

Integration of Rapid Ohia Death Research through Forest Pathology and Pathogen Genetics

Final FY17 Report
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Genetic studies and my forest pathology approach were proposed to fill in critical gaps and provide tools for a solid understanding of rapid ohia death (ROD). At the time of the proposal in 2016, more than 30,000 acres of ROD had been recorded, but the specific causes of the mortality had not been clearly identified. Two undescribed species of *Ceratocystis* (sp. A and B) were associated with ROD, and these two species were thought to be equally important. Together with other research efforts, we now have a much clearer understanding of the primary disease and *Ceratocystis* species associated with ROD. This understanding and newly developed research tools allowed for confirmation of the primary source of inoculum and spread of the *Ceratocystis* species, which in turn has led to the development of management strategies. The management of ROD continues to be guided and refined by on-going research that was initiated, in part, by this project.

Funding was requested for work to be conducted in or coordinated from Iowa, including genetic and other analyses on strategically collected isolates of *Ceratocystis*. These and other collaborative efforts were to provide tools for defining the diseases caused by *Ceratocystis* sp. A and sp. B, their mechanisms of dispersal, geographic distributions, projected pathways, and management strategies. These goals were to be addressed through four technical objectives, which have been fully and successfully completed. The main findings and management implications are listed in the following synopsis.

SYNOPSIS

In 2016, two species of *Ceratocystis* were both thought responsible for the massive loss of ohia trees on the Big Island. The single most important research contribution to our understanding of ROD has been the descriptions of *Ceratocystis* sp. A and B as *C. lukuohia* and *C. huliohia*, respectively, and the diseases they cause (Barnes et al. 2018). The descriptions were justified, in part, by genetic analyses conducted in Iowa. Laboratory mating tests with the ohia pathogens and their nearest relatives were conducted in Iowa, and these data also helped justify designation of

the two new species names. Descriptions of the symptoms associated with the two new *Ceratocystis* species suggested that the fungi cause two different diseases.

Isolates of the other *Ceratocystis* species recorded in Hawaii were collected and compared to the ohia pathogens in order to ensure that these really were distinct species and to see if they could mate with the ohia pathogens and produce even more aggressive strains that could potentially attack new hosts. The *Syngonium* strain in Hilo nurseries and the taro strain in Hawaii (now recognized as *C. uchidae*, Li et al. 2018) were shown to be very closely related to the respective ohia pathogens based on genetic analyses and mating studies. Somewhat surprisingly, we were able to successfully cross the *Syngonium* and taro strains, respectively, with *C. lukuohia* and *C. huliiohia* in the laboratory, though there was a reduction in the number of viable progeny in the interspecific crossings. Thus, these other strains or newly introduced strains of *Ceratocystis* could potentially lead to new recombinant strains of the ohia pathogens that could be more aggressive on ohia or on other hosts. However, the genomic analyses showed that the respective ohia pathogens did not arise directly from the *Syngonium* or taro strains, in contrast to what had been hypothesized in 2016. Nonetheless, care should be taken to limit the spread of these other *Ceratocystis* and to prevent new strains of *Ceratocystis* from being introduced to Hawaii through the nursery trade.

Based on the known biology of its nearest relatives (Caribbean subclade members of the Latin American Clade), *C. lukuohia* would be expected to be capable of causing a vascular wilt disease. As expected, it was the only *Ceratocystis* isolated from rapidly dying ohia trees that had deep, radial penetration of the host sapwood. This rapid wilting and systemic colonization were confirmed in a preliminary inoculation study (with Lisa Keith's Lab) of mature ohia trees, in which the fungus quickly moved up the sapwood of inoculated trees at up to 10 cm per day, causing wilt symptoms in a few months. This disease is now called Ceratocystis wilt of ohia.

The Asian relatives of *C. huliiohia* are not aggressive pathogens, and inoculation of ohia trees showed limited movement in the host and production of canker-like symptoms. In studies of fresh outbreaks of the dying ohia trees with ROD-like symptoms, *C. lukuohia* has been the only *Ceratocystis* species isolated from the newly-killed trees, and *Ceratocystis* wilt is now the main focus of management. Its current distribution on the island is well characterized and closely watched.

The precise pathways that the two ohia pathogens took into Hawaii are not clear, but they each appear to have arisen from a single genotype or spore based on the genetic analyses. Importation of live plants was the likely culprit. The identified center of *C. lukuohia* genetic diversity was shown to be in the Lower Puna, the likely source of diversification, if not the original introduction of the pathogen. This is consistent with the Pictometry imagery that showed dying ohia trees near Leilani Estates in 2009, before homeowners recognized ohia mortality in the area. More important for management and predicting the spread of the epidemic is the support for the hypothesis that the pathogens are airborne and *C. lukuohia* is moving north, most likely in the form of spores in ambrosia beetle frass.

The hypothesis that *C. lukuohia* and *C. huliiohia* are principally disseminated in the wind in the form of fungal spores in liberated ambrosia beetle frass could not be adequately tested with the

detection technology available in 2016. Therefore, we developed new genetic tests that allow for detection of small amounts of *Ceratocystis* DNA, less than that found in a single spore. The technology was a modification of a protocol developed for a related *Ceratocystis* species (Lumia et al. 2018). The extreme sensitivity of the new test was combined with deployment of air sampling technology that has been used with many other plant pathogens. This Rotorod sampling and the new detection protocol have been tested throughout the island and continue to be deployed under the direction of Wade Heller (USDA-ARS). The Rotorod sampling thus far indicates that DNA of these fungi can be sporadically abundant in the air at over a mile downwind from ROD trees. These protocols should prove valuable in evaluating management strategies and prioritizing sites for treatment.

From the extensive genome analyses of *C. lukuohia*, 21 specially designed genetic markers were developed to “fingerprint” isolates and trace the accumulation of rare mutations in populations of the pathogen. We fingerprinted 82 isolates that I collected and another 21 isolates from Lisa Keith’s Lab at PBARC. These isolates represented the known foci of *Ceratocystis* wilt on Hawaii Island, including the recent Kohala outbreak site. These data suggested two modes of spread from Lower Puna: 1) the gradual northerly expansion of new genotypes and their spread with trade winds to the southwest and 2) the apparent jumps of “Puna genotypes” northward to major outbreaks at Kamae’e Stream and Waipunalei on the Hamakua Coast via storms such as Tropical Storm Iselle in 2014. Such northward jumps up the east coast of the island have been the focus of management.

Consistent with the Rotorod spore sampling, the genetic fingerprinting studies indicate a gradual movement of *C. lukuohia* southwestward from Puna, Waiakea and Hilo towards Volcano National Park and to Ka’u. There appears to be a continuous dispersal of inoculum to these areas, and storms do not appear to be necessary for infection of trees. Many of the infected trees in these situations are wounded by humans or other animals. In such regions with remote and diffuse sources of inoculum, the most effective management might be to avoid wounding, fencing to exclude ungulates, and use of a fungicidal wound dressing on fresh wounds. Development of such wound dressings are sorely needed.

The strategy for slowing the spread of ROD northward up the east coast (and to Maui) in storm events has been to reduce major sources of airborne inoculum. Killed trees infested with ambrosia beetles have been felled and tarped to prevent dispersal of airborne ambrosia beetle frass. Alternatively, insecticides have been applied to the trunks of recently killed trees to lessen ambrosia beetle attacks or to already infested trees to slow down ambrosia beetle tunneling. Although initial trials showed promise for reducing production of ambrosia beetle frass, there has been little progress in pursuing this critical management tool.

For larger areas with diseased trees too numerous to treat by tarping, trees have been felled to keep the ambrosia beetle frass close to the ground and out of the wind. Large numbers of ROD-killed trees have been felled at three sites to see if this could prevent further spread. Isolates taken from trees at the three sites have been genetically fingerprinted to determine if these genotypes can spread and lead to new infections downwind from the felling operations. Thus far, these genotypes have not been detected in subsequent sampling of diseased trees in the area.

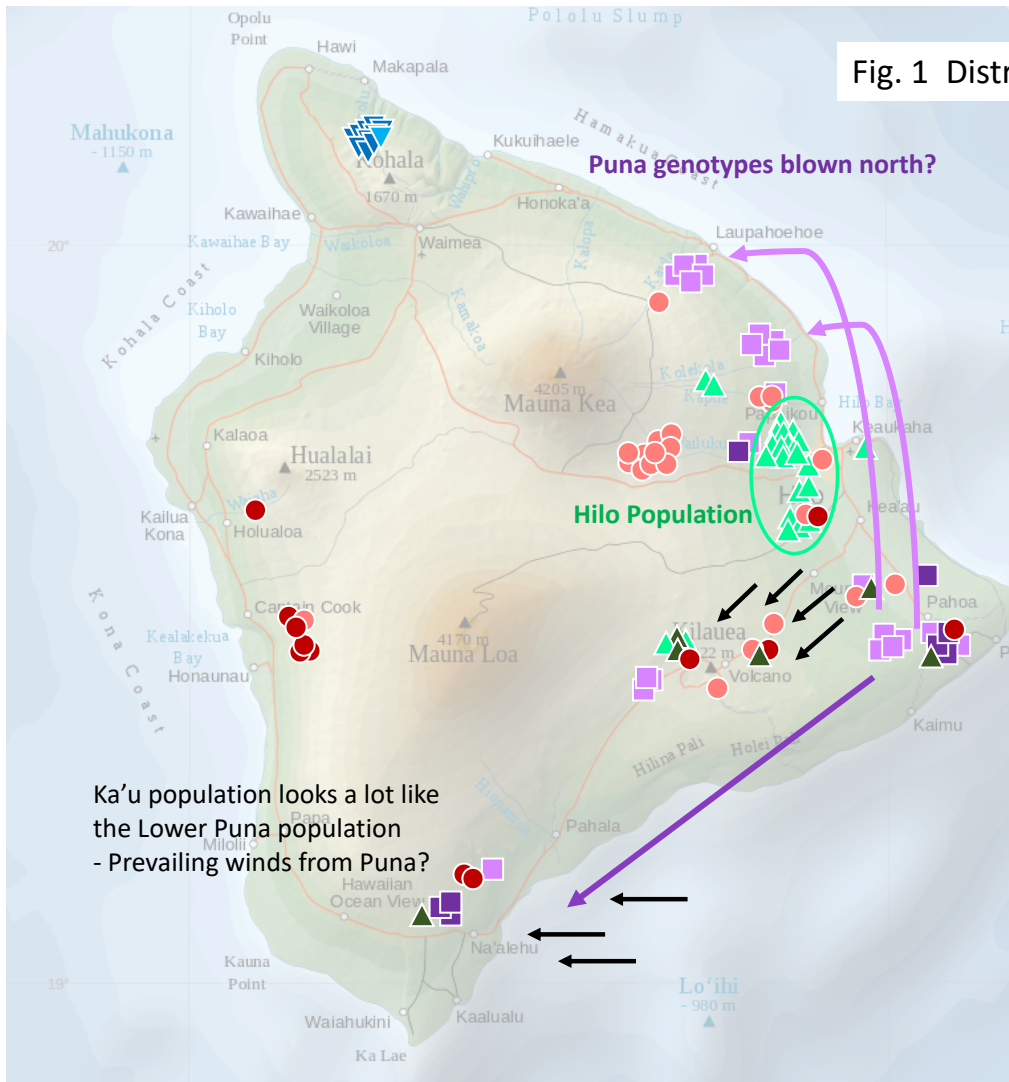
The DNA fingerprinting database of 103 isolates was designed to be easily built upon by others, and it is hoped that it will be used by scientists in the coming decades to trace movement of *C. lukuohia*. A similar fingerprinting database could be developed for *C. huliiohia*. When new outbreaks occur, it should be possible to trace its origins to upwind sources of inoculum. If the pathogen is detected at new sites on Hawaii Island or on Maui, the source of that inoculum could be identified and treated. For instance, the Kohala outbreak is associated with a unique genotype of *C. lukuohia*, and fingerprinting of an outbreak population on Maui could be traced back to Kohala or another diseased stand on Hawaii. Both the new outbreak site and its source of inoculum could be treated.

The HISC funding was important in the development of these management protocols, which should be improved upon by ongoing research studies and the new genetic tools and database developed from this study.

PUBLICATIONS RELEVANT TO THE PROJECT

- Barnes, I., A. Fourie, M. J. Wingfield, T. C. Harrington, D. L. McNew, L. S. Sugiyama, B. C. Luiz, W. P. Heller, and L. M. Keith. 2018. New *Ceratocystis* species associated with rapid death of *Metrosideros polymorpha* in Hawaii. *Persoonia – Molec. Phylog. Evolut. Fungi* 40:154-181. <https://doi.org/10.3767/persoonia.2018.40.07>
- Li, Qian, T. C. Harrington, D. McNew, and J. Li. 2017. *Ceratocystis uchidae* sp. nov., a new species on Araceae in Hawaii and Fiji. *Mycoscience* 58:398-412.
- Lumia V., V. Modesti, A. Brunetti, C. L. Wilkinson, G. Di Lernia, T. C. Harrington and M. Pilotti. 2018. Real-Time PCR for *Ceratocystis platani* detection: in-depth validation to assess the diagnostic potential and include additional technical options. *iForest*, 11:499-509.

Fig. 1 Distribution of microsatellite genotypes of *Ceratocystis lukuohia*



- Only the most common allele at each of the 21 loci (the most common and perhaps the original genotype, no accumulated mutations)
- Same as red, except for one or two unique alleles peculiar to a site (different genotypes independently derived from the red genotype)
- Differing from the red genotype by a single mutant allele that is dominant in Lower Puna (perhaps a mutation that developed early in the epidemic)
- Many different genotypes derived from the dark purple genotype (mostly Lower Puna, but Kamae'e and Waipunalei outbreaks, too)
- ▲ A rare genotype, differing from the red genotype by single mutant allele (in Puna, Volcano and Ka'u)
- ▲ Many different genotypes derived from the dark green genotype, most encountered in the Stainback Rd. to Pi'ihonua areas (from old canefields west of Hilo)?
- ▼ Kohala outbreak represented by 11 isolates, a distinct genotype, but most similar to green triangle genotypes (Pahoehoe Stream and Hilo area).

Ka'u population looks a lot like the Lower Puna population
- Prevailing winds from Puna?