Ti Leaf (*Cordyline terminalis* or *fruticosa*) Diseases in Hawaii's Commercial Orchards

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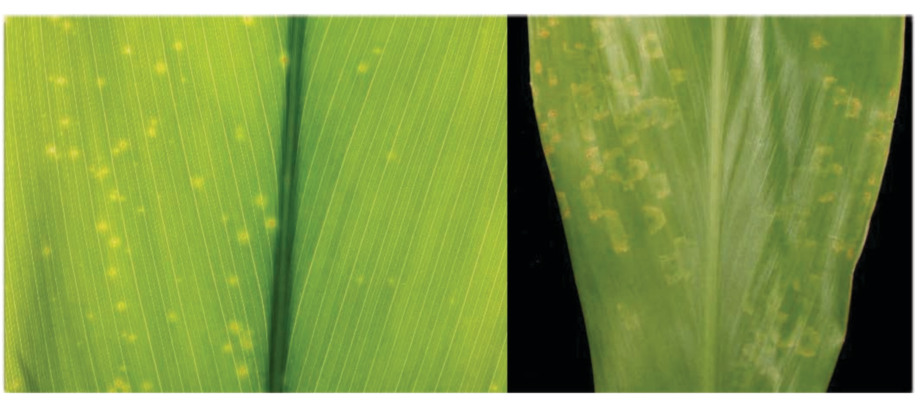
Ti leaf (*Cordyline* sp.) also known locally as *Kī* is an important agricultural crop to Hawaii. Many associate the ti leaf as an ornamental crop, commonly used for hula skirts, leis, garlands, or *pū´olo* (bundle or container) used to make an offering to the gods (Fig. 1), as greens in floral arrangements, and an important part of the tropical landscape with many new leaf sizes and colors. According to Waimea Valley, *Kī* was planted around the *heiau* (temple sites), used frequently in ceremonies, worn around the neck or on the body for protection, and is also a kinolau (plant body form) for *Laka* (the goddess of hula).

Figure 1. The significance of the pu'olo, which is made from the ki, is found both in the intention of the offering and the offering itself.

The leaves of the ti plant also serve as a food wrapper placed around or below edible products such as laulau (a Hawaiian dish consisting of a meat product wrapped in taro leaves) and also used to line pans for baking fish, other seafood and favorite local fruits such as breadfruit. A unique and delicious flavor is added to the cooked products.

Ti leaves used in food preparation are not consumed. They are used as food wrappers primarily due to its ability to provide moisture, tolerate high oven temperatures, and imparts a subtle flavor to many cultural dishes. As a result, the appearance of leaves was never a factor in ti production. Blemished leaves that could not be sold for ornamental purposes were sought after by food processors. In 2010, the issue of excessive spotting on leaves was raised by a major commercial food processor of laulau in Hawaii. Ti leaves with severe spotting were no longer acceptable by market standards.



Fungal growth (left), viral leaf spotting (right) Photo Credit: Chris Kadooka

Leaves that come in contact with an edible product are considered an agricultural food commodity and are subject to the same standards and regulations as other edible agricultural products. Local growers were faced with minimizing leaf blemishes using solely cultural and physical methods to secure their market share. Based on conversations with a cooperative of ti farmers, leaf diseases primarily from fungal and bacterial pathogens cause damage from 5% to as high as 50% during weather periods conducive to disease on Oahu. Infections caused by bacteria (*Xanthomonas* sp.) are difficult to control, due to constant wet weather conditions and non- acceptance of residues of copper-based products by food processors. The addition of a new viral disease, ti ringspot, increased producers' crop losses.

On Oahu, ti leaf is grown predominantly on the windward side of the island. Areas that have high rainfall and humidity are the best environment for *Cordyline* production. Unfortunately these conditions are ideal for fungal and bacterial pathogens. Crop protection chemicals are available for *Cordyline* sp., but the harvested product must not be used in food systems. Laboratory and field trials which evaluated promising crop protection chemicals for possible minor crop fungicide registrations have been completed with the assistance of CTAHR's IR-4 Minor Crop Pesticide Registration Program.

Brown streaking commonly associated with the pathogen, Xanthomonas sp.
Photo Credit: Chris Kadooka

Fungicide Screening

Three fungal pathogens were identified on ti by Uchida et al: 1) *Cercospora*, 2) *Colletotrichum*, and 3) *Phyllostica*. Brown streaks were positive for bacteria. However, due to the difficulty in managing bacterial pathogens and the low occurrence of the bacteria in the field, the focus of the project was on the management of fungal and viral diseases. Twelve fungicides (Heritage, Flint, Folicure, Luna Privilege, Pristine, Scholar, Switch, Procure, Rally, Reason, Tanos, and Tilt) were selected and evaluated for efficacy on the three fungal pathogens. Azoxystrobin (Heritage), propiconazole (Tilt), and trifloxystrobin (Flint) were selected, based on laboratory bioassay performance, by Uchida, Kadooka, Kawate et al. for field evaluations. Efficacy of the fungicides to control leaf spots caused by *Cercospora* sp., *Colletotrichum* sp., and *Phyllosticta* sp. on ti were conducted at a cooperator's farm in Kahalu'u, Oahu.

Leaf spotting was the result of fungal pathogens. Three fungal pathogens were identified on ti by Uchida et al: 1) Cercospora, 2) Colletotrichum, and Phyllostica. Photo Credit: Chris Kadooka

Early fungal spots Fungal sporulation on leaf Petiole and sheath rot

Field Trial #1: Azoxystrobin (Heritage), propiconazole (Tilt), and trifloxystrobin (Flint) were first applied for control of leaf spot diseases of ti caused by *Cercospora* sp. and *Phyllosticta* sp. in a grower's field located in Kahaluu, Oahu in February 2011. The experimental area was established in an existing ti field; plant heights varied from 3 - 8 feet in height. The treatments were: 1) untreated control; 2) azoxystrobin, 0.25 lb ai/A; 3) propiconazole, 0.11 lb ai/A; and, 4) trifloxystrobin, 0.094 lb ai/A. Each treatment area was a 10 ft x 20 ft block.



Twenty (20) plants within each block were selected for evaluation. Immediately prior to the first application, the oldest leaf on each selected plant (within each plot that did not have any obvious chlorotic spots) was tagged with flagging tape. Fungicide treatments were subsequently applied using a gas-powered backpack mistblower, Solo Port 423. All fungicide treatments were applied at 70 gal A-1 with Latron B-1956 at 16 fl oz 100 gal-1 on 02/24/11, 03/10/11, and 03/24/11. On 03/14/11, 03/23/11, 04/06/11, and 04/20/11, visual ratings of the severity of infection of each leaf was recorded. A 0 to 5 rating scale was used with 0 = no lesions, 1 = up to 20 pinpoint spots visible by backlighting leaf, 2 = up to 100 pinpoint spots visible by backlighting leaf or < 20 chlorotic spots, 3 = up to 100 chlorotic spots, 4 = over 200 yellow spots, and 5 = yellow and necrotic spots coalescing into larger spots and blights.

Data was analyzed using PROC GLM (SAS), in a completely randomized model, and means were separated using Tukey's Studentized Range Test. Results indicated that azoxystrobin and propiconazole were the most effective fungicides, followed by trifloxystrobin. All fungicides

Figure 1. Visual disease rating scale; 0 = no disease (not shown).

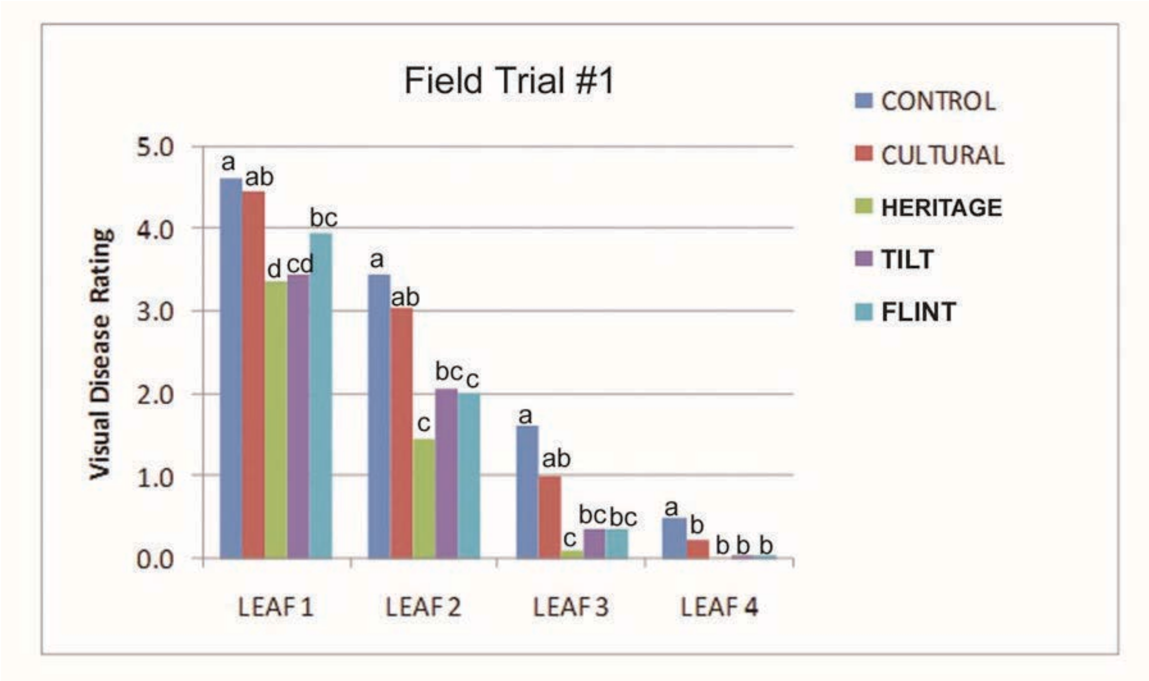


Figure 2. Visual disease ratings of ti leaves taken on 04/20/11. Columns within each evaluated leaf with a different letter are significantly different at α=0.05 using Tukey's Studentized Range Test.

tested had some level of effectiveness against leaf spots. No obvious phytotoxic symptoms were apparent in any of the treatments. No sanitation practices were implemented in field trial #1, aside from removal of collected treatment leaves and initial field clean up.

Field Trial #2: In June 2011, the experimental area was re-established in the existing ti field. Fungal pressure was reduced in comparison to the prior trial. Mancozeb was added as a treatment for this trial. Increased sanitation efforts were implemented, which included removal of all infected leaves, establishment of uniform plant heights, and increased spacing between plants were imposed. Selected treatment plants were 5 to 6 feet tall. Each treatment area was a 10 ft x 20 ft block. Twenty (20) plants within a block were selected for evaluation. Treatments were applied following the same procedures described in Field Trial #1. Treatment rates for Propiconazole, Azoxystrobin and Trifloxystrobin were the same as Field Trial #1. Three applications were made: 6/2/11, 6/30/11, and 7/26/ 11. Data was collected on 9/26/11, 10/25/11, 11/28/11. Preliminary visual rating data suggest, the use of cultural practices alone (no fungicide treatments) slightly reduced leaf spotting caused by *Cercospora* sp. and *Phyllosticta* sp., however, the differences were not statistically significant.

Start of program: 2/2011

September 2011

Field sanitation: Project

Field sanitation: Commercial practice

Based on the laboratory and field efficacy trials, Hawaii IR-4 submitted a project request at the 2012 IR-4 Food Use Workshop in St. Louis, MO to establish a tolerance for azoxystrobin (Quadris) and propiconazole (Tilt) in ti, leaves and roots, with existing residue data. In February 2013, IR-4 is proceeding with a no data tolerance petition for both azoxystrobin (Quadris) and propiconazole (Tilt) in ti.

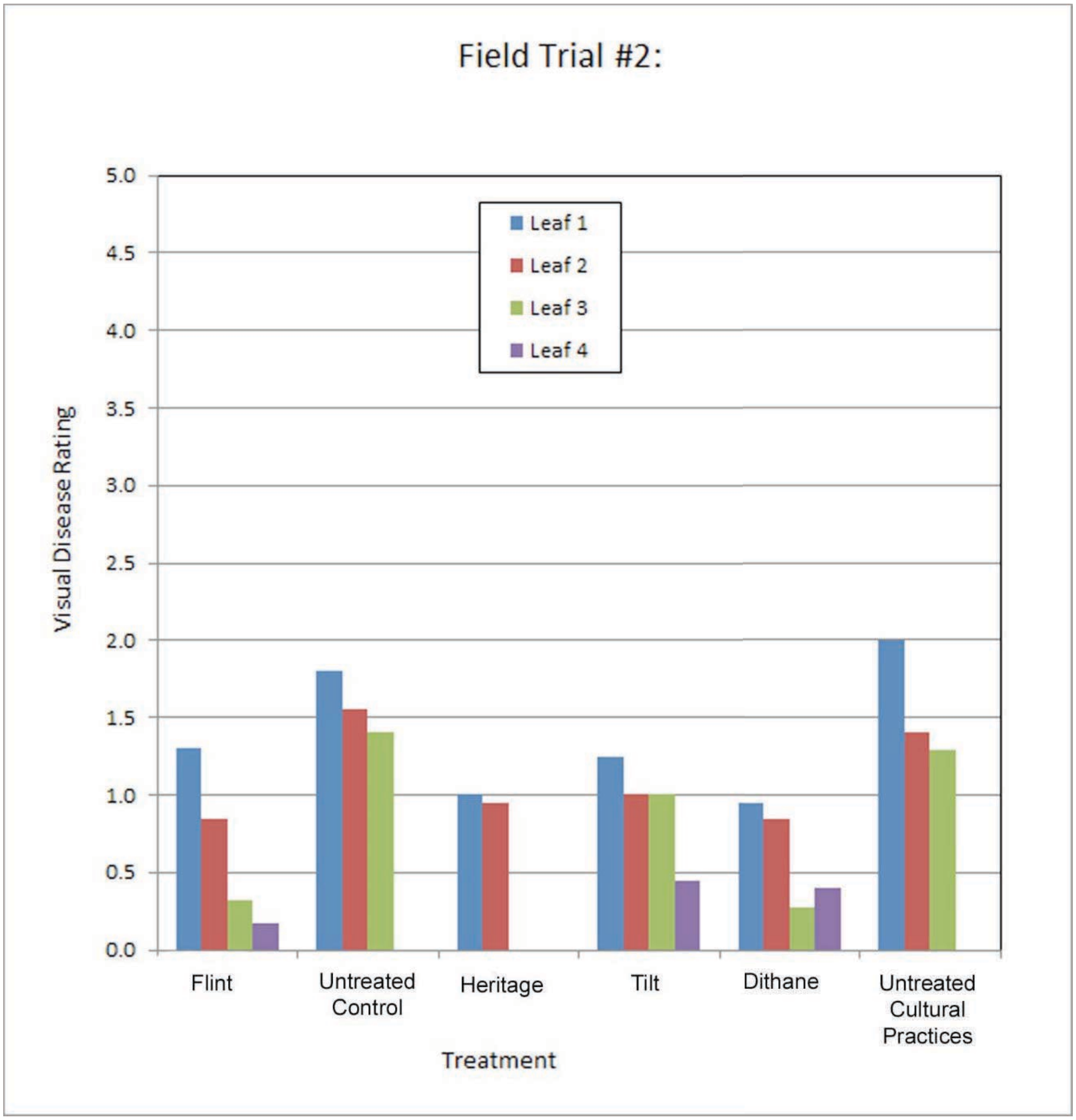


Figure 6. Visual disease ratings of ti leaves taken 179 days after treatment.

The project established new ti fields at UH experiment stations in anticipation for IR-4 residue work. However, if the no data petition is accepted then the field residue work may not be needed. The supporting data required for IR-4 to continue with the registration process has been completed by UH CTAHR via grant support by Hawaii Department of Agriculture. The final registration by Syngenta will take a few years to be finalized. Preliminary feedback from Syngenta indicates a combined product made up of azoxystrobin and propiconazole (Quilt) may be the final product to be registered for use on ti. There is a concern about the combination product having two registered active ingredients because it will limit the number of applications per year and reduce available rotational products for resistance management purposes.

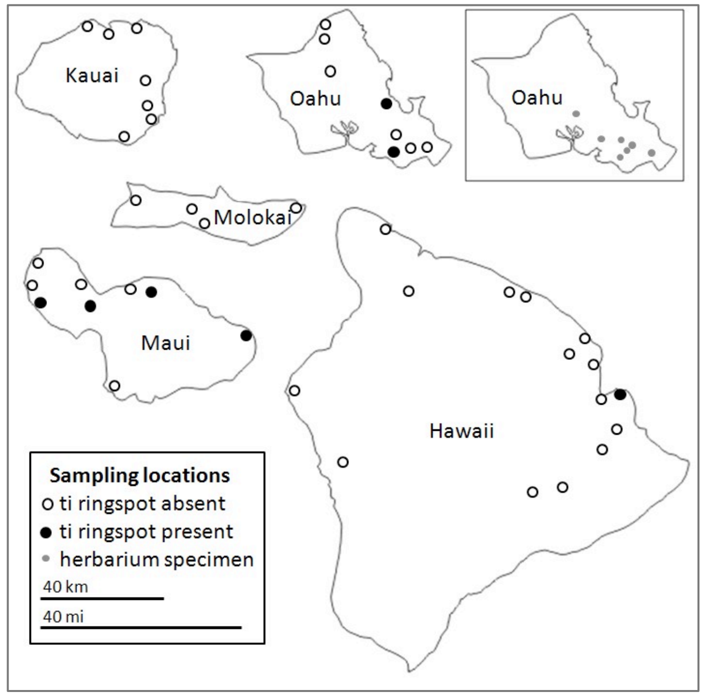
Examples of ti ringspot disease which is associated with a new emara-like virus that is possibility transmitted by eriophyid mites.

Ti Ringspot and Ti Viruses

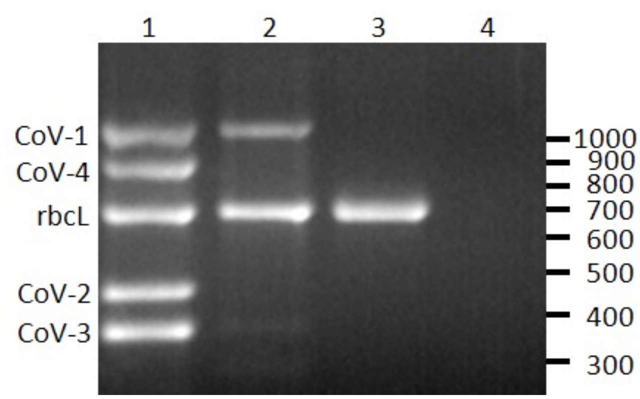
In 2009, growers reported an abnormal ring spotting symptom on ti plants located in windward, Oahu. Plants exhibiting ringspot symptoms were collected from a commercial farm on Oahu and brought to UH Manoa for examination. Common green ti plants in Hawaii have been propagated by cuttings almost exclusively for centuries, and over this period have accumulated a large number of plant viruses To characterize the virome (the viral genomes present in a host) in these plants, we isolated double-stranded (ds)RNA which represents the replicative form of most RNA-based viruses, but would not contain the genetic material of any DNA-based viruses. To search for DNA-based viruses, we also isolated total RNA which would contain the transcription products of DNA virus genomes, but also the transcription products of plant, RNA virus, and any microbial genomes present in the sample. These two samples underwent massively-parallel (next generation) sequencing to characterize the genetic material. For the dsRNA sample, over 1.07x105 reads were generated. Of these reads, most mapped to the genomes of four new viruses designated Cordyline virus 1 (CoV-1), CoV-2, CoV-3, or CoV-4. No viral sequences were detected in the total RNA sample.

The entire genome of CoV-1 and the majority of the genomes of CoV-2, CoV-3, and CoV-4 have been sequenced. Using these data, a reverse-transcriptase polymerase chain reaction (RT-PCR) assay was developed that can detect and distinguish these four viruses in ti plants. One hundred and thirty-seven common green ti plants with and without ti ringspot symptoms were sampled from 43 sites on five islands (Kauai, Oahu, Molokai, Maui, and the Big Island) and underwent the RT-PCR assay. Eleven asymptomatic ornamental ti varieties from Lyon Arboretum and ten asymptomatic common green ti herbarium specimens from Bishop Museum were also sampled and assayed. Based on this survey, it appears that the CoVs are very common in Hawaii's ti plants, but that none of these viruses are involved in the etiology of ti ringspot. It appears the CoVs do not cause obvious symptoms in the ti plants they infect, although it is possible that they reduce the overall vigor of the plant or may act synergistically with other ti pathogens.

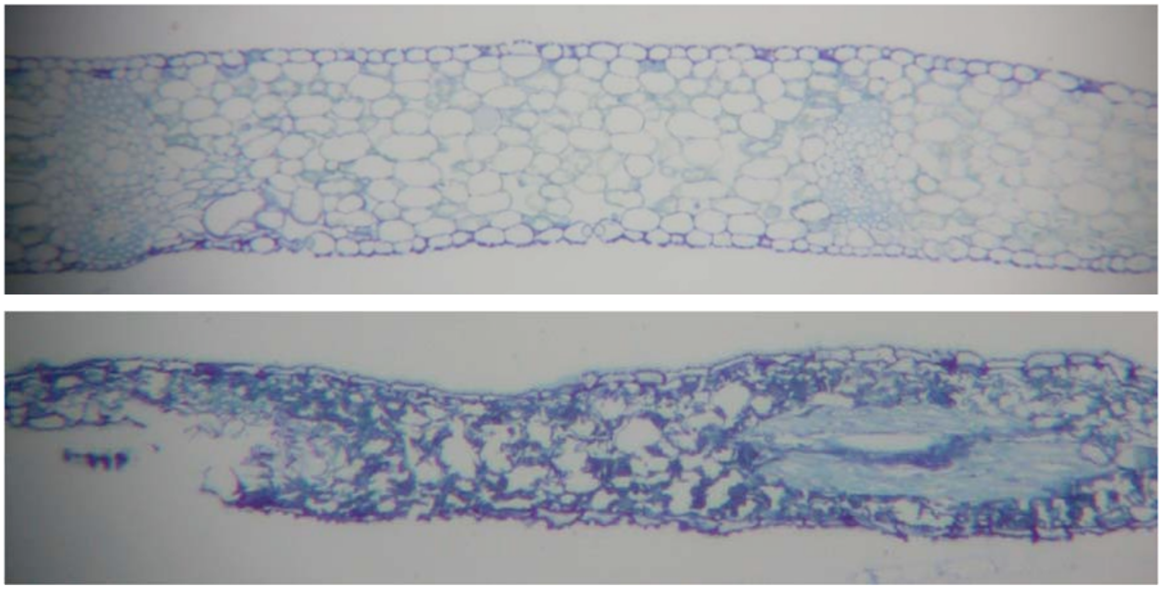
To search for additional pathogens that may be responsible for ti ringspot disease, we examined ultrathin sections of symptomatic and asymptomatic ti leaf tissue using light and transmission electron microscopy (TEM). We identified virus-like particles (VLPs) consistent with the morphology of an emaravirus virion in the early stages of ringspot lesion development. These VLPs were not observed in asymptomatic tissues or in mature ringspot lesions. Members of the genus *Emaravirus* are generally transmitted by eriophyid mites, which we suspect may be involved in ti ringspot. These viruses typically produce ringspot symptoms in their host, similar to ti ringspot. A recent research article describes a test for detecting the four known emaravirus species. We used this test to identify a novel emaravirus present in ti plants with symptoms of ti ringspot. This virus, which we are tentatively naming Ti ringspot virus (TiRV), is most closely related to *European mountain ash ringspot associated virus* (EMARaV) based on available sequence data. The amino acid sequence identity between TiRV and EMARaV is 46% in a region of the polymerase protein of these viruses. We strongly suspect that TiRV is responsible for ti ringspot disease. As such, we have developed, and are currently evaluating a TaqMan Probe-based qRT-PCR assay for the detection of this virus.



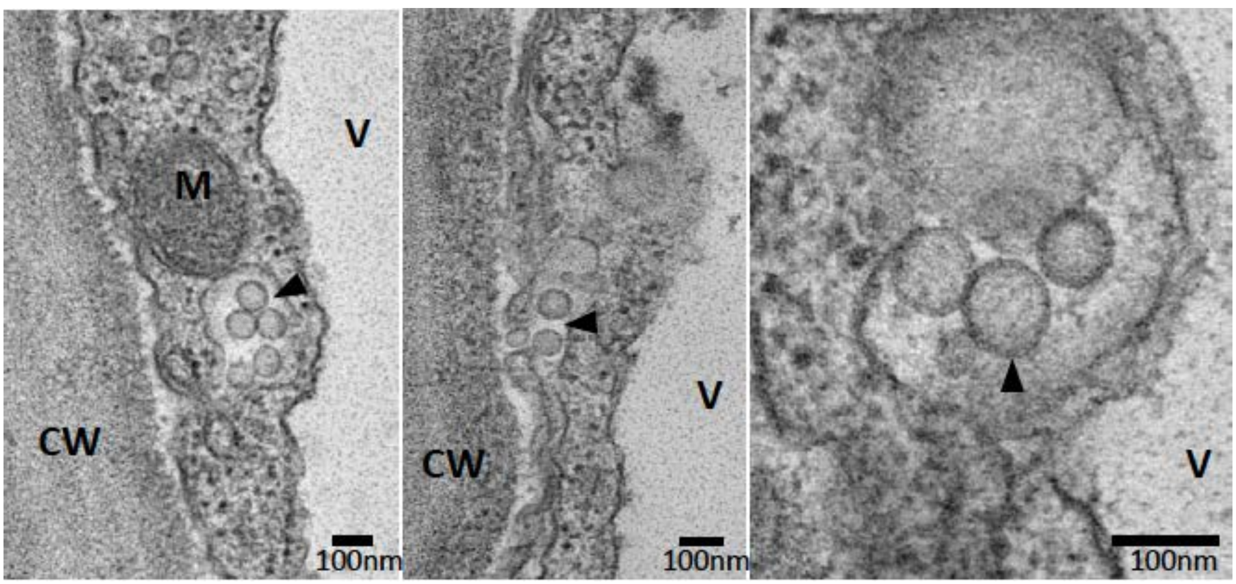
Locations of where common green ti plants were sampled on five of the major Hawaiian Islands and assayed for CoVs. The location of where some herbarium specimens were collected on Oahu (inset) is approximate due to a lack of detailed information. Relative sizes of the islands are to scale, but their geographic locations relative to each other are not accurate.



Reverse-transcription PCR detection of Cordyline viruses (CoVs) 1-4 infecting C. fruticosa. Lane 1, plant infected with all four CoVs; lane 2, plant infected with CoV-1; lane 3, CoV-free plant; lane 4, non-template control. The rubisco large subunit (rbcL) is an internal positive control for the assay. The scale on right indicates size in base pairs.



Cytopathology of leaf tissue with ti ringspot symptoms (lower) in comparison to healthy leaf tissue (upper) as observed using light microscopy.



Transmission electron micrographs of double-membrane, virus-like particles (arrowheads) approximately 85 nm in diameter present in young ti ringspot lesions. CW, cell wall; M, mitochondrion, V, vacuole.

To manage the viruses that infect ti, we generated virus-free germplasm using cryotherapy, a procedure that kills all but the meristematic cells (which are generally virus-free). These meristematic cells regenerate, producing a virus-free plant. Approximately ~100 ti plantlets underwent cryotherapy, and 12 survived the procedure. These plants were assayed and confirmed to be free of CoVs and TiRV. These plants will be critical in future studies on the biology of CoVs and TiRV, such as in vector transmission experiments. In addition, these plants will serve as a source of virus-free ti germplasm. These plants are currently being grown and propagated in a foundation block greenhouse at the University of Hawaii. Planting material has already been requested and sent to local nurseries due to their high leaf quality and vigorous growth characteristics.

Uchida et al compared the eriophyid mite population on symptomatic and healthy leaves, documenting that symptomatic leaves had higher mite numbers than healthy leaves or leaves with low number of spots. Epidemiologically, the disease moved into the clean plots beginning at the end of the field closest to the diseased, mite infested field. Tiny spots in tracks were also noted (photo on right).

Virus-free ti germplasm growing in a foundation block greenhouse at the University of Hawaii.

Sticky cards installed at the field test site detected leaf hoppers and eriophyid mites (photo on left).

The overall program goals were focused on meeting the immediate and emerging needs of Hawaii's ti leaf industry through research, extension and collaboration. We have identified the major economic pathogens on ti and have evaluated appropriate pest management strategies for long term disease management. We believe increased cultural management of the disease, in combination with registration of promising fungicides identified through laboratory and field research, assists in safeguarding the profitability and sustainability of Hawaii's ti leaf farmers; increases the efficiency and productivity of crop production practices; heightens the standard of quality and consistency of Hawaii grown ti leaves; and minimizes invasion of introduced pathogens from abroad (through increased ti imports). CTAHR's Pesticide Registration Program and the National IR-4 program will address the requirements necessary to obtain minor crop registrations for ti. An anticipated impact of this program would be the establishment of crop protection chemical registrations and identification of a new virus in support of Hawaii's ti industry.

Research Outputs

Melzer, MJ, Sugano, J, Uchida, JY, Kawate, MK, Borth, WB, Hu, JS. 201X. Ti ringspot disease is associated with a novel emara-like virus. Phytopathology (in preparation)

Melzer, M, Ayin, C, Sugano, J, Uchida, J, Kawate, M, Borth, W, Hu, J. 2013. Differentiation and distribution of Cordyline viruses 1-4 in Hawaiian ti plants (*Cordyline fruticosa* L.). Viruses 5:1655-1663

Melzer, MJ, Sugano, JS, Uchida, JY, Kawate, MK, Borth, WB, Sether, DM, Hu, JS. 2013. Molecular characterization of closteroviruses infecting *Cordyline fruticosa* (L.) in Hawaii. Frontiers in Microbiology 4:39 (doi: 10.3389/fmicb.2013.00039)

Melzer, MJ, Sether, DM, Borth, WB, Mersino, EF, Hu, JS. 2011. An assemblage of closteroviruses infects Hawaiian ti (*Cordyline fruticosa* L.). Virus Genes 42:254-260

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