BIOLOGICAL CONTROL POTENTIAL OF *MICONIA CALVESCENS* USING THREE FUNGAL PATHOGENS

Eloise M. Killgore
Biological Control Section, Hawai'i Department of Agriculture, P. O. Box 22159, Honolulu, Hawai'i 96823-2159, U.S.A.
Email: *eloisekillgore@yahoo.com*

Abstract. Biological control of miconia *(Miconia calvescens)* became a management option as soon as the severity of its threat to Hawaiian ecosystems was recognized. No weed in Hawai'i has received as much publicity, attention, and funding for control. Three fungal pathogens have been considered as potential agents. *Colletotrichum gloesoporioides* f. sp. *miconiae* was assessed within six months, the petition for release approved within eight months and the fungus released on the islands of Hawai'i and Maui in 1997. It is established on Hawai'i and has spread to other areas. Its effectiveness is under evaluation. *Pseudocercospora tamonae* causes extensive damage to leaves, attacks other melastomes and the seedlings of some Myrtaceae but only fruits on miconia. It is very uncertain whether this species will be approved for release. *Coccodiella myconae* produces large wart-like growths that deform leaves considerably. It appears to be an obligate parasite of miconia but hyperparasitized by another species tentatively identified as *Sagenomella alba*. It has proven difficult to transfer from one plant to another where it does not sporulate. Further work on this species is in progress.

Key words: Biological control of weeds, *Coccodiella myconae, Colletotrichum gloeosporioides* f. sp. *miconiae,* invasive species, Melastomataceae, *Miconia calvescens*, *Pseudocercospora tamonae, Sagenomella alba*.

THE TARGET WEED, *MICONIA CALVESCENS*

Early Considerations

Davis (1978) first expressed concern about the threat of *Miconia calvescens* DC (Myrtales, Melastomataceae) in Hawai'i. In January 1991, a miconia tree with seedlings was discovered in a plant nursery near Hana, Maui. By midyear, surveys revealed additional populations in the surrounding area including mature flowering trees (Gagné *et al.* 1992). The threat from this plant generated considerable publicity (Altonn 1991, Eager 1995, Tanji 1996a,b, Yohay & Fukutomi 1997). The resilience, dominance of emergent miconia plants, formation of monotypic stands, their fecundity, widespread dissemination by frugivorous birds, the erosion associated with the shallow-rooted miconia trees and its deleterious impact on native forests of Tahiti and Moorea were described by Meyer (Meyer 1994,1996). Medeiros *et al.* (1997) noted that the similarity in stature, composition, and requirements of climate of Hawaiian and Tahitian forests strongly suggested similar vulnerability. The publicity stimulated many private and public agencies to support control activities.

Melastome Action Committees

The miconia problem generated considerable support but it lacked leadership. Maui Land and Pineapple Company had similar concerns with another weed *Tibouchina herbacea* (DC) Cogn. (Myrtales, Melastomataceae). The combined interest led to the organization of the Melastome Action Committee (MAC) committee by Tri-Isle Resource Conservation and Development Office, U.S. Department of Agriculture (Medeiros

1998). Many private and government agencies participated: Biological Resources Division U.S. Geological Survey; U.S. Fish and Wildlife Service; U.S.D.A. Forest Service; National Park Service; Hawai'i Army National Guard; Hawai'i Department of Agriculture (HDOA); Hawai'i Department of Land and Natural Resources; University of Hawai'i; Maui County's Office of Economic Development; and, The Nature Conservancy (Conant 1996).

This proactive committee furthered public awareness and planned a broad control strategy against miconia principally on the island of Maui. MAC focused primarily on the containment of miconia using chemical and mechanical eradication because the bulk of the funds were dedicated for local use only. Other funding was obtained for initial exploratory work on biological control.

A somewhat similar committee was established later on Hawai'i with funding again focused on local control efforts. Different control strategies were taken because the principal infestations on Hawai'i were on small parcels of private property whereas those on Maui were on large areas of primarily state lands.

Within a few years MAC became the Maui Invasive Species Committee. The Hawai'i committee was later integrated with the Big Island Invasive Species Committee. The focus on the management of melastomes diminished in light of other problems resulting in a much less focused effort against miconia. Funds for biological control were decreased significantly and would have ceased but for the persistence of one or two people. Their efforts have established new funding for renewed exploratory and biological studies for potential invertebrate agents in Costa Rica and Brazil.

BIOLOGICAL CONTROL

The search for natural enemies of miconia initially was the responsibility of HDOA. Their staff included an experienced exploratory entomologist and many biological control researchers as well as limited insect and pathogen quarantine facilities.

Exploration

The initial exploratory studies between July 1993 and September 1995 were conducted in Costa Rica, Brazil, Paraguay, and Trinidad and Tobago. Many insect feeders and several diseases were collected and sent into quarantine in Hawai'i (Burkhart 1996). Burkhart recommended that biological control studies focus on the pathogens because, apart from some processionary caterpillars (Lepidoptera, Riodinidae), the insects did not appear to have much impact on the plant or they were probably not host-specific. In 1996, Barreto began exploration for diseases of miconia in Brazil. He shipped many fungi that were isolated from miconia plants into quarantine in Hawai'i. He also traveled to the Dominican Republic and Costa Rica to relocate three of the fungi mentioned by Burkhart. He later collected the same fungi in Brazil and recommended all three pathogens for further screening.

Further exploration was conducted in 1996 and 1998 in Guatemala where plants closer to the Hawaiian biotype were thought to exist (Killgore 1996, Ramadan 1998). Very sparse populations of miconia were found throughout the country. Failure to establish collaboration with Guatemalan scientists dimmed prospects for further research on several prospective biological control insects, particularly a *Margardisa* sp. (Coleoptera, Chrysomelidae), found in the Peten and Coban areas of the country. No new pathogens were found.

Barreto (2000) explored several regions of Ecuador for diseases of miconia but found nothing new. With little hope for new diseases the search for other collaborators to reconsider insects in Central America resulted in the establishment of a cooperative agreement with the University of Costa Rica. A psyllid has already been recommended and interest in at least one species in the Riodinidae rekindled.

Host Range Testing

The widely accepted protocol for selecting host range test plants follows the centrifugal phylogenetic method first proposed by Wapshere (1974). Members of the family Melastomataceae were challenged first, followed by species in other families within the Order Myrtales.

All members of the Melastomataceae in Hawai'i are naturalized alien species (Almeda 1990). Most are weedy and noxious, and include *Clidemia hirta* (L.) D. Don, *Tibouchina herbacea* (DC) Cogn., *T. longifolia* (Vahl) Baill. ex Cogn., *T. urvilleana* (DC) Cogn., *Arthrostema ciliatum* Pav. ex D.Don, *Oxsypora paniculata* (D.Don) DC, *Medinilla venosa* (Blume) Blume, *Dissotis rotundifolia* (Sm.) Trian, *Heterocentron subtriplinervium* (Link & Otto) A. Braun & C. Muell., *Melastoma candidum* D. Don, *M. sanguineum* Sims, *Pterolepis glomerata* (Tottb.) Miq. *Tetrazygia bicolor* (Mill.) Cogn., and *Trembleya phlogiformis* DC. Because none of these plants is endemic, indigenous, or economically important in Hawai'i except as ornamentals, a pathogen of *M. calvescens* capable of infecting these melastomes could still remain a candidate for biological control. A pathogen, that did not infect any other melastome, was not expected to infect any plant species outside of the family and was deemed highly specific and desirable for biological control purposes. The need for careful, thorough screening (Watson 1985) was confirmed by results with *Pseudocercospora tamonae* (see below).

Other than the family Melastomataceae, there are only 19 genera within the Order Myrtales that occur in Hawai'i (Wagner *et al.* 1990). Some are endemic, indigenous or have economic importance including *Terminalia* L. (Combretaceae), *Cuphea* P. Br. (Lythraceae), *Lythrum* L. (Lythraceae), *Eucalyptus* L'Her. (Myrtaceae), *Eugenia* L. (Myrtaceae), *Metrosideros* Banks ex Gaertn. (Myrtaceae), *Psidium* L. (Myrtaceae), *Syzygium* Gaertn. (Myrtaceae), and *Wikstroemia* Endl. (Thymelaceae). Susceptibility of any of the plant species belonging to these genera would necessitate further evaluation.

Host range tests are typically performed under the most advantageous conditions for the pathogen. For fungi, these conditions would normally include a very high concentration of spores of 1×10^5 per ml or higher, and a relative humidity of 100% for 16 to 48 hours. Host range testing under these conditions often indicates a broader host range than is found under field conditions (Watson 1991), and may distort the evaluation of maximum risk posed by a biological control candidate resulting in the rejection of agents that are really safe and useful.

The pathogens

Colletotrichum gloeosporioides (Penz.) Sacc. f. sp. *miconiae* (Phyllachorales, Phllachoraceae).—This fungus causes leaf spots on miconia resulting in leaf yellowing and premature defoliation. It reproduces by asexual spores, or conidia, produced in acervuli that arise on the abaxial leaf surface. Setae are sometimes observed in these structures. The conidia are 14.7 –17.5 µm long and 5.0 –6.25 µm wide, straight, cylindrical, with rounded ends. Conidia of *Colletotrichum* fungi are produced under high humidity conditions and are disseminated by "wind-driven rain" (Trujillo, pers. com.). All

test plants were inoculated using a spore concentration of 1 x 10^5 conidia/ml in sterile water and incubated for 48 hours in an enclosed chamber at 100% relative humidity. Host range testing, concluded in November 1996, showed that this fungus was restricted in pathogenicity at least to the genus *Miconia*. The most closely related species, *Clidemia hirta,* was not susceptible. This form of the fungus did not infect any other species in the Melastomataceae, which occur in Hawai'i, or any member of the other families in the order Myrtales. Based on the host specificity tests, the fungus was subsequently described as f.sp. *miconiae* of *Colletotrichum gloeosporioides* (Killgore *et al.* 1999).

The request to release *C. g. miconiae* from the containment facility was submitted in December 1996 to HDOA for state approval, which was granted on March 3, 1997, and forwarded to USDA APHIS PPQ. The Federal permit was signed on July 11,1997 (Killgore *et al.* 1998), and the fungus released on the island of Hawai'i shortly thereafter and on Maui later that year. Attempts to establish the disease on Maui have failed so far. The fungus requires rain and wind for spore dissemination and recent drought conditions may have limited its spread. Studies on the impact of the fungus are ongoing.

Under aseptic laboratory conditions the fungal pathogen attacked germinating miconia seeds and also kills newly emergent seedlings (Su Que Leong, pers. com.). If these observations are confirmed in the field they suggest that *C. g. miconiae* could play an even more important role as a biological control agent for *M. calvescens*.

Pseudocercospora tamonae (Chupp) Braun (Deuteromycotina, Dematiaceae).—This fungus was isolated and identified by Barreto from leaves of *Miconia phanerostila* Auth. (Myrtales, Melastomataceae) and also from *M. calvescens* from Rio de Janeiro, Brazil. The minute leaf spots did not appear to be damaging, but when pathogenicity tests on miconia were completed, the pathogen proved to be more aggressive than *C. g. miconiae*. Besides causing numerous leaf spots, infected leaves became deformed and defoliation occurred three weeks after inoculation.

The conidia are pale brown, cylindrical, tapering towards the apices, 53-90 x 3 –5 µm with 6-11 septa. They are produced on conidiophores, which emerge from diseased leaf tissue. High humidity favors spore production and spores are easily disseminated by air currents.

Unlike the *C. g. miconiae*, however, *Pseudocercospora tamonae* was not as host specific. It infected other species within the Melastomataceae including *Clidemia hirta*, *Arthrostema ciliata*, *Dissotis rotundifolia*, *Tibouchina urvilleana* and *T. herbacea*. It also infected members of the Myrtaceae, including *Metrosideros polymorpha* Gaud., *Psidium cattleianum* Sabine and *Syzygium malaccense* (L.) Merr. & Perry. More leaf spots developed on *M. calvescens* leaves than on any other susceptible plant species, and sporulation of *P. tamonae* was only observed on miconia. Leaf yellowing and premature defoliation occurred only on those susceptible genera of the Melastomataceae. Only immature leaves of *M. polymorpha, P. cattleianum* and *S. malaccense* became infected, and there was no defoliation. The request to release this pathogen has been submitted. It is expected that the wider host range suggested by the host range test conducted for this fungus will be accepted as representing an aberration due to unnatural experimental conditions. Infections are obtained in the test only when leaves are exposed under very high humidity. The pathology is not ecologically significant as the fungus was unable to complete its life cycle from spore to spore on the non-target plants.

Coccodiella myconae (Duby) Hino & Katuamoto (Phyllacorales, Phyllacoraceae).—*Coccodiella myconae* was collected in Costa Rica and Brazil Barreto, who noted that it should be transferred to *Coccodiella*. It is extremely common in Brazil but always associated with the hyperparasite provisionally identified as *Sagenomella alba* W. Gams & Soederstrom (Eurotiales, Trichocomaceae). Infection produces wart like growths on the adaxial leaf surface with a corresponding concavity on the abaxial side resulting in the infected leaf becoming deformed and falling prematurely. Dark brown to black stromata line the concavities in a somewhat circular pattern. Perithecia develop within the stromata and produce asci (80-110 x 6-9 μm) with unicelluar ascospores (6-9 μm).

Diseased samples were sent on numerous occasions to the HDOA pathogen quarantine facility in Honolulu but the fungus could not be cultured. Ascospores collected from diseased leaves were inoculated onto Hawaiian miconia plants, but the transfer was successful in only three of seven attempts. In each case, however, the fungus failed to develop mature fruiting structures and the fungus was never recovered.

C. myconae is almost certainly an obligate parasite and since most obligates are host-specific, the outlook for this fungus as a biological control agent is extremely favorable. Research into the susceptibility of the Hawaiian miconia biotype, the conditions for infection and disease development, and the life cycle of the fungus are underway in Brazil.

DISCUSSION

Concurrent research on the biological control of a weed at the same time as conventional control measures are being developed is unusual. The history of this species in Tahiti and its behavior in Hawai'i clearly illustrated its threat to Hawaiian forests (Gagné *et al.* 1992). Conventional control technologies were viewed as a containment strategy awaiting the development of biological control agents. Funding for each approach had to be sought from different agencies because of differing jurisdictions and constraints. Some weed control specialists believed that miconia had become too well established in Hawai'i and the eradication projects would require too many years of dedicated funding and leadership (Conant, pers. com.). Others felt that miconia could be eradicated in Hawai'i in part from a general opposition to biological control but also from a lack of appreciation of the difficulty of eradicating a species using conventional technologies. The lack of consensus delayed an aggressive approach to biological control.

Although *C. g. miconiae* can slow the growth of established miconia plants, and also cause dieback of young seedlings, it alone will not control this weed. Preliminary observations of field-release plots have shown that the pathogen may decrease population densities.

The lack of host specificity in tests with the miconia pathogen, *Pseudocercospora tamonae*, may be cause for its exclusion as a biological control agent. However, under laboratory conditions, this fungus is a more devastating pathogen than *C. g. miconiae* and it is not dependent on wind-driven rain for dissemination. Observations of disease development on all susceptible test plants showed that *P. tamonae* was a primary pathogen of miconia and other melastomes but not other families in the Myrtales where it causes minor disease symptoms. Since one of these hosts is *Metrosideros polymorpha*, an endemic dominant of most montane forests in Hawai'i, support for

releasing *P. tamonae* will be difficult to obtain. The validity of the concern should be evaluated. Infection only occurred under conditions of maximum humidity and very high spore densities over several hours' exposure, an improbable situation in the field. However, if infection occurred under normal field conditions then the pathogen might, through time, adapt to *Metrosideros*.

Research on the miconia pathogen *Coccodiella myconae* has not progressed due to difficulties in disease transmission. The potential of this fungus, however, precludes abandonment of this agent at this stage. Ongoing research into the life cycle and mechanism of infection of this fungus may lead to solutions to the transmission problems. Isolation of the fungus from its hyperparasite can be achieved.

Total eradication of miconia is and always will be the primary goal of environmentalists in Hawai'i (Conant *et al.* 1996). Biological control is basically a control mode and will not by itself eradicate miconia. At best, it will reduce the growth and spread of this weed to the extent that the conventional control means will be successful in critical areas. Meanwhile the search for additional biological control agents continues.

ACKNOWLEDGEMENTS

Thanks to Dr. C. W. Smith for continuous encouragement on biological control as the only viable long-term solution to miconia. The financial support of the following agencies is acknowledged: Government of French Polynesia, U.S. Geological Service Biological Resources Division, State of Hawai'i, Maui Water Board. Logistical support and other cooperation from the following has assisted the program enormously: U.S. Forest Service, National Park Service, U.S. Geological Service Biological Resources Division, Hawai'i Department of Agriculture, The Nature Conservancy Hawai'i.

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