Project Title: Evaluation of *Euselasia* spp. for biocontrol of *Miconia calvescens* in Hawaii

Principal Investigator: M. Tracy Johnson
Institute of Pacific Islands Forestry
Pacific Southwest Research Station, USDA Forest Service
P.O. Box 236, Volcano, Hawaii 96785
tel: 808-967-7122 fax: 808-967-7158
email: tracyjohnson@fs.fed.us

Cooperators: Dr. Paul Hanson and Kenji Nishida, Universidad de Costa Rica, San Jose

Amount Requested: $63,000

Project Leveraging: Project builds on past and existing funds from the state of Hawaii and other agencies, and long-standing collaboration with University of Costa Rica. Current non-federal matching and in-kind at UCR valued at $16,500. Additional match from existing Forest Service biocontrol program for project supervision and facility maintenance: $26,000.

Project Goals and Supporting Objectives:

Research in the native range of *Miconia calvescens* has progressed to the point that several natural enemy species have been identified that are likely to be host specific and effective as biocontrol agents in Hawaii. Further development of these agents requires rearing and host specificity testing in quarantine. Two closely related leaf-feeding enemies of miconia, *Euselasia bettina* and *Euselasia chrysippe* (Lepidoptera: Riodinidae), are among the most promising natural enemies. Further evaluation of these butterflies depends on overcoming the challenge of rearing them through their entire life cycle in captivity. In the short term, tests of larval feeding can proceed on Hawaiian plants using eggs collected and shipped from Costa Rica. Rearing methods used in Costa Rica need to be adapted to meet constraints on handling the adult butterflies under quarantine containment in Hawaii. If we can identify the right conditions for mating and egg laying in quarantine, then rigorous testing of adult host choice will be possible. Specific objectives of this project incude:

1) Collect egg masses of *E. chrysippe* and *E. bettina* from known populations in Costa Rica.
2) Refine rearing techniques in enclosures in Costa Rica.
3) Ship egg masses to Hawaii and test larval host specificity in quarantine.
4) Rear adult butterflies in quarantine.
5) Test adult host choice in quarantine.

Project Justification/Urgency:

Miconia calvescens is widely recognized as the most threatening invasive plant in Hawaii and in rainforest watersheds throughout the tropical Pacific (Medeiros et al. 1997). Its impacts in French Polynesia, where it has come to dominate forests on Tahiti since its introduction in 1937, have been devastating to native flora (Meyer 1998). Although spread of miconia in Hawaii has been contained with some success using herbicides and hand-pulling, and continuing this effort remains a high priority statewide, its costs are extremely high, approaching $2 million per year.

Complete eradication is not possible because of the large areas invaded and persistent seedbank (over 15 years), and therefore biological agents are necessary for sustainable, long term management (Smith 2002). A fungal pathogen of miconia was introduced and established in Hawaii in 1997 (Killgore 2002), however sustained control by this single agent appears unlikely.

Our current strategy for miconia is to develop a suite of agents that attack different parts of the plant, including stems, leaves and reproductive structures, with the goal of damaging miconia in multiple ways to lower its overall fitness. Research in Costa Rica and Brazil since the mid 1990s has generated a list of natural enemies which have been prioritized according to their potential for biocontrol. Based on qualitative observations of the damage they cause to plants in the native range, on their observed or expected host specificity, and on their potential to avoid biotic interference from their own natural enemies already present in Hawaii, eight insect species and two additional pathogens have been identified as high priorities for miconia biocontrol (Barreto 2005, Johnson 2010). Most promising among defoliating species are two *Euselasia* butterflies whose larvae feed gregariously, removing large areas of leaf tissue (photo). These species are well known from our detailed studies in Costa Rica (Allen 2007, Nishida 2010). We propose to hire a MS student who has extensive experience with these butterflies to continue their development as biocontrol agents.

Approach:

Collection of eggs of *Euselasia* species from field sites in Costa Rica have been a routine activity of our Univ. of Costa Rica research team for many years. Egg masses contain 40-80 eggs and are typically very lightly parasitized, so it is possible to obtain several hundred larvae from a concentrated field effort during the growing season. We already have obtained permits for export from Costa Rica and import to quarantine under USDA-APHIS oversight in the US. Shipment and rearing is facilitated by the long duration of the egg stage of these insects – a month or more depending on temperature. We have shipped egg masses successfully on several occasions and reared the larvae on potted miconia in quarantine with very high rates of survival to adulthood. Host specificity will be evaluated in Hawaii using standard no-choice larval feeding tests on an established list of approximately 30 test plant species, including numerous Melastomataceae and representative Myrtales and several unrelated species of ecological and economic significance. A total of 1000 eggs, or approximately 15 egg masses, will suffice for replicated testing of all plants with cohorts of 5-10 larvae per test. Survival and growth will be compared across test plants using analysis of variance. Results are expected to be very straightforward given the evidence of an extremely narrow host range for these species in Costa Rica.

We have reared many hundreds of *Euselasia* larvae to adulthood in Costa Rica, and established temporary populations on cultivated miconia outside their natural habitat (on miconia planted on the UCR campus). Our team in Costa Rica has also witnessed mating of reared adults in outdoor enclosures on a few occasions. Adults required nectar or decaying fruit as a food source and

horizontally sunlit open space near host plants for courtship and mating, which occurs in the early morning. With additional trials in Costa Rica, we expect to be able to have females mate and oviposit under conditions that can be reproduced in Hawaii. Conducting these trials initially in Costa Rica is much less expensive and more efficient in requiring lower numbers of insects. Having the same individual develop rearing techniques in Costa Rica and repeat them in our Hawaii quarantine is expected to be critical. Kenji Nishida has been selected to conduct this project because of his extensive experience with these particular *Euselasia* as well as rearing hundreds of other tropical Lepidoptera. By having two *Euselasia* species to work with, we expect will improve the chance that at least one will prove amenable to rearing in containment.

If we can successfully reproduce conditions for mating and oviposition of *Euselasia* in our Hawaii quarantine, we will also examine adult host selection behavior under choice and no-choice situations. Behaviors of interest will include oriented flight, landing, searching, and egg-laying. Tests will focus on the same plant species used for larval feeding trials and follow similar protocols for replication and analysis. Given that adults begin laying eggs after about one week and survive for up to one month, we can expect to conduct a large number of tests with a small but steady supply of emerging adults.

We propose to start collection and rearing of *Euselasia* in Costa Rica in April 2011 with the beginning of the rainy season (April - November). Egg masses will be shipped to Hawaii and larvae tested as numbers allow. After approximately 4 months in Costa Rica we expect to have enough egg masses and rearing experience to transfer Kenji to Hawaii for rearing trials and host specificity tests in quarantine. Additional travel back and forth to Costa Rica may be necessary, depending on supplies of *Euselasia* and success of rearing attempts in Hawaii. Duration of the project is expected to be 12 months, but could be extended to 18 months if an additional collection season in Costa Rica is required – depending on progress assessed at end of 2011.

Expected Products and Outcomes:

Host specificity data and rearing methods will be written up for peer-reviewed journal publication and combined with existing published studies on biology of *Euselasia* in its native range for a release petition to the state of Hawaii. Since all observations in their native environment indicate a very narrow host range – probably restricted to a few species of Miconia, one or both *Euselasia* should be found suitable as biocontrol agents if tests on Hawaiian plants result as expected.

If mating cannot be achieved under conditions in our quarantine, we will consider other options. One would be to try other containment environments in Hawaii, which would likely require special permitting and some delay. Another possibility is that a *Euselasia* species could be considered for biocontrol release on the weight of its larval host range alone since this is expected to be extremely narrow. In any event, we expect to have most of the data needed and a draft release petition written for either *Euselasia chrysippe* or *E. bettina* at the conclusion of this project.

Budget:

	Requested	Univ. Costa Rica Match	FS-IPIF Match
Salaries: Project technician (GS-7)	30,157	13,000	
UCR cooperator (2%)		1,000	
UCR student assistant	3,200		
Principal investigator (15%)			12,000
Quarantine technician (GS-7)	10,000		
Benefits: Project technician (GS-7)	9,271		
UCR cooperator			
UCR student assistant			
Principal investigator			4,000
Quarantine technician (GS-7)	3,474		
Supplies/Shipping	898		
Quarantine Facility maintenance			10,000
UCR lab/greenhouse		2,500	
Travel: Costa Rica-Hawaii (3 round trips)	6,000		
Total	**63,000**	**16,500**	**26,000**

Personnel:

Collections, rearing and experiments will be conducted by entomologist Kenji Nishida (MS Univ. of Costa Rica) hired to work approx. ¾ time on this project under direct supervision of PI Tracy Johnson, who will be responsible for coordination of activities between Costa Rica and Hawaii, oversight of experimental design, data analysis and write-up. A Hawaii-based quarantine technician will work ¼ time on this project, assisting larval feeding tests and rearing efforts. Our UCR cooperator is Paul Hanson, professor in the School of Biology at University of Costa Rica, who has sponsored our team of student researchers on Proyecto Miconia for the last 10 years. A UCR student under his supervision will work part-time to assist fieldwork.

Timetable

	2011										2012										
	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Hire project technician	x																					
Collect egg masses and ship		x	x	x	x																	
Rear in Costa Rica			x	x	x																	
Larval tests in Hawaii			x	x	x	x																
Rear in Hawaii						x	x	x	x	x	x	x	x	x	x	x	x	x				
Assess progress							x															
Test adults in Hawaii							x	x	x	x												
Additional egg collections?									x	x				x	x	x						
Additional Costa Rica rearing?											x	x										
Further larval/adult tests											x	x	x	x	x	x	x	x				
Write up													x	x	x	x	x	x	x			

Literature Cited

Allen, P. 2007. Demografía, patrón de supervivencia y efectos de tamaño de grupo en larvas gregarias de *Euselasia chrysippe (*Lepidoptera: Riodinidae), un potencial agente de control biológico de *Miconia calvescens (*Melastomataceae) en Hawai. MS Thesis. Escuela de Biología, Universidad de Costa Rica, San Jose, Costa Rica, 51 p.

Barreto, R.W., C.D.S. Seixas, E. Killgore, and D.E. Gardner. 2005. Mycobiota of *Miconia calvescens* and related species from the neotropics, with particular reference to potential biocontrol agents. Technical Report 132 pp. 24. Pacific Cooperative Studies Unit, University of Hawaii at Manoa, Honolulu, HI.

Burkhart, R.M. 1995. Natural enemies of *Miconia calvescens*. Hawaii Department of Agriculture, Honolulu, HI.

Johnson, M.T. *In press.* Miconia biocontrol: where are we going and when will we get there? *In:* 2009 International Miconia Conference Proceedings

Killgore, E.M. 2002. Biological control potential of *Miconia calvescens* using three fungal pathogens. Pp. 45-52 in: Proceedings of a workshop on biological control of invasive plants in native Hawaiian ecosystems, C.W. Smith, J. Denslow, S. Hight (eds.).. Pacific Cooperative Studies Unit, University of Hawai'i at Manoa. 122 pp. (Technical Report 129).

Medeiros, A.C., L.L. Loope, P. Conant, and S. McElvaney. 1997. Status, Ecology, and Management of the Invasive Plant, Miconia calvescens DC (Melastomataceae) in the Hawaiian Islands. Records of the Hawaii Biological Survey for 1996. Bishop Museum Occasional Papers 48: 23-36.

Meyer, J.-Y. 1998. Observations on the reproductive biology of *Miconia calvescens* DC (Melastomataceae), an alien invasive tree on the island of Tahiti (South Pacific Ocean). *Biotropica* 30: 609-624.

Nishida, K. 2010. Description of the immature stages and life history of *Euselasia* (Lepidoptera: Riodinidae) on *Miconia* (Melastomataceae) in Costa Rica. *Zootaxa* 2466:1–74. http://www.mapress.com/zootaxa/2010/f/zt02466p074.pdf

Smith, C.W. 2002. Forest pest biological control in Hawaii. Pp. 91-102 in: Proceedings of a workshop on biological control of invasive plants in native Hawaiian ecosystems, C.W. Smith, J. Denslow, S. Hight (eds.).. Pacific Cooperative Studies Unit, University of Hawai'i at Manoa. 122 pp. (Technical Report 129).