**Appendix C**

**Select W4185 Impact Statements**

**2016-2021**

An international effort to advance biological control of the spotted wing drosophila in Europe and North America identified *Ganaspis* nr. sp. *brasiliensis* as the best candidate for release. A national effort led to the successful USDA APHIS petition to release this parasitoid, with release permits acquired across the United States. Parasitoid production is underway at UC Berkeley and USDA Newark’s BioControl Unit, with the goal of developing regional insectaries throughout the US in 2022.

Since the start of a biological control program targeting ACP in California (2011), ACP densities in urban citrus have declined by over 70%. Introducing a key parasitoid from Pakistan and diligently educating the public about this pest program contributed to keeping some of the largest citrus producing areas in CA ACP free for more than 10 years (Milosavljevic, I. *et al,* 2021).

The giant reed leaf sheath midge, *Lasioptera donacis* is highly specific to the giant reed and is a good biocontrol agent.Following studiesthat showed this leafminer does not require a particular and exotic fungal symbiont to complete its development, TAG has approved its release by the US cooperator.

The assessment of long-term outcomes of leafy spurge biocontrol introductions affirms the value and ongoing effectiveness of these efforts, including the important contribution from the stem-boring beetle *Oberea erythrocephala* as well as the more widely recognized contributions from flea beetles.

Research has indicated the need for increased classical biocontrol programs targeting “legacy” pests infesting perennial tree crops. Legacy pests are non-native insects and are defined as those that have been present in the cropping system for more than 25 years, have not been fully researched for classical biocontrol potential, and are now considered “naturalized” while still causing significant and persistent crop production problems and economic losses.

The level of competence of plant quarantine workers, port inspectors, and customs officials within Micronesia is gradually being raised as more personnel are trained in annual PPQ workshops. Increased participation by Guam Customs officers appears to reflect heightened awareness of the threat to Guam posed by invasive species and the increased cargo and human traffic resulting from the ongoing military buildup.

Successful establishment of *P. spegazzinii* on *M. micrantha* on Guam will reduce the density of this weed on Guam, and reduce the amount of herbicides currently used to manage it.

In our study of the influence of symbionts on the biology of natural enemies, and in particular, whitefly parasitoids in the genus *Encarsia,* we found that temperature extremes may have a large impact on symbiont function, and thus *Encarsia* population dynamics.  In particular, we found that both high and low temperature extremes reduced *Cardinium* symbiont density in *Encarsia suzannae.* High temperatures reduced the strength of the reproductive manipulation (cytoplasmic incompatibility), while non-intuitively, low temperatures increased the strength of the phenotype caused by symbiont.  The temperatures at which this occurred were well within the range where no impacts would be seen on the wasp itself.  The impact of this research is that we find that temperature can de-stabilize symbiont function at temperatures that wouldn't influence the natural enemy itself.  Even symbionts that are reproductive manipulators can be required by the organism (for example because of metabolic redundancy, reliance on symbionts for reproduction).  We thus expect natural enemies with symbionts to be especially vulnerable to the effects of even mild changes in climate.

Study of infestation of the weed Dalmatian toadflax, *Linaria dalmatica*, in Utah by the introduced biocontrol agent *Mecinus janthiniformis*, a stem-mining weevil, determined that the weevil attacks the weed heavily and has low mortality rates within toadflax stems under the hot, dry summer conditions that prevail at locations in Utah where the weed is problematical.  Field study also resulted in increased understanding of weevil phenology, including as driven by degree-day accumulation that can be used for optimally timing collection and standardized-monitoring efforts for the weevil as it is redistributed widely and evaluated further for its long-term impact on toadflax in Utah.

The Arizona cotton IPM program has been a success.  It has added an estimated 9000 jobs since 1996 and $700M in annual contributions to the AZ economy. These are in addition to the direct savings of greater than $600M to cotton growers since 1996.

We have found that pesticide use in California crops is shaped, in part, by the need to control native insect herbivores that expand their host plant range from native plants to include the introduced crop plant.  This emphasizes an important opportunity for biocontrol to be targeting not just invasive pests species, but also native species as well.

Guam researchers received a small grant from USDA-APHIS-CAPS in 2017 to continue to survey for aphidiid parasitoids of aphids on Guam and Saipan, a project which initially began in September 2015. This project will continue until the 31 August 2018.

Research on biological control of the soybean aphid in Minnesota has led to new analytical tools to analyze the ability of the parasitoid *Aphelinus certus* to control populations of this invasive pest.  These tools include a stage-based matrix modeling approach that can be modified based on environmental and life-history characteristics for this or similar host-parasitoid systems.

Research results will be of great value to practitioners of biocontrol as they implement monitoring and redistribution programs for *M. janthiniformis*, a biocontrol agent of Dalmatian toadflax. Degree-day accumulation can be used for optimally timing collection and survey efforts for weevils during spring and summer when they occur on weed stems. Such timing will be aided by using the sex-specific results of degree-day modeling, given that male and female weevils differed predictably from each other in their phenology (with males arriving earliest in the spring on the host plant).

Approximately 235,600 *Aulacidea acroptilonica* were redistributed to field sites in CA, CO, ID, MT, NV, UT, WA and WY. The gall wasp is now established and increasing in population at least 25 sites in Montana. The gall midge *Jaapiella ivannikovi* is also established in Montana with populations dispersing at some establishments.

New agents are being investigated for the biological control of Russian knapweed, hoarycress, perennial pepperweed, common tansy, ox-eye daisy, invasive hawkweeds, and rush skeletonweed. The target weeds have either no biological control agents currently available or the agents already established are not effective over the range of the target weed. In addition, a better understanding of biological control and its implementation will be achieved by monitoring the impacts associated with these biological control agents.

Development and implementation of biological control based action thresholds for whiteflies in cotton have the potential to reduced risk to growers, enhance grower profits, reduced insecticide use, and enhance environmental benefits.

Bt cotton is a selective technology for caterpillar control and facilitates conservation of natural enemies important to biological control of cotton pests overall.

The African opiine braconid parasitoid, *Fopius ceratitivoru*, was imported to Hawaii, cleared through quarantine, established in laboratory colonies, and released in coffee fields to attack Mediterranean fruit fly (*Ceratitis capitata*).

Asian citrus psyllid (ACP) and natural enemy phenology and impact studies over 15 sites for approx. 3 years is nearing completion (31 Dec. 2017). These studies have been concentrated in urban areas with backyard citrus as the host plants being monitored for ACP activity and natural enemy presence/absence and impacts.

Argentine ants are a major impediment to Asian Citrus Psyllid (ACP) biocontrol as ants protect ACP nymphs from natural enemies. For this protection, ACP nymphs reward ants with honeydew, a sugary product ants harvest and return to the nest. A novel ant baiting strategy has been developed to kill Argentine ants, biodedgradable hydrogels,that act as small reservoirs containing sucrose solution and thiamethoxam. Ants drink from these beads and return the toxicant to the nest killing the queen. Results show that hydrogels provide 80-96% ant suppression in commercial citrus orchards.

The parasitoid, *Macroglenes penetrans*, was exported from Canada to Western Montana in 2014. Wasp adults were released in wheat midge infested fields at several locations of Golden Triangle, Montana, in 2014. From 2015-2017, adults were regularly monitored in several locations of Montana. Current surveys clearly showed that this parasitoid species is now well established in Golden Triangle, Montana.

Five extension articles on Asian Citrus Psyllid (ACP) biocontrol and invasives were published. A total of 20 extension presentations were made. Topics covered ACP and BMSB biocontrol, avocado pests, general overviews on invasions, IPM, and biocontrol. Two web pages on dung biocontrol and ACP biocontrol were developed as were blog posts on ACP natural enemies. Three extension conferences on invasive pests and their management were organized. Numerous interviews were given on biocontrol and these included newspaper interviews, radio interviews, and trade magazines.

Outputs include completion of host range and host specificity testing of the brown marmorated stink bug egg parasitoid, *Trissolcus japonicus*. This is a significant step forward towards the possible release of this parasitoid in California for biological control of BMSB. The high efficacy of biodegradable hydrogel beads was demonstrated for controlling the invasive Argentine ant in citrus orchards. These results suggest that insecticide sprays for citrus pests could be reduced if ants are controlled and natural enemy activity increases.

A two-year field study demonstrated no non-target impacts of Bt eggplant on arthropod natural enemies in Bangladesh. Bt eggplants provided high levels of target pest control with significant economic gains.

Farmer data was used to determine which insecticides currently used in citrus pest management are most disruptive to *Euseius tularensis* predatory mite populations, thereby interfering with biological control of either citrus red mite or citrus thrips. Results gave farmers recommendations for which materials to use to retain their key biocontrol agents within their groves.

A symposium, “Rapid evolution in biological control systems: Implications for safety and effectiveness’ was held at the joint meeting of the Entomological Societies of America, British Columbia, and Canada in Vancouver, BC November 2018.  The goal was to address concerns arising at the intersection of the science, technology, and policy arenas in biological control due to recent discoveries pertaining to rapid evolution in biological control systems. Talks addressed importation (goal A), conservation (goal B), and augmentation (goal C) biological control and included many of the arthropod and weed pests addressed by this W4185 Project.

The more expensive enhanced diet must be used to mass produce *Trichogramma brassicae* because *Ephestia kuehniella* females from the enhanced diet oviposit more and larger eggs, the wasps parasitize more of the largest host eggs, and significantly more female wasps are produced from the largest eggs.

*Aphthona* flea beetles redistributed on all significant leafy spurge populations in NM have reduced densities to non-economic levels throughout the state.

A new *in vitro* assay has been developed with potential usefulness in identifying candidate species of entomopathogenic nematodes to control specific arthropod pests.

Water and soil resources for agriculture, and habitat for native plant species, in rangelands, forests, wetland and aquatic systems, have been protected through biological and integrated control of alligatorweed, arundo, Cape-ivy, Dalmatian toadflax, French broom, Russian thistle, Scotch broom, water hyacinth, and yellow starthistle

*Gryon gonikopalense*, a host specific egg parasitoid of the cole crop pest *Bagrada hilaris*. It is an ideal biological control agent as its impact prevents any major damage to the host plants. The development cycle of the egg parasitoid is perfectly synchronized with its host and is able to attack a high percentage of host eggs on various species. It is effective at low host density, and targets eggs that are dominantly buried into the ground by the *Bagrada hilaris* females making this parasitoid an invaluable tool for pest containment.

The phylogeography study conducted on *Pyrrhalta viburni* parasitoids strongly suggests that there is only one egg parasitoid of *P. viburni* present in Europe, which provided important clues regarding the assemblage of natural enemies attacking it in its native range.

The genetic barcoding of olive psyllid, *Euphyllura olivine*, individuals collected in Spain, France and California revealed that the Californian population is closely related to one population in Southern Spain and one population in Southern France, indicating the potential origin of the Californian invasive populations and suggesting where to look for promising natural enemies.

In 2019, a UC-Berkeley laboratory conducted research and compiled results on invasive pests (spotted wing drosophila, olive psylla, brown marmorated stink bug, vine mealybug) and native pests (stink bugs and leaf footed bugs). The work resulted in numerous presentations to growers and researchers, 13 peer-reviewed publications, and two USDA APHIS petitions to release natural enemies of invasive species (spotted wing drosophila and olive psylla).

A large team of researchers are collaborating to build a global database that is being used to evaluate the influence of the agricultural and natural landscape on the success of biological pest control.

Growers were presented a greater number of control tools for invasive and native pests of vineyards, nut crops and various row crops, including hemp. This information has aided growers in more sustainable farming techniques, resulting in a reduction of the pesticide load in the environment, a reduction in pest damage and an increase in farm profitability.

Over 155,000 *Aulacidea acroptilonica* galls and adults were consigned or released in ID, MT, and NM. The gall wasp is now established and increasing in population at least 25 sites in Montana and dispersing over 8 km at some establishments. The gall mite, *Aceria drabae,* was also released in Montana. This is the first biological control agent to be released in North America against hoarycress.

Low summer mortality rates within Dalmatian Toadflax stems should promote weevil establishment under the hot, dry conditions typical of locations in Utah where the weed is problematical. Increased understanding of weevil phenology within host stems will facilitate development of standardized, summer monitoring for this biocontrol agent by stem dissection.

Control of invasive Argentine ants increases natural enemy impacts on sap sucking pests in citrus by more than 90% and colonies of some pest species are completely eliminated.

The importance of controlling the invasive Argentine ant in citrus orchards was demonstrated. The positive flow on effects for natural enemies and subsequent biocontrol of sap sucking citrus pests was significant. These results suggest that insecticide sprays for citrus pests could be reduced if ants are controlled and natural enemy activity increases. These beneficial effects could be amplified if flowering cover crops are planted around the margins of citrus orchards. Work demonstrated that natural enemies, especially hover flies, respond strongly to this resource and impacts on key citrus pests such as Asian citrus psyllid increase substantially. The results of this work were extended to hundreds of end users via talks, the web, field days/workshops, and media interviews.

Regional monitoring of Virginia creeper leafhopper allows us to follow the impacts of the *A. daanei* rear-release program that ran between 2015-2018. In 2018, the first year without releases, a rather drastic reduction in parasitism rates was noted. We are now considering the possibility of resuming *A. daanei* introductions, but first clarifying a few questions about the biogeography and behavior of this natural enemy. Multiple presentations were given on this leafhopper/parasitoid in 2019, including grower (EcoFarm, Santa Cruz Mountains Winegrowers Association, Wild Farm Alliance, CAPCA Fresno/Madera, CAPCA North Coast), professional talks (ESA Pacific Branch) and public talks (Cal. Academy of Sciences “NightLife!” event)

The effectiveness of canopy thinning of macadamia nut orchards was demonstrated to facilitate diversification of understory plant assemblages, and the impact on natural enemies of macadamia felted coccid (*Ericoccus ironsidei*).

Chalcidoidea are economically and biologically one of the most important groups of insects, and yet very little is known of their taxonomy (identification) or relationships. Research is identifying new potential biological control agents for use against pestiferous leafminers on citrus, whitefly on citrus, aphids on wheat and other crops, and for wasps attacking pestiferous ants. New research on cryptic species complexes (morphologically identical but reproductively and biologically distinct species) using molecular markers has tremendous potential for the identification of new biological control agents. This research is providing a better understanding of the wasp parasitoids attacking several pest groups in California including the Citrus Peelminer, Citrus Leafminer, sharpshooter parasitoids and the Asian Citrus psyllid. Identification keys and other products will help other researchers to better understand the impact of these groups, and identify gaps that aid in targeting new biological control agents.

Five years ago W-4185 project members released the first biological control agent to be used in North America against hoarycress. Over the past five years, projects have provided over 165,000 *Aulacidea acroptilonica* adults for redistribution. Gall wasps were utilized for research releases, but the majority of adults were sent to county, federal, and state cooperators in ten counties located Montana, and tribal lands (Crow, Northern Cheyenne, Ft. Belknap, Chippewa Cree, and Confederated Salish and Kootenai tribes). Consignments were also made to USDA-APHIS PPQ & CPHST, BLM, New Mexico State University, University of Wyoming, Washington State University, Wyoming Weed & Pest, Department of Agriculture in California, Oregon and Nevada, and the Nez Perce Biocontrol Center, and the biocontrol program at Whitehall High School, MT. We also released the gall mite *Aceria drabae* in Montana in 2019 and 2020, and had recovery of the mite at both of the 2019 releases.

*Orcytes nudivirus* is currently being disseminated throughout Guam and its impact monitored. New strains of *O. nudivirus* are being sought from coconut rhinoceros beetle infested countries in the Western Pacific Region.

A method of rearing bulb mites in the laboratory has been developed and published. This information will be of use to other scientists who are working on this pest, and who need to rear them for their biological control research.

Multiple presentations and factsheets were prepared and given on the value of habitat plantings, reaching at least 1,000 growers, many of whom have adopted this practice which benefits the environment by supporting beneficial arthropods.

*Gryon gonikopalense*, a host specific egg parasitoid of the cole crop pest, *Bagrada hilaris,* is an ideal biological control agent as its impact prevents any major damage to the host plants. The development cycle of the egg parasitoid is perfectly synchronized with its host. The parasitoid is able to attack a high percentage of host eggs on various species. The parasitoid is effective at low host density, and targets eggs that are dominantly buried below-ground by the *Bagrada hilaris* females. The parasitoid is an invaluable tool for pest containment

The microbiome study of *Arytinnis hakani* revealed the absence of phytopathogenic bacteria and thus ensures that this biocontrol agent will not vector plant diseases if released.

Evaluation of host specificity of *Psyttalia ponerophaga* for biological control of the olive fly, *Bactrocera oleae,* is helping to evaluate the potential of this biological control agent against *Pyrrhalta viburni*. Study of the impact of rearing on an alternative host on the host specificity of *Psyttalia ponerophaga* showed the safety of rearing this parasitoid on the alternative host *Ceratitis capitata*.

*Aphthona* flea beetles redistributed on all significant leafy spurge populations in New Mexico have reduced densities to non-economic levels throughout the state.

Russian knapweed gall wasp, *Aulacidea acroptilonica,* continues to establish much better than Russian knapweed gall midge, *Jaapiella ivannikovi,* under hot and dry conditions*.*

*Mecinus janthiformis* flea beetles have been released on all known populations of Dalmatian toadflax in New Mexico.

Chalcidoidea are economically and biologically one of the most important groups of insects, and yet very little is known of their taxonomy (identification) or relationships. Our research is identifying new potential biological control agents for use against pestiferous leafminers on citrus, whitefly on citrus, aphids on wheat and other crops, and for wasps attacking pestiferous ants. New research on cryptic species complexes (morphologically identical but reproductively and biologically distinct species) using molecular markers has tremendous potential for the identification of new biological control agents. This research is providing a better understanding of the wasp parasitoids attacking several pest groups in California including the Citrus Peelminer, Citrus Leafminer, sharpshooter parasitoids and the Asian Citrus psyllid. Identification keys and other products will help other researchers to better understand the impact of these groups, and identify gaps that aid in targeting new biological control agents.

Common mullein is an invasive weed in the USA that causes economic and ecological damage in pastures, rangeland, and disturbed and natural areas, especially in California and Hawaii, from sea level to more than 13,000 ft elevation. ARS researchers in Sidney, MT, using molecular tools, have determined that the invasion is mostly dominated by a single genotype that exists across the western states, with origins of Belgium and Germany. This information helps land managers protect against development of herbicide resistance in the invasion, and helps researchers ensure that future biological control agents will have highest control efficacy against the most common genotypes.

Study of parasitism of the cereal leaf beetle (CLB), *Oulema melanopus*, determined that introduction of the host-specific parasitoid *Tetrastichus julis* (Eulophidae) has been highly successful for biocontrol of CLB in Utah.  The wasp was found to parasitize the majority of CLB larvae in small grain fields each growing season, with rates of parasitism approaching 100% both early and late in the season.  Study of movements of wasps between individual fields indicated that time, money, and effort need not be spent by growers on redistribution of these wasps into newly planted fields, as the wasps are now widely established throughout the region and have strong dispersal capacity to colonize newly created habitat on their own.