Colorado Department of Agriculture, Tamarisk Coalition, and University of California-Santa Barbara

Updated June 27th, 2012

The goal of this biocontrol monitoring protocol is to define **when**, **where**, and **how many** *Diorhabda* spp. are in existence across an objective area. Within that we then want to have a good idea of where defoliating sized populations exist, where newly aggregated populations are rising, and where beetles have yet to colonize. This sweep protocol is designed to be fast and efficient and answer only simple questions about *Diorhabda* spp. distribution, density and abundance. It can be utilized on and off river corridors and should be a basic protocol for establishing the degree of beetle presence in a region. In general the amount of sweep samples taken within an area can vary, but the more sweeps the greater the resolution of the data. Due to reports stating that *Coniatus splendidulus* (tamarisk weevil) is being found within our monitoring area, we have incorporated a quick search to our monitoring protocols. In order to understand the population extent of the Tamarisk Leaf Beetle, TC relies heavily on the involvement of partner organizations and agencies to assist in data collection.

This protocol does not address the ecosystem impacts of the tamarisk leaf beetle such as mortality, vegetation response, efficacy of the beetle in controlling tamarisk, or any of the other many impacts. TC is working with partners to understand the feasibility of developing a Biocontrol Consortium to coordinate intensive monitoring efforts, coordinate with researchers to answer desired research questions, connect the current research to land managers and other researchers, and potentially conduct trainings on intensive monitoring protocols. For more information on the different programmatic areas of the Tamarisk Coalition's Biocontrol Monitoring Program, please visit: http://tamariskcoalition.org/programs/tamarisk-leaf-beetle-monitoring-program

There are <u>two data collection options</u> discussed below, sweep netting and visual presence/absence. Both options provide the necessary information for the yearly population distribution map, but the sweep netting protocol provides more accurate population estimates and is also more likely to pick up very low populations such as a single beetle in a large stand of tamarisk. We recommend using the sweep netting protocol, but recognize that not everyone has access to sweep nets and therefore we will also accept data collected by the visual presence/absence method.

Choosing a Site and Surveying Tips:

Since the ultimate goal of monitoring the tamarisk leaf beetle is to understand population extent on a landscape scale, it is important to choose monitoring locations that are appropriately spaced. As a rule of thumb, we select sites no less than 5 miles apart. Often times sites may be 20-30 miles apart. The distance between locations depends on many variables including, but not limited to, frequency of accessible tamarisk, time restrictions, safety, property ownership and scope of monitoring area.

When choosing a specific site, we try to find areas with a variety of tamarisk characteristics. For example, if you are driving along and there is an area that is green, an area that has been defoliated and an area that has both green and defoliated tamarisk, we would stop at the later location. But overall,

the most important factor is finding a large enough stand of tamarisk that is an appropriate distance from your previous point.

We have found in the past that the *Diorhabda* spp. are generally found in greater numbers on branches that are higher off the ground, dry, warmer from sunlight, full of green foliage and not over water. The beetles typically fall directly towards the ground when the branches are shaken or hit with the net.

Little is known about the characteristics of Tamarisk Weevil (*Coniatus splendidulus*); we do know that they hide from the observer and that they do not fall off the branches as easily during sweeping. The easiest way to note their presence on a tamarisk is by spotting their silken egg cages (Appendix A).

Sweep Netting Surveys:

Using standard 38 cm diameter cloth insect sweep nets (**Photo 1**) and doing ~1 m sweeps through the foliage, a total of 25 sweeps are done per sampling location/GPS point. All of these sweeps should be swept with an upward direction, so that the beetles will fall into the net. Between every 5 sweeps the contents of the net is recorded using defined parameters (**Table 1**), and every set of 5 sweeps is roughly ~ 5 m apart. Such that 5 sets of 5 sweeps, each roughly 5 meters apart will be done per sampling location/GPS point (1 Sweep x 5 = 1 Set...1 Set x 5 = 1 Sample...1 Sample/GPS pt).

If more than 25 sweeps are accomplished, 1 GPS point must still be recorded for each set of 25 sweeps. Thus sampling involving 100 sweeps in an area would result in 4 individual GPS points (or sample locations) attributed with a separate data record for every round of 25 sweeps/1 sample location. If marginal tamarisk is present at a sample location, sweeps should still be completed on whatever tamarisk is present.

When counting net contents take care to not let beetles escape or fly away when possible. Shaking the net vigorously or tapping the sides can sometimes knock beetles into the bottom of the net aiding in the count. All adult beetles should be counted. Larvae will be counted as either "Early" (1st and 2nd Instars: small & black with no yellow stripe) or "Late" (2nd & 3rd Instars: larger body, with noticeable yellow stripe).

During sweep sets observers should be observant for egg clusters laid near the end of leaves. If no eggs are spotted during the sweeps one 15 second "egg survey" should proceed scanning the canopy for the creamy pink/white clusters. After the "egg survey" conduct a 15 second *Coniatus splendidulus* survey looking for *Coniatus splendidulus* adults and their silken pupae cages. For photographs of *Diorhabda* spp. and *Coniatus splendidulus* please refer to the Appendix A.



Photo 1: Insect Sweep Net and GPS unit

Visual Presence/Absence:

Choose an appropriate site and spend 2-5 minutes visually examining tamarisk branches for adult beetles, larva, and eggs or hatched eggs. It is important to look at a variety of trees and all parts of the tree from the base to the top. While you just need to mark P or A in the P/A column, it is beneficial to use the comments area to record relative abundance of the beetles and beetle stage (i.e. adult, larva). While you are looking for beetles you can also look for evidence of *Coniatus splendidulus* and note P/A in the *Coniatus* column.

Defoliation and Refoliation:

Defoliation will be categorized for each sampling location as the average level of defoliation within the sample location (~0.25 km²) and recorded as a percentage in 10% increments. Percent defoliated is defined as % of canopy currently brown from tamarisk leaf beetle damage (See Photo 4A for 100% defoliated). Keep in mind that the bright yellow tinge that tamarisk get mostly in the later end of the season is leaf hopper (*Opsius stactogalus*) damage and should not be confused or recorded as *Diorhabda* defoliation (see Appendix A).

In concurrence with defoliation, refoliation will only be recorded as "Present/or Absent," this is to avoid confusing partially defoliated trees as fully defoliated trees that are partially refoliated. Refoliation is best noticed by the "fireworks puffs" that are the signature of tamarisk re-sprouting on the stems and branches. Note that refoliation will not occur until mid-season, and without previous records it can be hard to truly validate. If an area is revisited multiple times in a season and valid identification of refoliation is ensured, refoliation can be recorded as a percentage similar to defoliation. Thus the average % of refoliation within ~0.25 km² (recorded in 10% increments) can be recorded.

Data Sheet Recording:

When sweeping an area 1 GPS point should be taken for every 5 sets of sweeps (or equivalent 25 sweeps) or visual presence/absence location. Upon taking data at each GPS point, the date, GPS ID (including UTM coordinates in WGS84) should be recorded. In the Beetle Monitoring Method it is only necessary to record data in either the Sweeping Method or Presence/Absence section, for example if you use the Sweeping Method, you do not need to fill in the P/A column. Presence/absence of eggs, presence/absence of *Coniatus* spp., percent defoliation, and presence/absence of refoliation should be recorded in the appropriate columns. When possible photos of the sweep area should be taken at the GPS point location and recorded by their respective ID number with a compass bearing for the direction of the shot. At any data collection point written comments about geographical location, note worthy net samples, or other significant information should always be recorded. Anything is better than nothing.

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				Late Larvae (Stripe)												

 Table 1: Data recorded per GPS point

Mortality-driven Monitoring Protocol Addendum

In order to maintain consistency in presence/absence sampling methodology, this addendum for stands of tamarisk showing evidence of high mortality has been developed. As the tamarisk leaf beetle continues to defoliate in consecutive growing seasons, signs of plant mortality become apparent. Typically, standing dead tamarisk maintain the limb structure of the originally defoliated plant. These limbs often leaf-out in seasons immediately after initial defoliation, but in successive years the plant reverts to sending up new shoots in the interior. The extant dead branches no longer produce foliage, but remain in place and are surprisingly resilient. This resiliency makes the sweep netting surveys both impractical and detrimental to equipment (i.e. – sweep nets).

If established, sampling points should remain at the same GPS position recorded in previous years, unless mortality in that vicinity is so complete that nearby tamarisk needs to be sampled instead. Once the sampling point has been identified, the amount of vegetation will determine whether or not branch sampling or direct observation will be employed. If vegetation growth allows, pull live branches down into a sweep net and vigorously shake the branch for 15 seconds, then check the net contents according to the procedures given in "Sweep Netting Surveys". Repeat this with 4 other branches on the same or another nearby plant, to complete 1 set. Complete 4 other sets, for an entire sample. If the growth form is such that it is not practical to bend branches into a sweep net, follow the "Visual Presence/Absence" protocols listed above on 5 separate plants, or use a combination of branch and visual sampling to complete a 5 set sample. If the stand has attained great enough mortality that even the above methods are impractical for 5 sets, simply observe available vegetation to determine beetle presence.

If sampling is being conducted from a boat and mortality is so great that no green limbs are accessible, find shore access as close to the sampling area as possible and use the above protocols. If the dead plants are so thick that shore access is not possible, search for any green growth that may be observed from the boat and record whatever data are obtained, recognizing that the typical 5 sets may not be practicable.

All data should be recorded on the data collection sheet utilizing the "Sweeping Method" boxes for information rather than the "P/A" box, as 5 separate sets and not a single presence/absence will be recorded. Note in the "Comments" box that the "Mortality Addendum" was employed.

Data Sharing:

To ensure that the data you've collected will be incorporated into our maps and datasets, please email your field data to Ben Bloodworth (<u>bbloodworth@tamariskcoalition.org</u>) by **September 15**. If you know this is not possible for you, please let us know so we can make appropriate plans.

We would prefer the data to be sent to us as one of the three following options listed in order of preference:

- 1. Shapefile
- 2. Excel File
- 3. Scanned Data Sheets

This is purely based upon your capabilities. The better the data format that you can provide us, the quicker we can transfer your work to our datasets. If you require assistance in getting your information into a different format, please feel free to contact the Tamarisk Coalition.

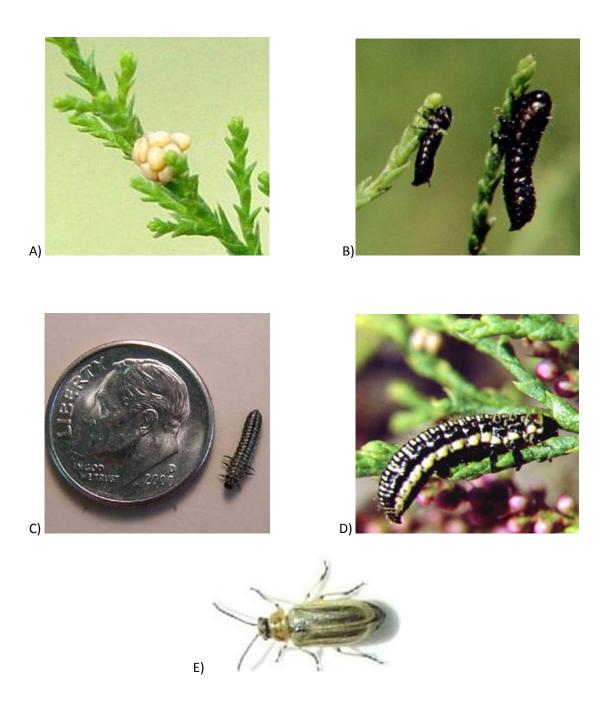
We'd also appreciate any high quality photographs you may have taken during your field season. Aside from any general photographs of the field work, we also find time series and before and after photographs very useful. With all photographs you provide use, please include the location and GPS coordinates.

For overall questions, please contact:

Ben Bloodworth Program Coordinator <u>970.256.7400</u> <u>bbloodworth@tamariskcoalition.org</u> www.tamariskcoalition.org

Appendix A

Diorhabda spp: (A) The pinkish egg masses of *Diorhabda* spp. usually laid near the ends of the branches. (B) 1st instar larvae compared the larger 2nd instar larval stage. (C) The much larger 3rd instar larvae compared to a dime. (D) Note the 3rd instars highly noticeable yellow stripe. (E) The adult beetle is yellow to straw colored with roughly 4 brown stripes running down their dorsal elytra.



Coniatus splendidulus: (A) The adult tamarisk weevil is approximately half the size of an adult *Diorhabda* beetle. This beetle is composed of multiple hues of colors, light green being the most dominant, but also has blotches of tan, speckles of black, all topped off with a shimmering metallic touch. (B) The globe-like pupae cages are about the same color as defoliated tamarisk leaves and are the easiest way to note weevil presence. (C)The cages are generally exposed on the foliage of the tamarisk; this picture gives you a good idea about the size of the cages. (D) A size comparison between the weevil and an empty pupae cage.









Tamarisk Defoliation: (A)Tamarisk leaf beetle defoliation is very brown colored with many straw like desiccated leaves still attached to the plant, the tree in Photo A is 100% defoliated. (B) Following defoliation many tamarisk will resprout largely from the along the stems of the tree. (C) Yellower senescent leaves found in the fall when leaf hopper damage is most apparent are very different compared to the much dryer/brown canopies of defoliated trees, these trees have no tamarisk leaf beetle damage.









Compiled by: Levi Jamison - Colorado Department of Agriculture, Tamarisk Coalition & University of California Santa Barbara. Updated 6/9/2010
Revised by: Ben Bloodworth - Tamarisk Coalition, 7/01/2014

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Recorder(s):

*Collect Data in WGS 84

**% Defoliation: % of canopy <u>currently</u> brown from tamarisk leaf beetle damage

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