

**PROSPECTIVE BIOLOGICAL CONTROL OF *MICONIA CALVESCENS*  
IN HAWAI'I WITH A NON-INDIGENOUS FUNGUS *COLLETOTRICHUM*  
*GLOEOSPORIOIDES* (PENZ.) SACC. F.SP. *MICONIAE*  
PERSPECTIVES DE LUTTE BIOLOGIQUE CONTRE *MICONIA*  
*CALVESCENS* A HAWAI'I AVEC UN CHAMPIGNON NON-INDIGENE  
*COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) SACC. F.SP.  
*MICONIAE***

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Host range tests of a fungal pathogen from Brazil, identified as *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *miconiae*, were concluded at the Hawaii Department of Agriculture's Plant Pathology Quarantine Facility in December 1996. Of nine different genera within the family Melastomataceae and 13 genera and 17 species representing other members of the families of the Myrtales, only *M.c.* was susceptible to infection by this pathogen. *C. gloeosporioides miconiae* causes anthracnose type of leaf spots followed by premature defoliation of *M.c.* When the pathogen is inoculated onto injured stems, cankers develop causing a dieback of the branch. The disease is disseminated via spores or conidia that are produced in fruiting structures called acervuli. Free moisture is required for sporulation as well as for germination of the conidia, hence, this biocontrol pathogen is expected to be most effective in wet and windy areas.

*Des tests de spécificité à l'hôte utilisant un agent pathogène fongique provenant du Brésil, identifié comme Colletotrichum gloeosporioides (Penz.) Sacc. f. sp. miconiae, ont été effectués dans le laboratoire de quarantaine pour les pathogènes du Département d'Agriculture de Hawaii en décembre 1996. Parmi les 9 genres choisis dans la famille des Mélastomatacées et les 13 genres choisis dans l'ordre des Myrtales, seul M.c. a été sensible à l'infection par cet agent pathogène. C. gloeosporioides miconiae provoque des tâches foliaires de type anthracnose suivies par la défoliation prématurée des feuilles de M.c.. L'inoculation du pathogène sur des branches endommagées provoque le développement de chancres et entraîne le dépérissement de la branche. La maladie est disséminée par les spores ou les conidies qui sont produites dans des structures fructifères ou des acervuli. Comme une humidité ambiante est nécessaire pour la sporulation ainsi que pour la germination des conidies, cet agent pathogène de lutte biologique est supposé être plus efficace dans des zones humides et ventées.*

The serious threat and invasion of *Miconia calvenscens* into the Hawaiian ecosystem (Gagné *et al.*, 1992) prompted an immediate response from all resource levels to seek ways in which this noxious weed could be eradicated in Hawai'i (Conant *et al.*, 1997). A biological control approach was recognized at this early stage of the *M.c.* control program as a complement to other eradication and control efforts. As a result, a cooperative biological control project was initiated by the Cooperative National Parks Resources Studies Unit at the University of Hawai'i at Manoa, between Robert Barreto, Plant Pathologist at the Federal University of Viçosa, Brazil, and the Biological Control Section of the Hawaii Department of Agriculture (HDOA). Barreto's contribution would be the search for pathogens of *M.c.* in Brazil where the plant species has its origins, and the HDOA's responsibility would include the testing and screening of any *M.c.* pathogen for biological control potential.

A result of this cooperative effort was the discovery of a fungal pathogen *Colletotrichum gloeosporioides*, which was isolated by Barreto from lesions on *M.c.* leaves collected from various locations in Brazil. In March 1996, several cultures of this organism

were sent to the HDOA's Plant Pathology Quarantine Facility, a high-level containment facility constructed to test non-indigenous plant pathogens. The pathogenicity of the fungal organism was confirmed according to Koch's postulates (proof of pathogenicity) soon after its arrival in Hawai'i. A host range testing program was initiated immediately.

### THE FUNGUS *COLLETOTRICHUM GLOEOSPORIODES*

The pathogen isolated from *M.c.* was identified using descriptions of type species by Mordue (1971), von Arx (1957), and Sutton (1980). The identification of the species was also confirmed by Barreto. The fungus belongs to the Subdivision Deuteromycotina (Fungi Imperfecti), Class Coelomycetes, Order Melanconiales.

*C. gloeosporioides* causes typical anthracnose type of lesions on leaves of *M.c.* (Fig. 1) six to eight days after inoculation, followed by leaf abscission in three to four weeks. If a branch or stem is wounded inoculated, the pathogen causes a canker or stem lesion (Fig. 2) which girdles the area, and ultimately causes dieback of the branch.

The fungus reproduces by means of asexual spores or conidia (Fig. 3) which are produced in fruiting structures called acervuli (Fig. 4). These acervuli appear on the surface of leaf spots under high humidity conditions. The conidia are dispersed by wind-driven rain, and germinate when there is free moisture on leaf surfaces. Upon germination a conidium produces a hyphal strand or mycelium, which forms an appressorium or pad-like structure. The adhesive quality of the appressorium keeps the hypha near the leaf surface. From the appressorium, the hypha then penetrates into the leaf epidermis. The lesion first appears as a small, dark, pinpoint-sized spot, which quickly expands. Acervuli are then produced within these lesions when conditions are favorable. Under artificial conditions, the fungus is easily cultured on 10% potato dextrose agar, sporulating moderately and producing a light pinkish-colored spore matrix.

The biocontrol potential of this *M.c.* pathogen was assessed on its leaf spotting and defoliating effect on *M.c.* plants and on the probability that the fungus was host specific to plants within the family Melastomataceae. Other races of *C. gloeosporioides* are well known for their host specificity and used as weed biological control agents. The *Clidemia* race of the same fungal species, *Colletotrichum gloeosporioides* f. sp. *clidemiae*, was identified and developed by Trujillo (1986) and Trujillo *et al.* (1986) as a biological control pathogen of *Clidemia hirta* (L.) D. Don, another noxious melastome weed in Hawai'i. The results of Trujillo's host specificity tests showed that *C. g. clidemiae* was very specific to *C. hirta* and did not infect *M.c.* although both genera belong to the same tribe Miconieae.

Another biocontrol agent, *C. gloeosporioides* f. sp. *aeschynomene*, the patented mycoherbicide "Collego" developed for the control of northern jointvetch, is also very specific in its host range. This biological control agent is only infective to members of the subfamily Faboideae (family Fabaceae or Leguminosae) (TeBeest, 1988).

Since previous races or *formae speciales* of the fungal species *C. gloeosporioides* have been documented, the prospects were optimistic that the *M.c.* pathogen possessed similar host specific characteristics.



**Fig. 1.** Anthracnose type of leaf spots on leaves of *M.c.* 14 days after inoculation with conidia of *C.g. miconiae*. The oldest leaf is beginning to turn prematurely yellow due to the infection by the fungus.

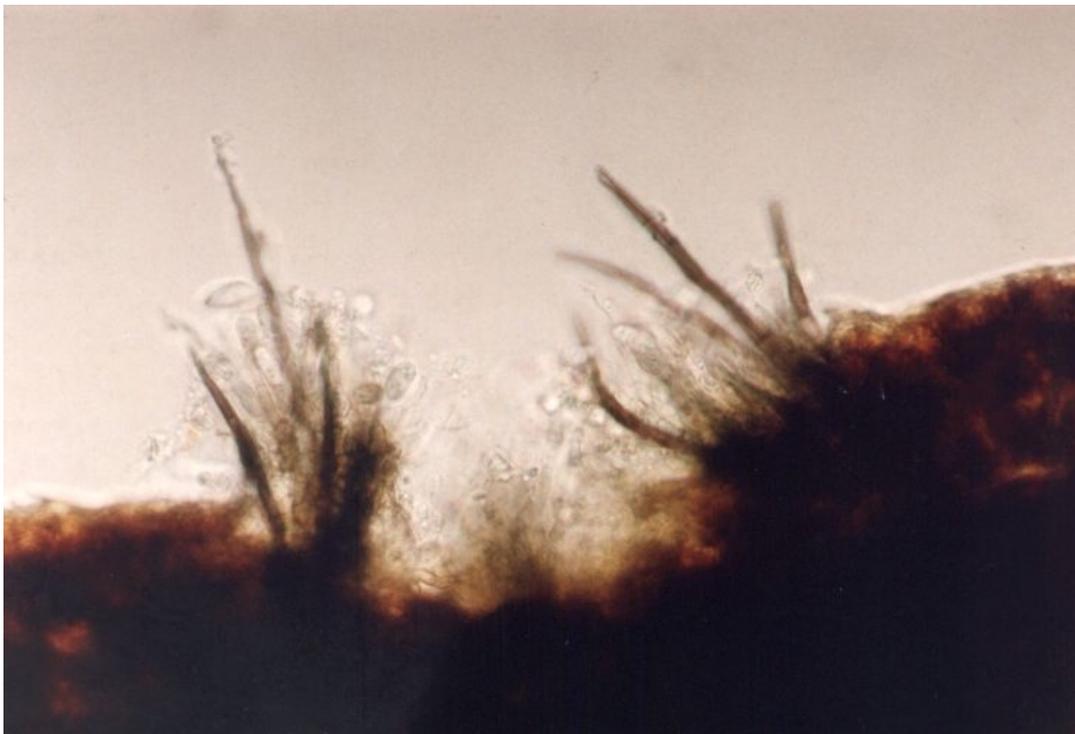
**Fig. 2.** Wilting of young *M.c.* plants due to infection by wounding of stem area with conidia of *C. g. miconiae*. Plants were d one month after inoculation.



**Fig. 3.** Asexual spores or conidia of *C. g. miconiae* average 6.5  $\mu$ m in width and 22-25  $\mu$ m in length. They are produced abundantly in fungal fruiting structures and each is capable of causing an infection site.



**Fig. 4.** Cross section of an anthracnose leaf spot showing a section of an acervulus or fruiting structure of *C. g. miconiae*. The acervulus is ringed by dark, sterile hairs or setae.



## HOST RANGE TESTING AND RESULTS

The centrifugal phylogenetic method of Wapshere (1974) was used in determining the host range of plants tested with the *M.c.* isolate of *C. gloeosporioides*. This method involves inoculating the potential biocontrol agent onto plant species closely related to the target weed, then moving on to species further removed and ending at that point where infection does not occur. In this case, plant species belonging to the family Melastomataceae were tested first. Of the other eleven genera in the family Melastomataceae, eight were tested. These included: *Clidemia hirta* (L.) D. Don; *Arthrostemma ciliatum* Pav.ex D.Don.; *Dissotis rotundifolia* (Sm.) Trian; *Heterocentron subtriplinervium* (Link & Otto) A. Braun & C.; *Pterolepsis glomerata* (Rottb.) Miq.; *Tibouchina herbacea* (DC) Cogn.; *Medinilla scortechenii*; and *Melastoma candidum* D. Don. Other test plants represented the remaining families within the Order Myrtales.

Of all the plants tested, *M.c.* was the only susceptible host of the isolate of the *Colletotrichum gloeosporioides* from Brazil. None of the other Melastomataceae or plants belonging to the other families within the Order Myrtales became infected. These families included: Combretaceae (one species); Lythraceae (two species); Myrtaceae (ten species); Onagraceae (two species); and Thymelaceae (two species).

Since host range test results clearly defined a high level of specificity, the authors consider the pathogen isolated from *M. calvescens* is a race or *forma specialis* of *C. gloeosporioides* and have proposed the scientific name, *Colletotrichum gloeosporioides* f. sp. *miconiae*.

## PERMITS AND RELEASE

Based on the results of the host range tests, the request for the field release of *C. gloeosporioides miconiae* was submitted to the State of Hawaii's Plant Quarantine Branch for approval on 31 December 1996. On 03 March 1997, the approval to release was granted. Shortly thereafter, another application was submitted to the federal or national agency for permission to release this fungus from the quarantine facility. The permit was granted on 11 July 1997.

The first field release occurred on 25 July 1997 on the island of Hawai'i. Due to the conditions attached to the permit, releases were made at only two sites. Fungal spore suspensions were sprayed onto *M.c.* plants in a designated area (6 m radial circle) at two locations, in Onomea and Pahoia. One month later, incipient disease spots appeared at both sites. After monitoring for disease development and spread for a one year period, additional areas may be targeted for releases.

## DISCUSSION

**Biological Control Prospects for Hawai'i**--Natural infection of *M.c.* by *C. gloeosporioides miconiae* will result in leaf spotting and defoliation which in turn will slow the growth and development of *M.c.* plants. Under controlled conditions, tests have shown that artificially wounding and inoculating this pathogen causes cankering of branches. Whether the fungus will naturally infect *M.c.* stems or branches remain to be documented. If cankers caused by the pathogen are observed under field conditions, *C. gloeosporioides miconiae* will be a more effective biological control agent.

Dissemination of *C. gloeosporioides miconiae* is dependent on climatic conditions. The production of conidia or spores requires high humidity conditions. Wind-driven rain will dislodge and disperse the spores over a wide range. Fortunately, *M.c.* plants thrive under this type of environment so that existing climatic conditions will favor disease development

and dispersal of the fungus. It is expected that the disease will spread from inoculation sites quite readily.

The first release of the *C. gloeosporioides miconiae* was limited to two sites on the island of Hawai'i. No other release sites were permitted for a one year period. This restriction was imposed on the HDOA by the United States Fish and Wildlife Service (USFWS) whose agents were not completely confident in the safety of the fungus. Although extensive tests were completed and the results were scrutinized by scientists from various fields of study, restrictions were still levied. It is certain that with only two release sites, the pathogen's effect on controlling *M.c.* will be curtailed. The restraint by USFWS will be in effect for the first year. After 25 July 1998, the pathogen will be released at many sites within the *M.c.* infestation on the islands of Maui and Hawai'i and biological control activity by this *M.c.* pathogen will commence.

**Prospects for Tahiti**--Host specificity tests on plants in the Melastomataceae, endemic or indigenous to Tahiti, would be the first step in clearing the way to release this pathogen. Additional host testing would include native or endemic plants within the Order Myrtales. These have been identified, and testing of these plants will commence as soon as they have been propagated and shipped to the HDOA's Quarantine Facility in Honolulu.

Although the classical approach to biological control of *M.c.* may be appropriate for Hawai'i, it may not be the best way to approach the problem in French Polynesia where the *M.c.* infestation is extremely widespread (Meyer, 1994; 1996). An alternative method of inoculum preparation and application may be more effective and economical. Formulating the fungus as a mycoherbicide (Bowers, 1982; Boyette *et al.*, 1991) will permit a more rapid means of pathogen production and release. For Hawai'i, this would be a major obstacle as herbicides (and mycoherbicides) are regulated by the U.S. Environmental Protection Agency and the process to clear any class of "herbicide" for public use would make it prohibitive. For Tahiti, this mode of pathogen application would be very advantageous.

**Conclusion**--For any biological weed control program, it is very difficult to predict the level of control that any one biocontrol organism will have on a target weed. For such a formidable weed as *M.c.*, one fungus, *C. gloeosporioides miconiae*, is not expected to eradicate this invasive weed. This pathogen will, however, reduce the weed's growth and vigor. Many biocontrol agents will be needed for a more effective control. A complex of natural enemies, including arthropods and other pathogens, would definitely increase the chances of controlling *M.c.* in Tahiti and Hawai'i.

The cooperative agreement between the governments of French Polynesia and Hawai'i to conduct the exploration for and the testing of natural enemies of *M.c.* will begin an international, biological control warfare against the invasive plant *M.c.*.

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