Dissemination and impacts of the fungal pathogen, *Colletotrichum gloeosporioides* f. sp. *miconiae*, on the invasive alien tree, *Miconia calvescens*, in Tahiti (South Pacific)

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Summary

Long-term monitoring of biological control agents in their areas of introduction is essential to assess their effectiveness. There is a need to monitor and evaluate agent dispersal and impacts so that the degree of success can be quantified or reasons for failure can be clearly understood. A fungal pathogen, *Colletotrichum gloeosporioides* f. sp. *miconiae* Killgore & L. Sugiyama (Melanconiales, Coelomycetes, Deuteromycetinae), found in Brazil in 1997 was released in the tropical oceanic island of Tahiti (Society Islands, French Polynesia, South Pacific) to control miconia, *Miconia calvescens* DC (Melastomataceae), a small tree native to Tropical America, which has invaded native rainforests. The plant pathogen, proven to be highly specific to miconia, causes leaf spots, defoliation and eventually death of young seedlings in laboratory conditions. Two permanent plots in Tahiti (Taravao Plateau and Lake Vaihiria) were monitored for a period of 6 years to assess the pathogen's dispersal and impacts on miconia in the wild. Leaf spots were observed approximately 30 days after inoculation. Percentage of infected plants reached 100% after 3 months, and between 90% and 99% of leaves were infected. Subsequent re-infection occurred after 3 months at Vaihiria and 18 months at Taravao. Mortality rate for monitored plants was 15% and reached 30% for seedlings less than 50 cm tall. Within 3 years, the fungus had disseminated throughout the island of Tahiti and had infected nearly all the miconia plants up to 1400 m in montane rainforests. It was also found on the neighbouring island of Moorea without any intentional release there. Leaf damage on miconia canopy trees increased from 4% to 34% with elevation in permanent plots set up between 600 and 1020 m. Our study showed that rainfall and temperature were two limiting environmental factors that affected fungal spread and disease development. Although this plant pathogenic agent is successfully established, has spread efficiently and has caused significant impacts on seedlings, additional biocontrol agents are still needed to fully control the massive invasion by miconia in the Society Islands.

Keywords: biological control, island, monitoring; rainforest.

Introduction

'The greatest weakness of biological control has been the failure to quantify success adequately and to monitor programs effectively' (Myers and Bazely, 2003: 171).

Long-term monitoring of biological control agents in areas of introduction is essential to evaluate their effectiveness, i.e. their establishment (or acclimatization), their reproduction (or replication), their dissemination and their impacts on the target invasive alien species. Success of biocontrol programmes against weeds in agro-ecosystems or invasive plants in natural ecosystems is considered to be achieved if there is a notable reduction in population density through a decrease in the plant vigour, growth rate, ability to reproduce or to germinate (Briese, 2000; Myers and Bazely, 2003). It is also important to verify that the released natural enemy, although proven to be host-specific in laboratory conditions, does not affect other non-target species in natural conditions (Barton, 2004).

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This paper summarizes the results of monitoring a fungal pathogen introduced in a tropical island ecosystem to control a dominant invasive tree in native rainforests. We documented the establishment and dissemination of the plant pathogenic agent, quantified its direct impacts on the target species in the wild and verified the absence of damages on non-target plant species over a 6-year period. Factors suspected to have influenced the dynamics and impacts of the plant pathogen are discussed, and its general efficiency is assessed.

Methods and materials

Miconia, the 'green cancer' of Pacific islands native forests

Miconia, *Miconia calvescens* DC (Melastomataceae), represents one of the most dramatic and devastating cases of a documented plant invasion into island ecosystems. A small tree 4–12 m tall (up to 16 m in its native range), miconia is native to tropical rainforests of Central and South America where it is an understory species in dense forest and a colonizer of small forest gaps (Meyer, 1994). It was introduced to the tropical oceanic island of Tahiti (French Polynesia, South Pacific Ocean) in 1937 as a garden ornamental plant because of its large, striking leaves with purple undersides (under the horticultural name *Miconia magnifica* Triana). Rapid vegetative growth (up to 1.5 m per year), early sexual maturity (reached in four to five years), self-pollination and independence from specific pollinators, three flowering and fruiting peaks per year, prolific fruit and seed production (millions of tiny seeds per tree), active dispersal of the small fleshy berries by frugivorous native and alien birds over long-distances, high rate of seed germination (90% in 15–20 days in laboratory conditions) even under very low light conditions, large size and persistence of the soil seed bank (up to 10,000 seeds/m^2 and longevity more than 15 years) and shade-tolerance make this species a particularly aggressive colonizer in undisturbed native forests and a competitor with native and endemic insular species (Meyer, 1994, 1996, 1998). In less than 50 years, miconia has successfully invaded all the native mesic and wet forests of Tahiti (rainfall >2000 mm/year) from sea level to 1400 m elevation and covers approximately 70% of the island. Between 40 and 50 species of the 100 plants endemic to Tahiti are believed to be on the verge of extinction due to the invasion of miconia (Meyer and Florence, 1996). Miconia is also invasive in the rainforests of Hawaii (Medeiros *et al.*, 1997), New Caledonia and north Queensland in Australia, and remains a potential threat for many other wet tropical Pacific islands.

Because conventional manual and chemical control methods have shown their limits in heavily invaded islands such as Tahiti and Moorea (more than 80,000 and 3500 ha, respectively), where miconia is forming dense monospecific stands on steep, mountainous slopes, biological control is viewed as the only effective alternative.

Cgm, a host-specific fungal plant pathogen

Colletotrichum (Order Melanconiales, Class Coelomycetes, Subdivision Deuteromycetinae) is one of the most important genera of plant pathogenic fungi worldwide, particularly in subtropical and tropical regions (Prusky *et al.*, 2000). There are several form species (or strains) of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. which are very specific to their plant hosts, including the well-known *C. gloeosporioides* forma specialis *aeschynomene*, which is used as a mycoherbicide registered as Collego® for control of the invasive legume, *Aeschynomene virginica* (L.) Britton, Sterns & Poggenb. (Fabaceae), in soybeans and rice in several Mississippi River delta states, and *C. gloeosporioides* (Penz) Sacc. f. sp. *jussiaeae* successfully used as a biological agent for control of winged water primrose, *Jussiaea decurrens* DC (Onagraceae) on rice fields of Arkansas (Templeton, 1982; TeBeest, 1991). In the Pacific Islands, *C. gloeosporioides* (Penz) Sacc. f. sp. *clidemiae*, found in Panama, was released in Hawaii in 1986 to control the invasive shrub, *Clidemia hirta* D. Don (Melastomataceae) (Trujillo et al., 1986; Trujillo, 2005).

C. gloeosporioides (Penz) Sacc. f. sp. *miconiae* Killgore & L. Sugiyama (hereafter, *Cgm*) was discovered in the State of Minas Gerais in Brazil in 1997 and isolated by Dr Robert Barreto (Universidad de Viçosa). It reproduces by asexual spores or conidia, 14.7–17.5 µm in length and 5.0–6.25 µm in width, which are produced in acervuli that arise on the abaxial surface leaf (Killgore *et al.*, 1999). Conidia of *Colletotrichum* fungi are produced under high moisture conditions and are disseminated by wind-driven rain. In the laboratory, test plants were inoculated using a spore concentration of 1×10^5 conidia millilitre in sterile water and incubated for 48 h in an enclosed chamber at 100% relative humidity (Killgore, 2002). The *Cgm* causes foliar anthracnose and necrosis, which lead to premature defoliation. When the pathogen is inoculated onto injured stems, cankers develop causing a dieback of the branch. Under aseptic laboratory conditions, the fungal pathogen attacked germinating miconia seeds and also killed emergent seedlings (Killgore, 2002). Mortality rate of very young seedlings (1 to 1.5 months old, less than 5 mm tall and with two leaves) in laboratory conditions (at room temperature between 24 and 30°C, hygrometry between 40 and 70%, 12 h light and 12 h darkness lightning regime) was 74% only 1 month after inoculation (Table 1).

Host-specificity tests were conducted at the quarantine facilities of the Hawaii Department of Agriculture in Honolulu following Wapshere's phylogenetic centrifugal method (Wapshere, 1974) on 28 plant spe-

Table 1. Mortality rate of young *Miconia calvescens* seedlings (1 to 1.5 months old) infected by *Colletotrichum gloeosporioides* f. sp. *miconiae* in laboratory conditions.

Treatment	Inoculated (*N* = 163)	Control (*N* = 166)
Number of dead seedlings 1 week after inoculation date (%)	99 (61%)	1 (0.6%)
Number of dead seedlings 2 weeks after inoculation date (%)	115 (71%)	6 (4%)
Number of dead seedlings 4 weeks after inoculation date (%)	120 (74%)	6 (4%)

cies in the botanical order *Myrtales* (species from the families Combretaceae, Lythraceae, Melastomataceae, Myrtaceae and Thymelaeaceae), including native and endemic Tahitian Melastomataceae (*Astronidium* spp. and *Melastoma denticulatum* Bonpl. ex Naudin). Repeated tests showed that the *Cgm* was highly specific to miconia (Killgore *et al.*, 1999). It was released in the Hawaiian Islands in June 1997 after approval by the US Department of Agriculture, the US Department of Interior and the Hawaii State Department of Agriculture and in Tahiti with the approval of the French Polynesian Government. The two release sites in Tahiti were the Taravao Plateau on the peninsula of Tahiti Iti in April 2000 and near Lake Vaihiria at the centre of Tahiti Nui in September 2002. Both sites are located at approximately 600 m elevation with an annual rainfall of 3300 and 7000 mm/year, respectively.

Monitoring impact and dissemination

Monitoring was conducted in two permanent plots of approximately 100 m^2 set up in the two release sites in Tahiti for a 6-year period (2000–2006). A total of 110 seedlings and juvenile miconia plants between 20 cm and 1.5 m tall on Taravao and between 10 cm and 2.8 m tall on Vaihiria were tagged per plot. It was almost impossible to study smaller seedlings and larger plants for technical reasons (size of the tags and access to higher branches and leaves). Miconia plants were inoculated by spraying a solution of *Cgm* spores (concentration of 1×10^8 conidia millilitre in sterile water), which were mass-cultured in laboratory conditions (on 10% V8® juice agar and under constant fluorescent illumination at 20–24°C) at the Institut Louis Malardé in Tahiti from an inoculum sent by the Hawaii Department of Agriculture.

Every week for the first 3 months after the release, then every 6 months during the monitoring period, we recorded the number of dead plants per plot and the percentage of infected leaves (with one or more foliar lesions) for each plant; we counted the number of leaf spots on the most infected leaf for each plant and visually estimated the percentage of leaf damages (i.e. leaf area loss due to necrosis) on the most infected leaf for each plant (called 'maximum damages'). A visual check for non-target damage was done at the same time, in and around the two permanent plots for disease symptoms. In particular, species belonging to the genus *Astronidium*, an endemic shrub of the same family as miconia, were examined.

A total of 20 transects, 10 m long, were set up in 2002 and 2003 on different parts of the island of Tahiti (leeward side, windward side, peninsula of Tahiti Iti) between 70 and 1100 m elevation to evaluate *Cgm* dispersal across the island. Observations on isolated plants were also made at higher elevation (up to 1400 m) and in other mountainous areas above 1000 m elevation in the centre of Tahiti during field surveys in 2003 (Fig. 1). Maximum leaf damages on mature, reproductive miconia trees (called 'canopy leaves') was measured in 2005 and 2006 on 28 cut trees (three to eight trees per site for a total of 1571 leaves) in eight other permanent plots located in different sites on the island of Tahiti between 600 and 1020 m elevation.

Results

Establishment and reproduction

Typical symptoms of *Cgm* infection appeared between 21 (Taravao) and 33 days (Vaihiria) after the initial inoculation. Subsequent re-infection by the fungus on miconia plants located outside the release plot (i.e. fungal reproduction and dispersal) varied between 3 (Vaihiria) and 18 months (Taravao) after inoculation. The difference in time to onset of re-infection between the two sites (located at the same elevation) may be explained by a much lower mean annual rainfall in Taravao (3300 mm/year) compared to Vaihiria (7000 mm/year) and by a serious drought period, which occurred in Tahiti when the pathogen was released on Taravao in 2000 (rainfall of 144 and 216 mm during the months of September and November 2000, respectively, vs a mean annual rainfall of 212 and 355 mm in September and November during the last 10 years. Data received from Météo-France in French Polynesia, personal communication).

Dissemination

Within 3 years (2003), the fungus was detected 15 km from the release sites and spread throughout the island of Tahiti. It has infected almost all the miconia trees, juvenile plants and seedlings between sea level and 1400 m elevation. Miconia plants found on the neighbouring island of Moorea (located approximately

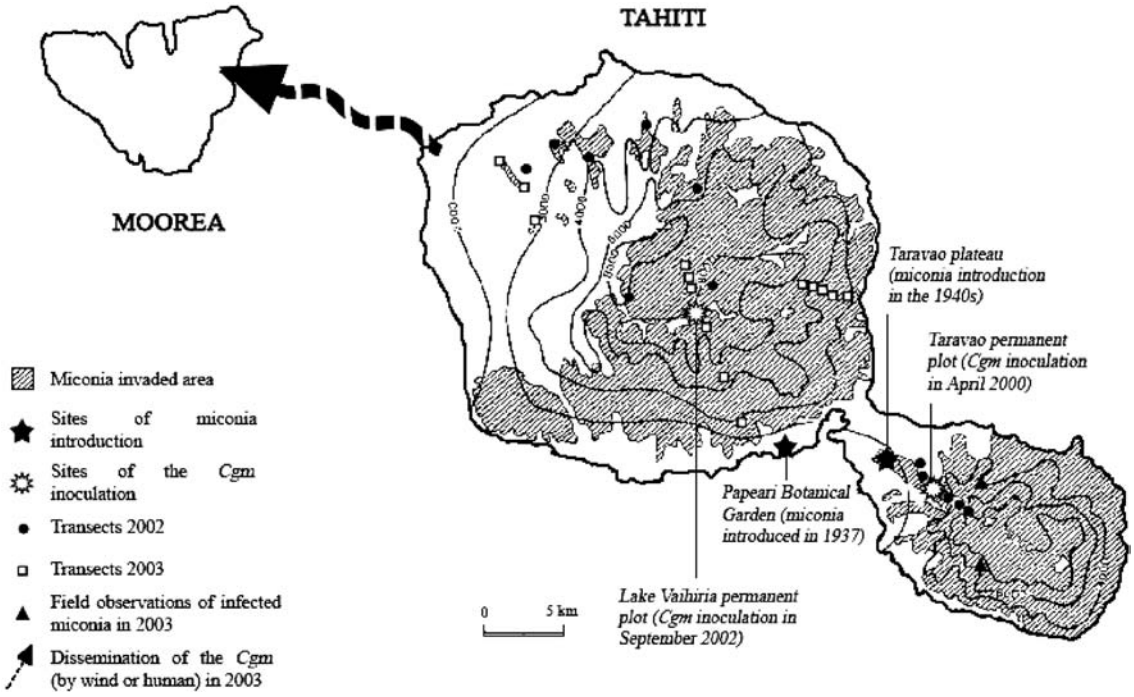

Figure 1. Map of *Miconia calvescens* invasion in Tahiti and the evolution of *Colletotrichum gloeosporioides* f. sp. *miconiae* dissemination from the two release sites.

20 km from Tahiti) were also infected by *Cgm* in 2003 without being intentionally introduced (Fig. 1). Spores may have been carried there by the wind or on contaminated clothing or equipment.

Impacts

Three months after inoculation, 100% of the tagged plants and between 90% and 99% of their leaves were infected by *Cgm* (i.e. presence of leaf spots) in the two permanent plots (Figs. 2 and 3). The mean number of leaf spots on the most infected leaf increased from 10 (1 month after the inoculation) to 90 (6 years after the inoculation) in Taravao, and from 10 (1 month after the inoculation) to 55 (4 years after the inoculation) in Vaihiria. In 2006, the mean mortality rate was approximately 15% for the monitored plants (14.1% in Taravao and 16.7% in Vaihiria), reaching approximately

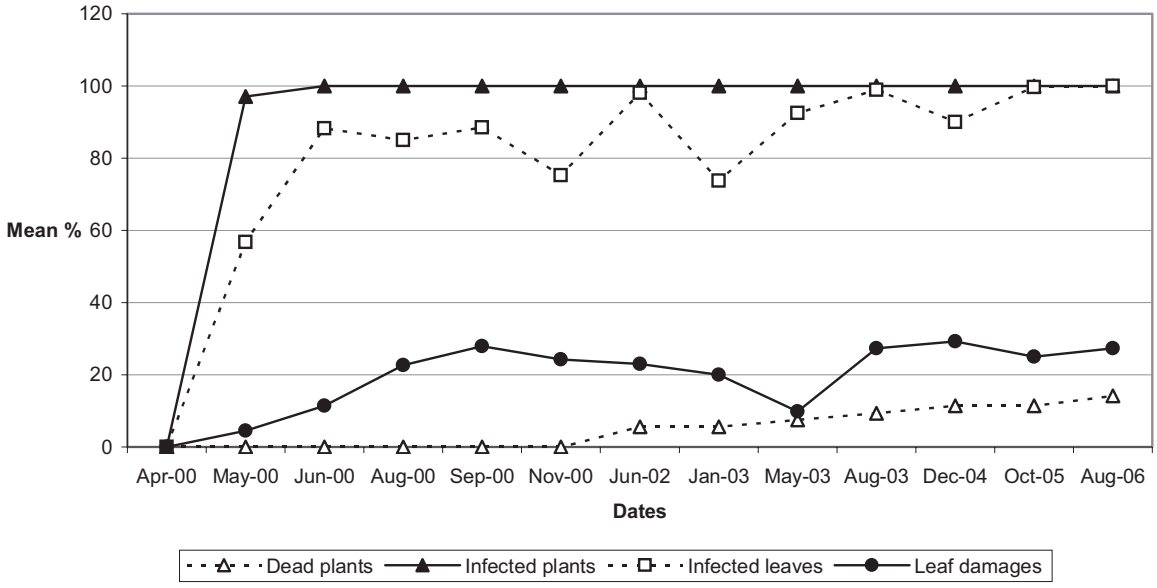

Figure 2. Evolution of *Colletotrichum gloeosporioides* f. sp. *miconiae* impacts on monitored *Miconia calvescens* plants in the Taravao release site (2000).

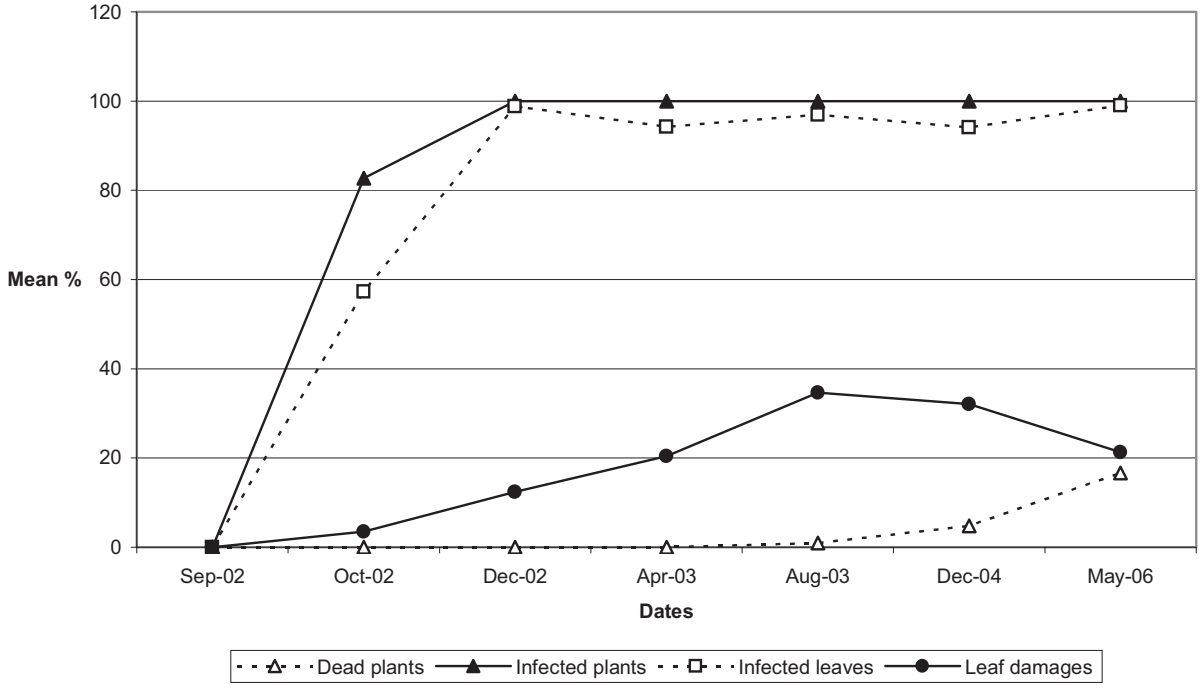

Figure 3. Evolution of *Colletotrichum gloeosporioides* f. sp. *miconiae* impacts on monitored *Miconia calvescens* plants in the Vaihiria release site (2002).

30% for small seedling less than 50 cm tall (28.6% in Taravao and 32.3% in Vaihiria). Multiple damages on the surviving tagged miconia plants, including rotting stems and deformed leaves, ranged between 14% (Taravao) and 47% (Vaihiria). Mean maximum leaf damages on the tagged miconia plants in the two monitored sites was approximately 25% (27.4% in Taravao and 21.3% in Vaihiria), and maximum damages on miconia canopy leaves in eight other permanent study sites (100 m^2 quadrats) varied between 4% (at 600 m elevation) and 34% (at 970 m elevation; Fig. 4). None of the non-target alien and native plants, located within or outside the permanent plots, displayed any symptoms of *Cgm* infection after 6 years of monitoring.

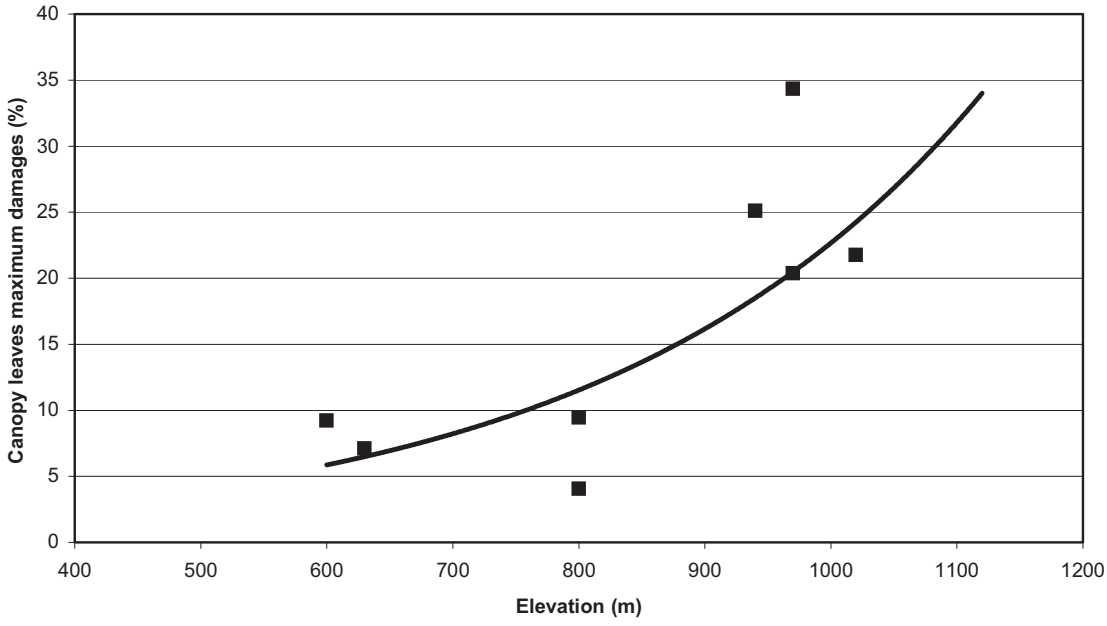

Figure 4. Mean percentage of *Miconia calvescens* canopy leaves maximum damages caused by *Colletotrichum gloeosporioides* f. sp. *miconiae* according to elevation in eight different plots set up in Tahiti in 2005–2006 (N = 1571 leaves).

Discussion

Only a very few biocontrol programmes using plant pathogens with the goal of preserving tropical island native forest ecosystems are documented (Gardner, 1992; Smith *et al.*, 2002; Trujillo, 2005) compared to their common use in agriculture ecosystems (see, e.g. Templeton, 1982). In natural areas, the need to carefully monitor and evaluate agent establishment, dissemination and impact is particularly important, so that the degree of success can be quantified or reasons for failure can be understood (Briese, 2000).

The *Cgm*, a fungal pathogen proven to be highly-specific to miconia in laboratory conditions, was successfully introduced to the tropical oceanic island of Tahiti in 2000 (Meyer and Killgore, 2000). Our results show that in 3 years, it has established, reproduced and spread throughout the island of Tahiti and even to the neighbouring island of Moorea, located approximately 20 km away, without any intentional release there. The *Cgm* has succeeded in infecting almost all the miconia plants on both islands without causing any apparent harm to non-target plant species. It was capable of distributing itself by natural means, particularly at high elevation in montane rainforests or cloudforests (up to 1400 m elevation), and to infect both dense monospecific stands and isolated miconia plants. Several biocontrol programmes elsewhere in the world have been considered unsuccessful (or only partially successful) because of the large habitat range of the target species but the narrower ecological range of their natural enemies (e.g. *Lantana camara* L. in Hawaii; Broughton, 2000).

Leaf damage caused by *Cgm* is more severe in high-elevation areas of Tahiti (Moorea and Raiatea, unpublished observation) where cooler and wetter conditions prevail, suggesting that temperature and moisture (as humidity or free water) are important factors for disease development. The reproduction and dissemination of the pathogen was delayed at the Taravao site due to a drought period, which occurred when the pathogen was released in 2000. The same pattern was observed in Hawaii after the release of the *Cgm* in 1997. The importance of air temperature was demonstrated for other *C. gloeosporioides* with an optimum temperature for many form species at about 28°C. Disease development was severely limited at 36°C (TeBeest, 1991). Defoliation of *C. hirta* caused by *C. gloeosporioides* f. sp. *clidemiae* over contiguous areas only occurs when weather conditions are favourable, i.e. cool (16–24°C), windy and rainy (Conant, 2002; Trujillo, 2005; R. Hauff, personal communication).

Mortality rate was high for very young miconia seedlings (74% 1 month after inoculation) in laboratory conditions but was relatively low and slow for larger miconia plants in the field (15% for plants between 10 cm and 2.80 m tall, up to 30% for seedlings less than 50 cm tall, 4 to 6 years after the release). Although the plant pathogen may slow the growth of established miconia plants (between 17% and 35 % of the surviving miconia plants showed multiple damages, especially rotten stems and curved leaves) and cause the dieback of young seedlings, it alone will not control the massive invasion of miconia. Partial defoliation of reproductive canopy trees (up to 35%) favoured the recruitment of native plants, including rare threatened endemic plants, by enhancing the light availability in the understory (Meyer *et al.*, 2007).

The *Cgm* was released on the island of Raiatea (Society Islands) in 2004, where manual and chemical control operations have been conducted since 1992 on a 450-ha infested area, and in Nuku Hiva (Marquesas Islands) in 2007, where small miconia populations (<1 ha) were recently discovered. On these islands, manual and chemical control will be necessary to achieve complete eradication, but the fungal pathogen is expected to reduce the number of miconia plants, especially those at the seedling stage. Additional biological control agents are still much needed to fully control the massive invasion by miconia in the Society Islands and the Hawaiian Islands.

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