

## **Genetic Markers for Post-release Monitoring of Knotweed Psyllid, Evaluation of Knotweed/Tree Seedling Competition, and Other Activities Supporting Biological Control of Japanese Knotweed**

**Roy Van Driesche (PI)**, an entomologist at UMASS, specializes in biological control. Current projects include emerald ash borer and Japanese knotweed. He will administer the project's finances, lead field research activities, and be responsible for local natural enemy releases.

**Nick Mills, with Jeremy Andersen.** Nick Mills is a professor of Entomology at UC Berkeley and Jeremy Anderson is a Ph.D. student in his lab. This group will be responsible to develop a working set of genetic markers to separate the two psyllid strains and their hybrid.

**Christian Marks** is a TNC research scientist, heading the floodplain forest conservation initiative for the CT River Program. He is a plant ecologist specializing in invasive species and riparian plant community dynamics. He will participate in field knotweed research activities, excluding actual release of the biological control agent.

**Fritzi Grevstad** at Oregon State University has led the knotweed biocontrol program since 2004. She will be in charge of seeing the project through the regulatory process and holding colonies of the psyllid. She will provide a starter colony in MA after release is approved, provide insects for genetic marker studies, and advise on psyllid rearing and sampling.

**AMOUNT REQUESTED \$76,050 total federal dollars, plus \$25,360 state match (25%)**

### **GOALS AND OBJECTIVES**

The goal of is to support biological control of Japanese knotweed in the eastern United States.

**Objective 1:** Develop genotyping markers to identify the northern and southern races of the knotweed psyllid and the hybrid (2013) (Mills/Andersen)

**Objective 2:** Development of techniques to measure competition between knotweed seedlings and native tree seedlings on river bars (2013-2014) (Marks/Van Driesche).

**Objective 3:** Continue collection of pre-release plant performance data (Marks/Van Driesche)

**Objective 4:** Release and establish two races of knotweed psyllid (2014 or 2015) (Van Driesche)

### **PROJECT JUSTIFICATION**

**WEED.** Invasive *Fallopia* spp. in the US are *F. sachalinensis* (giant knotweed) from Hokkaido, *F. japonica* from southern Japan, and their American hybrid, *F. x bohemica* (Pridham and Bing, 1975; Patterson, 1976; Conolly, 1977). After introduction, knotweeds became problem weeds in the US, as in the UK and Europe where they have been present since the 1840s (Beerling et al., 1994). In the NE US, all 3 forms exist, although the hybrid *F. x bohemica* is the most widespread. A genetic survey of the knotweeds at our research sites was done in 2012 to determine if all sites are the hybrid or if variation exists (under analysis, by John Gaskin).

**DAMAGE.** Large stands of knotweed occur in many areas of PA, MA, NY, and VT along higher velocity rivers, on thousands of acres of wetlands, stream banks, and hillsides. It is also a serious problem in NJ, MD, and VA, along river banks, wetlands, roadways, and disturbed areas (Muenscher, 1955; Conolly, 1977; Beerling, 1990; Mehrhoff, 1997; Virginia Native Plant Society, 1999). Knotweed thickets exclude native species and are of little value to wildlife

(Holzner, 1982) and damage invaded riparian communities (Maerz et al. 2005, Siemens and Blossey 2007). Rhizomes reach 15-20 m (Locandro, 1973) and allow for early emergence and great height, shading out other vegetation, and reducing native species diversity (Sukopp and Sukopp, 1988; Gerber et al., 2008; Maerz et al., 2005; Aquilera et al., 2010). Allelopathic chemicals exuded by knotweed also help suppress native plants (Murrell et al., 2011).

In floodplain forests, establishment of trees on newly formed river bars is believed to be inhibited by knotweed stands, retarding afforestation of sediment bars, a potential important detriment to afforestation of these new river-edge habitats. Experimental data on competition between knotweeds and native plants have been obtained by transplanting tree or herb seedlings into mature knotweed stands. In Washington, this was done with conifers and alder (Urgenson et al., 2012) and in New York with silver maple and boneset (*Eupatorium perfoliatum*) (Siemens and Blossey, 2007). In both cases, established knotweed stands significantly reduced growth of test plants. Indeed, the rarity of seedlings growing inside mature knotweed stands supports these conclusions. In our proposal, we focus on competition as it would likely occur on river bars, which are newly formed habitat that would be gradually colonized by both tree seedlings and knotweed seedlings. It is very important to determine if knotweed seedlings outcompete tree seedlings, as this would likely be one of the major ecological impacts of this invader. To our knowledge, no experiments have addressed this point. We proposed to do so through long term study plots selected to be in the early phase of such competition. Use of such plots at sites with and without the psyllid will provide information not only on the ecological impact of the weed on native tree seedlings, but on the potential of the biological control agent to change the outcome of this interaction, making knotweed less competitive against native tree seedlings.

## **BIOCONTROL PROGRAM.**

**Pre-release surveys.** Surveys for existing knotweed natural enemies in North America (conducted in the Northeast, Pacific Northwest and Alaska) found no herbivores with any potential for control (McIver and Grevstad, 2010).

**Agent.** In the native range (Japan, China, Korea, and Taiwan [Beerling et al., 1994]), herbivore and pathogen damage to the plant is high (Grevstad and Shaw, pers. obs.). Over 186 species of insect herbivores and 40 fungal species were recorded in Japan (Shaw et al., 2009). Of four species imported into quarantine in Oregon, the psyllid *Aphalara itadori* (**Fig. 1-end of document**) has emerged as the candidate of choice. It has been released in the UK, where its effects are now being monitored (Djeddour & Shaw, 2010). A TAG petition for its release in the US is pending and is provided with this proposal as a separate file (TAG petition-separate file).

**Races and hybrids of the knotweed psyllid.** *Aphalara itadori* feeds on sap, depleting the plant's energy supply. High psyllid densities cause leaf twisting (**Fig. 2-end of document**). In the lab, psyllids can kill potted knotweed plants within one psyllid generation (about one month). There are two host races of the psyllid being petitioned for release: **Southern strain**—from Kyushu that feeds well on all three forms of knotweed and a **Northern strain** --- from northern Japan (Hokkaido) whose performance is best on *F. sachalinensis* but which is more damaging in the short term to all three knotweeds than is the southern strain. In the laboratory, hybridization between the two strains has been observed and the crossed line reproduced well on all three knotweed species (Rob Bouchier, Per. Obs.). Because both strains have desirable features, both are intended for release. Because they are not visually distinct and because hybrids are possible, genotyping markers must be developed for strain and hybrid recognition in post-release monitoring. Releases are anticipated to begin in the western US in 2013 or 2014 (depending of

speed of petition review) and in the eastern US in 2014 or 2015. In advance of release, efforts are needed to develop practical molecular markers to separate strains and hybrids.

### **Project History (efforts in New England, 2011-2012)**

*Site selection-2011.* In 2011, 5 release and 5 paired control sites were selected, 3 pairs in MA, and 1 pair each in NH and VT. All sites support dense stands of knotweed along rivers. Control sites are upstream of release sites by <5 miles and in a westerly direction (i.e., upwind of prevailing wind direction). This placement will slow the invasion of the control sites by the psyllid, post release, via either wind movement of adults, or river movement of infested plants.

*Pre-release data on plants-2012.* In 2011, we tested potential methods of collecting data, but plots were destroyed mid-summer by Hurricane Irene, which caused strong floods at all sites, destroying plants before measurements were completed. A full set of pre-release data using modified protocols was collected in 2012 (**Figs. 3-7, end of proposal**), including (1) measurement of dia and height of all stems in eight 1 m<sup>2</sup> plots at each of the ten field (80 plots total); (2) establishment of plots on river bars to assess competition over time between knotweed seedlings and tree seedlings via photography (3 plots per site at 3 pairs of sites; total: 9 release and 9 control plots). This activity required plot selection, sketching, and note taking on types and relative densities of plants by species on bars, plus photographing plots from fixed vantage points for future reference; (3) hemispherical sky photos (once per site in mid summer) from a fixed point in the center of each of the 80 knotweed plots to assess canopy cover (as % sky); (4) collection of knotweed seeds and measurement of total lengths and weights of knotweed inflorescences on three random stems from each of 80 plots; and (5) Collection of plant tissue samples from all sites (from inside plots) for DNA analysis to determine genetic status of plants at study sites. Data analysis is in progress by John Gaskin at USDA lab, Sidney, Montana.

## **APPROACH**

### **Objective 1: Develop Genotyping Markers for the Northern and Southern Races of Knotweed Psyllid and their Hybrids (2013) (Mills, Andersen).**

Development of markers to study genetic variation within and between populations has been greatly simplified by advances in Next-Generation Sequencing (NGS) technologies (Metzker, 2010; Angeloni et al., 2012), which permit several possible approaches for genotyping individuals. One approach allows for both the simultaneous discovery of markers and the genotyping of individuals (e.g., RADtag or RADseq) (Baird et al., 2008; Hohenlohe et al., 2011; Peterson et al., 2012). However, this approach is currently limited by the large amounts of DNA required, and its utility for multi-year programs remains unclear. Another approach allows for the rapid identification and development of markers, followed by traditional genotyping analyses using either single-nucleotide polymorphisms (SNPs) (Helyar et al., 2012), or repeat regions of DNA (microsatellites) (Cerna and Straka, 2012; Keller et al., 2012). Microsatellites allow researchers to identify target regions of DNA, and in some cases perform statistics to optimize the usability of markers, reducing time and expense. Individuals can then be genotyped at a later date, allowing for more flexibility in the number of individuals and loci examined.

We will extract DNA from individuals of both the northern and southern races of psyllids, as well as known hybrid individuals. From each DNA extract, an individual library will be constructed with a unique barcode attached. This barcode will allow for the pooling of individuals during NGS sequencing. We expect, based on our experience with the Illumina NGS

platform, and using the size of the Asian citrus psyllid genome as a reference, that this approach will allow us to pool up to four individuals per sequencing run while achieving excellent coverage (~10X). Therefore with only a limited number of runs we will be able to examine enough individuals to identify independent loci from each race and known hybrids. DNA extraction, library preparation, sequencing, discovery of polymorphic markers, and analysis will be performed at the University of California Berkeley in the lab of Dr. Nicholas Mills. In addition, this process will identify large amounts of genomic data during the *de novo* genome assembly process. While this approach is unlikely to reconstruct the entire genome from each race, these assemblies will be a valuable resource for any future genetic studies in this group.

Once a list of target SNPs has been generated, we will then test the validity and utility of a subset of these loci to ensure that these results are reproducible, and that we can accurately identify known hybrids. During this process we will test 93 individuals (31 from each race and 31 laboratory crossed hybrids) at 144 SNP loci using the Sequenom MassARRAY® MALDI-TOF genotyping instrument at the University of Arizona Genetics Core Facility. This platform is highly flexible, and thus ideal for future resurvey efforts as it allows a large number of individuals (382) to be genotyped simultaneously with very small amounts of DNA per individual. This approach will minimize the labor and costs associated with the genotyping of individuals from post-release sampling events, allowing high-resolution tracking of gene flow within and between these two races.

**Objective 2: Development and Use of Techniques for Measuring of Competition between Knotweed Seedlings and Native Tree Seedlings on River Bars (2013-2014) (Marks, Van Driesche).**

Measuring competition between invasive plants and native species is a critical part of biological control projects, because it demonstrates the ecological impact of the pest on native plants and, after establishment of the agent, can document the reversal of those impacts under the influence of biological control. Rayamajhi et al. (2009) showed an inverse relationship between melaleuca (*Melaleuca quinquenervia* [Cav.] S. T. Blake) density and plant biodiversity over time in the Florida Everglades as a biological control reduced densities of this invasive wetland tree. Similarly, Meyer et al. (2012) documented the recovery of native plant biodiversity and increase in % cover by native plants in understories of forests dominated by the invasive tree *Miconia calvescens* DV in response to the effects of a classical fungal biocontrol agent (*Colletotrichum gloeosporioides* f. sp. *miconiae* Killgore and L. Sugiyama). Modern weed and insect biocontrol projects must work cooperatively with conservation biologists and, for projects in wild lands, must document both the initial ecological damage caused by the invasive pest and the ecological recovery and benefits to the invaded ecosystem. The above two projects are good examples of that. By working with Christian Marks, a plant ecologist of The Nature Conservancy, we hope to do the same in our knotweed project. It is not going to be sufficient in the future to simply assume that invasive plants are ecologically damaging based on their dominance of habitat area, nor will it be sufficient for a claim of success simply to document reduction in the invasive plant's % cover in the target habitat. Therefore, our bar plot competition study is very important because it is our project's tool to both measure the impact of the weed on riparian forest regeneration and to measure a reduction in that damage over time due to the biocontrol agent.

Methods to document knotweed impacts on tree seedling establishment and growth have to be long term (because the process unfolds slowly) and simple enough to implement on a meaningfully large number of plots (since rivers are dynamic, some plots may be lost). We have

elected to have an experimental format (sites with and without the biocontrol agent), with 3 release and 3 control sites (6 distinct rivers) with 3 sampling plots within each site. Data are recorded through the use of annual plot photographs and annotated diagrams (**see example, Fig. 8 A-D at end of document**). Annually, we will relocate plots on river bars that were initially selected because seedling trees were growing in close proximity to young knotweed plants. Each year, vegetation on bars will be re-described and re-photographed from a fixed vantage point. We expect to see suppression of tree seedlings by knotweed at a higher rate at control sites (no psyllids) than at psyllid release sites. This process resembles the use of before and after photography (commonly used in weed biocontrol projects) with the added aspect of documentation not only of decline of the target weed, but also an increase in native plants. This additional element is new and greatly increases the value of this approach by generating the data on ecological benefits that our conservation biologist colleagues will expect to see. So while this technique does not break new ground technically, its design does.

### **Objective 3: Continue Collection of Pre-release Plant Performance Data (2013).**

All plant performance data collected in 2012 will be collected in 2013 using the same set of 10 sites (80 plots), being stems/m<sup>2</sup>, stem diameter, stem height, % sky above plant canopy, and reproductive output per m<sup>2</sup> at all sites.

### **Objective 4: Release/Establish Knotweed Psyllid (*Aphalara itadori*) (2014-15) (Van Driesche).**

***Insect source and release pattern and rate.*** After APHIS and state release permits have been obtained, Fritzi Grevstad will supply psyllids for rearing to support field release. Unsexed adults (1-10 thousand per site depending on rearing success) will be free-released and 500 will be placed inside temporary field cages over knotweed stems (5 cages per site; 100 per cage). APHIS petition for TAG review (see Appendix I) was submitted October 1, 2012 by Fritzi Grevstad. If approved in time, releases in Oregon will start in 2013 and in MA in 2014.

***Assessment of reproduction and establishment.*** Same-year psyllid reproduction will be determined by examining foliage for eggs and nymphs. Next-year recoveries will be by searching foliage for eggs or nymphs. Psyllid density will be quantified as #/stage (E, N, A) per unit of leaf area. Post-establishment, adults will be sampled in April, after their emergence, when plants are small via direct counts in release plots. In May-October, eggs and nymphs will be counted on the top 0.5 m of randomly selected shoots, 3 times/yr. Knotweed stem #/plot, dia, and height will be assessed once per yr (July, August) and reproductive output per m<sup>2</sup> will be estimated by measuring production of flowering structures (in September) for which a weight-to-seed relationship ( $R^2 = 0.95$ ) has been determined (Fig. 6).

## **EXPECTED PRODUCTS AND OUTCOME**

1. Development of genotype markers for two strains of knotweed psyllid and the hybrid.
2. Photographic method for assessing knotweed/ tree seedling competition on river bars.
3. Establishment of the knotweed psyllid in NY, MA, NH and VT
4. Enhanced involvement of TNC in early stages of classical biocontrol projects against environmental pests (invasive weeds).

## BUDGET (YRS 1+2)

### Federal dollars

**1. salaries and fringe**--\$43,410 is requested for salary student hourly or technician help, as follows:

- a. technician--\$25,050 for 2yrs at 0.5 time at \$15/hr, 40 hr/wk, unbenefitted
- b. student hourly--\$18,360 for 2yrs at 0.375 (4.5 mo/yr), \$12/hr, 40hr/wk, unbenefitted

**2. supplies.** \$5000 is requested for cages, tools for cutting and measuring plants, and for rearing psyllids in the laboratory

**3. equipment.** No equipment is requested.

**4. travel.** \$6000 (\$3000 per yr) is requested for travel

**5- subcontract** \$21,640 is requested for subcontract\* to develop genotyping markers for psyllid identification

**Total federal dollars \$76,050**

**Non-federal match (25% of total project) (based on totals for 2 yrs of work)**

Lost indirect (20% of federal dollars) \$15,210

Salary of PI (Van Driesche) \$10,150

**Total match dollars \$25,360**

**Total Project (Federal + UMASS) \$101,410**

### \*Budget for subcontract with UC Berkeley

Salary - GSR IV (4 mo @ \$1796.50/mo)	\$7,186
Benefits - GSR IV (1.8%)	\$129
Genomics facility use fee	\$3,700
Supplies - extraction and library construction for 12 psyllids	\$2,374
Supplies - Next gen sequencing and bioinformatics	\$4,754
Supplies - extraction and testing of SNPs for 96 psyllids	\$3,497
Supplies - total	\$10,625
Total direct costs	\$21,640

## TIMETABLE FOR WORK

### 2013

**Objective 1.** Develop Genotyping Markers for the Northern and Southern Races of Knotweed Psyllid and their Hybrids

**Objective 2** Development and Use of Techniques for Measuring Knotweed Competition with Native Tree Seedlings on River Bars.

### 2014

**Objective 2** Development and Use of Techniques for Measuring Knotweed Competition with Native Tree Seedlings on River Bars.

**Objective 3:** Release and Establish Knotweed Psyllid (*Aphalara itadori*)

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## CV FOR ROY VAN DRIESCHE

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**Education:** BA, BS (Entomol.) Oregon State Univ. 1970; Ph.D. (Entomol.) Cornell Univ. 1975

### Professional Experience

2012-joined Dept of Environmental Conservation, UMAS

2007-present- no longer Chair, just Full Professor in dept.

2004-2007, Chair of Entomology Division within merged PSIS Department

2001-2004-Chair of the Department of Entomology, University of Massachusetts

1986-present -Extension Prof., Dept. Entomology, Univ. Massachusetts, Biological Control Specialist, with emphasis on (1) floriculture pests, (2) classical biological, and (3) parasitoid host range estimation and non-target effects of biological control

1976-1986--Professional Staff--Department of Entomology, University of Massachusetts; Pesticide Coordinator; duties included: (1) development of extension programs for pesticide applicator training (2) conduct of research on parasitic Hymenoptera, especially methods of field evaluation of impact on host populations

1975--Oregon Department of Agriculture; Pesticide Mis-Use and Registration Specialist

### Selected Publications 138 (110 in referred journals)

Van Driesche, R.G. 1983. The meaning of "percent parasitism" in studies of insect parasitoids. *Environ. Entomol.* 12: 1611-1622.

Bellows, T.S. Jr., R.G. Van Driesche and J.S. Elkinton. 1992. Life-table construction and analysis in the evaluation of natural enemies. *Annu. Rev. Entomol.* 37: 587-614.

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Van Driesche, R. G., K. Idoine, M. Rose, and M. Bryan. 1998. Evaluation of the effectiveness of *Chilocorus kuwanae* (Coleoptera: Coccinellidae) in suppressing euonymus scale (Homoptera: Diaspididae). *Biological Control* 12: 56-65.

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## CV FOR FRITZI S. GREVSTAD

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### EDUCATION

1998. Ph. D. Cornell University, Ithaca, New York (Ecology and Evolutionary Biology)

1989. Bachelor of Science. University of Washington, Department of Zoology

### PROFESSIONAL EXPERIENCES

**2010-present Assistant Professor/Senior Research.** Department of Botany and Plant Pathology, Oregon State University. Currently serving as the lead scientist in the development of biological control programs for invasive knotweeds (*Fallopia* spp.) in North America and gorse (*Ulex europaeus*) in the Pacific Northwest. Responsibilities include pre-release investigations of candidate biocontrol agents, monitoring of the distribution and impacts of released agent populations, research to enhance biocontrol effectiveness, writing of scientific papers and reports, mentoring students, proposal development, and supervising research staff.

**2000-2010. Biological Control Specialist.** Olympic Natural Resources Center, Univ. of Washington, Seattle, WA. Lead scientist for the development and implementation of a biological control program for *Spartina* spp. in intertidal estuaries in Washington. Initiated pre-release investigations for biological control programs against invasive knotweeds and gorse.

**1998-1999. Postdoctoral Research Associate.** Department of Entomology, Oregon State University, Corvallis, OR.

**1989-1990. Research Technician** Zoology Department, University of Washington, Seattle, Washington.

### KEY PUBLICATIONS

Grevstad, F.S., R. H. Shaw, R. Bouchier, P. Sanguaneko, G. Cortat, and R. Reardon. 2012. A comparison of the efficacy and host specificity of two populations the psyllid *Aphalara itadori* for biological control of invasive knotweeds in North America. In press for *Biological Control*.

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## CV FOR Jeremy Andersen

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### Education:

2009 – Present University of California Berkeley, Ph.D. anticipated 2014  
2006 – 2009 University of Massachusetts Amherst, M.Sc., Organismic and  
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2001 – 2006 University of Massachusetts Amherst, B.Sc. (Honors), Biology

### Awards and Fellowships:

2010 to present EPA STAR Fellow – “Revisiting the success of natural enemies to  
provide sustainable ecosystem services and reduce pollution.”  
2010 and 2011 Margaret C. Walker Fund recipient

### Recent Presentations:

2011 International Meeting, “Non-invasive DNA extraction techniques for parasitic  
hymenoptera.” Second Entomophagous Insects Conference. Antibes, France.  
June 20th-23rd.  
2010 International Meeting, “Cryptic diversity in *Nuculaspis californica* and its  
implications for theories on local host adaptation.” Joint Meeting of the Society  
for the Study of Evolution, Society of Systematic Biologists, and American  
Society of Naturalists. Portland State University, June 25th – 29th

### Publications:

Andersen, J.C. and N.J. Mills. Accepted. DNA extraction from museum specimens of parasitic  
hymenoptera. PLoS ONE. DOI: 10.1371/journal.pone.0045549  
Gwiazdowski, R.A., I.M. Veal, J.C. Andersen, and B.B. Normark. 2011. Discovery of cryptic  
species among North American pine-feeding *Chionaspis* scale insects (Hemiptera:  
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*aguacatae* and several congeners (Hemiptera: Diaspididae). Annals of the Entomological  
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**Education:** *University of East Anglia, Norwich, Norfolk, England*  
1975 B.Sc. (Hons) Biological Sciences, 1st Class  
1979 Ph.D. Predator-prey interactions

**Employment:** *Oxford University, Oxford, England*  
1978-82 Junior Research Fellow, Lincoln College  
*CAB International, Institute of Biological Control*  
1982-87 Senior Forest Entomologist, European Station, Switzerland  
1988-90 Scientist-in-Charge, UK Station, Silwood Park, England  
*University of California at Berkeley*  
1990-96 Assistant Professor of Entomology  
1996-02 Associate Professor of Entomology  
2002- Professor of Entomology

**Honors and Awards:**  
1975 Final Examination Prize in Biology, University of East Anglia, UK  
1978-82 Cook Junior Research Fellowship, Oxford University  
1989-90 Hon. Lecturer, Dept. Applied Biology, Imperial College, London  
1997 DANR Distinguished Service Award, Outstanding Faculty  
2002 CNR Distinguished Teaching Award  
2012 ESPM Outstanding Mentorship of Graduate Students Award

**University and Professional Service:**  
1997-00 Advisory Committee, USDA National Biological Control Institute  
1997-03 Head Advisor, ESPM Graduate Program, UC Berkeley  
1998- Co-Director, Center for Biological Control, UC Berkeley  
1999-04 Chair, Biological Control Workgroup, UC Statewide IPM Project  
2001- Curator, Essig Museum (Entomology), University of California, Berkeley  
2002-04 Vice-President, International Organization for Biological Control (NRS)  
2004-07 Department Chair, ESPM, University of California, Berkeley  
2009 Member, Light Brown Apple Moth Review Committee, National Research Council of the National Academies

**Editorial Service:** Associate Editor, *Ecological Entomology*  
Editorial Boards of *PLoS ONE* and *Biological Control*

**Research Interests:** Invasive species, biological control, ecology of natural enemies

**Publications:** 139 refereed publications in scientific journals and books

## CV for Christian O. Marks

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**Current Position.** As floodplain forest ecologist for the Nature Conservancy, I study how physical and ecological processes affect floodplain forests of the Connecticut River basin. I apply this knowledge to identifying the most resilient sites for conservation, developing improved methods for restoring floodplain vegetation and managing invasive species, quantifying the magnitude of river flows needed to restore natural floodplain forest dynamics, and developing measures of conservation activity effectiveness.

### Education

(1) **McGill University** (Montreal, Quebec, Canada). Ph.D. 2005, Biology. Thesis title: "Evolution of functional diversity in tree seedlings." Dean's honor roll for PhD thesis (top 10% of theses at McGill University). J. Stan Rowe award from the Canadian Botanical Association for the best paper in ecology published by a student at a Canadian university 2007. Runner-up for the ESA Theoretical Ecology Section Sixth Annual Alfred J. Lotka and Vito Volterra prize for best student talk at ESA conference 2005.

(2) **Massachusetts Institute of Technology** (MIT, Cambridge, MA, USA) S.M. 1997. Mechanical Engineering. Thesis title: "Removal of hexavalent chromium from a contaminated soil by electroremediation".

(3) **University of New Brunswick** (UNB, Fredericton, NB, Canada) B.Sc.E. 1995. Mechanical Engineering. Thesis title: "Experimental measurement of gas compressor valve dynamics". Samuel Peters prize for top mechanical engineering graduate from UNB 1995.

### Professional experience

2007-present. Applied Research Scientist, The Nature Conservancy, Connecticut River Program, Northampton, MA.

2005-2007. Postdoctoral Research Associate, Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, USA.

1998-2005. Research Assistant, McGill University, Biology Department, Montreal, Quebec, Canada.

1997-1998. Project Engineer, Thyssen Production Systems, Ravensburg, Germany, and Detroit, USA.

1995-1997. Research Assistant, Mechanical Engineering Department, Fluid Mechanics Lab, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA.

### Selected Publications (of 9)

(1) Nislow, K.H., Lutz, K.A., **Marks, C.O.** (in press) Aquatic Conservation Planning in the Northern Appalachian Ecoregion. In *Landscape-scale Conservation Planning*, editors S.C. Trombulak and R.F. Baldwin. Springer, NY: 99-120

(2) **Marks, C.O.**, and Muller-Landau, H.C. (2007). Comment on "From Plant Traits to Plant Communities: A Statistical Mechanistic Approach to Biodiversity". *Science* 316: 1425c

(3) **Marks, C.O.**, and Lechowicz, M.J. (2007). The ecological and functional correlates of nocturnal transpiration. *Tree Physiology* 27: 577-584

(4) **Marks, C.O.**, and Lechowicz, M.J. (2006). Alternative functional designs and the evolution of diversity. *The American Naturalist*. 167: 55-66

## Figures



Fig. 1. The psyllid *Aphalara itadori*



Fig. 2. Damage caused by *A. itadori*.

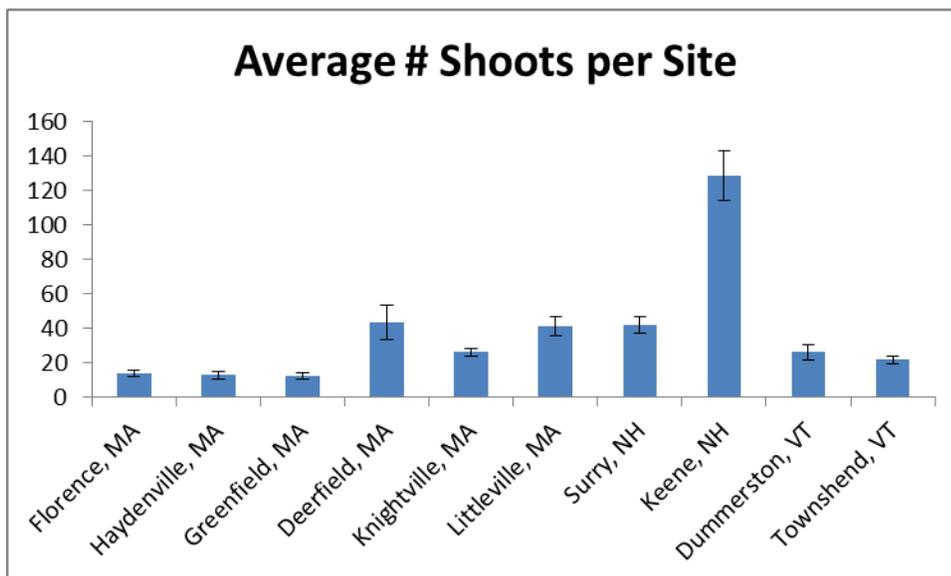


Fig. 3. Knotweed stem densities per m<sup>2</sup> at the ten research plots in 2012.

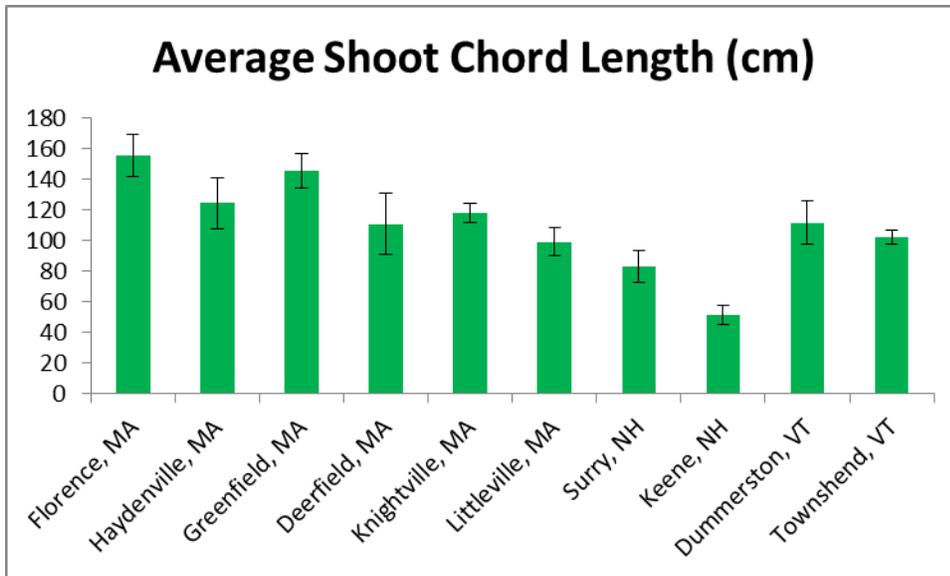


Fig. 4. Average chord length (cm) of knotweed stems in plots at ten research sites in 2012.

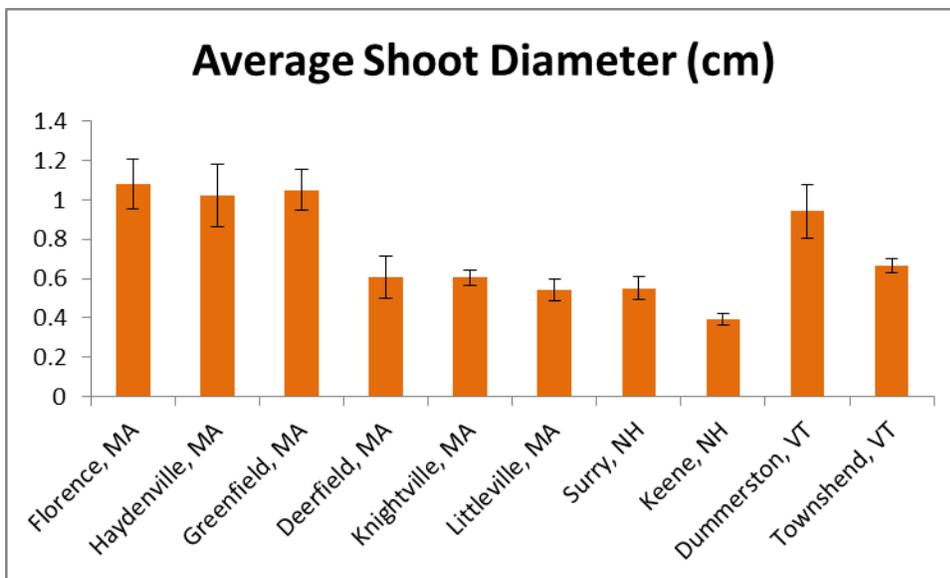


Fig. 5. Average diameter (cm) of knotweed shoots at ten research sites in 2012.

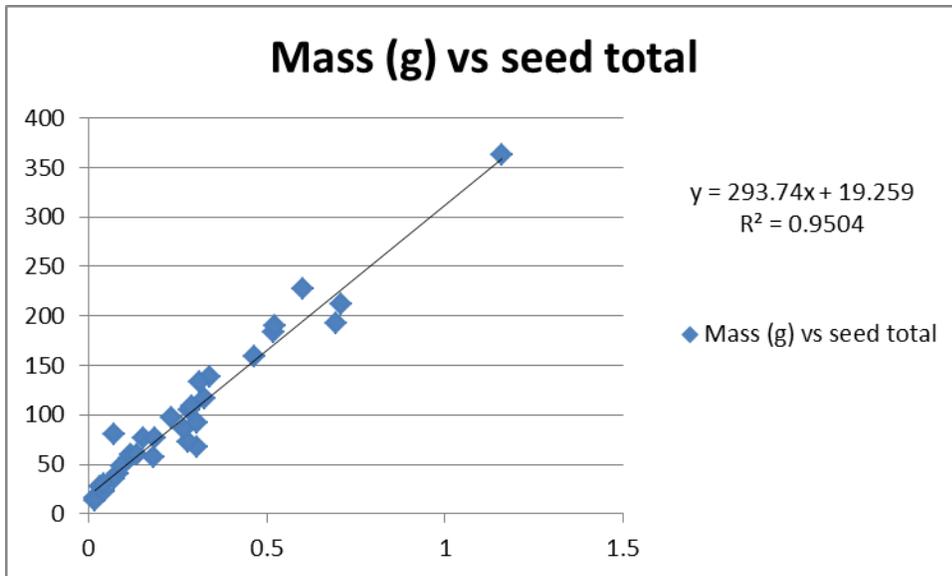


Fig. 6. Total inflorescent weight (g) on Y axis (after seed removal) to total number of seeds (X axis) for samples taken at peak seed abundance for knotweed at Greenfield, MA, full sun research site in 2012.

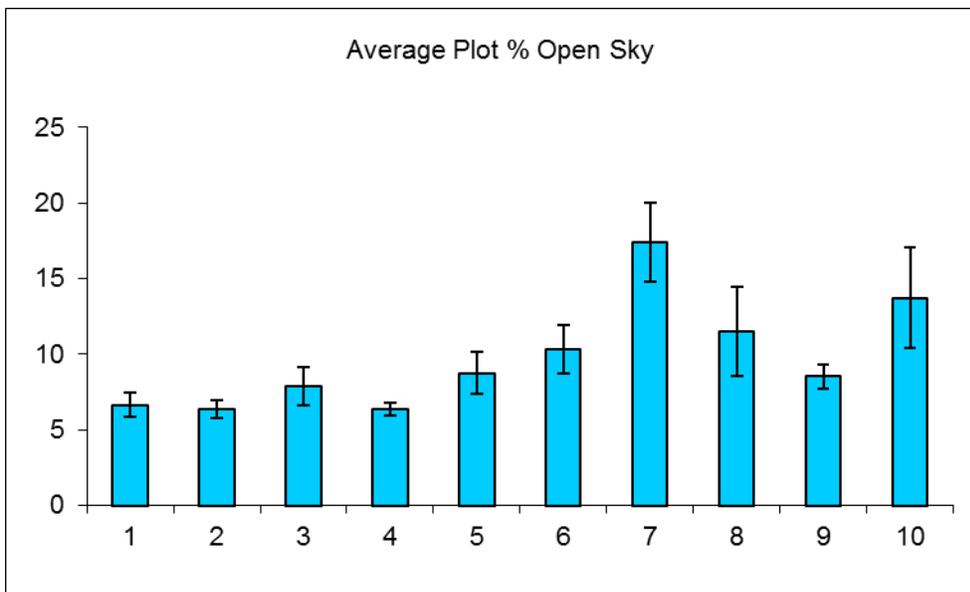


Fig. 7. Percentage of open sky as measured with hemiphotos under knotweed canopies in plots at the ten research sites in 2012, taken on overcast days after maturity of knotweed stands, showing that with one exception knot weed canopies reduce light at ground level by more than 85%.

# Plot 1



Fig 3A

# Plot 1 - Highlights



Fig 1B

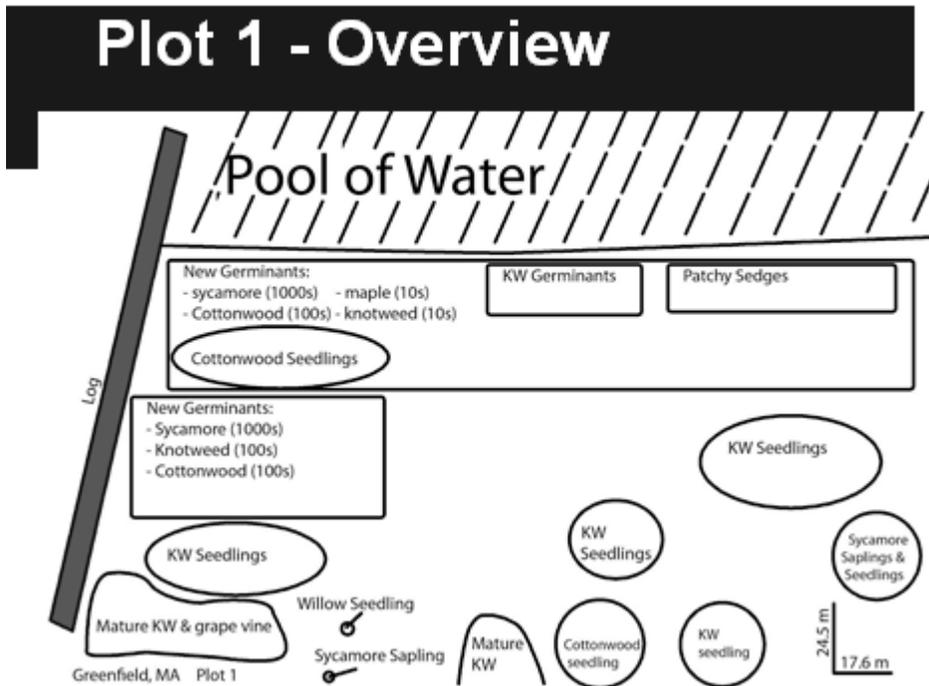


Fig 3C

## Plot 1 - General Information

Time:	11:00am
Weather:	blue skies
Approx. % Vegetation:	60
Approx. % Silt:	40
Approx. Area (m):	24.5m by 17.6m
GPS area Coords:	18T 0695205 UTM 4721298
GPS area error (ft±):	16.4
GPS photo Coords:	18T 0695205 UTM 4721311
Compass Direction to plot:	212
GPS photo error (±ft):	14.6
Camerman postion:	from across pool of water
<b>Note:</b> Human disturbance in the form of a fire pit and atv tracks	

Fig 3D

Fig 8. Example of visual records for bar plot competition studies between Japanese knotweed seedlings and seedlings of native trees: A (yearly photo of plot, here 2012), B (annotated and colored photo to show types of plants), C (further annotation of number of plants in plot by type), and D (location and description of plot).