

Development of Potential Biological Control Agents for Invasive Plant Species Using Native Pathogens in South Florida

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Abstract

One of the greatest threats to the native ecosystems in any part of the world is invasion and permanent colonization by non-native species. Florida is no exception to this biological invasion, and currently colonized by an extensive variety of exotic plant species. The unrestricted growth of many of these nonnative plants jeopardizes the survival of an array of native plants in Florida. Originally imported from Asia over thirty years ago, Old World Climbing Fern (*Lygodium microphyllum*) has become one of the most invasive and destructive weeds in southern Florida. To date different effective control measures of its growth and spread has not been successful. Fire and herbicide application is currently in practice, however they are not cost effective and environmentally friendly approach. Biological control is proposed as a tool useful for ecosystem restoration and management. Most of the potential hazards of classical exotic introduced biocontrol agents can be avoided by selecting pathogens that are already endemic in the area where they are to be used. We have initiated studies to discover and develop potential native microbial biocontrol agents of invasive plant species. Brazilian pepper. We have identified sicklepod fungus *Myrothecium verrucaria*, as a possible bioherbicide against *Lygodium microphyllum*. Series of greenhouse studies demonstrated the pathogenicity of *Myrothecium verrucaria* on *Lygodium microphyllum*. Brazilian pepper an exotic invasive hardwood tree species is now covers hundreds of thousands of acres in south and central Florida, as well as many of the islands on the east and west coasts of the state.

Brazilian pepper (*Schinus terebinthifolius* Raddi) is native to Brazil, Argentina and Paraguay. Significant infestation of Everglades National Park (ENP) by Brazilian pepper has occurred in an area known as the "Hole-in-the-Donut", (HID) covering over 3,000 ha that were previously agricultural lands. Periodic field survey for occurrence of disease were made, putative fungal and bacterial pathogens were isolated and tested for pathogenicity using detached leaf assay and seedling inoculation. We have found that native microbial pathogens of Brazilian pepper trees do occur with capability to cause significant damage. Three fungal isolates were tested in a greenhouse spray inoculation study. All three isolates caused necrosis of apical buds and only one isolate caused leaf spots and chlorosis on young leaves. Among the bacterial isolates from the pepper tree disease samples, two were found to be pathogenic. Further, evaluation of the two bacteria in a controlled greenhouse spray inoculation study showed only one of the isolates was able to induce leaf spot symptoms on leaves. Another potential bacterial pathogen was isolated from pepper trees showing inflorescence blight with complete absence of drupes. Limited inoculation studies using the bacterial isolate from the blight on pepper inflorescence caused flower discoloration, absence of drupe development and tip dieback. This type of biocontrol method can be incorporated as a valuable tool in the over all management strategy to restore native ecosystems in a cost effective and environmentally friendly manner.

Introduction

The fragile natural ecosystems in the South Florida are being threatened by an invasive old World climbing fern, *Lygodium microphyllum*. The Old World climbing fern, is native to tropical and subtropical areas of Africa, southeastern Asia, northern and eastern Australia, and the Pacific islands. It was first found to be naturalized in Florida 1965 and is spreading at an alarming rate and already over 100,000 acres have been found to be infested by this aggressive weed. Old World climbing fern occurs in a variety of forested and nonforested sites in southern Florida, including hardwood hammocks, cypress swamps, savannas, woodlands, marshes, and wet prairies. Brazilian pepper (*Schinus terebinthifolius*) is an evergreen invasive exotic hardwood tree species native to South America. The infestation within the Everglades National Park-HID area has reached a monospecific stand stage of succession due to dense colonies. Biological control is the deliberate use of one or more organisms to suppress the growth or reduce the population of another organism to a level at which it is no longer an economic problem. Displacement of non-target organisms by the use of a native bioherbicide is not generally considered to be a significant risk compared with potential displacement of non-target organisms by exotic biological control pathogen release. The occurrence of disease among introduced plant species due to native phytopathogens is not uncommon. Therefore, it may be possible to develop effective biological control agent/s of invasive plants by developing locally available and adapted native phytopathogens.



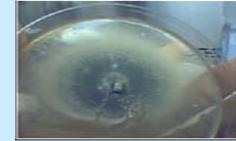
Close-up of tree branch showing leaf spots with chlorosis



Die back symptoms on growing branches



Extensive Lygodium infestation of ground and canopy



Myrothecium verrucaria on potato dextrose agar



Close-up of *Exserohilum* sp. mycelia growth showing clusters of spores on inoculated leaf



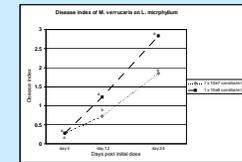
Leaf spots one week after bacterial inoculation



Extensive necrosis two weeks after bacterial inoculation



Leaf necrosis and death of *Lygodium* plants due to *M. verrucaria* infection



Effect of *M. verrucaria* inoculation on disease index



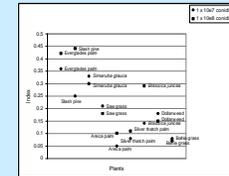
Inflorescence blight



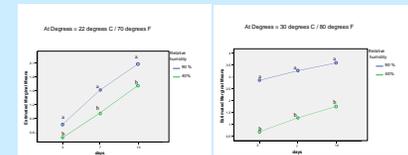
Browning and shriveling of flowers following bacterial inoculation



Asymptomatic healthy flowers on non-inoculated control



Pathogenicity of *M. verrucaria* on selected native plant species as determined by disease index.



Effects of humidity and temperature on pathogenicity of *M. verrucaria* on *Lygodium* as determined by disease index.

Materials and Method

Periodic surveys were made on Brazilian pepper trees/plants in the highly infested HID areas of Everglades National Park, Homestead, Florida. During the survey plant sample materials from Brazilian pepper with disease symptoms were collected and brought to the laboratory. Fungal and bacteria pathogens of pepper are isolated by carrying out Koch's postulate steps. Replicated detached leaf assay were conducted in the lab and in greenhouse inoculation studies were done on pot grown pepper seedlings. Inoculation application of bacteria or fungi was done by spray inoculation (1×10^8 cells/ml) in 0.02% (vol/vol) Silwet L-77 + calcium carbonate solution. The plants were then enclosed in a plastic bag to maintain high humidity. Typical symptoms were observed in 6 to 10 days in inoculated plants and systemic progression of the infection was also noted. The mycoherbicide isolate of *Myrothecium verrucaria* (Dr. Douglas Boyette, USDA-ARS, Stoneville, Mississippi - a common soil fungus present in the Southeast U.S.) was tested on *Lygodium* plants. Inoculum was prepared by mixing the spore stock with vegetable oil (1:1, vol/vol), with concentrations of 1×10^7 and 1×10^8 conidia per ml. The number of infected leaves and the severity of infection were recorded for each plant in the study. Disease severity was rated each of the 3 day intervals before inoculation using 0 to 4 rating scale to indicate disease severity. Further a disease index (DI) score was calculated by (sum of [severity rating x number of plants in that class] / total number of plants no symptoms).

Results and Discussion

Severe foliar diseases have been found to affect the Brazilian pepper trees in the HID. In some trees heavily infected leaves fell prematurely, resulting in severe defoliation. Occasionally trees with die back symptoms on young growing branches and inflorescences were also observed. Brazilian pepper is susceptible to native microbial pathogens. Localized epidemics or plants with severe symptoms are good source of potential biocontrol agents. Periodic foliar disease survey of Brazilian pepper trees in the HID area and isolations efforts have so far resulted in the collection of several fungal and bacterial isolates with potential for suppressing Brazilian pepper growth and reproduction. *M. verrucaria* inoculation of *Lygodium* plants resulted in successful disease development and high disease index demonstrating the fungal growth suppressive effects on *Lygodium*. The results suggest that there is potential for using *M. verrucaria* as a biological control in the field as it does not seem to have a significant negative effect on the native species tested. The research area of new association of native pathogens on exotic invasive species needs to be further explored, so that the potential for developing more diverse combinations of biocontrol agents can be realized.

Acknowledgment

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