1. **Project Title:** A Biological Control Feasibility Study of the Invasive Weed Japanese Stiltgrass, *Microstegium vimineum* (Trin.) Camus

2. **Principal Investigator:**
   Judith A. Hough-Goldstein, Ph.D.
   Professor, Department of Entomology & Wildlife Ecology
   University of Delaware
   531 South College Ave.
   Newark, DE 19716-2160

3. **Cooperators and Other Participating Institutions:**
   Jianqing Ding, Ph.D.
   Research Scientist and Program Leader, Invasion Ecology and Biocontrol Lab
   Wuhan Botanical Garden/Institute, Chinese Academy of Sciences
   Moshan, Wuhan, Hubei Province, 430074 China
   
   William L. Bruckart III, Ph.D.
   Plant Pathologist
   USDA, ARS, FDWSRU
   1301 Ditto Ave.
   Ft. Detrick, MD 21702

4. **Amount Requested (yearly and total), Project Leveraging**
   2014: $25,000 (April 1, 2014 – May 31, 2015; current proposal)
   2015: $25,000 (anticipated, to continue support of graduate student)
   Total: $50,000

5. **Project Goals and Supporting Objectives**

   **Goal/Objective #1:** To gather and evaluate information on topics relevant to the feasibility of pursuing biological control for *Microstegium vimineum*.

   Feasibility studies conducted prior to or early in a biological control project can identify potential risks and help assess the suitability of a weed species for biological control, before significant research and monetary investment. Wheeler et al. (2007) recommended gathering information on the following topics, all of which we propose to address: nature of ecological and economic damage of the target weed; area of origin and geographic distribution; identification, taxonomy, and analysis of the target and related plants using traditional and molecular data; potential conflicts of interest with economically important species; potential risks to native plants; secondary plant chemistry; level of specificity needed to avoid non-target effects; and areas surveyed and natural enemies found. Results should allow an informed decision or summary recommendation concerning whether the project should be pursued.
A preliminary review of the literature suggests that substantial information is available on all of these topics except the area of origin of Microstegium vimineum; its secondary plant chemistry; and preliminary survey of the plant in its native range for natural enemies. Therefore this project will focus particular attention on these three areas.

**Goal/Objective #2: To develop a test-plant list for submission to the Technical Advisory Group for Biological Control Agents of Weeds (TAG).**

Before host-range testing is conducted with potential agents, a test-plant list must be approved by TAG. This will require review of the most recent phylogeny of *M. vimineum* and its botanical relatives. This will be a practical outcome of the overall feasibility summary. The TAG recently acted favorably on a petition for release of the first-ever grass-feeding biological control agent, the delphacid bug *Prokelisia marginata* Van Duzee, for control of cordgrass, *Spartina alterniflora* Loisel. The test-plant list for that species included related species (within the same tribe and subfamily of the target); unrelated grasses with similar morphology to the target; grasses found in the same habitat as the target; and economically important turfgrasses (four species) and crop grasses (oats, barley, wheat, and corn; Grevstad et al 2003). This list may provide a good model to follow for the *M. vimineum* test plant list.

**Goal/Objective #3: To determine whether potential biocontrol agents of Japanese stiltgrass are present in China.**

Current information suggests that China may be the center of origin of *Microstegium* spp., and several species of Lepidoptera and Hemiptera (including aphids and scales) have been reported on plants in that genus in China (Zheng et al. 2004). However, substantial scope remains for exploration and literature review. Preliminary research is necessary as an important part of this feasibility study.

**Goal/Objective #4: To establish sites in North America for pre-release monitoring and eventual natural enemy release.**

Many sites in Delaware and surrounding states have been heavily invaded by Japanese stiltgrass and would benefit from biological control if agents become available. A challenge with biological control is to show effectiveness of agent release when other natural processes are constantly causing plant populations to shift from year to year. Therefore, ideally well before agent release, invaded sites should be identified and monitored, and upon release control sites and release sites should be compared. Many sites in Delaware State Parks could be used in this effort; potential sites are close to the University of Delaware, and consist of sites in all types of habitat, including sunny locations, deep shade, wet or dry locations, in all State Park properties north of the C&D Canal (Rob Line, Manager, Environmental Stewardship Program, Del. Division of Parks and Recreation, personal communication).

6. **Project Justification/Urgency**

Japanese stiltgrass is one of the most aggressive weed invaders of the forest understory, with proven
severe effects in reducing the diversity of woodland herbaceous communities and inhibiting forest regeneration and succession (Barden 1987, Pemberton 2002, Oswalt et al. 2007, Baiser et al. 2008, Flory and Clay 2010a, 2010b). It is currently reported in 27 states (EDDMapS 2013) and it is continuing to spread. Dr. Marc Imlay, Chair of the Biodiversity and Habitat Stewardship Committee for the Maryland Chapter of the Sierra Club, states that “Japanese stiltgrass has destroyed 10% of the natural environment in our region according to the surveys done by the National Park Service several years ago,” and notes that the problem may be increasing: “I have found that many groups that have succeeded in removing Japanese stiltgrass each year from a natural area are no longer successful. Suggested causes include climate change with bigger specimens and more late emergents.” *Microstegium vimineum* is listed as a noxious, banned weed in Alabama, Massachusetts, and Connecticut (USDA, NRCS 2013). Invasions create dense, monospecific stands that reduce native plant diversity and productivity, inhibit forest regeneration, and alter forest species composition and successional trajectories (Flory 2010), and these areas are not easily recolonized by native species (Barden 1987). Japanese stiltgrass invades sites in a wide range of environmental conditions, including deep shade, and may have the greatest detrimental effects on native plants under part-shade conditions (Flory et al. 2007).

Japanese stiltgrass was first introduced to the USA from south-east Asia in the early 1900s, and had spread to at least 14 eastern states by 1972 (Fairbrothers and Gray 1972). There was a substantial lag time between the introduction of *M. vimineum* into the US and its recognition as an aggressive invader in the 1980s (Barden 1987). The species was not deliberately introduced, and there are no records of its origins from the native range or when and where it was introduced. It was formerly used as packing material for imported Chinese porcelain, and it has been proposed that discarded packaging material containing seeds may have been the original source of introduction (Swearingen 2009). Molecular markers for population genetic studies could shed light on the origin (e.g. Goolsby et al. 2006, Selkoe and Toonen 2006). Novy et al. (2012) characterized 17 microsatellite loci for *M. vimineum*, which should be useful for tracking of invasion dynamics and further population genetics studies, but so far further studies using these loci have not been published.

Pemberton (2002) stated that Japanese stiltgrass “appears to be a good candidate for biological control,” noting that it has no congeneric native or economic species in the United States. A taxonomic revision of the genus *Microstegium* was recently published by Chen et al. (2012), incorporating observations of morphology as well as molecular data. This work describes 16 species in the genus, mostly tropical or subtropical, and all native to the Old World, and confirms that there are no other species of *Microstegium* other than *M. vimineum* currently found in North America.

Flory et al. (2011) showed that on average, introduced populations of *M. vimineum* outperformed native range populations from China over a range of common gardens with diverse environmental conditions. These results are compatible with the evolution of increased competitive ability (EICA) hypothesis (Blossey and Notzold 1995), which suggests that an introduced population will increase its investment in various growth parameters, and is able to do this because it can reduce investment in defenses due to
relative lack of natural enemies in the introduced range. Thus if EICA is true for *M. vimineum*, the implication is that natural enemies occur and cause significant amounts of damage in the native range and are not currently found in the introduced range, suggesting that biological control could be effective in controlling this important pest.

Invertebrate herbivore damage rates on *M. vimineum* in North America are very low, estimated at 0.4 to <10% of leaf tissue removed (Sanders et al. 2004, Morrison et al. 2007). In plots that were experimentally invaded by *M. vimineum*, arthropod abundance was reduced by 39%, and species richness of arthropods declined by 19% compared to uninvaded plots (Simao et al. 2010). *Microstegium vimineum* is not browsed by white-tailed deer, which are overabundant in much of its range, and in forests where *M. vimineum* produces a dense understory, both white-tailed deer herbivory on native plants and *M. vimineum* can work together to cause complete failure of forest regeneration (Baiser et al. 2008, Webster et al. 2008). A recent study reported that five indigenous (native or naturalized) orthopteran species and three hemipteran species were feeding on *M. vimineum* in study plots in Georgia, an apparently new phenomenon in 2008, approximately 15 years after establishment of the invading plant in this area (Bradford et al. 2010). In addition, a pathogen, *Bipolaris* sp., has been found infecting *M. vimineum* in some areas, and may suppress populations, although it is not host-specific, infecting a wide range of grasses (Kleczewski et al. 2012). The long-term effects of accumulating native pathogens and/or arthropods on stiltgrass and other invasive species is unknown but warrants further study (Flory and Clay 2013).

In China, *M. vimineum* occurs in at least 15 provinces, and 15 other species in the same genus are also found in China. One aphid species and 7 Lepidoptera, all in the family Satyridae (now subfamily Satyrinae of the Nymphalidae), have been reported feeding on the genus *Microstegium* (Zheng et al. 2004). The species-rich grass-feeding subfamily Satyrinae contains seven multivoltine and 108 monovoltine species worldwide (Cizek et al. 2006). Numerous other insect species feed externally on grasses, including chewing insect in the Orthoptera, Lepidoptera, Coleoptera (mainly Chrysomelidae and Curculionidae), Hymenoptera (Syrphidae), and Phasmida. Sap-feeders on grasses include Hemiptera (Auchenorrhyncha, Sternorrhyncha, and Heteroptera, mainly Miridae), and Thysanoptera. Stem-borers are also found on grasses, from the Diptera (mainly Cecidomyiidae and Chloropidae), Hymenoptera (Cephidae, Eurytomidae), Homoptera (mainly Pseudococcidae), Lepidoptera (mainly Pyralidae, Noctuidae), Coleoptera (mainly Cerambycidae, Mordellidae, Chrysomelidae), and Acari (mites) (Tscharntke and Greiler 1995). Little is known about the communities of endophagous insects feeding inside the shoots of grasses, although these species might be expected to be more host-specific than ectophages (Tscharntke and Greiler 1995). A review of a study in Germany noted that fewer insect species were found on annual than on perennial grass species; however, one endophage and about 30 ectophages were found on five annual grass species (Tscharntke and Greiler 1995).

So far there have been no systematic surveys of insects or pathogens feeding on the genus *Microstegium* in Asia. No information could be found concerning secondary chemistry or other defenses of *Microstegium* against herbivory. Silica-based defenses are typically the most important defense mechanism
identified in grasses (Vicari and Bazely 1993, Tscharntke and Greiler 1995). However, some grasses also contain a variety of hydroxamic acids (such as DIMBOA in maize), phenolics and polyphenolic compounds such as tannins, cyanogenic glycosides, alkaloids, and fungal endophytes that may produce ergot and other alkaloids (Vicari and Bazely 1993). All of these compounds can act as anti-herbivore defenses, and host-specific herbivores that have overcome these secondary metabolites and use these compounds as behavioral cues to seek out and exploit the correct host may also exist (Wheeler and Schafner 2013).

Grass weeds have rarely been targets of biological control, both because of the large number of important crop grasses that might be at risk from agent importations, but also because of the concern that grass insects may not have the same levels of specificity as insects feeding on other plant families (Pemberton 2002). This perception may be incorrect, however, and may be due more to lack of detailed knowledge of secondary compounds in grasses rather than to reality. Recently a host-specific delphacid bug, Prokelisia marginata Van Duzee, native to the eastern United States, was introduced into the state of Washington for control of cordgrass, Spartina alterniflora Loisel, a native of eastern North America that has invaded salt marshes on the west coast (Grevstad et al. 2003, 2012). Programs are also underway for two other grasses, Phragmites australis (Cav.) Trin. ex Steudel (Blossey et al. 2002) and cogon grass, Imperata cylindrica (L.) P. Beauv. (Van Loan et al. 2002), although to date no deliberate releases have been made against these targets.

7. **Approach**

Hough-Goldstein will conduct a review of *M. vimineum* suitability as a target of biological control and conduct preliminary research on use of molecular tools to identify the area of origin of *M. vimineum*, and its secondary plant chemistry (Objective 1). Much of this will involve literature review, but exploratory work will also be conducted to determine feasibility of producing primers, PCR, and collections worldwide to determine the origin of the North American population through molecular neutral markers; and identifying likely plant chemistry and chemists willing and interested in conducting analyses. Further work on potential pathways determined in year one will be pursued in year two.

Hough-Goldstein will also prepare a proposed test-plant list (Objective 2). This will be based on current taxonomic summaries (Chen et al. 2012), with additional species added using the work of Grevstad et al. (2003) as a model. The test-plant list will be submitted to TAG at the end of the second year.

Although not included in the funding request for this proposal, Ding will conduct searches in China for potential agents (Objective 3). He and his students will systematically search herbivorous insects on the plant across China. Preliminary no-choice host-range studies will be conducted in his laboratory on any insects collected. In addition, possible pathogens will be sent to Bruckart for further screening for pathogenicity on *M. vimineum* and for host specificity.

Pre-release monitoring sites will be established in Delaware and surrounding states by Hough-Goldstein
(Objective 4). Paired sites (treatment/release of agents, and control sites) with similar light conditions, humidity, soil, and surrounding vegetation will be established in a variety of state park lands. Monitoring of *M. vimineum* percent cover will be conducted.

8. **Expected Products and Outcomes**

At the completion of this two-year project we should have a good idea of the chance of success of a major effort at biological control of Japanese stiltgrass. Products will include (1) a publication summarizing risks and suitability of targeting *M. vimineum* for biological control; and (2) a test plant list for submission to TAG. If the outcome of the feasibility study is promising, then additional funding will be sought to pursue host-range testing of potential agents.
**Additional Information**

Short (1-page) CV for PI: see attached

Budget: see attached

**Timetable**

2014

Hough-Goldstein:
- Conduct review of *M. vimineum* suitability as a target of biological control and conduct preliminary research on use of molecular tools to identify the area of origin of *M. vimineum*, and its secondary plant chemistry (Objective 1).
- Prepare a proposed test-plant list (Objective 2).
- Survey Delaware parks for potential pre-release monitoring sites (Objective 4).

Ding:
- Conduct searches in China for potential agents (Objective 3). Conduct preliminary host-plant range no-choice tests. Send any pathogens found to Bruckart.

2015

Hough-Goldstein:
- Complete review of *M. vinimeum* suitability as a target of biological control and write up feasibility study results for publication (Objective 1).
- Complete proposed test-plant list, and submit to TAG (Objective 2).
- Set up and monitor pre-release monitoring sites (Objective 4).

Ding:
- Conduct further searches in China for potential agents (Objective 3). Conduct preliminary host-plant range no-choice tests. Send any pathogens found to Bruckart.

**Literature Cited**


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EDUCATION
1-81 Cornell University, Ithaca, NY: PhD (Entomology), 1981; MS (Entomology), 1977  
1968-72 Harvard University, Cambridge, MA: BA (Biology), 1972

POSITIONS HELD
1997-present  Professor, Dept. of Entomol. & Wildlife Ecol., Univ. Delaware, Newark, DE.  
Research (approx. 60%): biological control of invasive weeds, insect behavior, pest management  
Teaching (approx. 40%): biological control, insect pest management, entomology laboratory
1996-2002  Chair, Dept. of Entomology & Wildlife Ecology, Univ. Delaware

PEER REVIEWED PUBLICATIONS AND BOOK CHAPTERS (most recent only; total 65)

GRANT SUPPORT (current only; total $850,000)
V. D’Amico, L. Hanks, J. Millar, and J. Hough-Goldstein. 2012 - 2017. Developing new methods to predict the invasive potential of exotic wood-boring beetles. USFS (FHP-STDP) $84,000 (to date).

PROFESSIONAL ACTIVITIES/AWARDS (selected)
NE1032, Biolog. Control Arthropod Pests and Weeds. 2008-18 (member-at-large, Regional Project Executive Committee, 2013 (Secretary in 2014, Chair in 2015).
Peer Review: active in reviewing manuscripts and serving on program and grant review panels
Nominated for IOBC-NRS Distinguished Scientist Award, June 2013
USDA-APHIS-PPQ Eastern Region Biological Control Programs Recognition Award, 2010
Delaware Invasive Species Council Research Award, 2009
Subject Editor for Environmental Entomology, 2003 – 2007
Univ. Delaware Outstanding Master’s Graduate Student Advising and Mentoring Award, 2004
Graduate Women in Science Award for Excellence (Runner-up), Cornell University, 1981