Biological Control Program Annual Report 2018

California Department of Food and Agriculture

Biological Control Program

2018 Summary

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The CDFA Biological Control Program greatly appreciates the many biologists and agriculture commissioners throughout the state whose co-operation and collaboration made this work possible.

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Preface

C. H. Pickett and M. J. Pitcairn

This edition of the Biological Control Program Annual Report is the first since the program was refunded in 2017. Due to severe budget reductions in fiscal year 2011-12, the California Department of Food and Agriculture (CDFA) discontinued support for biological control activities, therefore eliminating a stand-alone Biological Control Program. Biological control work continued on a smaller scale for federally funded projects within California during that break. Recently, representatives of the agricultural industry in California asked for a renewal of biological control activities for California agriculture.

The Program now comprises two Senior Environmental Scientist Supervisors, Dr. Charles Pickett and Dr. Michael Pitcairn. Dr. Bill Roltsch retired in 2017. Ms. Viola Popescu, previously a part-time employee with CDFA, and Dr. Chris Borkent were recently hired as full-time Environmental Scientists. Dr. Borkent is trained in systematics and came to the program with a great deal of insect collecting expertise. He previously worked with CDFA's Plant Pest Diagnostics Center. Ms. Popescu has her Master's degree in Agronomy and a great deal of field experience working with the Biocontrol Program.

Highlights of insect projects

Most of the Program's activities the past two years have centered around two stink bugs new to California. The bagrada bug was first detected in California in 2008, recently entering central California. This bug represents a serious problem to cole crops, especially those produced under organic certification. The second stink bug of concern is the brown marmorated stink bug, which was confirmed as established in Los Angeles County in 2006. The species has now spread to 17 additional counties and has been detected in 18 others. Until recently it has only been a nuisance pest, residing in people's homes during winter months. Now it has become an agricultural pest with reports of damage to almond and peach. The candidate parasitoid for this stink bug, *Trissolcus japonicus*, was just discovered in southern California.

Highlights of weed projects

Scotch Broom is an invasive plant found along California's central, coastal and Sierra Nevada foothill regions. The weed displaces native vegetation, burns readily, and makes reforestation difficult. A non-native gall mite, *Aceria genistae*, that causes severe damage to Scotch broom has been found in California. Field surveys show that the mite occurs in 11 counties, from Siskiyou County south to Amador County. Research by the United States Department of Agriculture, Agricultural Research Service's Invasive Species and Pollinator Health Research Unit (USDA-ARS-ISPH) is examining its host specificity in the field and documenting its impact on Scotch broom.

The Biological Control Program has been assisting the USDA-ARS-ISPH Research Unit in the mass production of the plant hopper, *Megamelus scutellaris*. This plant hopper is a biological control agent for water hyacinth, a plant that has caused serious problems with navigation and water management in California's water system. In 2018, over 65,000 planthoppers were released in the Sacramento-San Joaquin River Delta, of which 40% were produced by CDFA.

The gall wasp, *Aulacidea acroptilonica*, forms galls in stems of Russian knapweed reducing flower and seed production. Russian knapweed is an exotic weed of pastures throughout California. Releases of the gall wasp have resulted in establishment at field sites in Siskiyou and Lassen counties. Efforts are underway to release and establish this beneficial insect on Russian knapweed infestations in Central Valley of California.

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Weed Projects

Biological Control of Cereal Leaf Beetle in Northern California

Charles H. Pickett, Viola Popescu, Chris Borkent, and Rob Wilson[1]

Cereal leaf beetle (CLB), *Oulema melanopus* (L.) (Coleoptera: Chrysomelida) is a serious pest of small grain crops and forage grasses. Feeding by adults and larvae can cause up to 25% yield loss, if left unchecked. CLB, native to Eurasia, was first reported in North America in 1962 in Michigan. It has since spread throughout most of the United States (US) and Canada. However, no findings were reported in California until 2013. It has since spread throughout the Klamath Basin area of northern California, apparently moving south from Oregon. The larval parasitoid, *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae) and egg parasitoid *Anaphes flavipes* (Foerster) (Hymenoptera: Mymaridae) were first imported from Europe into mid-western US and provided good to excellent control of this pest in the 1970's. The former parasitoid, introduced into the Pacific Northwest around 2000 has provided excellent control of CLB (Roberts and Rao 2012). The main goal of this ongoing project is to establish nursery sites along the leading edge of the expanding CLB population in northern California. The immediate benefit is the permanent establishment of an excellent biological control agent for CLB in California. This is the first step in achieving successful biological control of this pest. The benefits of successfully CLB biologically are numerous: higher yields, significantly lower production costs and substantially lower pesticide use.

Results

Objective 1. Survey northern California for cereal leaf beetle.

Weekly surveys beginning late May 2018, continuing through late July, in Modoc, Lassen, and Siskiyou Counties were conducted for presence of CLB (Table 1). The goal of this survey is to find additional release sites for parasitoids of CLB, to determine southern and western limits of the expanding CLB population, and if previously released parasitoids successfully overwintered and spread. Sampled grains included wheat, oats, and barley. Modoc, Lassen, and Siskiyou counties were sampled with the assistance from county staff: Cheryl Lauristen, Lassen Co.; Jolene Moxon and Gary Fensler, Modoc

Co.; and Tony Orr, Siskiyou Co. Two to four sets of 100-400 sweeps were made in most fields (Table 1). Adults and larvae were again found in Modoc County.

Table 1. Sweep net collections of cereal leaf beetle in 2019 from northern California.								
						#cereal leaf beetle		
Date	**grain**	**Name of site (county)[1]**	**Latitude**	**Longitude**	**#Sweeps**	**Adult**	**larvae**	**% parasitized**
June 6	mixed grasses	Oilar – near McArthur (L)	N38.40285	W121.37972	100	0	0	.
	triticale	Buckman (L)	N41.00793	W121.31993	200	0	0	.
	triticale	Buckman (L))	N41.00221	W121.32148	100	0	0	.
	wheat	Ross (L)	N41.03857	W121.33137	200	0	0	.
	pea/triticale mix	Dahle (L)	N41.00551	W121.17807	0	0	0	.
	pea/triticale mix	Dahle (L)	N41.00551	W121.17807	0	0	0	.
	triticale	Dahle (L)	N41.00551	W121.17807	110	0	0	.
	wheat	Near Bieber (L)	N41.11695	W121.12421	200	0	0	.
	wheat	Across from Wild Life Center (L)	N41.14635	W121.09600	200	0	0	.
	wheat	Near Wild Life Center (L)	N41.14660	W121.07766	200	0	0	.
June 13	beardless wheat	Nr. MacDoel near Plant Sciences (S)	N41.81509	W121.99342	300	0	0	.
		Plant Sciences S. PSI-04 (S)	N41.80020	W122.00561	300	0	0	.
	wheat	Nr. MacDoel (S)	N41.80796	W122.05531	300	0	0	.
June 19	wheat	Alturas Ranch 1 (L)	N40.92567	W120.53070	300	0	0	.
	organic wheat	Alturas Ranch 2 (L)	N40.93347	W120.55315	400	0	0	.
	triticale	Kemp Ranch (L)	N41.09444	W120.91721	440	0	0	.
	wheat	Across from Wild Life Center (L)	N41.14635	W121.09600	300	0	0	.
June 20	wheat	David King (M)	N41.83407	W121.34481	400	12	24	78
	winter wheat	Hemp Hill field (M)	N41.84469	W121.34564	250	15	140	8
	winter wheat	Baley Collingsly (M)	N41.86381	W121.36466	150	0	10	73
	wheat	Baley, Stronghold field (M)	N41.89304	W121.41394	350	1	6	57
	wheat	Seus, Tule Shores field (M)	N41.90764	W121.34908	200	5	4	75
	wheat	Akley Ranch, Center Pivot (M)	N41.90764	W121.34912	400	1	3	33
	beardless wheat	Plant Sciences 2 (S)	N41.81509	W121.99342	300	0	0	.
June 27	wheat	Akley Ranch,Center Pivot (M)	N41.90764	W121.34912	200	1	5	80
	winter barley	Huffman (M)	N41.85174	W121.32359	400	0	11	22
	wheat	David King (M)	N41.83407	W121.34481	250	2	77	88
	winter wheat	Hemp Hill field (M)	N41.84469	W121.34564	200	1	9	11

	wheat	Baley Collingsly (M)	N41.86381	W121.36466	300	1	4	0
	wheat	Baley, Stronghold field (M)	N41.89304	W121.41394	200	0	1	0
	Barley	Federal Wild Life Refuge (M)	N41.89337	W121.41909	200	1	1	0
	winter wheat	Federal Wild Life Refuge (M)	N41.89948	W121.41878	200	0	6	0
	wheat	Seus, Tule Shores field (M)	N41.90764	W121.34908	200	0	14	29
July 2	wheat	Akley Ranch,Center Pivot (M)	N41.90764	W121.34912	300	0	3	33
	wheat	David King (M)	N41.83407	W121.34481	200	0	54	84
	winter wheat	Hemp Hill field (M)	N41.84469	W121.34564	200	0	7	17
	wheat	Baley Collingsly (M)	N41.86381	W121.36466	200	0	2	50
	wheat	Baley, Stronghold field (M)	N41.89304	W121.41394	200	0	1	100
	barley	Federal Wild Life Refuge (M)	N41.89337	W121.41909	200	0	1	0
	wheat	Federal Wild Life Refuge (M)	N41.89948	W121.41878	200	0	2	0
	wheat	Seus, Tule Shores field (M)	N41.90764	W121.34908	200	0	2	50
July 10	wheat	Akley Ranch,Center Pivot (M)	N41.90764	W121.34912	300	0	2	100
	wheat	David King (M)	N41.83407	W121.34481	200	0	53	100
	winter wheat	Hemp Hill field (M)	N41.84469	W121.34564	200	0	6	100
	wheat	Baley Collingsly (M)	N41.86381	W121.36466	200	0	0	.
	wheat	Baley, Stronghold field (M)	N41.89304	W121.41394	200	0	0	.
	barley	Federal Wild Life Refuge (M)	N41.89337	W121.41909	200	0	6	100
	wheat	Federal Wild Life Refuge (M)	N41.89948	W121.41878	200	0	1	100
July 19	wheat	Akley Ranch,Center Pivot (M)	N41.90764	W121.34912	300	0	0	.
	wheat	David King (M)	N41.83407	W121.34481	200	0	3	100
	winter wheat	Hemp Hill field (M)	N41.84469	W121.34564	200	0	1	100
	wheat	Baley Collingsly (M)	N41.86381	W121.36466	200	0	0	.
	wheat	Baley, Stronghold field (M)	N41.89304	W121.41394	200	0	0	.
	barley	Federal Wild Life Refuge (M)	N41.89337	W121.41909	200	0	0	.
	wheat	Federal Wild Life Refuge (M)	N41.89948	W121.41878	200	0	0	.

[1](L) represents Lassen County, (M) County, and (S) Siskiyou County

No CLB were found in Siskiyou County, near the town of Macdoel, where this pest was first found last year, and releases were made of *T. julis*. No CLB have yet been found in Lassen County. The parasitoid *T. julis* was found attacking CLB at all sites where CLB was present.

Objective 2. Develop nursery sites for *Tetrastichus julis* in northeastern California.

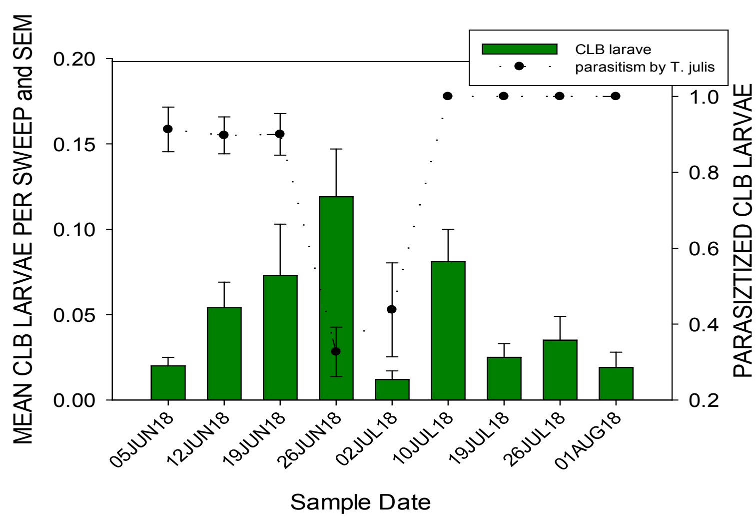
Each year since 2014, a field insectary has been maintained at the University of California Intermountain Research and Extension Center (UC IREC). It is designed to produce the CLB parasitoid *T. julis*. Winter wheat is planted in fall, followed in spring by an adjacent, sequential planting of oats capable of supporting high populations of CLB (Figure 1). At any one time, the optimal stage of oats is available for CLB reproduction. The larger the beetle population, the more parasitoids will be produced for dispersal and release into surrounding small grain farms. The buildup of CLB larvae, and thus parasitoids, is from the first planted grains to most recent. The adults tend to oviposit during the pre-bloom stage. *T. julis* populations are capable of rapid buildup due to a short life cycle and multiple eggs laid within each beetle (Table 2). From 2017 to 2018, those CLB attacked by *T. julis* averaged 6.2 parasitoids each, with a maximum of 25 found in a single host. The insectary also provides a pesticide-free environment to measure changes in CLB and *T. julis* populations. Winter wheat was planted October 31, 2017, first oat planting was April 26, 2018 followed by plantings on May 14th and 31st. Oat and wheat plots were 42 ft by 250 ft, roughly quarter of an acre each.

Figure 1. Panoramic view of UC IREC field insectary, June 12, 2018. Oats1, Oats2, Oats3 refer to the sequence in which oats were planted, Oats1 being first. Respective planting dates, above. Arrow shows movement of CLB population.

Table 2. Number of *T. julis* larvae or eggs per host cereal leaf beetle.				
Year	**Plot**	**Mean larvae per host**	**Maximum number per CLB**	**Number CLB dissected**
2017	Oat1	4.7	11	26
	Oat2	8.5	17	24
	Oat3	5.7	15	53
	Wheat	3.8	8	16
2018	Oat1	8.1	21	76
	Oat2	5.5	13	49
	Oat3	5.7	28	94
	Wheat	7.6	25	26
	average	**6.2**		

The 2018 buildup of CLB at the UC IREC followed a similar pattern with past populations in oat plots peaking in late June, then decreasing (Figure 2). Parasitism of CLB larvae was >80% throughout the sampling period except for a drop in late June early July. A similar dip in parasitism was observed in previous years, which was explained by an uncoupling of the parasitoid and host population as a result of unusually warm periods in early spring (Evans et al. 2012), thereby allowing the CLB population to

Figure 2. Cereal leaf larvae and proportional parasitism, UC Intermountain Research and Extension Center, 2018



briefly out-reproduce the parasitoid. The most striking find was that larval parasitism reached 100% and stayed at this level for the last 4 weeks of sampling. All 2nd-5th instar larvae dissected had one or more developing parasitoids.

Objective 3. Move parasitoids to additional 'hot spots' of cereal leaf beetle.

Regional parasitism in commercial small grains (usually some variant of wheat) demonstrates that *T. julis* is now well-established in the Klamath Basin of Modoc County (Table 1). Although some fields had zero parasitism, many had 100% (Table 1). No additional releases of *T. julis* were made in 2018 because all new sites had high levels of CLB parasitism when first sampled (Table 1). Annual trends at UC IREC in CLB seasonal parasitism show a rapid increase since 2014 (Figure 3). Many of the developing parasitoids likely dispersed into surrounding areas. Parasitism has reached exceptionally high levels at the UC IREC, however CLB continues to persist on oats. We chose oats for the insectary since they are known to be highly susceptible to CLB infestation. Despite the buildup of CLB in 2018, population numbers did not result in visible damage to the crop. CLB on winter wheat grown at the insectary have remained at low levels, never averaging more than 0.02 larvae per sweep over the season while the same larvae exceeded 0.06 per sweep on oats in 2015. Regional levels of CLB on small grains recorded from surrounding commercial farms show CLB numbers are trending to lower levels than recorded from oats in the insectary (Table 3, Figure 3).

Figure 3. Seasonal trends in cereal leaf beetle and *T. julis*. Field Insectary. UC Intermountain Research and Extension Center. Oats combined.

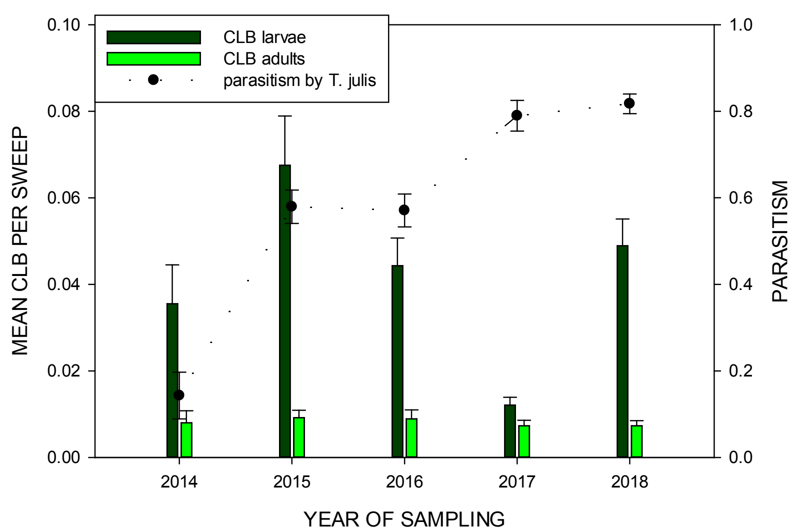


Table 3. Seasonal number of cereal leaf beetle per sweep in survey samples of wheat and related commercially grown grasses, and associated parasitism by *T. julis*. 2018 in Modoc and Siskiyou counties.					
	Cereal leaf beetle			**Parasitism by *T. julis***	
Year	**Larvae Mean (SEM)**	**Adults (SEM)**	**N = number sweep samples**	**Proportion parasitized CLB (SEM)**	**N = locations with CLB present**
2016	0.20 (0.070)	0.03 (0.026)	23	0.05 (0.022)	7
2017	0.04 (0.009)	0.013 (0.009)	63	0.66 (0.050)	9
2018	0.06 (0.011)	0.004 (0.001)	123	0.38 (0.050)	13

Summary

The 2018 results demonstrate that *T. julis* is well-established in the Klamath Basin region of Modoc County in northern California. Data from the field insectary at UC IREC illustrate the potential for this parasitoid to respond quickly to increasing numbers of CLB, and to persist on beetle populations year after year. The ability for this parasitoid to control CLB in this part of the country can be attributed to its host specificity, and ability to rapidly reproduce and increase overall population size. Although CLB has repeatedly decreased in activity mid-season at UC IREC, it has the propensity to rapidly increase. Furthermore, a suite of predators can attack CLB in small grains and contribute to its control (Kheirodin et al. 2019). Regional surveys have yet to find CLB in Lassen County, although wheat is located ~50 miles south of known infestation areas in Modoc County. *T. julis* is likely responsible for slowing the spread of CLB south. Additional survey work is needed to determine long-term impacts of *T. julis* on CLB populations throughout the Klamath Basin area.

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Host Specificity Testing for *Trissolcus japonicus*, a Parasitoid of BMSB

Jesús R. Lara[1], Charlie Pickett, Mark Hoddle[1]

Brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Penatomidae), is native to Asia and is an invasive insect pest in the United States (US) where it is currently established in 44 states, including California. BMSB is known to feed on a variety of fruit, vegetable and nut crops and direct crop damage is visible in the form of external necrosis or discoloration, or internally as corking damage. This results when the stink bug penetrates plant tissue with piercing-sucking mouthparts. In the US, BMSB has been collected from >100 host plant species representing 56 families, including commercial crops. Serious feeding damage by BMSB to specialty crops, in some cases resulting in economic losses, has been reported for apples in the Mid-Atlantic region as well as hazelnuts, apples, pears, blueberries, and cherries in the Pacific Northwest.

Documented BMSB crop damage across the US raised concerns regarding the economic threat BMSB poses to California agriculture. In California, BMSB was confirmed established in 2006 (Los Angeles County). One of the first pest management priorities in California was to determine the distribution of BMSB and assess its threat for crop damage. Presently, based on trap-based (passive) surveillance, field scouting and reported finds from the public, 17 counties have known reproductive populations and 18 additional counties have had confirmed detections. Initial climate-based models predicted a vastly limited distribution of BMSB in the San Joaquin Valley, but contrary to initial expectations, University of California, (UC) field studies documented feeding damage by established BMSB populations on commercial peach and almond, two important specialty crops. In 2018, BMSB was declared an official pest of almonds in California and laboratory research conducted at UC Riverside suggests BMSB can exhibit similar feeding damage to developing Kerman pistachios, which overlap to some extent with almond acreage in California.

In response to the growing BMSB threat in California, lab and field studies led by UC Davis and the California Department of Agriculture (CDFA) researchers are examining more carefully the response of BMSB development to California temperature/humidity conditions. The results from these studies could improve the calibration of predictive BMSB phenology and distribution models to promote cost-effective BMSB integrated pest management (IPM) in California. In support of IPM efforts, complementary research led by CDFA and UC Riverside seeks to develop a classical biological strategy using the candidate natural enemy *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae) to prevent the spread of BMSB into vulnerable agricultural acreage in California (Figure 1). Per state and federal regulations, natural enemy safety testing and submission of an environmental impact statement are required before approved parasitoid releases can be made for BMSB control. In preparation for this work, permits were secured to propagate *T. japonicus* under quarantine conditions at Riverside with initial colony specimens provided by Kim Hoelmer (USDA- Beneficial Insect Introduction Research Unit, Newark, DE).

Figure 1. *T. japonicus* emerging from BMSB egg**.** Photo by Lara Artiga.

Planned lab safety testing experiments exposed egg masses from BMSB and several non-target stink bug species to *T. japonicus* under quarantine conditions (Figure 2). A total of 452 replicates were completed as part of the safety testing of the BMSB parasitoid, *T. japonicus*. These efforts account for >12,000 stink bug eggs. These data are being compiled for analyses and preparation of a final CDFA report. The results from these experiments are critical to define the physiological host range and host specificity of *T. japonicus* in California, an area with a problematic BMSB infestation and a prime candidate for future *T. japonicus* releases. Current laboratory results indicate that *T. japonicus* successfully parasitize several native non-target pentantomid species from California under quarantine exposure. However, *T. japonicus* consistently parasitized BMSB host eggs at higher levels. Furthermore, it is worth noting that laboratory conditions maximized the level of non-target exposure. In the field, exposure of non-target eggs to *T. japonicus* may be limited by environmental factors and therefore non-target parasitism levels may be lower than those recorded from laboratory studies. This important aspect will be part of future studies comparing baseline BMSB and non-target stink bug egg parasitism levels recorded in the lab and the field.

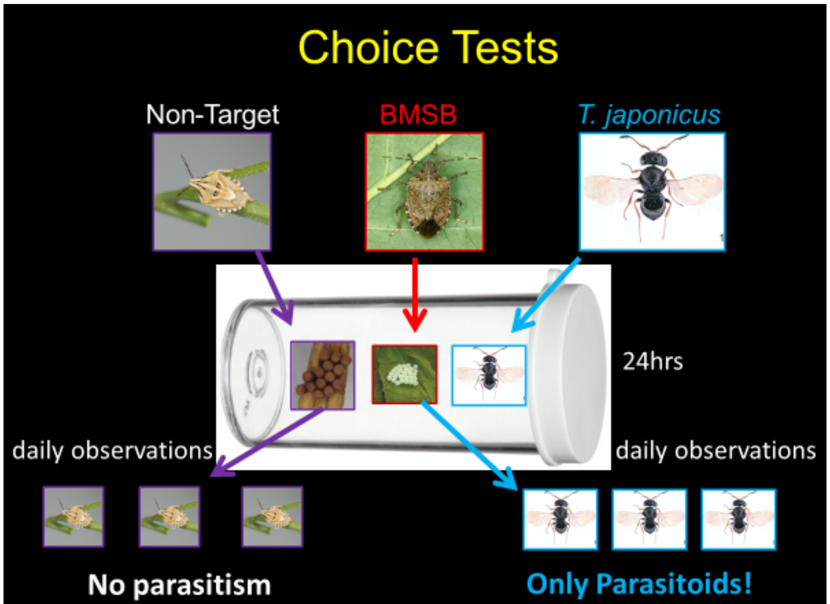

Figure 2. Graphic showing how choice tests are conducted for T. japonicus. Created by Lara Artiga.

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Survey in California for Resident Natural Enemies of the Brown Marmorated Stink Bug

Charles Pickett, Chris Borkent, Viola Popescu, Michael Calacsan, Ricky Lara-Artiga[1], Mark Hoddle[1], and Sateur Ham

A survey has been conducted in California to determine resident natural enemies of brown marmorated stink bug (BMSB) eggs. Frozen, sentinel eggs attached to cards were placed on known host trees to measure egg predation and parasitism. To identify key predators feeding on eggs, a time lapse, rain resistant field camera was employed. Cameras were placed at a subset of 4-6 study sites each year since 2014. Using results from imaging and sentinel egg remnants, unique 'fingerprints' were developed to identify predator taxa feeding on eggs. Within urban areas, the carabid *Laemostenus complanatus* is the most common predator, followed by earwigs (*Forficula auricularia and Euborellia annulipes*), oriental cockroach *Blatta orientalis*, and spiders (i.e. *Trachelas*). Predation of egg masses reached 26% in 2017 for cards placed on the trunk of trees (chest height),but was only 8% for cards placed on the underside of leaves. In 2017 BMSB predators were imaged attacking sentinel egg cards, on average, 1.29 times per day, while during the same year predators of bagrada bug, *Bagrada hilaris*, in a related study were imaged 6.5 times per day. Parasitism of BMSB eggs during the same year was 3.6% and 13%, for cards placed on trunks and leaves, respectively. These values fall within the range measured across the United States (US) for eggs that have been frozen prior to placement in the field. The native parasitoids emerging from sentinel egg cards were two species of chalcids, *Anastatus* sp. and *Ooencyrtus,* and 3 species of scelionids, *Trissolcus euschisti*, *T. hullensis*, and *T. brochymenea*. Most of the emerging parasitoids were *T. euschisti*.

Northern California.

Although the BMSB average trap catch has dropped slightly in northern California over the last 3 years, from 0.77 per trap per date in 2016 to 0.24 in 2017, its population statewide continues to expand. We have now found it well established in 15 counties, up from 10 reported in fall of 2016. In northern California, a total of 513 sentinel egg cards were placed in the field. In most locations we now attach cards to both the tree trunk and one of its leaves. Egg parasitism averaged 3.5% for cards on tree trunks and 13% when placed on a leaf. BMSB are far more likely to lay their eggs on leaves than the bark of the tree, and these parasitoids appear to search leaves far more readily than the trunks of trees. The target parasitoid *Trissolcus japonicus* was never found attacking these eggs. It is currently under testing for safety by CDFA/UC Riverside, and others across the country. In addition to inland locations with known BMSB populations, cards were placed on the northern California border shared with Oregon. *Trissolcus japonicus* is now being actively released and established in the southern part of Oregon. The sentinel cards have also been deployed in the San Jose area, an area with a high BMSB population and near areas of high commerce where BMSB eggs originating from China (and parasitoids) are more likely to be present. The native parasitoids emerging from sentinel egg cards were two species of chalcids, *Anastatus* sp. and *Ooencyrtus,* and 3 species of scelionids, *T. euschisti*, *T. hullensis*, and *T. brochymenea*. Most of the emerging parasitoids were *T. euschisti*.

Southern California.

BMSB is established in two southern California counties, Los Angeles and Orange. Trap captures have been consistently relatively low, compared to the situation on the east coast, with less than 100 adults per field season for all sites beginning in 2014. However, BMSB has noticeably been spreading within established Southern California counties and this is clear from field collections and compiled reports from the public. Approximately 312 egg cards were deployed in the field sites across Los Angeles, Fresno and Madera. There is evidence of limited predation and parasitism at these sites. Recovered parasitoids are being identified, with a primary focus on *Trissolcus* sp. Presently, *T. euschisti* have been recovered and some *Anastatus* sp. as well. The goal will be to combine pooled sentinel card California datasets to provide stakeholders with greater perspective of the natural enemies that could be used to enhance biological control practices at the orchard/field level.

The results show that there is a serious lack of natural control of BMSB eggs by resident parasitoids in Northern and Southern California which only attack from somewhere between 3.5% and 13% of eggs, and these values are inflated because those used on sentinel cards were frozen. Studies have shown that native parasitoids are less likely to successfully develop from viable BMSB eggs. Predation of egg masses has been as high as 26%, but that was recorded from egg masses placed on trunks. This last year (2017) we found only 8% of sentinel eggs placed on leaves attacked by predators. Much higher levels of biological control will be needed to provide significant control in urban and farm landscapes, highlighting the need for importing and releasing *T. japonicus*, known to prefer BMSB eggs over most other non-target host eggs. Unusually warm summers over the past 5 years have likely helped curb the buildup of BMSB in California, but that will not help during years with closer to normal temperatures, especially near coastal areas and foothills, and production areas of northern California.

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Biological Control of Bagrada Bug: Foreign Exploration and State Survey for Resident Natural Enemies

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The primary objective of this project is to import into California highly specific, co-evolved parasitoids that attack the bagrada bug. *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), is a serious exotic stinkbug pest of cole crops. Importation of such parasitoids can provide critical suppression to this pest. Three species of egg parasitoids have been collected from Pakistan and are now in culture at a United States Department of Agriculture (USDA) Agricultural Research Services (ARS) laboratory in the United States (US) and an overseas laboratory in southern France. Most likely there are more to be discovered within the natural distribution of bagrada bug which includes South Africa, north through eastern Africa and into Pakistan and India. This report focuses on ongoing foreign exploration for additional, unknown parasitoids that could specialize in attacking eggs of bagrada bug. It also focuses on survey work in California on resident natural enemies, as required for a federal field release permit, that may feed on eggs of bagrada bug. Completion and approval of a petition for field release can take three to five years or more for a single biocontrol agent. Host specificity testing for parasitoids found associated with bagrada bug eggs in Pakistan is ongoing and reported elsewhere in this annual report (see Hogg et al.).

Foreign Exploration.

Although three species of egg parasitoids have been collected in Pakistan, nothing is known about bagrada bug's natural enemies in Africa. It is known to occur from South Africa to Kenya, as well as in the Indian subcontinent. The USDA-ARS European Biological Control Laboratory (EBCL) in France has established cooperative agreements with collectors in South Africa and Kenya, the former under an agreement between EBCL and the University of Stellenbosch (under signature), whose main local investigator is Dr. Pia Addison. The University is located in the western Cape region, 50 km east of Cape Town. The latter agreement is between the Kenya Agricultural and Livestock Research Organization (KALRO) in Kenya, to collect parasitoids attacking bagrada bug eggs. In general, egg parasitoids are considered to be the most effective biological control agents of stink bugs and will be the focus of collecting efforts.

In 2018, cooperators in Kenya were trained and later collected samples of bagrada bug. Three sites in January were sampled with evidence of bagrada bug. As a result, additional sites were sampled through the first part of 2018. A bagrada bug colony was established and a technician and a student were trained to produce sentinel egg cards to collect parasitoids in 2018. In February, the sites visited in Meru County showed very high populations of bagrada bug. Consequently, multiple sentinel egg cards were placed in additional sites. As of August 18, 2018, 10 parasitoids emerged from cards and are undergoing identification. Preliminary results suggest species of *Gryon* and *Trissolcus*. Samples of bagrada bug have also been shipped to EBCL for genetic analysis.

Survey for resident natural enemies in California.

The California Department of Food and Agriculture (CDFA), the USDA-ARS Albany, and University of California, (UC) Davis have been surveying for resident natural enemies of bagrada bug in central and northern California during the latter half of 2018. Sentinel egg cards were used for this purpose, consisting of killed (frozen) bagrada bug eggs glued to waterproof index cards. The CDFA work has also included imaging eggs for identification of predators from a subset of sites where sentinel cards have been used in capturing parasitoids and in measuring egg predation. These activities have included organic and commercial farms willing to work with us. Sentinel cards were also placed in patches of known weedy hosts of bagrada bug, or trap crops of alyssum, *Lobularia maritima* within or near commercial fields of cole crops. Up to 35 locations across 13 counties in central and northern California were used in the 2018 season. Weeds included shortpod mustard, *Hirschfeldia incana*, or perennial pepperweed, *Lepidium latifolium*. These weeds appear to be the primary hosts of bagrada bug in spring and early summer.

Sentinel cards with bagrada bug eggs were placed either on the ground or attached to leaves at each of the study sites, roughly once every two weeks from May through December of 2018. Pheromone baited traps were placed at most sites, near the sentinel cards to help in tracking the bagrada bug population. Because we have been doing imaging of sentinel eggs placed in fields, the cards were modified to include a slight tan color paper, with three matrices of eggs (Figure 1) to help with reflected light during imaging. Sand was added to measure its impact on egg predation (Figure 1). The use of a camera was included in survey studies to aid in the identification of arthropods eating sentinel eggs. Identification was further aided by addition this year of pitfall traps along with cameras (Figure 2). The USDA-ARS and UC Davis continued to use the same design, but with lighter colored, thicker card stock. Both types of card stock are water resistant and purchased from *Rite in the Rain* ®.

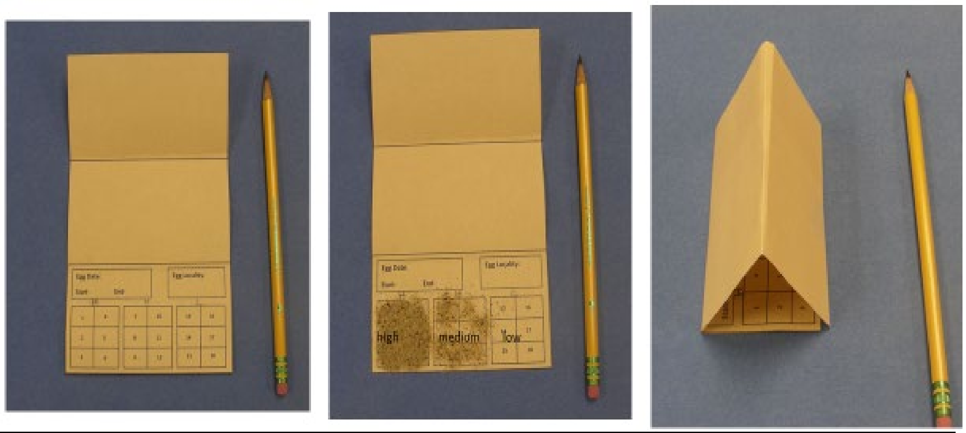

Figure 1. Sentinel egg card used by CDFA to measure predation and parasitism. Only the bottom third of card with matrices were attached to leaves, while the delta shaped card was used on the ground. A similar trap design was used by cooperators.

Figure 2. Pitfall trap (arrow) and field camera on stand for imaging.

In addition to measuring predation, the role of sand interfering with either predation or parasitism was examined. All eggs were frozen prior to use, and glued to cards using Gorilla Glue®, three sets of six eggs, one each in every matrix cell (Figure 2). Results from 2017 suggest that sand did not interfere with predation (Table 1); ANOVA model p-value for testing differences between means was greater than 0.05. These data support imaging results which clearly showed ants, and other predators clustering around eggs placed on sentinel cards but buried below a 1 or 2 mm layer of sand, as well as on eggs not under sand.

Table 1. Impact of sand on egg predation. Shown is proportion of predated eggs on sentinel cards. Means preceded by same letter are not significantly different. Model p value = 0.4124.

Waller Grouping	Mean	Depth of sand	N = number of cards
A	0.71	0 mm	58
A	0.72	1 mm	40
A	0.65	2 mm	58

To measure location effects on placement of sentinel cards, they were placed both directly on the ground at study sites, and on the lower side of plant leaves, either cole crops or weedy host plants. Sites in the Sacramento region did not show a significant difference ($\alpha = 0.05$) between predation of eggs placed on leaves and ground (Table 2). However, significant differences were seen at sites near the central coast and South Bay Area, 34% of eggs attacked on ground and 24% on leaves (Table 2). These results may highlight a difference in the overall level of predation as measured in the Sacramento Region (plus Kern County) versus the more coastal areas. Between 62% and 69% of eggs were attacked in the former regions as compared to 24% to 34% for the latter. Many of the sites in the coastal areas experience only natural rainfall (non-farm, ephemeral weedy patches), while in the Sacramento Valley all the sites were on or next to irrigated crops. More soil moisture and higher plant diversity on small organic farms could result in greater predator diversity. In the Sacramento area most sites were at small, organically registered farms. Why more predation was recorded on the ground than plant leaves in the coastal and Bay Area sites is unclear.

Table 2. Impact of card location on egg predation, 2017. Shown is proportion of predated eggs on sentinel cards. Means preceded by the same letter are not significantly different.							
Sacramento Region and Kern Co.				**Central Coast and South Bay Area**			
Duncan grouping	**Mean**	**Location**	**N**	**Duncan grouping**	**Mean**	**Location**	**N**
A	0.69	ground	57	A	0.34	ground	91
A	0.62	leaf	56	B	0.24	leaf	90

A total of 11 eggs on sentinel cards were parasitized in 2017, all placed on leaves, and represent less than 1% of eggs placed in the field. Five of these completed development, emerged from eggs and were identified as *Ooencyrtus* sp. and *Trissolcus* sp. The most common predator observed feeding on eggs was the native ant *Solenopsis xyloni* followed by what appears to be a species of collembola (Table 3). *Seira* nr. *lusitanica* belongs to the family Entomobryidae, and like ants, many were seen on cards and feeding on eggs, an unusual record (Figure 4).

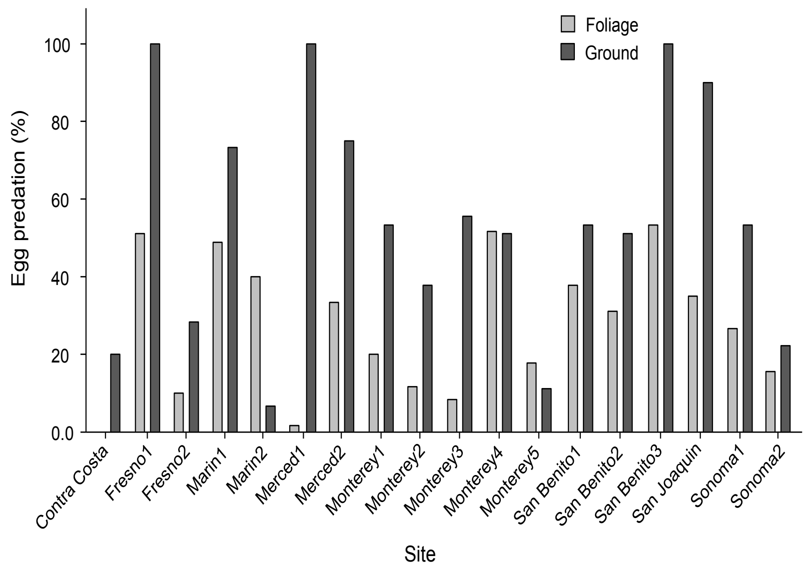

Figure 3. Predation of sentinel bagrada bug eggs in August 2017. Central to northern California.

Table 3. Most common arthropod predators imaged at two locations in northern California, summer and fall 2017.		
Common name	**Family or Order**	**Taxa**
ants	Formicidae	*Solenopsis xyloni*
collembola	Entomobryidae	*Seira* nr. *lustanica*
pill bugs	Isopoda	unknown
big eyed bugs	Geocoridae	*Geocoris sp.*
earwigs	Dermaptera	*Forficula auricularia, Euborellia annulipes*
cockroach	Blattellidae	unknown
harvestmen	Opilliones	unknown

Figure 4. Images of collembola coming from survey sites in the Sacramento Valley: (A) possibly *Seira* nr. *lusitanica* (Entomobryidae, det. F. Janssens) on sentinel card and (B) seen through microscope, unknown collembola.

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Host Specificity Testing of Parasitoids for Bagrada Bug

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Bagrada bug, *bagrada hilaris* (Burmeister), was discovered near Los Angeles in 2008, and has become an important pest of cole crops in California. Bagrada bug has rapidly spread from southern California northward into the central valley and along the coast north to Monterey Bay and beyond. The pest can reach high numbers and destroy plants, and its ability to persist on weedy mustards and grasses enhances its distributive capacity. Conventional pesticides are the primary control means, leaving organic growers with few options. A long-term solution is introduction of specialized parasitoids. No specialist natural enemies of bagrada bug are known to occur in California.

Cultures of two species of bagrada bug hymenopteran parasitoids collected in Pakistan (*Trissolcus hyalinipennis* Rajmohana and Narendan, and *Gryon* sp. - Scelionidae) are now in quarantine at a United States Department of Agriculture (USDA) Agricultural Research Services (ARS) laboratory in Albany, California (Figure 1). These

Figure 1. Parasitoid rearing in the quarantine conditions at the USDA-

cultures were expanded in winter/spring 2017 and subsequently in 2018 in preparation for host specificity testing. Many non-target stinkbug colonies in the laboratory (Figure 2) declined during the winter, and additional specimens were collected in spring and summer 2017 and 2018 to augment laboratory colonies of the non-target stinkbug species

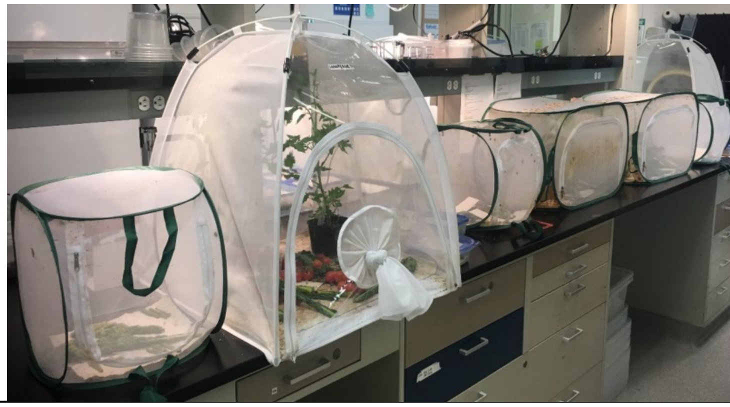

Figure 2. Rearing cages for non-target stinkbug species at the USDA-ARS facility in Albany, CA.

Murgantia histrionica (harlequin bug), *Nezara viridula* (southern green stink bug), *Euchistus conspersus* (consperse stink bug), *Euchistus servus* (brown stink bug; collected in 2017 only), *Thyanta pallidovirens* (red-shouldered bug), *Banasa sordida* (banasa stink bug), *Chlorochroa ligata*, *Chlorochroa uhleri* and *Holcostethus* species. *Podisus maculiventris* (spined soldier bug, a beneficial predator) was obtained from a commercial insectary.

Host specificity tests for *T. hyalinipennis* and *Gryon* sp. were conducted inside quarantine facilities at the USDA-ARS, Albany (*Ooencyrtus* sp. - Hymenoptera: Encyrtidae is being tested at University of California, (UC) Riverside; T. Perring pers. comm.). Tests thus far for *T. hyalinipennis* have used a no-choice design, whereby the parasitoid is exposed to just one species of host egg. For these tests, clusters of 10-15 fresh stink bug eggs (<24 h old) were glued onto card stock and placed in glass vials, and one 24- to 48-hour-old, mated female parasitoid was then released into each vial and removed after 24 hours. At least one vial containing bagrada bug eggs was also exposed to parasitoids during most trials, to compare the suitability of non-target stink bugs and bagrada bug to the parasitoids. Eggs were then monitored, and numbers of parasitized eggs and emerging stink bugs and parasitoids were recorded. Eggs were then dissected after approximately 30 days, to record numbers of parasitoid larvae that failed to complete development.

Thus far, both *T. hyalinipennis* and *Gryon* sp. have been tested on nine non-target stinkbug species (Table 1). For each parasitoid species, 2-23 replicates of each stinkbug species were completed in 34 trials in 2018 (in both 2017 and 2018, 2-26 replicates of each stinkbug species were completed in 52 trials). Although both species parasitized (i.e., successfully attacked or emerged as adults) bagrada bug eggs at high rates, results thus far indicate that both parasitoid species are also capable of parasitizing eggs of non-target stinkbug species to varying degrees (Table 1). Adults of both parasitoid species emerged from eggs of *Thyanta pallidovirens and Holcostethus* sp. at relatively high rates., although dissections from 2017 indicated that high percentages of *Gryon* sp. failed to emerge, suggesting that these are suboptimal hosts for this species (dissections from 2018 remain in progress). *Trissolcus hyalinipennis* also emerged at relatively high rates from *Euschistus conspersus* and *Banasa sordida*. Neither species was capable of completing development on *Murgantia histrionica*, *Nezara viridula*, *Chlorochroa ligata* or *Chlorochroa uhleri*.

Only a limited number of replicates have been completed for several of the non-target species, and more are being initiated, particularly for *Gryon* sp. In 2018 improvements were made for rearing protocols for non-target species such as *Chorlochroa uhleri* that proved difficult to rear in 2017. Other species such as *Banasa sordida* have yielded only small numbers of eggs, and one species (*Euschistus servus*) was not obtained in 2018. Future plans are to conduct 'choice' tests, whereby each parasitoid is exposed to eggs of two stink bug species simultaneously: bagrada bug and one of the non-target stink bugs. Choice tests will be limited to non-targets that have proven susceptible to parasitism under limited, no-choice tests. Results of the no-choice tests thus far indicate there could be significant differences in host specificity between the two biocontrol candidates. Although *Gryon* sp. attacked several of the non-targets in question, it did so to a lower degree than *T.* hyalinipennis. Additional replication is required to make this determination with a high degree of certainty.

Table 1. Mean emergence rates of candidate parasitoid species on non-target stinkbug species in laboratory trials (n = number of clusters with 10-15 eggs each).						
Parasitoid species	**Stinkbug species**	***n***			**Emergence (%)**	
		2017	**2018**	**total**	**2017**	**2018**
Gryon sp.	*Bagrada hilaris*	9	30	39	37.23	50.34
Gryon sp.	*Banasa sordida*	0	2	2	-	10.00
Gryon sp.	*Chlorochroa ligata*	0	4	4	-	0.00
Gryon sp.	*Chlorochroa uhleri*	1	11	12	0.00	0.00
Gryon sp.	*Euschistus conspersus*	5	11	16	0.00	0.91
Gryon sp.	*Euschistus servus*	2	0	2	0.00	-
Gryon sp.	*Holcostethus* sp.	1	2	3	0.00	90.00
Gryon sp.	*Murgantia histrionica*	2	14	16	5.26	0.00
Gryon sp.	*Nezara viridula*	2	6	8	3.33	0.00
Gryon sp.	*Podisus maculiventris*	0	15	15	-	4.67
Gryon sp.	*Thyanta pallidovirens*	4	13	17	17.65	20.00
T. hyalinipennis	*Bagrada hilaris*	14	42	56	40.52	51.46
T. hyalinipennis	*Banasa sordida*	0	2	2	-	45.00
T. hyalinipennis	*Chlorochroa ligata*	0	17	17	-	0.00
T. hyalinipennis	*Chlorochroa uhleri*	0	8	8	-	0.00
T. hyalinipennis	*Euschistus conspersus*	4	11	15	57.41	36.36
T. hyalinipennis	*Euschistus servus*	7	0	7	47.83	-
T. hyalinipennis	*Holcostethus* sp.	2	16	18	82.14	88.13
T. hyalinipennis	*Murgantia histrionica*	3	23	26	0.00	0.00
T. hyalinipennis	*Nezara viridula*	9	15	24	0.00	0.00
T. hyalinipennis	*Podisus maculiventris*	0	4	4	-	0.00
T. hyalinipennis	*Thyanta pallidovirens*	1	4	5	0.00	35.00

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Olive Psyllid: Host Specificity Testing for *Psyllaephagus euphyllurae* (Encyrtidae)

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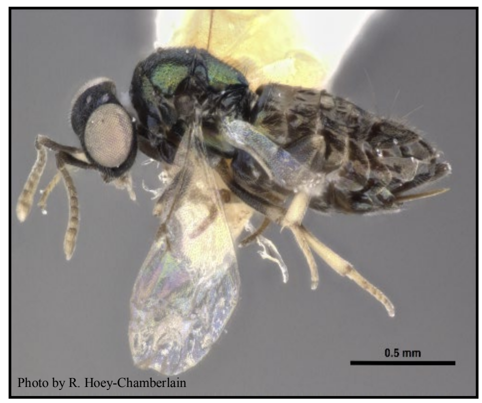
The olive psyllid*, Euphyllura olivina* (Costa), is a new invasive pest to California as well as North America. First reported in 2007 infesting olive trees in Newport Beach (Orange County), this psyllid has spread throughout southern California, moving north to Los Angeles County, south to San Diego County, inland to Riverside County, and most recently northward to Santa Barbara and Monterey counties. Although there are no reports of it infesting olives in major production areas of central and northern California, olive psyllid is spreading in the state. The encyrtid parasitoid *Psyllaephagus euphyllurae* (Masi) is the most common primary parasitoid associated with the olive psyllid in its native range, and CDFA proposes releasing and establishing permanent populations in California. No records of natural enemies specializing in attacking and reproducing on olive psyllid have been recorded during surveys of infested olive trees in California. Permanent establishment of a parasitoid specializing on olive psyllid in California would provide an efficient, clean, and cost-effective means of helping manage this pest, and perhaps slow its spread.



Psyllaephagus euphyllurae

Healthy and mummified olive psyllid

During 4 years of testing, *P. euphyllurae* has been exposed to seven non-target psyllids to determine its host specificity. During the first three years of testing, 2013-2015, studies were conducted at University of California, (UC) Riverside, while in 2018, studies were done at UC Berkeley. Three treatments have been used to measure host specificity: two sequential, no-choice tests, and a choice test. *Psyllaephagus euphyllurae* demonstrated a high degree of host specificity throughout these tests. The parasitoid was unable to develop into an adult on 255 non-target psyllids to which it was exposed, including a related non-target, native species, the manzanita psyllid *Neophyllura arctostaphyli* (same subfamily as olive psyllid). *Psyllaephagus euphyllurae* only developed from olive psyllid hosts. Parasitism under test conditions using naïve females was relatively low at 17.4%. Video imaging during choice tests conducted at UC Berkeley demonstrated that probing by *P. euphyllurae* occurred on 100% of exposed target olive psyllids. Only one singular probing attempt was made on manzanita indicating *N. arctostaphyli* did not provide necessary host cues for host discovery and acceptance. The same imaging showed that host feeding of non-targets, or the target, by young (1 to 3-day old adults) or old (16 to 24 day old) adult *P*. *euphyllurae* parasitoids does not occur. This behavior involves probing, then imbibing of insect haemolymph. Patch time was significantly shorter for the parasitoid on non-target plants than target species in two of three non-targets tested (*Ceanothus* sp. (t=2.69, df = 6.7, P=0.032) and *Rhus trilobata* (t=4.57, df = 6, P=0.004). Parasitoid host finding, attack, and oviposition was not observed on all three non-target test plants (plus psyllids).

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Discovery of a New Gall Mite, *Aceria genistae*, on Scotch broom in California*

Paul D. Pratt[1], Michael J. Pitcairn, and Viola Popescu

*The information presented here is a summary by Pratt PD, Pitcairn MJ, Oneto S, Brent Kelly M, Sodergren CJ, Beaulieu F, Knee W, Andreas J (2019) Invasion of the gall mite *Aceria genistae* (Acari: Eriophyidae), a natural enemy of the invasive weed *Cytisus scoparius*, into California, U.S.A. and predictions for climate suitability in other regions using ecological niche modelling. Biocontrol Science and Technology Vol. 29(5): 494-513.

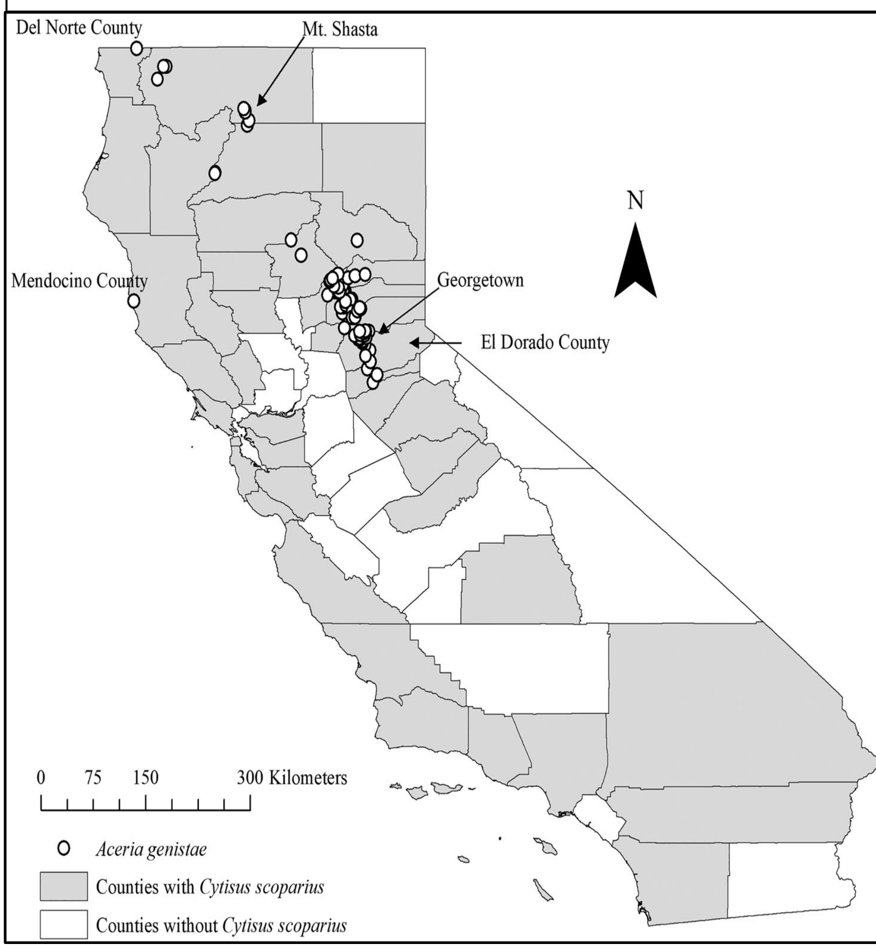
Scotch broom (*Cytisus scoparius* (L.) Link) in an invasive leguminous shrub that occurs throughout forests of northern California. It grows 3-8 feet tall and produces yellow pea-like flowers that develop into 1-2 inch long seed pods. In California, Scotch broom is common along roadsides, disturbed forest clearings, and road cuts but it can invade undisturbed grass and forested systems. Two biological control agents were introduced on Scotch broom in California in the 1960s: *Exapion fuscirostre* (Fabricius) (a seed weevil) and *Leucoptera spartifoliella* (Hubner) (a stem-mining moth). While both agents established and are now widespread, neither has caused the amount of damage sufficient to reduce Scotch broom populations. Recently, a gall mite, *Aceria genistae* (Nalepa), was discovered infesting broom plants in the western United States (US) including California. The feeding by immature and adult mites causes growing buds to develop into small abnormal fuzzy growths, which are a mass of distorted leaves and hundreds of mites (Figure 1). An individual mite is minute, barely visible to the naked eye but despite the small size, feeding damage is noticeable. The small fuzzy galls can occur

Figure 1: Galls caused by the European mite, *Aceria genistae*, on its host plant Scotch broom, *Cytisus scoparius*.

along the length of a stem and become quite numerous on a single plant (Figure 2). The Scotch broom gall mite was first discovered near Tacoma, Washington, and Portland, Oregon, in 2005. Reports from cooperators in Washington and Oregon reported its spread southward and in 2013, it was reported in Ashland, Oregon. Surprisingly, it was subsequently found in El Dorado County in 2014. Since then, surveys have been conducted for other occurrences in California. The mite has been subsequently found in several other locations, from Siskiyou County south to Amador County and areas in between (Figure 3). The gall mite has now been found in 11 counties with a widely scattered distribution. A geographic distribution model based on climate suitability suggests that the gall mite will find the Scotch broom in California occurringin areas highly suitable for colonization. Expectations are that the gall mite will continue to spread, especially into the northern Coastal Range. Areas of central and southern California are predicted to be

Figure 2. Scotch broom, *Cytisus scoparius*, infested with galls from the mite, *Aceria genistae*.

Figure 3. Geographic distribution of the adventive European gall mite, *Aceria genistae*, in California.



less suitable for the gall mite, particularly along the coast and the southern extent of the Sierra Nevada Mountains where Scotch broom occurs in limited abundance.

The Scotch broom gall mite was considered for use as a biological control agent and following host specificity testing was released in New Zealand in 2007 and Australia in 2008. Because the gall mite is adventive in the western US, there currently is no permit to move it or re-distribute it locally. Research efforts are underway to examine its host specificity in the field and, when completed, to request a permit for its use as a classical biological control agent. Prior host testing in New Zealand and Australia provided strong evidence that the mite is highly host specific. However, additional data documenting host specificity in relation to California native species, such as native lupines, are needed and will be obtained in future studies.

Currently, gall abundance is generally low throughout its current range, usually just 1 to 5 galls per plant at most locations. However, it is expected that gall abundance will steadily increase. Plants can be significantly stunted by galls forming year after year and result in reduced flowering and seed pod production and can even kill whole plants. At a couple of locations, whole patches have been seen with severe galling and no flower or pod production.

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Release and Establishment of *Megamelus scutellaris*, a New Biological Control Agent for Water Hyacinth in California*

Patrick J. Moran[1], Michael J. Pitcairn, Viola Popescu, and Baldo Villegas[2]

*Includes information from Moran PJ, Pitcairn MJ, Villegas B (2016). First establishment of the planthopper, *Megamelus scutellaris* Berg, 1883 (Hemiptera: Delphacidae), released for the biological control of water hyacinth in California. The Pan-Pacific Entomologist 92(1): 32-43.

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms) is an exotic emergent aquatic plant that infests waterways of the Sacramento – San Joaquin River Delta. Through rapid growth, the plant forms dense mats that obstruct passage by boats, restrict flow for irrigation and domestic uses, and alters aquatic ecosystems. In the early 1980s, two weevil biological control agents, *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache, and a moth, *Niphograpta albiguttalis* (Warren), were released in the Delta. Of these, only *N. bruchi* became established. *Neochetina eichhorniae* occurs at low numbers in areas south of the Delta (e.g. in the Merced River) and the moth failed to establish anywhere in California. The impact of *N. bruchi* feeding damage has been insufficient to reduce water hyacinth populations in the Delta. Beginning in 2011, a fourth biological control agent, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a planthopper whose nymphs and adults feed on the leaves and petioles of water hyacinth, was released into the Delta. Heavy feeding damage by *M. scutellaris* leads to premature leaf death, reduced growth rate, and eventually whole plant death. This planthopper has multiple generations per year and in areas of Florida where it was first released, can quickly build up high populations.

Figure 1: Colony of water hyacinth and the planthopper, *Megamelus scutellaris*, at the California Department of Food and Agriculture Facility in Sacramento.

In 2011, colonies of the planthopper were initiated at the California Department of Food and Agriculture (CDFA) Meadowview Facility (Figures 1 & 3) with material provided by Dr. Philip Tipping, United States Department of Agriculture (USDA) Agricultural Research Services (ARS) Invasive Plant Research Laboratory in Ft. Lauderdale, Florida. The colonies in Florida were initiated with insects collected in Argentina in 2006. In 2012, the planthopper colony was transferred from CDFA to the USDA-ARS Exotic and Invasive Weeds Research Unit in Albany, California, and a second colony was started at the USDA-ARS Aquatic Weed Research Laboratory in Davis, California. Insects for field release were provided by all three colonies. Releases of the planthopper occurred at three locations from 2011 through 2013 (Table 1): Whiskey Slough in San Joaquin County (n=1,500); Sevenmile Slough in Sacramento County (n=375); and Folsom (Willow Creek Ponds, Sacramento County) (n=31,444). The Folsom release location is a collection of dredger ponds through which Willow Creek flows before emptying into Lake Natoma, an impounded reservoir of the American River. Releases were accomplished by transporting water hyacinth plants infested with planthoppers (Figure 2)

Figure 2. Planthopper nymphs on a water hyacinth leaf and petiole.

Figure 3. *Megamelus scutellaris* nymphs on a water hyacinth leaf blade.

from the production colonies and placing them in between plants growing at the field sites. All stages of planthoppers were released (approximately 80% nymphs, 20% adults).

Results

Following the initial releases in 2011, a few adults and nymphs (density not quantified) were observed at the Folsom and Whiskey Slough locations in December 2011. No planthoppers were observed at Sevenmile Slough. No nymphs or adults were observed at any of the three sites in May 2012. In May 2013, no planthoppers were found at Whiskey Slough and Sevenmile Slough, suggesting that the insect failed to establish at these locations. In contrast, the planthopper persisted at low levels at Folsom, where the average peak density of planthoppers steadily increased each year: 0.8 insects/plant (2012), 1.8 insects/plant (2013), 2.0 insects/plant (2014), and 6.0 insects/plant (2015).

Table 1. Releases of *Megamelus scutellaris* in California.

Location	Date	Number
Delta, San Joaquin County		
Whiskey Slough	July 7, 2011	750
	July 19, 2011	750
Delta, Sacramento County		
Sevenmile Slough	July 18, 2011	375
Folsom, Sacramento County		
Willow Creek Ponds	July 8, 2011	750
	July 20, 2011	2,150
	Aug 9, 2012	1,732
	Oct 1, 2012	2,812
	July 23, 2013	24,000

Discussion

The initial releases of *M. scutellaris* from 2011-2013 appear to have failed to establish on water hyacinth at two locations in the Sacramento-San Joaquin River Delta. In early 2012, frost killed above-water portions of plants at these sites, however planthoppers survived the frost and plant damage at the Folsom site. The number of insects released at the two Delta sites (6% of total number insects released) was substantially less than numbers released at Folsom and may be one possible causes of failure. Establishment at the Folsom site may have been aided by abundant cover provided by shrubs and trees on the shore and by aquatic reeds and rushes which were absent at the two Delta locations. Cover vegetation may have buffered the hot and cold temperature extremes that could limit *M. scutellaris* survival. Densities of the planthopper are still well below levels needed to impede growth and reduce survival of host plants.

Releases after 2013

Mass production of the planthopper continues at the Albany and Davis USDA-ARS locations and at the CDFA Meadowview Facility (colony restarted in 2015). In 2016 and 2017, a total of 36,031 planthoppers were released at a field site near Hopeton (Merced County). In 2018, 65,244 planthoppers were released at 19 release sites within the central Delta (Figure 4, yellow dots). The CDFA planthopper colony contributed 40% of the total insect number released in 2018. Efforts for 2019 will be directed at increasing the number of release sites to 28 locations (Figure 4, 2019 new releases purple dots) to expand the release area within the Sacramento–San Joaquin River Delta.

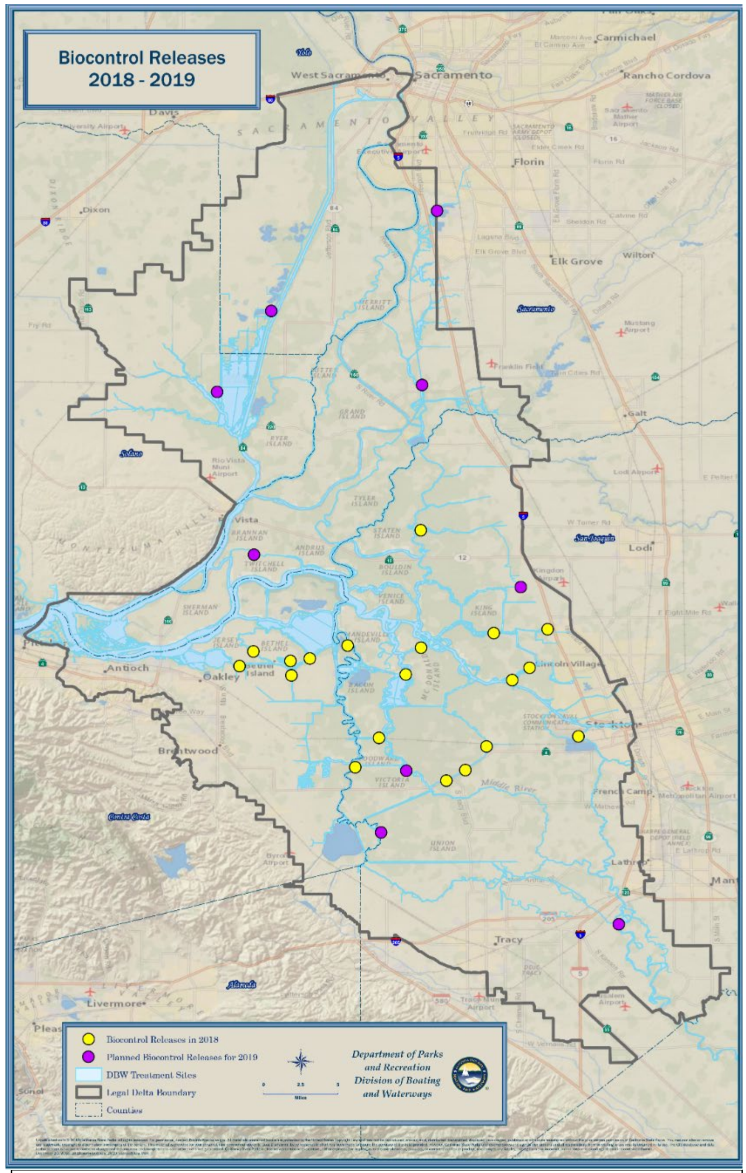

Figure 4. Locations of past (yellow) and future (purple) releases of *Megamelus scutellaris* in the Sacramento-San Joaquin River Delta.

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Biological Control of Russian Knapweed in Northern California: Release of Gall Midge *Jaapiella ivannikova* (Diptera: Cecidomyiidae)

Michael J. Pitcairn, Viola Popescu, Baldo Villegas[1], Jodi Aceves[2], and Caroline Gibbs[3]

Russian knapweed, *Rhaponticum repens* (L.) Hildago (formerly *Acroptilon repens*), is an exotic noxious weed that originates in central Asia and occurs throughout California. As a relative of yellow starthistle (*Centaurea solstitialis* L.), *R. repens* is known to be poisonous to horses, causing a chewing disease that can eventually end in death. In general, the plant is distasteful to cattle but grazing occurs if more palatable forage is unavailable. Russian knapweed is a perennial with an extensive root system that spreads in a widening circle. Above ground rosettes are formed in the fall and early spring, with one-to-two feet tall, seed-producing flower stalks developing in the spring and early summer. An exotic midge, *Jaapiella ivannikova* Fedotova, has recently been approved for use against Russian knapweed in the United States. Herein we report on efforts to introduce the gall midge into Northern California.

The adult female gall midge lays its egg on the buds and growing tips of the main and lateral shoots. The larval feeding produces a gall which comprises silky webs or hairs from leaves that grow together and fuse. The larvae develop through three instars and pupate in the gall. In Uzbekistan, its area of origin, *J. ivannikova* may have as many as four generations per year with each generation lasting approximately one month. In Montana and Wyoming, it has two generations per year. The gall midge overwinters as a mature larva in the gall.

Figure 1. Bouquet of Russian knapweed stems galled by *Jaapiella ivannikova* which were place at field sites in California.

Releases of *J. ivannikova* in California began in 2011 and continued through 2015 at four locations in Lassen and Siskiyou counties (Table 1). The release material was provided by Dr. Richard Hansen, United States Department of Agriculture (USDA) Animal and Plant Health Inspection Services (APHIS), Ft. Collins, Colorado, as immature fly larvae in bouquets of galled plant stems. Bouquets had 16 to 20 galls. According to Dr. Hansen, on average, 20-25 adults would emerge per gall with a female bias of 55-60%. Thus, on average, a bouquet of 20 galls was estimated to produce 400-500 adult flies. Successful emergence of adults from the gall is dependent on many factors, especially weather , so the actual number of emerged adults may be lower.

Table 1. Release information for *Jaapiella ivannikova* in Northern California**.**

Site name	Elev (m)	Release date	No. galls	No. Adults*
Lassen County				
Standish/Lambert Lane	1240	5/27/2011	40	800
Siskiyou County				
Little Shasta/Harry Cash Rd.	876	6/23/2011	32	640
		6/28/2013	40	800
Dorris/Indian Point Rd.	1296	6/13/2012	80	1600
(2 sites 50 meters apart)		5/28/2015	40	800
Dorris/E. Butte Valley Rd.	1433	7/11/2012	36	720
(2 sites 500 meters apart)		7/10/2014	40	800

*assuming 20 adults per gall (Richard Hansen, pers. comm.)

Results - Lassen County

<u>Standish/Lambert Road</u> **-** Releases of the gall midge (n=800) occurred on May 27, 2011, at one location near Standish, a small community about 15 miles east of Susanville. Two bouquets of Russian knapweed stems, each with 20 galls, were placed together within the knapweed infestation and adult flies were allowed to emerge under ambient conditions. The site was revisited on June 21, 2011 and plants near the bouquets and progressively away from the release point were examined for galls. Approximately one dozen galls were found on plants near the location of the galled bouquets. The site was monitored again on November 1, 2011 and no increase was found in the initial number of galls suggesting an additional generation did not occur. The site was revisited in July

2012 and April 2014 and no galls were found on either date suggesting that the gall midge failed to establish. At the April 2014 visit, it was discovered that the property had a new owner and the pasture was enclosed by a new fence and the owner intended to place animals in the pasture. Given this change, this site was not revisited after 2014 and no additional releases were made here.

Results - Siskiyou County

<ins>Little Shasta/Harry Cash Road</ins> **–** Releases (n=1,440) occurred in 2011 and 2013 near the community of Little Shasta. On both occasions, two bouquets of galled stems (n=16 galls/bouquet in 2011, n=20 galls/bouquet in 2013) were placed within a patch of Russian knapweed. This location was significantly drier than the Lassen County site. The plants were scattered and smaller in size. The placement of the two bouquets were made in the same way as in Lassen County but the plant canopy did not give much shade protection so branches of nearby rabbit brush plants were cut and placed over the bouquets to provide some sun protection. The site was monitored in July, August, and October 2011 but no galls were found on any visit. No galls were found when the site was revisited in September 2013, October 2015, November 2016 and August 2017. In 2013, the amount of rainfall was unusually low and even though plants developed shoots with flowers, the shoots dried up before setting seed.

Figure 2: Adult *Jaapiella ivannikova* on a Russian knapweed leaf. Photo by Joel Price, Oregon Department. of Agriculture.

Figure 3. Galls produced by *Jaapiella ivannikova* on a Russian knapweed stem.

<ins>Dorris/Indian Point Road</ins> – Releases (n=2,400) occurred in 2012 and 2015, in two clonal patches of knapweed approximately 100 meters apart (800 in one patch, 1,600 in the other). In September 2013, several galls were counted (n=28 galls on 19 plants) in one

of the clonal patches. No galls were found in October 2015, November 2016, and October 2017.

<u>Dorris/E. Butte Valley Road</u> **-** Releases (n=1,520) occurred in 2012 and 2014, in two open meadows infested with knapweed approximately 500 meters apart (760 in each meadow). The two release sites were monitored in September 2014, October 2015, and October 2017 and no galls were found on any date.

Discussion

In its native area, *J. ivannikova* goes through multiple generations within a season on plants that stay green late into the Summer and early Fall. In North America, the gall midge has established in Montana, Wyoming, Idaho and Oregon, in areas that have a continental climate similar to central Asia (summer rainfall and a late growing season). The climate of California is Mediterranean where rainfall is limited to the months of winter and early spring and the summer is hot and dry. In this climate, the flowering stalks of Russian knapweed plants dry up by early-summer, preventing the fly from producing multiple generations within a season. Thus, adult flies emerging after the first generation are unable to find green plants on which to deposit eggs and subsequently the population dies out. The requirement for additional generations late into the year appears to prevent this beneficial insect from establishing in Northern California.

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[2]Siskiyou County Agricultural Commissioner's Office, Yreka, California
[3]United States Bureau of Land Management, Susanville, California

Biological Control of Russian Knapweed in Northern California: Release of Gall Wasp *Aulacidea acroptilonica* (Hymenoptera: Cynipidae)

Michael J. Pitcairn, Viola Popescu, Jeffrey Littlefield[1], Thomas Getts[2], and Jodi Aceves[3]

Russian knapweed, *Rhaponticum repens* (L.) Hildago (formerly *Acroptilon repens*), is an exotic noxious weed that originates in central Asia and occurs throughout California. As a relative of yellow starthistle (*Centaurea solstitialis* L.), *R. repens* is known to be poisonous to horses, causing a chewing disease that can eventually end to death. In general, the plant is distasteful to cattle but grazing occurs it if more palatable forage is unavailable. Russian knapweed is a perennial with an extensive root system that spreads in a widening circle. Above ground rosettes are formed in the fall and early spring, with one-to-two feet tall, seed-producing flower stalks developing in the spring and early summer. A stem-galling wasp, *Aulacidea acroptilonica* Tyurebaev, has recently been approved for use as a biological control organism for Russian knapweed in the United States. Herein we report on the efforts to introduce the gall wasp into Northern California.

Adult wasps emerge early in the spring when the plant is just beginning to develop flowering shoots. The adult female lays its egg near the growing tips of the main and lateral shoots and feeding by the developing larvae produces a swelling in the stem that hardens into a gall. The immature wasp develops through three larval instars then aestivates through the summer and winter until spring when it pupates and emerges as an adult wasp from the gall. The gall wasp has only one generation each year.

Figure 1. Adult female gall wasp, *Aulacidea acroptilonica*. Photo by Joel Price, Oregon Department of Agriculture.

Initial releases of *A. acroptilonica* in California began in 2014 and continued through 2017 at five locations in Lassen and Siskiyou counties (Table 1). The release material for 2014 and 2015 was provided by Dr. Richard Hansen, United States Department of Agriculture (USDA) Animal and Plant Health Inspection Services (APHIS), Ft. Collins, Colorado, as adult wasps shipped in glass vials. Release material for 2016 and 2017 was provided by Dr. Jeffrey Littlefield, Montana State University, as immature wasps in galls. For 2016 and 2017, stem galls were collected in March from a field site near Toston, Montana, shipped to Sacramento, California and immediately placed in a refrigerator. In 2016, the galls were removed from cold storage on May 20 and placed in an emergence cage maintained at room temperature and natural photoperiod. Adult wasp emergence began June 11 and releases at field sites occurred June 13-22 (Table 1). In 2017, plants with galls were removed from cold storage on May 1 and placed in an emergence cage maintained at room temperature and natural photoperiod. Adult wasp emergence began May 15 and releases at field sites occurred May 18 – June 6 (Table 1).

Table 1. Release information for *Aulacidea acroptilonica* in Northern California

Site name	Elev (m)	Release date	No. adults	Galls within season
Shasta County				
Dorris/E. Butte Valley Rd.	1433	7/10/2014	109	0
(2 sites 500 meters apart)		8/19/2015	94	0
		6/17/2016	80	--
		6/22/2016	200	no data
		5/25/2017	200	--
		6/1/2017	200	0
Dorris/Indian Point Rd.	1296	7/9/2015	79	0
(2 sites 50 meters apart)		6/17/2016	80	--
		6/22/2016	200	0
		5/18/2017	100	27/sq. m
Little Shasta/Harry Cash Rd.	876	6/14/2016	50	0
Lassen County				
Karlo Road	1356	5/19/2017	100	--
		6/6/2017	175	25/sq. m
Trumball Road	1353	5/19/2017	100	0
Standish/Lambert Lane	1228	5/19/2017	100	2 in 50 plts

The intention in 2017 and 2018 was to better time the releases of adult wasps with the early emergence of Russian knapweed in California.

Recovery Results - Siskiyou County

Dorris/E. Butte Valley Road - Releases (n=883 adults) occurred annually from 2014 through 2017, in two open meadows infested with knapweed approximately 500 meters apart (approximately 440 adult wasps were released in each meadow). The two release sites were monitored in September 2014, October 2015, and October 2017 and no galls were found on any date.

Dorris/Indian Point Road – Releases (n=459 adults) occurred in 2015 through 2017, in two clonal patches of knapweed approximately 50 meters apart. Releases in 2015 and 2017 occurred in only one of the two patches. No galls were found in October 2015 and November 2016, but several galls were found in October 2017 and many more in September 2018. In 2017, the occurrence of galls was found up to 50 meters from the release point. In 2018, galls were found up to 75 meters from the initial release point.

Little Shasta/Harry Cash Road **–** Releases of the gall wasp (n=50 adults) occurred once in June 2016. No galls were found in November 2016 and August 2017.

Recovery Results - Lassen County

Karlo Road – Releases (n=275 adults) occurred in May and June 2017. This area is grazed by cattle so the release area (20 m x 5 m) was protected from grazing by an electric fence. Several plants with galls were found in October 2017 and many more in September 2018.

Trumball Road – Releases (n=100 adults) occurred in May 2017. The release occurred in a fenced yard near a secondary residence not subject to grazing. No galls were found in October 2017 or September 2018.

Standish/Lambert Road - Releases (n=100 adults) occurred in May 2017 at one location near Standish, a small community about 15 miles east of Susanville. A total of two galls were found in October 2017 and many more galls were found in September 2018.

Monitoring Results

Beginning in 2017, plant and gall abundance was measured at the Indian Point Road site in Siskiyou County and the Karlo Road site in Lassen County. At Indian Point Road, the knapweed patch (17 m x 18 m) is approximately 300 square meters in size. At Karlo Road, the knapweed patch (5 m x 20 m) is approximately 100 square meters in size. At both locations, transects were placed parallel (approximately 1 meter apart) through the knapweed patch where the releases occurred. A quadrat was placed every meter along the transects and all knapweed plants in a quadrat were removed and bagged separately. In 2017, a quadrat of 20 cm by 20 cm was used at the Karlo Road site but at Indian Point, a quadrat of 20 cm by 10 cm was used. The smaller quadrat was used at both sites in 2018. All plants were returned to the laboratory in Sacramento and measured for plant height, number of galls, number of flower heads, number of seeds per flower head, and dry weight. Weights were obtained after drying plants at 60^{o} C for 48 hours. The results show a significant increase in gall abundance in 2018 at both locations despite no additional releases occurring that year (Table 2).

Figure 2. Russian knapweed plant with *Aulacidea acroptilonica* galls

Discussion

The failure to find galls following the releases in June and July of years 2014 through 2016, followed by successful gall formation after the releases in May 2017 suggests that release timing for this beneficial insect is critical. The releases in 2014 were on plants fully formed and with flower buds about to open. In contrast, in May 2017 plants were just emerging from the soil, approximately 2-5 inches tall. Female adult wasps appear to prefer to oviposit on young meristematic tissue.

The level of impact the gall wasp may be having on its host plant is difficult to determine as there was a severe drought in 2018. The reduction in the number of flower heads and viable seed at both sites in 2018 may be due, in part, to the lack of rainfall but it was particularly severe at the Indian Point site where all plants dried up in early flower stage and none of the flowers produced viable seed. It will be interesting to see if results from 2019, during which there has been above normal rainfall, show additional increases in gall abundance and similar reductions in plant fecundity. Most encouraging, however, are the observations of initial establishment and overwintering of *A. acroptilonica* in Northern California.

Table 2. Plant and gall abundance at two Russian knapweed locations where the gall wasp, *Aulacidea acroptilonica* was released.

Lassen County Karlo Road	**# quadrats (# of plants sampled)**	**Mean plants per sq meter**	**Mean plant height**	**Mean flw hds per sq meter**	**Mean galls per sq meter**	**Mean seeds per sq meter**	**Mean galls per plant**
2017	14 (52)	92.9	40.4	953.6	25.0	4728.6	0.27
2018	24 (51)	106.2	65.0	316.7	660.4	1325.0	6.22
Siskiyou County Indian Point Rd	**# quadrats (# of plants sampled)**	**Mean plants per sq meter**	**Mean plant height**	**Mean flw hds per sq meter**	**Mean galls per sq meter**	**Mean seeds per sq meter**	**Mean galls per plant**
2017	31 (50)	80.6	38.0	1245.2	27.4	5758.1	0.34
2018	24 (50)	104.2	50.2	0.0	491.7	0.0	4.72

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[2]Weed Ecology and Cropping Systems Advisor, University of California Cooperative Extension, Susanville, CA
[3]Siskiyou County Agricultural Commissioner's Office, Yreka, California

List of CDFA's Biological Control Program Publications (2012-2018)

2012

Pena, J. E., R. E. Duncan, **W. J. Roltsch**, and D. Carrillo. 2012. Mortality factors of the avocado lace bug, *Pseudacysta perseae* (Heteroptera: Tingidae) in Florida. Florida Entomologist 95(1): 179-182.

Johnson, M. W., K. M. Daane, X-G Wang, V. Y. Yokoyama, **C. H. Pickett**, K. A. Hoelmer, and A. A. Kirk. 2012. Biological control of olive fruit fly. Integrated Protection of Olive Crops, IOBC-WPRS Bulletin 79: 63-69.

2013

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Cline, A. R., **B. Villegas, M. J. Pitcairn**, and C. O'Brien. 2013. The status of *Euhrychiopsis lecontei* (Dietz) (Coleotpera: Curculionidae), with notes on other weevils associated with *Myriophyllum* (Saxifragales: Haloragaceae) milfoil in California. The Coleopterists' Bulletin 67(2): 75-80.

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Swezey, S. L., D. J. Nieto, **C. H. Pickett**. 2014. Spatial density and movement of the Lygus spp. parasitoid *Peristenus relictus* (Hymenoptera: Braconidae) in organic strawberries with alfalfa trap crops. Environmental Entomology 43(2): 363-369.

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