Project title: Suitability of the gall forming mite *Aceria genistae* for redistribution as a biological control agent of Scotch broom (*Cytisus scoparius*) in the western USA: Is the enemy of my enemy a friend?

Principal investigator:
Paul D. Pratt, Ph.D., USDA/ARS, Exotic and Invasive Weeds Research Unit, 800 Buchanan St., Albany CA 94710, Ph. 510-559-6127, Fax: 510-559-5963, Cell: 925-222-9483, Email: Paul.Pratt@ars.usda.gov

Cooperators and other participating institutions:
David Bakke, Invasive Plants Program Manager, USFS, 1323 Club Drive, Vallejo, CA 94592, Ph. 707-568-8916, dbakke@fs.fed.us.
Michael J. Pitcairn, Ph.D. California Department of Food and Agriculture, Biological Control Program, 3288 Meadowview Road, Sacramento, CA 95832. Ph. 916-262-2049, Email: mike.pitcairn@cdfa.ca.gov
Steven A. Nadler, Ph.D. University of California Davis, Department of Entomology and Nematology, Briggs Hall 367, Davis CA 95616. sanadler@ucdavis.edu

Amount requested: $66,596 ($33,298 per year for two years). Project leveraging: $128,488 per year (USDA-ARS: 84,130; UC Davis: 35,252; CDFA: 9,106)

Project goals:

1. Determine the host specificity of the Scotch broom mite *Aceria genistae*.
   a. Quantify non-target use under field conditions in the few areas that the mite has colonized in California forests.
   b. Conduct host range tests to quantify environmental safety of this mite.
2. Submit an APHIS permit request to redistribute the mite to Scotch broom infested forests.
   a. Compile data and prepare a redistribution proposal for consideration by regulatory agency (TAG, APHIS).

Project justification and urgency:

The European shrub *Cytisus scoparius* (L.) Link (or Scotch broom) was first introduced to the Pacific Northwest of the United States in the 1850s as an ornamental (Gilkey 1957, Bossard and Rejmánek 1994) but was also intentionally planted for erosion control (Geickey 1957, Schwendiman 1977). Like other exotic broom species in the region, *C. scoparius* is a leguminous shrub with erect stems that typically grow 1-3 m tall (Coombs et al. 2004). Naturalized populations of *C. scoparius* often form dense thickets and, as a result, the weed is regarded as a noxious pest in forests and natural areas throughout the Pacific coastal region of North America from British Columbia to central California.

Scotch broom displaces native and important forage species as well as complicates reforestation efforts following disturbance. The exotic shrub invades undisturbed shrub and open forested systems below 1300 m elevation in the Pacific coastal states but is also common in disturbed areas like river banks, road cuts, and reforested lands. Competitive superiority over most native flora is facilitated by high reproductive output and long-lived seed bank. Moderately sized plants can produce in excess of 12,000 seeds a year, which can be self-dispersed through explosive dehiscence from the pea-shaped pods or moved by ants, rain or machinery (Bossard 1991). Economic analyses indicate that Scotch broom invasions cost reforestation efforts an estimated $47 million annually in Oregon alone (Coombs et al. 2004).

Classical weed biological control of *C. scoparius* in the United States was first implemented in the 1960s. Two specialist natural enemies, originating from the weed's native range, were released into the USA: the stem miner *Leucoptera spartifoliella* (Hubner) (Lepidoptera: Lyonetiidae) released in California in 1960 and the seed beetle *Exapion fusciostre* (Fabricius) (Coleoptera: Brentidae) released in California in 1964, in Oregon in 1983, and in Washington in 1989 (Coombs et al. 2004, Hosking et al. 2012). At least 10 other exotic herbivores also attack *C. scoparius* in the USA (enumerated in Coombs et al. 2004) but most notably *Bruchidius villosus* Fabricius (Coleoptera: Chrysomelidae), which was later intentionally released in Oregon and Washington after being discovered in North Carolina where it was not intentionally released (Coombs et al. 2004). This suite of natural enemies, while increasing the amount of stress on plant growth and thereby limiting the annual seed crop, do not collectively provide sufficient population regulation to alter the prevalence or invasion trajectory of *C. scoparius* in the United States.

A biological control program targeting *C. scoparius* was conducted by New Zealand and Australian scientists, resulting in the development of the European broom gall mite *Aceria genistae* (Nalepa) (Acari: Eriophyidae) (Figure 1). Following nearly a decade of improving rearing methodologies, host range testing, and clarifying taxonomic uncertainty (Saligocco et al. 2011, Xue et al. 2015), *A. genistae* was deemed sufficiently host specific for introduction and was subsequently released in New Zealand in 2007 and Australia in 2008-2010 (Paynter et al. 2012, Hosking et al. 2012). The gall mite established in both countries, with 100s of individual galls developing on attacked plants in some areas and early evidence of stem dieback on plants with high gall densities (Sagliocco et al. 2011).

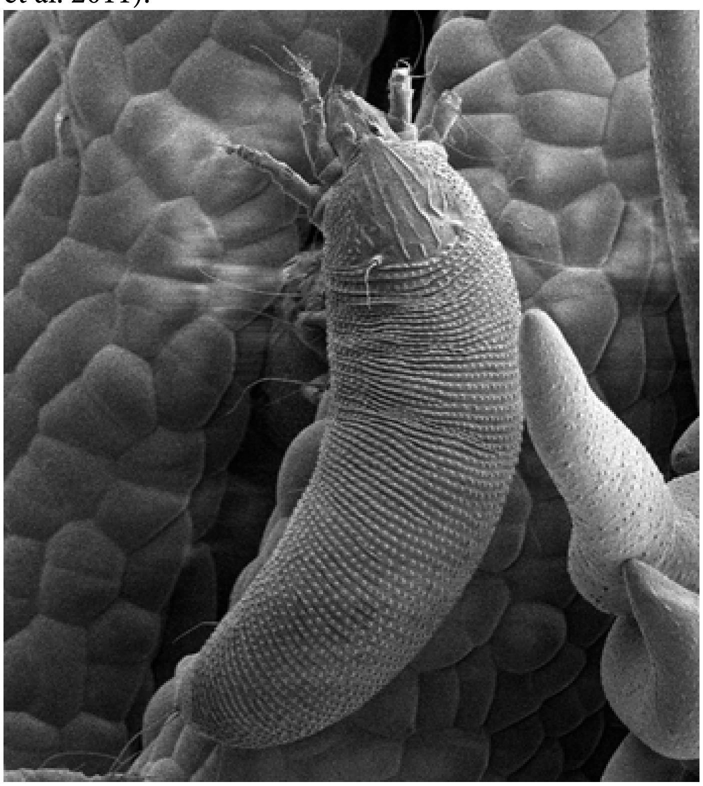

Figure 1. The Scotch broom gall mite, *Aceria genistae*

Surprisingly, an adventive population of *A. genistae* was discovered in 2005 near the city of Tacoma, in western Washington, USA. This extension of the herbivore's geographic range inspired surveys for the mite in *C. scoparius* infested lands from British Colombia, Canada, through the states of Washington, Oregon, and California. Feeding by *A. genistae* transforms meristematic buds into diagnostic galls that consist of deformed foliar tissue that collectively forms a round, pubescent mass arising from a foliar bud (Sagliocco et al. 2011; Figure 2). In California, Department of Food and Agriculture scientists surveyed select *C. scoparius* stands in El Dorado and Siskiyou Counties periodically from 2010 through 2014 but no *A. genistae* galls were discovered. A population of *A. genistae*, however, was discovered nearly 300 km south of the Oregon border in April 2014 when a land manager discovered abnormal growth on *C. scoparius* plants near Georgetown, El Dorado County, California and contacted the regional United States Forest Service office and University of California Cooperative Extension service for diagnosis. A site visit revealed galls visually similar to those produced by *A. genistae* as reported in the literature. In June 2014, a second population was discovered near the town of Happy Camp in Siskiyou County, near the Oregon border. Additionally, reports from land managers and foresters were collected by UC Cooperative Extension (Scott Oneto) and a database of field observations was developed. Systematic surveys of *C. scoparius* stands in 2015 and 2016 revealed a relatively small distribution of the mite in California (Figure 3). In these areas, however, the damage caused by the mite was striking, with some individual plants possessing 100s of galls and others killed outright by what appears to be an exceptionally high density of mite galls. The mite's impact and limited range have led several land managers to request that the USDA and CDFA redistribute the mite to other *C. scoparius* infested areas. Unfortunately, this is currently not an option as the mite is not permitted for release or redistribution in the US.

While the mite's diet breadth was studied previously, its safety in relation to the flora of California remains unknown. I propose quantifying the host range of *A. genistae* by monitoring for non-target use in areas where the mite is already established and has developed large densities. My research team will also conduct laboratory-based host range tests for species that are not sympatric with *C. scoparius*. Data collected from these studies will be integrated into a release proposal that will be submitted to APHIS' Technical Advisory Group for consideration.

Figure 2. *Aceria genistae* gall formed on Scotch broom (*Cytisus scoparius*) at Georgetown, CA

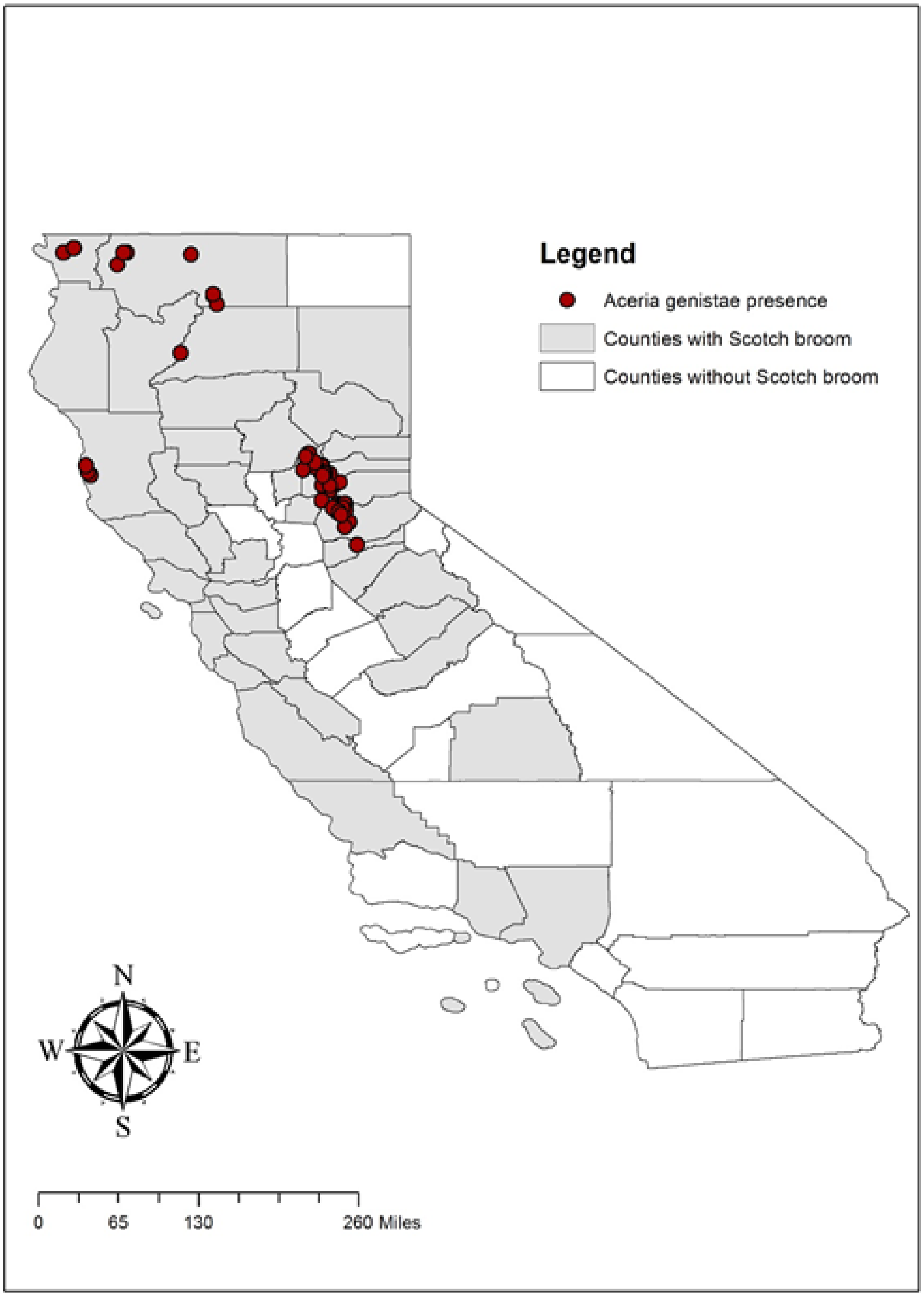

Figure 3. Current geographic distribution of the Scotch broom gall mite (*Aceria genistae*) in California

Preliminary field observations suggest that this mite is a very effective agent that, at long last, will provide effective control of this serious invasive shrub. It is critical to obtain a field-release permit so that the mite can be effectively incorporated into the integrated weed management and revegetation protocols now used by local land managers.

Approach:

This study will build upon the growing body of literature that documents non-target risks from herbivores with narrow host ranges is greatest for species that are closely related (phylogenetically) to their primary or preferred host (Pemberton 2000, Pratt et al. 2009). The methods used herein will investigate the use (i.e., galling or feeding damage) by *A. genistae* of species that are close relatives of Scotch broom and thus at greatest risk, including other broom species (exotics) and native *Lupinus* species. Two experiments will be conducted:

1. **Survey of non-target use in-situ:** The distribution of *A. genistae* in California is being monitored by USDA and CDFA scientists. Current populations are limited to select Scotch broom patches in Mendocino, Del Norte, Siskiyou, Shasta, Nevada, Amador, El Dorado and Placer Counties (Figure 3). Areas of high mite densities will be surveyed for brooms and native Lupinus species and in-situ assessments of host use will be quantified. This will be done by searching for non-target species within 100 meters of a heavily infested Scotch broom stands. Searches will take advantage of known herbaria records (Consortium of California Herbaria, Calflora), expert opinions from local land managers, and personal observations to locate brooms and Lupinus. Surveys will be done in March through June when many of the native Lupinus species are in flower. Non-target plants discovered near *A. genistae* infestations will be searched for signs of deformed growth or surface feeding that may be attributed to mites. Five randomly selected plants, or cuttings in the case of rare species, will be collected and examined further in the laboratory (microscopy may be needed). Herbarium specimens will be prepared to document the collection site and used to confirm identification of the test plant with *Lupinus* taxonomic specialists.

   Mites collected on the non-target plants will be preserved in 95% EtOH and submitted to USDA's Systematic Entomological Laboratory for identification. Data collection will include plant species evaluated, distance to *A. genistae* population, number of individuals or patch size assessed, proportion of non-target individuals damaged or galled, plant identification, and GPS coordinates of the site. While much of these data are observational and simple means of non-target use will be reported, the influence of distance from an *A. genistae* population on host plant use as well as proportion of plants attacked will be compared with ANOVA (Pratt et al. 2003). Evidence of host use in the field will inform which plants will be used in host range testing (see below). It should be noted that the gall forming midge Dasineura lupini also attacks lupine species but these galls are morphologically distinct from *A. genistae* galls so there is no risk of misidentification.

2. **Host range testing:** Host range testing will be conducted outdoors under natural environmental conditions at the Exotic and Invasive Weeds Research Unit facility in Albany, CA. Test plants will be placed on one of six bench tops in a randomized complete block design, with the position of each plant species within a group assigned randomly. Plant height and width will be measured at the start of the experiment in order to obtain an estimate of average plant size per species. Drip irrigation will be provided, with the number of emitters per pot dependent on plant size and water requirements.

   Inclusion of test plants will be influenced on species discovered in proximity to field surveys described above. However, three broom and four perennial lupine species have already been acquired for the study based on known sympatric distributions: Scotch broom (*Cytisus scoparius*), French broom (*Genista monspessulana*), Spanish broom (*Spartium junceum*), big leaf lupine (*Lupinus polyphyllus*), silver bush lupine (*Lupinus albifrons*), Parish's stream lupine (*Lupinus latifolius* var. *parishii*), and yellow coastal bush lupine (*Lupinus arboreus*). Five additional annual lupines have been identified and will be acquired or propagated when funding is secured.

   Scotch broom galls containing *A. genistae* mites will be collected from field sites in the Sierra foothills (near the towns of Volcano, Amador County, and Garden Valley, El Dorado County) by clipping stem sections containing galls, and transporting to the lab in zip lock bags within coolers. In an effort to standardize mite inoculations, galls will be measured with calipers (length and width), so that a roughly equal volume of gall material can be attached to each test plant with twist ties. Regression analysis will be used to quantify the number of mites per gall volume from previously collected monitoring samples, thus estimating the number of mites that each test plant receives. Inoculations will continue approximately once per month, as long as fresh galls are available in the field.

Approximately 3 weeks after inoculation and at monthly intervals thereafter, test plants will be visually assessed for any signs of discoloration or deformity that could be attributed to *A. genistae* feeding. Photographs will be taken of any plants with galls or deformed foliage. A short section of stem will be clipped from each plant and viewed under a microscope to assess for mite presence. The number of mites present will be counted at monthly intervals following inoculation, and pertinent behavioral observations recorded (i.e. mites crawling on stem or settled on developing leaf buds) which may help determine relative host plant acceptance. Counts of mites per sample and galls per plant will be compared with ANOVA, using the randomized complete block design.

Expected outcomes:

Preliminary (unreplicated) surveys have already been conducted in the field, and initial results indicate that *A. genistae* does not exploit French (*Genista monspessulana*) or Spanish (*Spartium junceum*) broom species, even when vegetation is intermingled. Casual observations of *Lupinus albifrons* and *Lupinus bicolor* also revealed no *A. genistae* host use. These initial assessments are very encouraging and support host range testing results from Australia and New Zealand (Paynter et al. 2012). However, formal host range testing of US species will be required to obtain an APHIS permit to redistribute the gall mite. This preliminary evidence provides strong support that funds used to investigate the host use patterns of *A. genistae* will result in finding of a narrow host range and no direct risk to other non-target plants (Pratt et al. 2009). Therefore, the expected outcomes of this research include acquiring an APHIS permit to redistribute and possibly inundatively release the mite in Scotch broom infested sites throughout the Western US. We expect these releases to markedly decrease the invasion potential of Scotch broom, kill or facilitate mortality of the weed, and aid with integrated pest management of the weed in forests of the Western US. This will ultimately reduce the amount of space and resources taken up by Scotch broom and save land managers time and limited funds that can be redirected to other priorities.

Budget: Year 1 (April 1, 2017 through May 30, 2018

Cost elements		USFS contribution	ARS contribution	UC Davis contribution	CDFA contribution
Direct costs					
Salaries					
Technical assistance (Clayton Sodergren)	1 FTE for 12 months	22,008		14,672	
Technical assistance (Matthew Perryman)	0.25 FTE for 12 months	10,290		20,580	
Technical assistance (John Herr)	0.5 FTE for 12 months		41,160		
Technical and supervisory assistance (Michael Pitcairn)	0.05 FTE for 12 months				8,606
Fringe benefits					
Sodergren	USDA benefits rate = 30% of salary	0	15,720		
Perryman	USDA benefits rate = 30% of salary	0	22,050		
Total salary and benefits		32,298	78,930		
Travel (field site surveys, some overnight travel, fuel, etc.		1,000	4,000		
Supplies (potting soil, irrigation system, shade cloth, etc.		0	1,200		
Totals		**33,298**	**84,130**	**35,252**	**9,106**

Timetable for Year 1 funding. Note that, as a two year project, these efforts will be replicated in the second seadon (2018 - 2019).

Research activity	**2017**									**2018**					
	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June
Survey of non-target in situ															
Field surveys	X	X	X	X	X						X	X	X	X	X
Confirm taxonomy					X	X									
Analyze data						X	X								
Host Range Testing															
Acquire test plants	X	X	X												
Inoculate test plants			X	X	X	X	X	X	X	X	X	X	X	X	
Evaluate host use				X	X	X	X	X	X	X	X	X	X	X	
Analyze data													X	X	X

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