lab

The use of genetic markers can be used to swiftly differentiate between different strains of a fungus, but it only requires the examination of a small amount of a fungus sample's DNA in order to function.

In Brazil, which led the world with the first sequencing of the DNA of a forest tree, (via Dario Grattapaglia's momentous study of *Eucalyptus grandis*), there is also a

push to learn about the entire sequence of *Puccinia psidii*, a major nemesis of the eucalypt.

In the upcoming months, Alexandro Alves' laboratory will be undertaking the sequencing exercise for Brazil's main strain of *Puccinia psidii*.

Strengthening the capacity of the Janice Uchida Laboratory, in Hawaii, to contend with this rust

There have already been a number of very significant rust-related activities that have taken place out of Janice Uchida's laboratory. The easiest ways to see some of the activities that have gone on at the University of Hawaii are to look at the list of references in this report or to simply google guava rust and check out many of the contributions that came from the University of Hawaii. Some of the salient developments that have been made in connection with this particular project are as follows: the documentation of the existence of *Puccinia psidii* in Hawaii (Uchida *et al*, 2006); the documentation on the distribution of *Puccinia psidii* in Hawaii (part of this documentation includes Rob Anderson's PhD thesis on this subject); and, the development of the genetic markers to distinguish between different strains of *Puccinia psidii* (Zhong, 2007).

Recently there has also been a significant amount of training on methods to collect *Puccinia psidii* DNA, run the genetic markers and interpret the resulting data. This training was provided to Chris Kadooka (of Janice's lab) by Mee-Sook Kim (of the Rocky Mountain Research Station). This was necessary because Shaobin Zhong, who had done an amazing job of developing these protocols, took a different job on the mainland. This training included three weeks of Chris' time visiting Mee-Sooks' lab in Moscow and Mee-Sook spending two weeks with Chris in Hawaii, in Janice Uchida's lab.

Additional activities (see ahead) will also, undoubtedly, contribute to the University of Hawaii increasing in its capacity and recognition as a central point for *Puccinia psidii* rust research.

The International *Puccinia psidii* Forest Pathology Conference in Hawaii

As Acelino and the author were talking in Brazil, it became apparent that this rust is spreading fast and that it would be unfortunate if the many individual countries, with vast extensions of myrtaceous forests, were stuck trying to fend for themselves against the imminent onslaught of this fungus. From this realization, the idea of having an international conference on this disease occurred spontaneously.

Without going into much detail, we felt that holding this conference in Hawaii, in the rainy season, in about 12 to 18 months, would be about right. This would probably mean about February of 2010.

The author has spoken with over a dozen scientists working on some aspect of this rust in Hawaii, California, Idaho, Washington DC, Australia and Brazil and all were very, very interested in having the event take place.

There are still an awful lot of details to work out for this conference: who all should be invited, the exact location, how big it should be, field trips, possible sponsors, etc.? Some queries will be made in the upcoming months to resolve these questions.

The risk analysis for rust in Hawaii

Over the past four years, at least two risk analyses have been written on the threat that *Puccinia psidii* poses to Hawaii (Kliejunas, La Rosa and Loope, 2006; Loope and La Rosa, 2008) and this risk has also been the theme of many meetings held by concerned officials in the state of Hawaii. On the basis of these analyses and this discussion, the State of Hawaii made the decision to install the "Interim Measure for Myrtaceaous species into Hawaii". However, at the time of this writing, the year-long duration of this interm measure is almost up, and there is a lack of decision about the need for continuing this measure (personal communication with Rob Hauff).

The most important piece of information that these Hawaiian decision-makers need to be able to decide whether to make this measure permanent, or to abandon it, is knowledge about what levels of damage to expect in Hawaii if new strains of this fungus were to be introduced into the Hawaiian islands.

The objective of this current study is to obtain the information that will be necessary to be able to predict this level of damage. Once this information is obtained, it will be the responsibility of this same group of concerned Hawaiian officials to meet again and decide what legislation (if any) is appropriate to enact for the future.

Connections with Forest Pathologists in South Africa, Australia, New Zealand

Puccinia psidii is a fungus that does not need a passport to work its way into every niche of the world where conditions are favorable for its development, and wherever hosts (ie myrtaceous species) are growing. Thus a very large portion of the humid tropics and sub-tropics will be climatically conducive for the disease caused by this fungus.

Because of this situation, and because their countries are especially loaded with myrtaceous species, a number of forest pathologists in Australia, New Zealand and South Africa should be especially concerned. Furthermore, as more is learned about this fungus and its potential impact their pathologists are, indeed, getting engaged.

By working on the rust with Brazil, as we are now doing, we have a very good entry mode into this international community of rust pathologists. Some of the key

contacts will be Morag Glen, Jack Simpson, Darren Kriticos and Ken Old of Australia, Mike Wingfield of South Africa and Margaret Dick of New Zealand. The author met with Morag while she was visiting a Berkeley lab shortly after returning from Brazil. He knows Mike and Margaret from time spent in South Africa and New Zealand earlier in his career. He overlapped with Ken Old on an assignment in Burma and he has been in recent contact with Darren Kriticos on a paper he is writing to model the environmental conditions of New Zealand for their ability to support the development of guava rust. Morag Glen and Jack Simpson have already been to Hawaii to do some initial work there and Mike Wingfield has been to a few locations in South America where he has searched for this rust.

The UFV Exchange program with NC State.

Murilo Zurbini, Dr. Eduardo Mizubutil and Dr. Elizabeth Pacheco Batista Fontes have recently visited NC State University to see if there were possibilities for exchanges of graduate students. Over dinner, with Eduardo and Acelino, we discussed several of the exchanges that might be especially productive (Note: the author received his PhD from NC State (plant pathology with minors in soils, forest genetics, forest management and statistics)).

Agriculture, experimental design, soils (all aspects), horticulture, molecular genetics, crop breeding, plant protection, plant physiology, plant propagation, agronomy, forest measurement, management, science and protection were among the leading disciplines that we discussed. Both NC State and Vicosa are among the leading universities in these disciplines for their respective nations.

Unfortunately, these Brazilian ambassadors for this exchange felt that there is a perception that Brazilian students would stand to gain more by going to the US than US students might gain by going to Vicosa.

Perhaps one of the hardest aspects of selling a stint in Brazil is that a US student would need to learn Portuguese. The fact of the matter is that few institutions in the US are teaching Portuguese while a huge proportion of those Brazilians, especially those who might aspire to a college education, have already had several years of English. This circumstance, particularly, limits the size of this exchange since it is predicated that each student going to the US must be balanced by a student coming to Vicosa. Still it could be argued that Vicosa has a great deal to offer. It has many excellent professors, top rate facilities and a vast number of agricultural and forest science topics for study that are world class in their significance. A few NC State students could be enticed.

Personal Development-

During this trip, the author kept his focus firmly on the purpose of this trip, (namely to work with Brazilian colleagues to develop studies that will lead to an accurate assessment of the risk that new strains of guava rust might pose to ohia in the state of Hawaii). Never-the-less, there were also several serendipitous opportunities for personal development as well. Among these were the following:

a) Catching up on all of the major diseases affecting Brazil's eucalypt forests. Partly this came about just as a result of visiting all of Acelino's laboratories and greenhouses. Also, Dr. Acelino Alfenas gave the author several books that he had authored or co-authored on the diseases of eucalypts in Brazil (these made for good reading on the 20 hour plane ride back to the states).
b) Catching up on intensive industrial forestry in Brazil. The author has been following this industry in Brazil over 30 years (this was his 5th trip to the country). This trip allowed the author to see the world's largest and most recently constructed pulp mill (Veracel), tour a state-of-the-art clonal eucalypt nursery, see many current forest operations, and catch up on several of the latest developments in forest genetics.
c) Getting familiar with his second of the three major agricultural/forestry research universities in Brazil: (1) Piricicaba, (2)Vicosa and (3)Lavras.
d) Seeing how Brazil manages its natural resources. The 3,600 km traveled while in Brazil were almost all down two lane roads traversing land in the states of Minas Gerais and Bahia. (Land use is mainly for agriculture, industrial forest plantations, grazing or mining. Along the route taken, most of the primary forest land has already been converted to other land uses although a few secondary forests remain.)
e) Brushing up on Portuguese and getting reacquainted with Brazilian culture; always an immense treat.

Conclusions-

This trip was imminently successful in terms of getting all of the planning and basic groundwork done that will be needed to enable Dr. Acelino Alfenas' forest pathology team to explore the genetic variability of the rust, *Puccinia psidii*, and to conduct the multi-strain guava rust pathogenicity tests on a wide range of ohia families. The results of these tests will, in turn, allow Hawaii's natural resource authorities to make informed decisions about whether or not to continue with the prohibition of myrtaceous species into their state.

There were many additional positive spinoffs of this trip. Several of these were also documented in this report, as well.

The author is extremely grateful to the International Activities Team for funding the travel costs for this trip.

Selected References

Alfenas, A.C., E.A.V. Zauza, R.G.Mafia, T.F. deAssis. 2004. Clonagem e doencas do eucalipto. Universidade Federal de Vicosa. 442 p.

Booth, T.H., Old, K.M. and T. Jovanvic. 2000. A preliminary assessment of high risk areas for *Puccinia psidii* (Eucalyptus rust) in the Neotropics and Australia. Agriculture, Ecosystems & Environment 82:295-301.

Glen, M. , A. C. Alfenas, E. A. V. Zauza, M. J. Wingfield and C. Mohammud. 2007. *Puccinia psidii*: a threat to the Australian environment and economy- a review. Australian Plant Phytopathology 36, 1-16.

Killgore, E. M. and R.A. Heu. 2005 A rust disease on ohia. New Pest Advisory No. 04-05, July, 2005. Dept. of Agriculture. State of Hawaii. 2 p.

Kleijunas, J., A.M.LaRosa and L. Loope (unpbl. 2006) Eucalyptus rust (A risk analysis for Hawaii) 13 pp.

Langrell, S.R.H., M. Glen and A.C. Alfenas. 2008. Molecular diagnosis of Puccinia psidii (guava rust)-a quarantine threat to Australian eucalypt and Myrtaceae biodiversity. Plant Pathology 57:687-701.

L. Loope and A.M.LaRosa (unpbl. 2007). `Ohi`a Rust (Eucalyptus Rust) (*Puccinia psidii* Winter) Risk Assessment for Hawai`i . 12 pp.

Simpson, J.A., K. Thomas and C. A. Grgurinovic. 2006. Uredinales species pathogenic on Myrtaceae. Australian Plant Pathology, 35: 549-562.

Friday, J.B. and D.A. Herbert. 2005. *Metrosideros polymorpha* ('ohi'a). Species profiles for Pacific Island Agroforestry. www.traditional tree.org 27 pp.

Uchida J, S. Zhong and E. Killgore. 2006. First report of a rust disease on Ohi'a caused by Puccinia psidii in Hawaii. Plant Disease 90, 524

Zhong, S. 2007. Development of microsatellite markers for Puccinia psidii, a rust fungus threatening ohia (*Metrosideros polymorpha*) and other Myrtaceae in Hawaii. 2007 North American Cereal Rust Workshop. http://www.ars.usda.gov/SP2UserFiles/Place/36400500/2007Workshop/Zhong_Hawaii.ppt

Figure 9. The author *en route* to Bahia.