BCIP 2014 Proposal

1. <u>Project Title</u>: **Using an aggregation compound formulation to strategically focus *Diorhabda carinulata* (Desbrochers) on *Tamarix* spp.: Enhancing the impact of a defoliating herbivore and improving the operational efficiency of monitoring establishment and population expansion.**

2. <u>Principal Investigator</u>:

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3. <u>Cooperators, Collaborators, and Other Participating Institutions</u>:

Tom Dudley, Marine Science Institute, University of California, Santa Barbara, CA

Justin Russak, Chemistry Department, University of California, Santa Barbara, CA

Dan Bean, Colorado Department of Agriculture – Palisade Insectary, Palisade, CO

Agenor Mafra-Neto, ISCA Technologies, Inc., Riverside, CA

Alexander Gaffke (graduate student) – LRES Department, MSU, Bozeman, MT

Sharlene Sing – USDA FS, RMRS, Bozeman, MT

Liz Hebertson (BCIP contact) – USDA FS, FHP, Ogden, UT

4. <u>Amount Requested (yearly and total), Project Leveraging</u>**:**

Annual request- $64,700; Total request- $64,700; matching funds- $21,552 (25%)

Match: $12,940 - foregone 20% F&A (IDC) charges; $8,612 PI (Weaver) salary + benefits;

This project leverages an additional $9,300 and another 10% is anticipated

5. <u>Project Goals and Supporting Objectives</u>:

Our goal is to optimize the deployment of male-produced aggregation pheromone of the tamarisk leaf beetle, *Diorhabda carinulata* (Dc), introduced for the biological control of *Tamarix* spp., to better develop, implement and assess the biocontrol program in western North America.

The project directly addresses two of the Priorities for the 2014 FHP/FHTET Biological Control of Invasive Plants program, specifically to: 1. Develop improved **rearing, distribution and post-release monitoring techniques** for a biological control agent; and 2. **Develop and implement technologies for rapid quantitative assessment** of biological control impacts. It also indirectly addresses the final FHTET Priority, 3. Integrated weed management with a **biological control component that is part of a methods development approach to determine efficacy** and is not considered an operational treatment.

Elements of the project are directed at enhancing the effectiveness of *Tamarix* biocontrol programs in FS Regions 1, 2, 4 and 6 where Dc has been historically released, and to evaluating its dispersal and establishment in FS Regions 3 and 5, and portions of Region 4, where population expansion is occurring and federally listed wildlife populations are potentially at risk via defoliation of nesting habitat.

The project also combines the efforts of two research programs into a unified theme of optimizing efficacy of biocontrol of *Tamarix* spp. by Dc and by monitoring its continued expansion and establishment. The core component of this is the strategic use of commercially formulated aggregation pheromone (in SPLAT™). The following objectives are centered on this technology.

1. Produce the alcohol component of the aggregation pheromone, (2*E*, 4*Z*)-2,4-heptadien-1-ol ("pheromone") first reported in Cossé et al (2005) in large quantities (150 g) suitable for an expanded strategic focus;

2. To do so, further modify methods for ramping up pheromone production to the most efficient and cost-effective capacity, including developing formulations highly suited for field longevity and storage;

3. Expand field trials exploring increased efficacy using pheromone formulations to: a) specifically target heavy defoliation of *Tamarix* spp. by Dc populations at four northern sites, and

    b) facilitate retention of northern Dc populations in areas experiencing heavy pheromone-mediated defoliation the previous year, and

    c) assessing Dc establishment at both historical <u>and</u> new release sites using pheromone-baited sentinel trees;

4. Implement a pheromone-mediated procedure using sentinel trees for monitoring dispersal and establishment of Dc in the U.S. southwest to:

    a) yield an early detection tool for tracking population expansion into tributaries of the lower Colorado River to the Mexican border, including several adjacent drainages, and

    b) to work closely with local cooperators to establish sentinel tree stations using pheromone formulations for detection of new colonization into regional watersheds such as the Verde/Salt/Gila system, Mojave and Owens, or upper Rio Grande;

5. Ultimately provide an operational framework for future application to other systems by land managers using a familiar technology for deployment while maintaining a low level of environmental risk to sensitive riparian areas.

6. <u>Project Justification/Urgency</u>

The invasion of western landscapes by non-native tamarisk or saltcedar, *Tamarix* spp., constitutes one of the most extensive and damaging changes to riparian and wetland ecosystems. Its biology and impacts are well-known and controversial (Busch and Smith 1995, Dudley et al. 2000, Shafroth et al. 2005), including displacement of native riparian vegetation and riparian-dependent wildlife species (Ellis 1995, Dudley and DeLoach 2004), alteration of flood and erosion dynamics (Graf 1978, Manners et al. 2013), depletion of groundwater resources (Shafroth et al. 2005), poor forage quality for livestock (Lesica and Miles 2001) and increased frequency and extent of wildfire (Drus et al. 2012).

In response, specialist agents to suppress *Tamarix* spp., including Dc, were approved for release in heavily-invaded riparian locations (DeLoach et al. 1996). At the same time the Southwestern Willow Flycatcher (*Empidonax traillii extimus*) was designated an Endangered Species that was known to use tamarisk as breeding habitat (Sogge et al. 2008). While not unexpected given its dominance within structural elements of riparian woodlands, the risk of *Tamarix* spp. defoliation and subsequent nest exposure during breeding season raised concerns that biocontrol could result in deleterious outcomes for the threatened species (Dudley and Bean 2012). After federal consultation, Dc was permitted for release in sites beyond 200 miles from known nesting sites in *Tamarix* spp., and in 2001 it was open-released in seven states (Dudley et al. 2001).

Results from initial releases were mixed (DeLoach et al. 2004, Dudley et al. 2012). In some sites populations established and within two to four years defoliated several thousand hectares of tamarisk (Pattison et al. 2010, Meng et al. 2012). Groundwater savings (Pattison et al. 2010), improved wildlife food resources (Longland and Dudley 2008) and target mortality along with evidence of a recovery process (Hudgeons et al. 2007, Bean et al. 2012) were documented, but **establishment failed at most sites** (Bean et al. 2007, Dudley et al. 2012). These need to be re-evaluated for locations which have not been visited in nearly a decade. It is possible that low level survival of the species was not observed and then sites were no longer visited.

Reasons for failures included avoidance of some host taxa that were not sympatric in locations of origin (Dudley et al. 2012) and predation by ants and other generalist predators (Strudley and Dalin 2013). Developmental synchrony with climate and host plants was also a key factor in establishment success or failure of southern releases (Bean et al. 2007). Critical day length that triggered diapause before the overall metabolic capacity to survive the prolonged interval before new vegetation was available initially prevented adult survival at southern latitudes and populations could not establish (Dalin et al. 2010). This biological safeguard against overlapping with Southwestern Willow Flycatcher habitat has faltered due to selection for a shortened critical day length in Dc (Bean et al. 2012). This forces an immediate need to effectively and strategically monitor expansion of Dc populations over a larger area southward: with no reason to assume that the risk of Dc utilizing an even larger area of critical habitat is unreasonable if the critical day length requirements of Dc continue to become shorter (McLeod and Koronkiewicz 2009). We require strategic capacity and protocols using pheromones for this.

Recent findings using controlled release formulations. In 2012, we began the practice of using sentinel plants (Britton et al. 2010) to better assess establishment and more readily measure impact by using trees baited with lures releasing species-specific aggregation-stimulating compounds in *Tamarix* spp. stands in WY. In 2013, both pheromones and host plant attractants were deployed using a commercially available sustained-release formulation (SPLAT™). The results were very encouraging. The aggregation pheromone worked better than the reported aggregation facilitating plant compounds (Cossé et al. 2006), their combination or a control at a pair of fully replicated, randomized complete block sites. These sites were located along the Big Horn River southwest of Lovell, WY and had received Dc from Fukang, China in 200. The Dc population was initially successful on local *Tamarix* spp. invasions. However, in 2012 there was a very marginal population of Dc due to a population crash when the area flooded heavily in 2008. These small populations were used as surrogate 'initial release' sites in 2013. Greater beetle capture in sweep nets and more rapid defoliation occurred quite rapidly on all pheromone treated trees. The longevity and effectiveness of the formulations was far superior to initial proof of concept trials with rubber septa in 2012, where only pheromone impacted beetle capture with no effect of the plant volatiles reported in Cossé et al. (2006), but all treated septa lost activity very quickly. In 2013, cumulative adult capture at a site with high beetle density was 4 times greater when pheromone was present relative to the blank, while cumulative larval numbers at a low beetle density site were more than double for the same comparison. Heavy feeding on pheromone treated trees resulted in early defoliation and beetles migrated to nearby trees (other treatments) to continue feeding. Thus, over time, the numbers on the less successful treatments inflate due to the success of the pheromone treatments!

Equally impressive was the longevity of the pheromone product in the SPLAT™ formulation. After 1 week under harsh field conditions the release rate was about 10,000ngd^{-1} from a 1g 'dollop' (Lapointe et al. 2011), which was equivalent to the release rate under controlled conditions in our laboratory at 25°C. Recent laboratory trials suggest that initial pheromone release rates from a 4g dollop were double that from a 1g dollop while a 2g dollop was intermediate at 3500ngh^{-1}. But the differences declined rapidly after 3 days and release rates were virtually identical at day 8. The dollop sizes were chosen from mass trapping (4g) Vargas (2009); lure and kill (2g) Vargas et al. (2009); mating disruption (1g) Lapointe et al. (2011).

Further deployment of these 1g formulations can be used to control subsequent dissemination and spread of biological control agents throughout a release location by targeting specific sentinel plants to measure spread or focus herbivory. It has also become apparent that the use of this technology in monitoring is quite critical, especially when tracking of populations provides critical environmental benefit. **It is remarkable and fortunate that pheromone treated sentinel trees draw beetles in effectively so that they can be captured in passive commercial delta traps hung near the treated tree.**

7. **Approach:**

1. **Pheromone Synthesis – production of gram scale amounts.** A limitation in this type of project is the supply of costly pheromone needed. The published synthesis of (2*E*, 4*Z*)-2,4-heptadien-1-ol and (2*E*-4*Z*)-2,4-heptadienal (Petroski 2003) has been modified and will be scaled up by an organic chemist to produce 150 g of (2*E*, 4*Z*)-2,4-heptadien-1-ol. This is a direct funding line in the budget.

2. **Formulation.** We will provide pheromone to ISCA Technologies, Inc., Riverside, CA to produce a 2.5% formulation in a specifically designed matrix. This will require product formulation costs that are also a direct funding line in the budget. Development costs will be mitigated by our field and laboratory experiments.

3. **Field study one – deploying products to focus herbivory**. We will target the two sites in the area near Lovell, WY again. At each site, the existing array of trees will be divided into 3 blocks, each containing at least 20 trees. Tree positions within each block will be randomized and UTM coordinates of each tree will be logged using a GPS. Within these blocks, there will be three treatments with 5 trees for each treatment. Treatments will be 1) SPLAT with aggregation pheromone, 2) SPLAT as a blank formulation and 3) a random untreated tree. Temperature and weather data will be monitored on-site using 3 HOBO dataloggers per block. We will also add two new sites along the west shore of Boysen Reservoir. The site near Lovell (44°46'30", 108°11'8") received beetles from initial releases made in 2001. The shoreline site at the Boysen Reservoir (43°17'12", 108°12'16") is located west of Shoshoni, WY Beetles were introduced from the site near Lovell in 2005, 2006, and 2007. It currently supports a population of beetles that is comparable to the parent site. Categorical data will be taken at the time of first deployment of lures, as will pre-counts. We will take weekly sweep counts and measure defoliation, but SPLAT™ products will be deployed only at the time of emergence of 1) overwintering, 2) F1, and 3) reproductive diapause adults based on temperature records, previous experience and the development rates reported by Herrera et al. (2005).The experiments will be a CRD design with the three treatments. Analysis appropriate for comparing local abundance of insect numbers, density, life stage and damage will be used. A mixed-model MANOVA will be used to quantify overall effects. The data collected will be part of a Ph.D. student project and will be published in scientific journals and in FHP numbered publications, where appropriate.

4. **Field study two – dispersal monitoring**. The current distribution of Dc in North America is available (http://www.tamariskcoalition.org/BeetleMonitoring) but has numerous geographic inconsistencies owing to survey methods not suitable for detecting insect presence early when population size is small. **Proposed surveys using the pheromone formulation associated with delta traps are intended not simply to fill gaps, but to target systems south, west and east of the mapped area that are likely avenues of beetle dispersal** during the next two years. A strategic network of pheromone based sentinel trees will be established in spring 2014 and maintained throughout the season to track dynamics of colonizing insects. Because dispersal occurs following epidemic population increases at sites where high *Tamarix* biomass supports high population growth rates, we will be especially vigilant during such periods based on routine inspection of currently established monitoring locations such as dense growths surrounding Lake Mohave, and similarly in newly-colonized areas on the Rio Grande in the region north and south of Albuquerque.

With this sentinel monitoring system, particular attention will be directed to dispersal pathways toward, and locations inhabited by Southwestern Willow Flycatcher in the Gila/Verde/Colorado system as well as the Rio Grande, in response to concerns regarding potential risk to nesting birds (Sogge et al. 2008). We work closely with colleague Matthew Johnson, who has previously been supported by FHP for *Tamarix* spp. biocontrol in relation to critical nesting habitat and has been assessing Southwestern Willow Flycatcher populations in the New Mexico and Arizona regions. In a parallel project he and colleagues will construct habitat models for Colorado Basin watersheds. Similarly we will work closely with Bureau of Reclamation biologists charged with assessing impacts to Southwestern Willow Flycatcher populations in the regions of the Lower Colorado, including identification of locations where restoration efforts should be focused. This conforms to on-going work supported by the Walton Family Foundation to conduct ecohydrological assessments with the goal of enhancing native plant habitat for Southwestern Willow Flycatcher in the context of anticipated *Tamarix* biocontrol (Dudley and Bean 2012). Likewise, pheromone based monitoring information will be linked to the Middle Rio Grande Endangered Species Collaborative Program for chronicling status of biocontrol and riparian vegetation in the three reaches of that river designated as Critical Habitat, particularly regions associated with Sevilleta and Bosque del Apache National Wildlife Refuges and Elephant Butte Reservoir.

A Rapid Assessment approach (Dudley and Brooks 2011, Bateman et al. 2010) will also be applied to monitor condition of *Tamarix* plants in relation to insect herbivory, including *Diorhabda* spp. and other specialist *Tamarix* herbivores, the long-established *Opsius stactogalus* leafhoppers and the recently detected tamarisk weevil *Coniatus spendidulus*. A congener, *C. tamarisci*, was approved previously through the APHIS-TAG process (Dudley and Bean 2012). Data from this approach, already in use at sites in western states for several years, plus geographic information on plants and herbivores will be logged into a GIS base map to be maintained on a project website (http://RIVRLab.msi.ucsb.edu/). **Numerous managers and researchers have expressed need for formulated pheromone and a protocol for its use.** Incorporating this as part of a dedicated research program will allow us to maintain cohesion for recording results from our own work and those of other stakeholders across the western states.

8. <u>Expected Products and Outcomes</u>: (products and how they will be used)

Demonstration that controlled release technology is readily implementable for weed biological control applications using sentinel trees and semiochemicals. Specific products:

I. Aggregation pheromone thoroughly tested in controlled release formulation for use by researchers, land managers and other stakeholders

II. Strategy for deployment of formulations to: 1) direct, 2) retain, 3) monitor and 4) potentiate biocontrol agents in targeted locations or across large landscapes.

III. Incorporation of facile monitoring of Dc expansion into critical habitat that builds on existing infrastructure available to disseminate information to stakeholders.

IV. Strategic field demonstration using a model weed biocontrol system that could be developed for other target weed biological control systems.

V. Future development of the commercial potential of the technology by seeking grants supporting small business development for this specific technology.

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BUDGET - One Year	
Salary - Graduate student (0.4 FTE)	$12,600
Benefits @ 10%	1,260
Undergraduate student @ $10.5/hr x 300 hr	3,150
Benefits @ 10%	315
Pheromone synthesis - 150g @ $165/g	24,750
Pheromone formulation - 80 syringes with 60g @ 2.5%	4,995
Subcontract - Dudley (itemized below)	11,632
Supplies - Field items	400
Supplies - Lab items	600
*Travel (project vehicle)	4,998
TOTAL REQUEST	**$64,700**
*Travel details	
Fuel *(650 mi @ 13 mpg X $3.25 gal) - $162.50 (12 trips)*	$1,950
Lodging *$90/night for 2 per room (12 trips)*	1,080
Per diem *(meals per day trip @ out-of-state rate) ($41/person x 2 days x 2 people - $164 (12 trips)*	1,968
Sub-total	$2,880
Subcontract Details - Dudley	
Salary - Co-PI (0.05 FTE)	$4,000
Benefits @ 28%	1,120
Technical assistant (0.25 FTE for 4 mo. @ $15/hr)	2,400
Benefits @ 18%	432
Supplies - Field items	500
Supplies - Lab items	300
*Travel (private vehicle)	2,880
TOTAL REQUEST	**11,632**
*Travel details	
Vehicle - (800 mi @ $0.44 per mile) - $352/trip (6 trips)	2,112
Lodging - (rental house or camping expense - contributed)	0
Per diem (meals per day trip @ out-of-state rate) - ($322/person x 24 days x 1 person - $XXX (6 trips)	768
Sub-total	$2,880

TIMETABLE

April - May 2014:	Delivery of pheromone; formulation
May - June 2014:	Begin field trials on targeted herbivory in WY
June - September 2014:	Begin field monitoring in the Southwest
June - August 2014:	Field assessment of longevity and release rates
September 2014 - April 2015:	Analyze data, prepare reports and publications

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Professional Experience

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Relevant Publications (of >100)

D. Piesik, D. Pańka, M. Jeske, A. Wenda-Piesik, K. J. Delaney, and D. K. Weaver. 2013. Volatile induction of infected (*Fusarium* spp.) and neighboring uninfected barley and wheat may influence attraction/repellence of a cereal herbivore. Journal of Applied Entomology. 137: 296-309.

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Publications (of >90):

Dudley, T.L. 2000. *Arundo donax*. Pp. 53-58 *In*: Bossard, C.C., J.M. Randall and M.C. Hoshovsky (eds), Invasive plants of California's wildlands. Univ. of Calif. Press, Berkeley.

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Relevant Publications:

Cossé, A.A., Bartelt, R.J., Zilkowski, B.W., Bean, D.W. and Petroski, R.J. (2005) The aggregation pheromone of *Diorhabda elongata,* a biological control agent of saltcedar (Tamarix spp.): identification of two behaviorally active components. J. Chem. Ecol. 31:657-670

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Bean, D.W., D.J. Kazmer, K. Gardner, D.C. Thompson, B. Reynolds, J.C. Keller and J.F. Gaskin (2012) Molecular genetic and hybridization studies of *Diorhabda* spp. released for biological control of *Tamarix*. Invasive Plant Science and Management (in press)

Bean, D.W., T.L. Dudley and K. Hultine. 2012. Chap. 22 Bring on the beetles: The history and impact of tamarisk biological control. P. 377-403 In: Sher, A. and M. Quigley (eds). Tamarix: A case study of ecological change in the American West. Oxford Univ. Press.

Dudley, T.L. and D.W. Bean (2012) Tamarisk biocontrol, endangered species risk and resolution of conflict through riparian restoration. BioControl 57: 331-347

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Honors

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B. PUBLICATIONS (from 49)

1. Ye, L.; Wang, X.; Keogh, E. J.; Mafra-Neto, A. 2009. Autocannibalistic and Anyspace Indexing Algorithms with Application to Sensor Data Mining. In: Proceedings of the SIAM International Conference on Data Mining. April 30 - May 2, 2009. Sparks, NV SDM 2009: 85-96.

2. Lapointe, S. L.; Stelinski, L. L.; Evens, T. J.; Niedz, R. P.; Hall, D. G.; Mafra-Neto, A. 2009. Sensory Imbalance as Mechanism of Orientation Disruption in the Leafminer Phyllocnistis citrella: Elucidation by Multivariate Geometric Designs and Response Surface Models. J. Chem. Ecol. 35: 896-903.

3. Vargas, R. I.; Pinero, J. C.; Mau, R. F. L.; Stark, J. D.; Hertlein, M.; Mafra-Neto, A.; Coler, R.; Getchell, A. 2009. Attraction and Mortality of Oriental Fruit Flies to SPLAT-MAT-Methyl Eugenol with Spinosad. Entomol. Exp. Appl. 131: 286-293.

4. Vargas, R. I.; Pinero, J. C.; Jang, E. B.; Mau, R. F. L.; Stark, J. D.; Gomez, L.; Stoltman, L.; Mafra-Neto, A. 2010. Response of Melon Fly (Diptera: Tephritidae) to Weathered SPLAT-Spinosad-Cue-Lure. J. Econ. Entomol. 103 (5): 1594-1602.

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6. Patt, J. M.; Meikle, W. G.; Mafra-Neto, A.; Sétamou, M.; Mangan, R.; Yang, C.; Malik, N.; Adamczyk, J.J. 2011. Multimodal cues drive host-plant assessment in Asian citrus psyllid (Diaphorina citri). Environmental entomology 40 (6), 1494-1502.

7.Batista, G. B.; Hao Y.; Keogh, E.; Mafra-Neto, A. 2011. Towards Automatic Classification on Flying Insects. 10th International Conference on Machine Learning and Applications Workshops. 364-369.

8.Suckling, D. M.; Sullivan, T. E. S.; Stringer, L. D.; Butler, R. C.; Campbell, D. M.; Twidle, A. M.; Allen, W. J.; Mafra-Neto, A.; El--Sayed, A. M. 2012. Communication Disruption of Light Brown Apple Moth (Epiphyas postvittana) using Four Component Sex Pheromone. Crop Protection. In Press.

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10. Vargas, R. I; Piñero, J. C.; Souder, S. K.; Gomez, L. E.; Spafford, H.; Stoltman, L. and Mafra-Neto, A. 2013. Field Trials of SPLAT MAT-Spinosad ME and SPLAT MAT-Spinosad ME/CL Mixtures for Fruit Fly (Diptera:Tephritidae) Suppression of Bactrocera dorsalis (Hendel) and Bactrocera cucurbitae (Coquillett) in Papaya Orchards. In Press.

11. Vargas, R. I; Piñero, J. C.; Souder, S. K.; Gomez, L. E.; Spafford, H.; Stoltman, L. and Mafra-Neto, A. 2013. Field Trials of SPLAT MAT-ME Against Bactrocera dorsalis, Bactrocera carmbolae, and Bactrocera zonata. Pest Management Science. In Press.

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Selected publications

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Sing, S. E. and R. K. D. Peterson. 2011. Assessing risks for established invasive weeds: Dalmatian (*Linaria dalmatica*) and yellow (*L. vulgaris*) toadflax in North America. International Journal of Environmental Research and Public Health. 8: 2828-2853.

Schat, M., S. E. Sing, R. K. D. Peterson, F. D. Menalled and D. K. Weaver. 2011. Growth inhibition of Dalmatian toadflax, *Linaria dalmatica* (L.) Miller, in response to herbivory by the biological control agent *Mecinus janthinus* Germar. Journal of Entomological Science. 46: 232-246.

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