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Pest risk analysis of tamarisk leaf beetles, *Diorhabda* spp., with an evaluation of its potential to spread into southwestern willow flycatcher, *Empidonax traillii extimus*, critical habitats and evaluation of control options



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Executive Summary

Tamarisk, or saltcedar, (*Tamarix* spp.) is an invasive plant that occurs in riparian areas. It has replaced native vegetation in many places and is reportedly responsible for increasing soil salinity, water loss, wildfire frequency and intensity, and flood frequency and intensity and for inhibiting the germination and growth of other plants. In an effort to control tamarisk, USDA-APHIS introduced tamarisk leaf beetles [*Diorhabda* spp. (Coleoptera: Chrysomelidae)] into the western and southwestern United States for biological control. The beetle defoliates tamarisk, which can lead to plant mortality and has proven to be highly effective for biological control.

As the leaf beetles defoliate and kill tamarisk, however, they are impacting the southwestern willow flycatcher, *Empidonax traillii extimus*. With the replacement of its native nesting habitat by tamarisk, this endangered bird has adapted to using tamarisk for nesting in some western riparian habitats. Unfortunately, as the beetle eliminates tamarisk, suitable habitat for the SWFL is decreased, at least for the short term. This species of bird was listed as endangered by the U.S. Fish and Wildlife Service in 1995, and habitat throughout the southwestern United States was designated as critical. *Diorhabda* beetles are naturally spreading into these critical areas.

A pest risk analysis was prepared on August 9, 2017 to help inform decision makers of the spread potential of *Diorhabda* beetles and the potential control options available within the authority of APHIS to limit impacts to the SWFL and designated critical habitat. This document is an update to the previous risk analysis that was produced on August 9, 2017. We updated the analysis in response to a remedial order from the United States District Court for the District of Nevada on June 19, 2018. This version contains the following changes: 1) the APHIS *Diorhabda* spread model was updated with 2017 detection data and its efficacy was evaluated, 2) recent research on beetle herding using new *Diorhabda* aggregation and repellent pheromones was added, 3) recent research on modeling tamarisk distribution, southwestern willow flycatcher (SWFL) abundance and habitat occurrence, associated *Diorhabda* interactions, and the effects of controls and ecological events on SWFL populations was added, and 4) additional knowledge gaps and research needs have been added.

Four species of *Diorhabda* species, *D. elongata*, *D. carinata*, *D. sublineata*, and *D. carinulata*, have been introduced into the United States since 2001. These leaf beetles can aggregate in large numbers and infest tamarisk trees, leading to complete defoliation. Multiple defoliations ultimately lead to tree death. In this analysis we predict the climatically suitable areas for establishment of *Diorhabda* spp. and confirm that most parts of the flycatcher habitat (approximately 75 percent) are climatically suitable for the beetle species.

The beetles are spreading in the United States both naturally and through human activity. We modeled the spread of *Diorhabda* spp. along riparian corridors into all of the SWFL critical habitats in the United States and determined how long it is likely to take for the beetle to spread to those areas. Our model predicts the number of years until beetle entry into SWFL critical

habitats rather than the number of years until such habitats are completely infested. Based on our model, the expected number of years until entry varied between 0 for habitats already colonized and 20 for the flycatcher habitats farthest away from *Diorhabda* spp. populations. The mean years until entry into flycatcher habitats by state were 6.5 years for Arizona, 12.6 years for California, 4.0 years for Colorado, 2.3 years for New Mexico, and 10.7 years for Nevada. The mean time until entry was significantly higher for California than for Arizona, Colorado, and New Mexico. The number of years until entry for Arizona and Nevada were significantly higher than for New Mexico.

The addition of the 2017 *Diorhabda* detection data indicated that the 25-km annual spread rate used in the APHIS model captured the majority of the beetle dispersal. The 2017 *Diorhabda* detections also revealed new spread routes for beetle entry into SWFL habitats due to increased proximity and indicated that people may be facilitating long-distance beetle spread in some cases.

We also evaluated several potential treatment or management options to prevent or slow the spread of *Diorhabda* spp. into the SWFL critical habitat. Due to the widespread distribution of *Diorhabda* species and their ability to spread, it is unlikely that we will be able to prevent the beetles from entering the critical habitat. Of the options evaluated, only host removal (i.e. selective thinning) combined with chemical control currently appears likely to be feasible in slowing the spread of the beetle. We note that recent research on beetle herding using dispersion pheromones has shown promise in directing *Diorhabda* movement. Consequently, beetle herding may provide an additional method for slowing *Diorhabda* spread in the future. *Diorhabda* spp. are not typically controlled anywhere in the world, so data on the efficacy of several treatment options is severely lacking.

Lastly, we discuss research and technology needs with regard to *Diorhabda* control and biology, SWFL population and habitat modeling, tamarisk occurrence modeling, and evaluation of control options.

The results of our analysis can be used to inform operational and policy decisions regarding the spread of *Diorhabda* spp. into SWFL habitats. Our results also provide decision makers with information to limit impacts to the SWFL and designated critical habitat that are within the authority of APHIS.

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1. Introduction

The Plant Epidemiology and Risk Analysis Laboratory of the Center for Plant Health Science and Technology, USDA Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), prepared this document at the request of PPQ Plant Health Programs (PHP). This is an update to a previous risk analysis that was produced on August 9, 2017. We updated the previous analysis in response to a remedial order from the United States District Court for the District of Nevada on June 19, 2018 (Boulware, 2018; Caraher, 2018). This version includes the following updates: 1) the APHIS *Diorhabda* spread model was updated with 2017 detection data and its efficacy was evaluated, 2) recent research on beetle herding using new *Diorhabda* aggregation and repellent pheromones was added, 3) recent research on modeling tamarisk distribution, southwestern willow flycatcher abundance and habitat occurrence, associated *Diorhabda* interactions, and effects of controls and ecological events on flycatcher populations was added, and 4) additional knowledge gaps and research needs have been added.

Tamarisk, or saltcedar, (*Tamarix* spp.) was introduced in the United States in the 1800s and is an invasive plant that occurs in riparian areas. Invasive tamarisk has replaced native vegetation in many places (de Gouvenain, 1996). Because it increases soil salinity, it inhibits the germination and growth of other plants. Other reported impacts include increased water loss, increased wildfire frequency and intensity, and increased flooding frequency and intensity (Wiesenborn, 1996).

As part of the control strategy for tamarisk, the *Diorhabda* spp. *D. elongata*, *D. carinata*, *D. sublineata*, and *D. carinulata* (Coleoptera: Chrysomelidae), referred to as *Diorhabda* for the remainder of the document for brevity, were released into the United States as biological control agents beginning in 2001 (DeLoach et al., 2004). Since that time, *Diorhabda* beetles have become widely established in western and southwestern states and have been very successful at reducing populations of tamarisk (Carruthers et al., 2008).

Unfortunately, the success of the biological control program may negatively impact the habitat of the endangered southwestern willow flycatcher (SWFL), *Empidonax traillii extimus*. SWFL is listed as endangered by the U.S. Fish and Wildlife Service (Sogge, 2010). Within the United States, SWFL occurs in southern California, Arizona, New Mexico, southwestern Colorado, and extreme southern portions of Nevada and Utah (Sogge, 2010), and some of these areas are designated as critical habitat (USFWS, 2013). SWFL has adapted to using tamarisk for nesting in some western riparian habitats (Dudley and Bean, 2012). As the leaf beetles defoliate and kill tamarisk, they are impacting the SWFL by causing short-term reduction of available nesting substrate (York et al., 2011). *Diorhabda* beetles are naturally spreading into critical areas.

On September 30, 2013, the Center for Biological Diversity (CBD) filed a complaint against APHIS and the U.S. Fish and Wildlife Service. CBD alleged that the APHIS saltcedar biological control program violated the National Environmental Policy Act (NEPA) and the Endangered

Species Act (ESA). On May 3, 2016 the Judge granted the plaintiff's second of five claims, finding that APHIS did not comply with ESA section 7(a)(1) that requires federal agencies to carry out programs for the conservation of protected species.

In response to the plaintiff's remedy proposal, APHIS prepared a declaration describing the authority and decision-making processes of the agency. The declaration informed the court of the timelines needed to ensure that a section 7(a)(1) conservation program for SWFL includes feasible alternatives that make prudent use of agency funds. This document was prepared to help inform decision makers of the potential spread of *Diorhabda* beetles and provide potential control options within the authority of APHIS to limit impacts to SWFL and designated critical habitats.

In this analysis we characterize *Diorhabda* spp., predict the potential for them to establish in SWFL habitat, model the spread rate into this habitat, evaluate potential management options, and identify pertinent research and knowledge gaps.

2. Background information

The genus *Tamarix* is one of the oldest and most dominant plant genera. It originated in central Asia (DeLoach et al., 1996) and comprises many species, a number of which occur in the United States and are infested by *Diorhabda* beetles (Table 1). Several species of tamarisk have been intentionally introduced into North America, beginning in the 1800s (Carruthers et al., 2008), as ornamentals, wind breaks, and stabilization for eroding stream banks (de Gouvenain, 1996). Currently, *Tamarix africana*, *T. aphylla*, *T. aralensis*, *T. chinensis*, *T. gallica*, *T. parviflora*, and *T. ramosissima* are reported to occur in the United States (NRCS, 2017). The most widespread and damaging species are *T. ramosissima* and *T. chinensis* and their hybrids (Gaskin and Schaal, 2002). Invasive tamarisk has replaced native vegetation in many areas (de Gouvenain, 1996) and is reportedly responsible for increasing soil salinity, water loss, wildfire frequency and intensity, and flood frequency and intensity and for inhibiting the germination and growth of other plants (Drus et al., 2013; Wiesenborn, 1996).

Table 1: Known tamarisk species infested by at least one species of *Diorhabda* beetle and tamarisk species presence in the United States.

Known species of <i>Tamarix</i> that are considered hosts for at least one species of <i>Diorhabda</i> beetle	Reported in the United States according to NRCS (2017)
<i>T. africana</i> [Tracy and Robbins, 2009, citing Peyerimhoff (1926) and Jolivet (1967)]	Yes
<i>T. androssowii</i> [Tracy and Robbins, 2009, citing Sha and Yibulayin (1993)]	No
<i>T. aphylla</i> (Tracy and Robbins, 2009)	Yes
<i>T. aralensis</i> (Tracy and Robbins, 2009)	Yes
<i>T. arceuthoides</i> (DeLoach et al., 2003; Kulinich, 1962)	No
<i>T. aucheriana</i> (Tracy and Robbins, 2009)	No

<i>T. boveana</i> (as <i>T. bounopaea</i>) [Tracy and Robbins, 2009, citing Peyerimhoff (1926)]	No
<i>T. cf. indica</i> (as <i>T. cf. troupii</i>) (Tracy and Robbins, 2009)	No
<i>T. chinensis</i> [DeLoach et al., 2003; Tracy and Robbins, 2009, citing Sha and Yibulayin (1993)]	Yes
<i>T. chinensis × canariensis</i> , syn.: <i>T. gallica</i> (Tracy and Robbins, 2009)	Yes
<i>T. elongata</i> (DeLoach et al., 2003)	No
<i>T. gallica</i> (Laboissière, 1914; Hopkins and Carruth, 1954; Lundberg et al., 1987; Tracy and Robbins, 2009)	Yes
<i>T. hampeana</i> (Tracy and Robbins, 2009)	No
<i>T. hispida</i> (Kulinich, 1962)	No
<i>T. hispida</i> var. <i>hispida</i> (DeLoach et al., 2003)	No
<i>T. hispida</i> var. <i>karelinii</i> (DeLoach et al., 2003)	No
<i>T. kansuensis</i> [Tracy and Robbins, 2009, citing Sha and Yibulayin (1993)]	No
<i>T. laxa</i> (DeLoach et al., 2003)	No
<i>T. leptostachya</i> (Tracy and Robbins, 2009)	No
<i>T. meyeri</i> (Samedov and Mirzoeva, 1985)	No
<i>T. parviflora</i> (Dudley et al., 2006; Tracy and Robbins, 2009)	Yes
<i>T. ramosissima</i> (DeLoach et al., 2003; Tracy and Robbins, 2009; Kulinich, 1962)	Yes
<i>T. ramosissima</i> , syn.: <i>T. chinensis</i> (Tracy and Robbins, 2009)	Yes
<i>T. senegalensis</i> (Tracy and Robbins, 2009)	No
<i>T. smyrnensis</i> (Regalin, 1997; Gök and Çilbirçoğlu, 2003; Gök and Çilbirçoğlu, 2005; Gök and Duran, 2004; DeLoach et al., 2003; Samedov and Mirzoeva, 1985)	No

Investigation of biological control options to control invasive tamarisk began in 1986 (DeLoach et al., 2000). *Diorhabda* from central Asia and China was identified as a potential candidate, and field releases were planned for June 1995 (DeLoach et al., 1996; DeLoach et al., 2000). In 1995, however, SWFL was placed on the endangered species list. SWFL is a small bird in the family Tyrannidae. It nests in relatively dense tree and shrub communities near rivers, swamps, and other wetlands. Historically, SWFL nested in native cottonwood, willow, and other similar plant communities, but the introduction of various invasive species, including tamarisk, has led to the loss of much SWFL natural habitat (Tibbitts et al., 1994; USFWS, 2014). The SWFL still nests in native vegetation but now also uses thickets dominated by non-native tamarisk and Russian olive (*Elaeagnus angustifolia*), as well as mixed native and non-native stands (USFWS, 2014).

Because SWFL now nests in introduced tamarisk, concerns were expressed that the proposed biological control program would cause further harm to SWFL (DeLoach et al., 2000; Durst et al., 2006; Stenquist, 1999). Biological and environmental assessments were undertaken to assess all potential impacts of the biological control program, but it was determined that the negative impacts caused by tamarisk to many native species and to water supplies outweighed its few benefits (DeLoach et al., 2004). In 1999, APHIS-PPQ determined that the proposed program was unlikely to cause significant impact, allowing for the first permits for release of *Diorhabda* (DeLoach et al., 2000).

Diorhabda was released into field cages in 1999 and 2000 and into the open field in 2001 (DeLoach et al., 2004). Multiple populations of *Diorhabda* have since been released and become established throughout the continental United States (Carruthers et al., 2008) (Figure 1). In 2010, APHIS PPQ officially ended the APHIS-PPQ saltcedar biological control program due to concerns about the potential effects on the critical habitat of SWFL (PPQ, 2010).

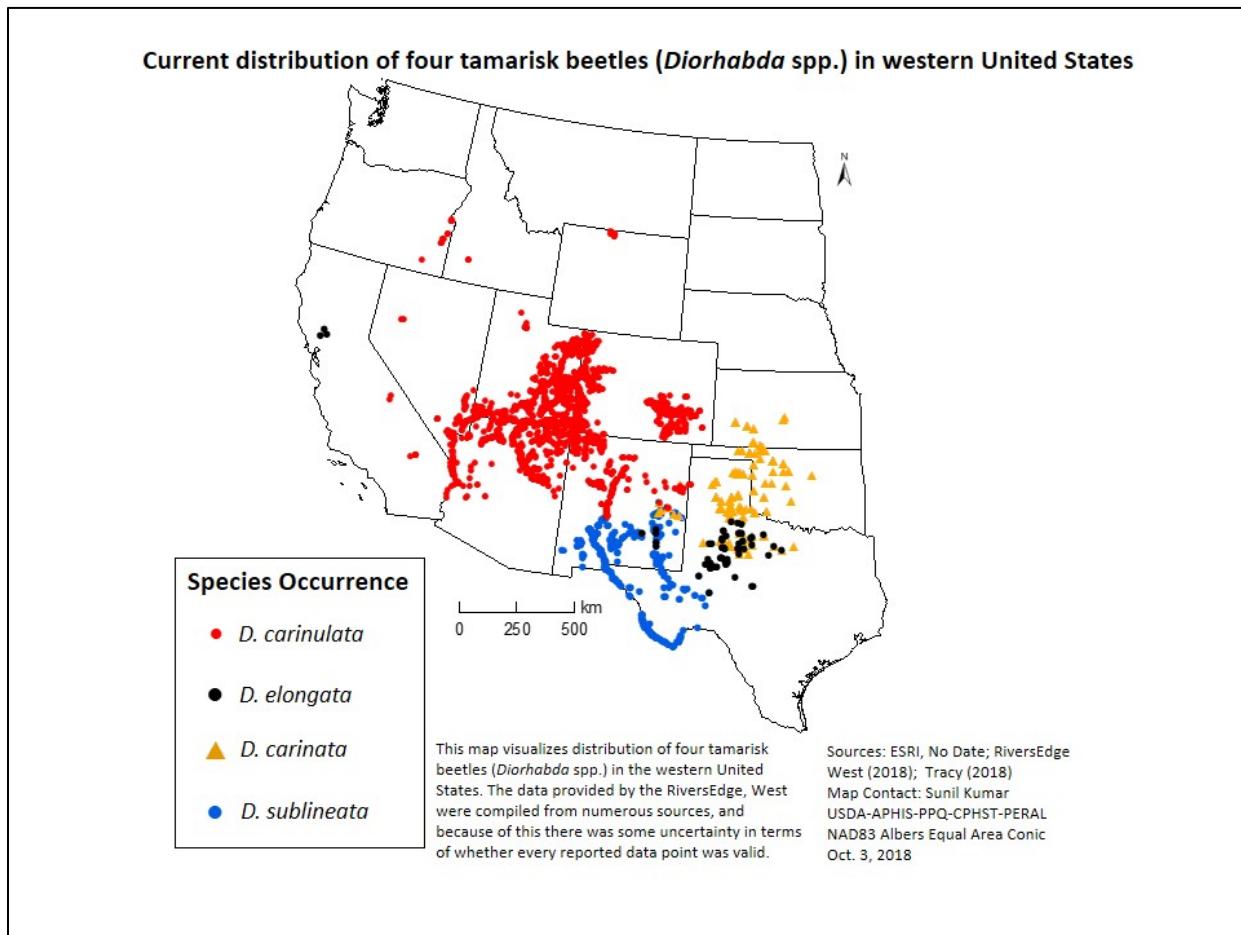


Figure 1: Current distribution of four tamarisk beetles (*Diorhabda* spp.) in the western United States.

3. *Diorhabda* biology and taxonomy

3.1 *Diorhabda* basic biology

Adult beetles overwinter under leaf litter on the soil surface (Cossé et al., 2005a) or in clumps of grass (Moran et al., 2009). Adults emerge when new foliage is available (Cossé et al., 2005a) and feed on the leaves of tamarisk trees (Tracy and Robbins, 2009). Females lay eggs in clusters on leaves (Cossé et al., 2005a). They can lay varying numbers of eggs per cluster (Lewis et al., 2003) and average 194 to 281 eggs, depending on the species (Moran et al., 2009; Tracy and Robbins, 2009). Larvae feed primarily on the leaves of host plants (Cossé et al., 2005a). Young larvae make small holes in the lower epidermis and parenchyma, while later instar larvae can consume entire leaves (Tracy and Robbins, 2009) and may also de-bark small twigs (Carruthers

et al., 2008). They also occasionally feed on shoots and flowers (Herr et al., 2009). Larvae mature through three instars (Herr et al., 2009) and pupate in loose cocoons under leaf litter or in the soil near defoliated trees (Cossé et al., 2005a; Herr et al., 2009; Tracy and Robbins, 2009). Pupae may diapause; the length of diapause depends on temperature and photoperiod (Bean et al., 2007a; Bean et al., 2007b; Herr et al., 2009).

Adults live for several months in the field (Carruthers et al., 2008). *Diorhabda* beetles can produce up to five generations per year (Tracy and Robbins, 2009), although three to four generations are commonly reported in most areas of the United States (Moran et al., 2009; Lewis et al., 2003; Tracy and Robbins, 2009). The number of generations per year is driven by temperature-dependent developmental rates (Herr et al., 2009). Populations of *Diorhabda* can grow quickly, with a population doubling time of 6.2 days (Lewis et al., 2003).

Diorhabda can disperse rapidly into new areas and in at least one study were estimated to disperse up to 25 km annually (Nagler et al., 2014). They appear to spread along riparian corridors throughout the summer and may disperse in a series of short flight events (Nagler et al., 2014). They may also disperse longer distances as hitchhikers.

Beetles can aggregate in large numbers. Male aggregation pheromones (Cossé et al., 2005a; Cossé et al., 2011) and green leaf volatiles have been identified and field tested as attractants for *D. elongata* (Cossé et al., 2006b) and *D. carinulata* (Cossé et al., 2011; Weaver, 2011; Weaver, 2014). It is likely that the pheromone will attract all *Diorhabda*, as the male aggregation pheromones appear to have the same components in different ratios among species of *Diorhabda* (Cossé et al., 2006b).

3.2 *Diorhabda* taxonomy and identification

The taxonomy of the genus *Diorhabda* was not fully understood during the early years of the tamarisk biological control program, and the leaf beetles released were classified as a single species, *D. elongata*, with a range across North Africa and Eurasia into China and Mongolia (Bean et al., 2013; Tracy and Robbins, 2009). Initial releases of *Diorhabda* had mixed results, with some populations failing to establish (DeLoach et al., 2004; Bean et al., 2007b). It was originally thought that distinct biotypes or ecotypes existed within *D. elongata* that had adapted to different climates and tamarisk hosts within the native range of the beetle (Dalin et al., 2009; Dalin et al., 2010). For example, the initial collections of *D. elongata* from the interior of central Asia did well against *T. ramosissima* and *T. chinensis* and their hybrids in Nevada, Utah, Colorado, and Wyoming but failed to thrive in coastal California, Texas, or southern New Mexico (DeLoach et al., 2004; Bean et al., 2013). The failures appeared to be a result of mismatches in the photoperiodic requirements for reproduction and diapause (Bean et al., 2007a; Lewis et al., 2003) and in target species of tamarisk (Dalin et al., 2009).

The initial failures led to the collection of new biotypes and ecotypes from the Mediterranean basin, Uzbekistan, and China (DeLoach et al., 2004). While at the time these beetles were not considered separate species, they were initially treated as if they were distinct species and were

held under quarantine conditions and tested for host range specificity (Milbrath and Deloach, 2006a; Milbrath and DeLoach, 2006b; Herr et al., 2009). All beetles were shown to be specific to tamarisk species (Bean et al., 2013), and populations from different regions displayed clear differences in their ability to use different tamarisk hosts (Thomas et al., 2010).

Upon closer examination, these beetles, initially thought to be biotypes or ecotypes of *D. elongata*, were determined to be distinct species (Bean et al., 2013). Five species of *Diorhabda* are currently recognized within a species complex that is specific to tamarisk (Tracy and Robbins, 2009). Four of those species have been released in the United States (Bean et al., 2013) (Table 3). The species only have a few visually apparent differences (Bean et al., 2013; Tracy and Robbins, 2009) (Figure 2), but using a stereomicroscope, Tracy and Robbins (2009) were able to differentiate species of *Diorhabda* by differences in the reproductive structures of both males and females.¹

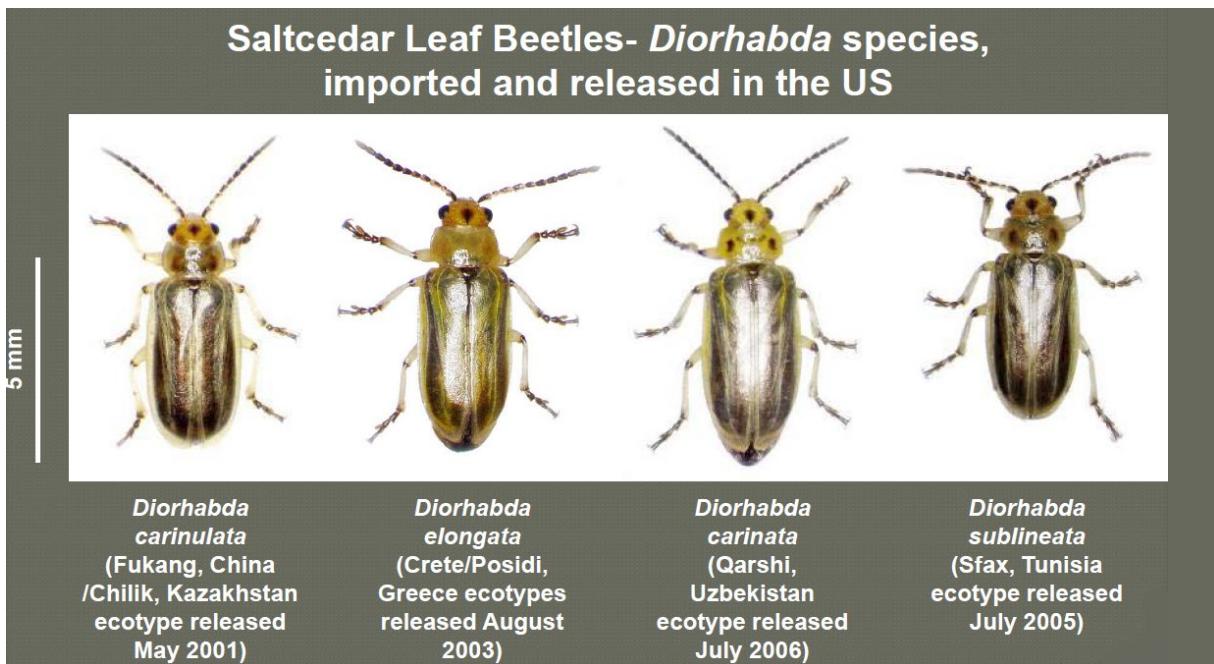


Figure 2: The four species of *Diorhabda* released in the United States (Source: Knutson, 2013).

The classification of the beetles as five distinct species is further supported by molecular, genetic, and hybridization studies (Bean et al., 2013). Furthermore, the species are associated with different tamarisk hosts in the literature (Table 2).

¹ Specifically the male endophallic sclerites and female vaginal palpi

Table 2: Species of *Diorhabda* present in the United States and the tamarisk hosts they are reported to infest.

Species	Tamarisk hosts present in the United States
<i>Diorhabda elongata</i>	<i>Tamarix gallica</i> (Lundberg et al., 1987; Tracy and Robbins, 2009) <i>T. parviflora</i> (Tracy and Robbins, 2009) <i>T. chinensis × canariensis</i> , syn.: <i>T. gallica</i> (Tracy and Robbins, 2009)
<i>Diorhabda carinata</i>	<i>Tamarix ramosissima</i> (Tracy and Robbins, 2009; Kulinich, 1962) <i>T. ramosissima</i> , syn.: <i>T. chinensis</i> (Tracy and Robbins, 2009) <i>T. aphylla</i> (Tracy and Robbins, 2009) <i>T. aralensis</i> (Tracy and Robbins, 2009) <i>Tamarix</i> sp. (Samedov and Mirzoeva, 1985; Tracy and Robbins, 2009)
<i>Diorhabda sublineata</i>	<i>Tamarix africana</i> [Tracy and Robbins, 2009, citing Peyerimhoff (1926) and Jolivet (1967)] <i>T. gallica</i> (Laboissière, 1914; Hopkins and Carruth, 1954) <i>T. aphylla</i> [Tracy and Robbins, 2009, citing A. Kirk (personal communication)] <i>Tamarix</i> spp. (Boehm, 1908; Alfieri, 1976; Tracy and Robbins, 2009)
<i>Diorhabda carinulata</i>	<i>Tamarisk ramosissima</i> (DeLoach et al., 2003) <i>T. chinensis</i> (DeLoach et al., 2003; Tracy and Robbins, 2009) <i>T. aralensis</i> (Tracy and Robbins, 2009) <i>T. parviflora</i> (Dudley et al., 2006)

While the species are similar in appearance, four of the five were recognized in the early 1800s as distinct species but were at some point all reported under the name *D. elongata* in the literature (Tracy and Robbins, 2009), adding to the confusion between species. Furthermore, each species of tamarisk beetle exhibits some degree of sympatry with at least one of the other species (Tracy and Robbins, 2009).

In their native range, *Diorhabda* do not appear to hybridize. Differences in genitalia between species are evidence of strong reproductive isolation in their native range (Tracy and Robbins, 2009). For example, *D. carinulata* and *D. carinata* co-occur in western Asia and are reported together (Tracy and Robbins 2009), but no naturally occurring hybrids have ever been reported (Bean et al., 2013). Hybridization studies in the laboratory have demonstrated the potential for successful gene flow between some but not all of the *Diorhabda* species (Bean et al., 2013). *Diorhabda carinata*, *D. elongata*, and *D. sublineata* appear to be able to hybridize with each other, while *D. carinulata* cannot hybridize with any of them. Crosses involving *D. carinulata* result in male sterility of the hybrids, incompatible genital morphology and low egg viability of the offspring if the hybrids are crossed with each other or with the parent species (Tracy and Robbins, 2009; Bean et al., 2013).

In the United States, however, hybrid populations have been detected in the field. Before the recognition that four distinct species of *Diorhabda* existed, caged field tests in Texas

inadvertently produced *D. elongata* x *sublineata* hybrid beetles that were then released (Moran et al., 2009). *Diorhabda elongata* x *sublineata* hybrids are reported to remain fully fertile for several generations (Moran et al., 2009).

Diorhabda carinata and *D. elongata* have also been shown to produce fertile hybrids under laboratory conditions (Tracy and Robbins, 2009; Bean et al., 2013). Hybrids of these two species have also been detected in the field (Michels Jr et al., 2013). In one unpublished laboratory study that performed crosses among *D. carinata*, *D. elongata*, and *D. sublineata*, all three species were reproductively compatible and consistently produced viable offspring through at least two generations (Bitume et al., 2017). Furthermore, the study reported that life history traits of the offspring were either unchanged from the parental species or improved with hybridization (Bitume et al., 2017).

It is unclear how common or fit hybrids are in the field, but the likelihood of hybrids naturally occurring will likely increase over time in the areas where these species co-occur.

4. Predicted area of establishment for species of *Diorhabda*

The introduced populations of *Diorhabda* will continue to spread in the United States. In this section we use quantitative spatial analyses to estimate the climatic suitability throughout the western United States for four tamarisk beetle species, *D. carinulata*, *D. sublineata*, *D. elongata*, and *D. carinata*, in order to predict the areas where these beetles can potentially establish. Critical habitat for SWFL as defined by the USFWS includes riparian and littoral areas in southern California, Arizona, New Mexico, southwestern Colorado, and extreme southern portions of Nevada and Utah (Sogge, 2010; USFWS, 2013).

4.1 Maxent modelling

Environmental suitability is one of the prerequisites for any species to establish in new areas after initial introduction. Spatial modeling of environmental suitability for a species can be performed using a number of spatial and statistical methods such as species distribution modeling or ecological niche modeling algorithms (Peterson et al., 2011; Jarnevich et al., 2015).

We used maximum entropy modeling (Maxent)² (Phillips et al., 2006) to predict areas likely to be environmentally suitable for four species of tamarisk beetle in the western United States. Maxent models the potential distribution of a species based on occurrence records (locations where the organism has been detected in the field) and environmental data such as temperature, precipitation, and elevation (Phillips et al., 2006, Phillips et al., 2017) (Figure 3). It has been

² Maxent is a software program that performs very well with species presence data (absence or abundance data are not required) (Evangelista et al., 2008; Kumar et al., 2009; Phillips et al., 2017). It is flexible and can use a diverse set of environmental variables, such as temperature, precipitation, and elevation, as predictors at varying spatial and temporal scales. Additionally, it has a built-in regularization multiplier (RM) that controls model overfitting.

widely used for modeling the environmental suitability for numerous invasive species, including insects, plants, and pathogens (Evangelista et al., 2008; Kumar et al., 2015; Kumar et al., 2016; Flory et al., 2012). Maxent automatically extracts a sample of background locations from the study area and then contrasts it against the presence locations. The software then integrates species occurrences and background locations with environmental variables and generates an environmental suitability (or probability of species establishment) raster with values varying from 0 (unsuitable) to 1 (highly suitable).

4.2 Species occurrence data

We used occurrence data (Figure 1) provided by the Tamarisk Coalition (2017a), RiversEdge West (2018), and Tracy (2017) encompassing the time period between 2007 and 2017 for the four species of *Diorhabda*. The data provided by the Tamarisk Coalition and RiversEdge West was compiled from numerous sources, so it was not certain whether every reported data point was valid. We removed duplicate records [more than one presence point within a square kilometer (km^2) grid cell] and performed ‘graduated spatial filtering’ using SDMToolbox (Brown, 2014; Kumar et al., 2016) to reduce spatial autocorrelation in occurrence data. Using the “Spatial Analyst” tool in ArcMap, we generated Gaussian Kernel Density layers to account for potential sampling bias in beetle occurrence data.

4.3 Climatic data

We used data for 19 bioclimatic variables (Karger et al., 2016). These bioclimatic variables were generated using monthly temperature and precipitation data from approximately 1979 to 2013 and represent average temperature and precipitation, seasonal variables, and climatic extreme indices (Kumar et al., 2016; Hijmans et al., 2005). We used approximately 1- km^2 spatial resolution for the climate data layers to account for the overall uncertainty of the model, including that due to species occurrence data. In addition, we obtained digital elevation model data at approximately 1- km^2 resolution (Hijmans et al., 2005). Since *Tamarix* is a riparian genus, we generated distances to streams and water bodies in ArcMap using the “Euclidean distance” tool and included the distance as an additional predictor in Maxent models.

We processed all 21 environmental variables (listed in Appendix 1) in ArcGIS to match Maxent requirements and examined them for cross-correlations using the Pearson correlation coefficient, r . Only one variable from a set of highly correlated variables ($|r|$ greater than 0.75) was used in our Maxent modeling; the others were dropped to account for multicollinearity. The number of variables in models for the four species varied from 7 to 11 (Appendix 1). A description of the Maxent modeling process is shown in Figure 3, and the process is detailed in Appendix 1.

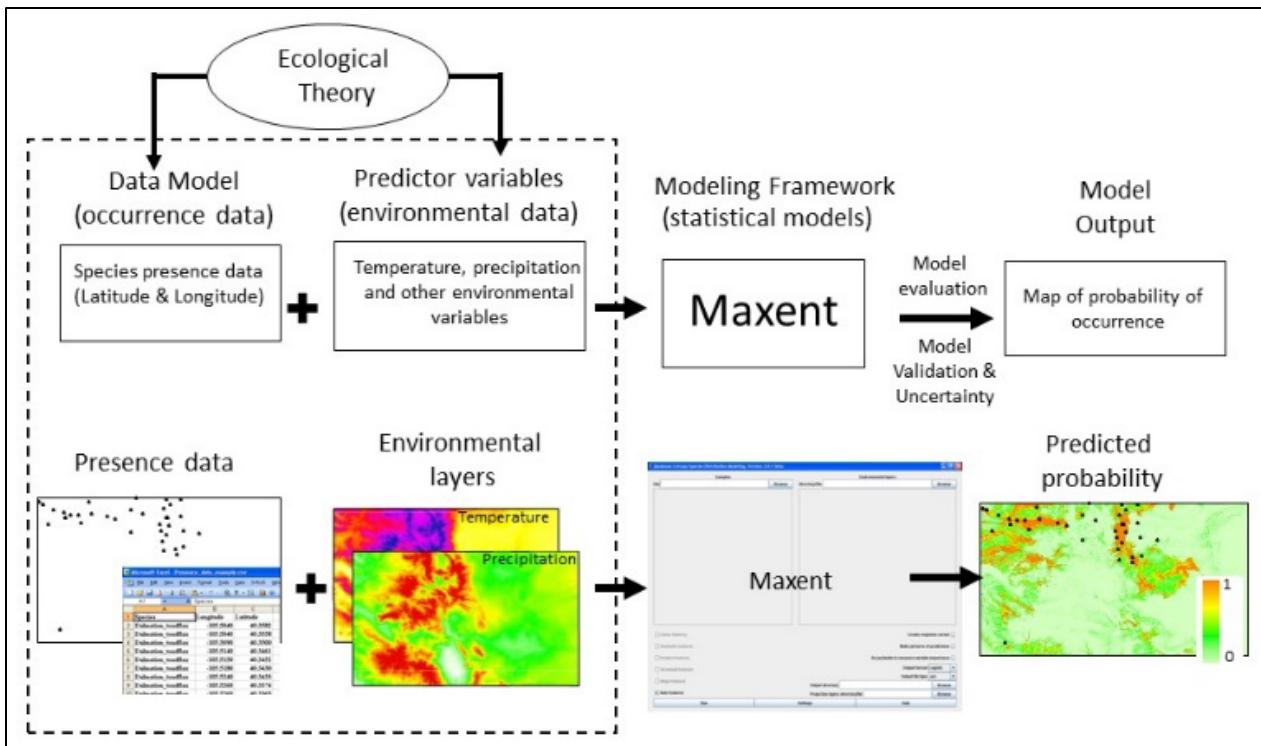


Figure 3: Generalized schematic of species distribution modeling or climatic or environmental suitability modeling from points to probabilities (Barnett et al., 2017).

4.4 Predicted areas of establishment

Based on the results of the Maxent model (see Appendix 1), we determined the following:

- The predicted highly suitable areas for *D. carinulata* occur mostly in central part of the study area (Figure 4).
- The predicted suitable areas for *D. elongata* occur in western Texas along the Rio Grande and Pecos Rivers and in parts of the central valley of California (Figure 5).
- The predicted suitable areas for *D. carinata* occur in northern Texas; western Oklahoma; and parts of Colorado, Kansas, and eastern New Mexico (Figure 6).
- For *D. sublineata*, highly suitable areas were predicted in western Texas, southern and southeastern New Mexico, and parts of Idaho and Arizona (Figure 7).
- Twenty five percent of SWFL critical habitat (primarily in southwestern California) was not environmentally suitable for establishment of any of the *Diorhabda* spp. (Figure 8).

As illustrated by the figures, the beetles are already present in much of the area most conducive to the establishment of each species. The models also predicted suitability, however, in areas where none of the beetles currently occur, such as in Idaho, central Washington, southwestern Montana, northeastern Colorado, southeastern Wyoming, and southwestern Arizona (Figure 8).

We note that these are areas where SWFL does not occur and therefore spread into this areas will have no impact on SWFL. The environmental habitat suitability for the *Diorhabda* in and around

the critical habitat for SWFL is shown in Figure 8. The analyses showed that 75 percent (64,000 ha) of the total 85,000 ha of SWFL critical habitat was environmentally suitable for *Diorhabda* spp. establishment; the remaining 25 percent (21,000 ha) was unsuitable (Figure 8).

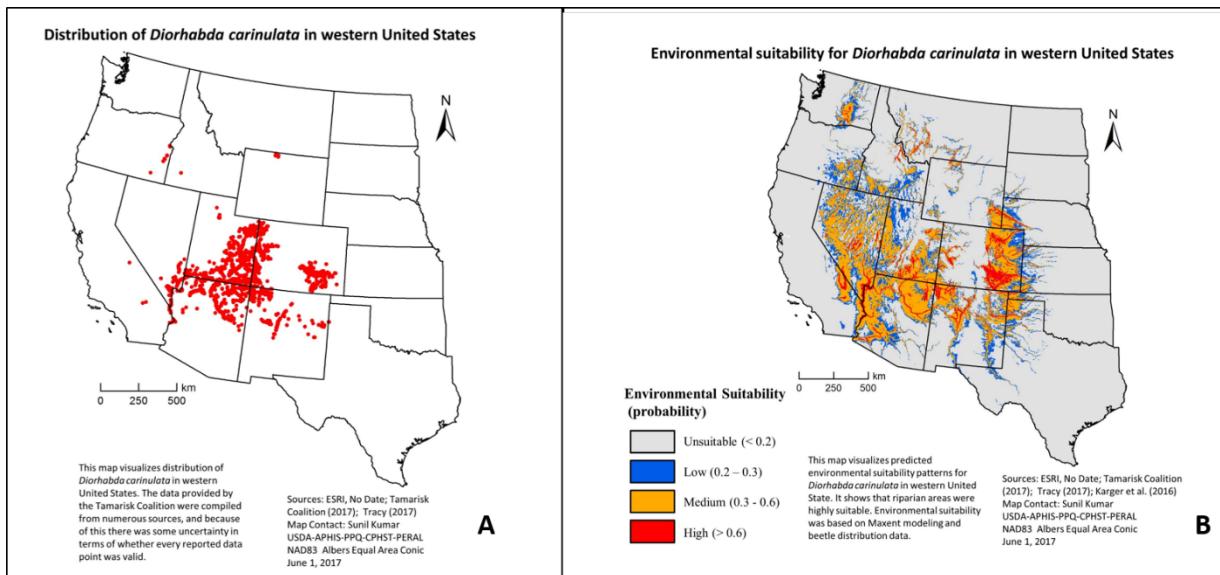


Figure 4: A) Occurrences of *Diorhabda carinulata* in the western United States. B) Predicted environmental suitability for *Diorhabda carinulata* in the western United States.

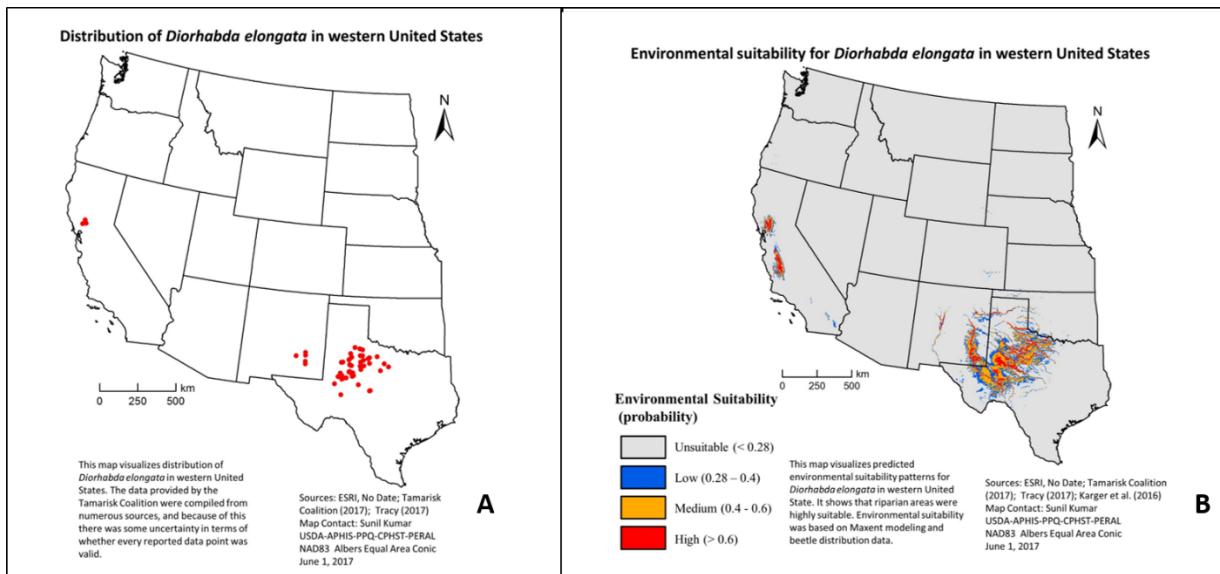


Figure 5: A) Occurrences of *Diorhabda elongata* in the western United States. B) Predicted environmental suitability for *Diorhabda elongata* in the western United States.

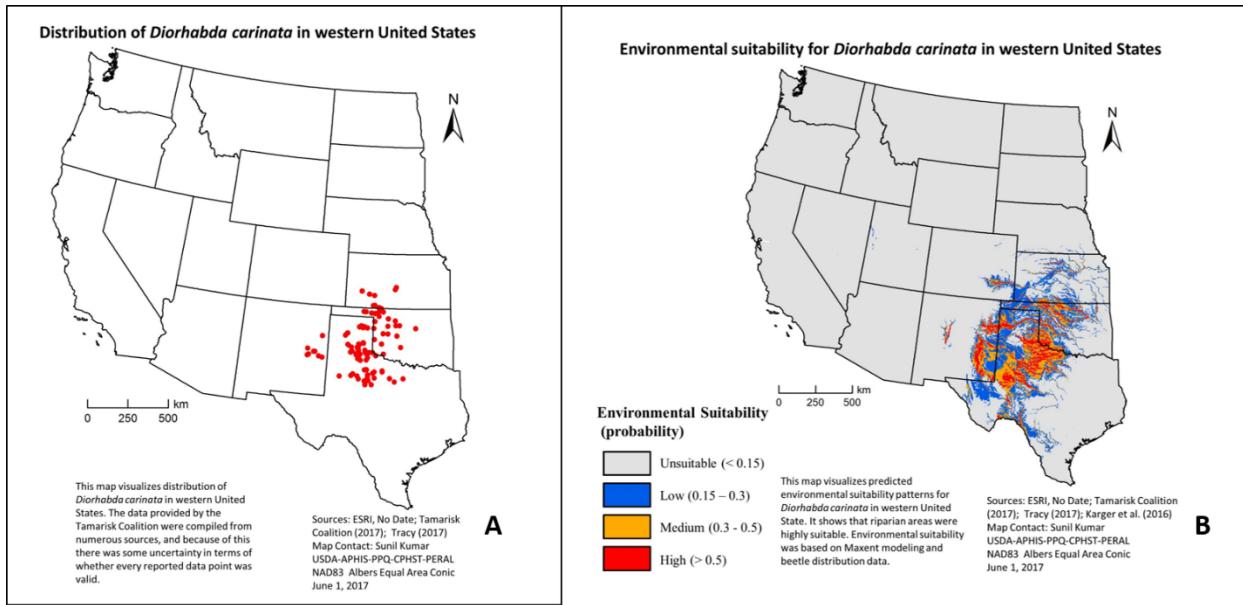


Figure 6: A) Occurrences of *Diorhabda carinata* in the western United States. B) Predicted environmental suitability for *Diorhabda carinata* in the western United States.

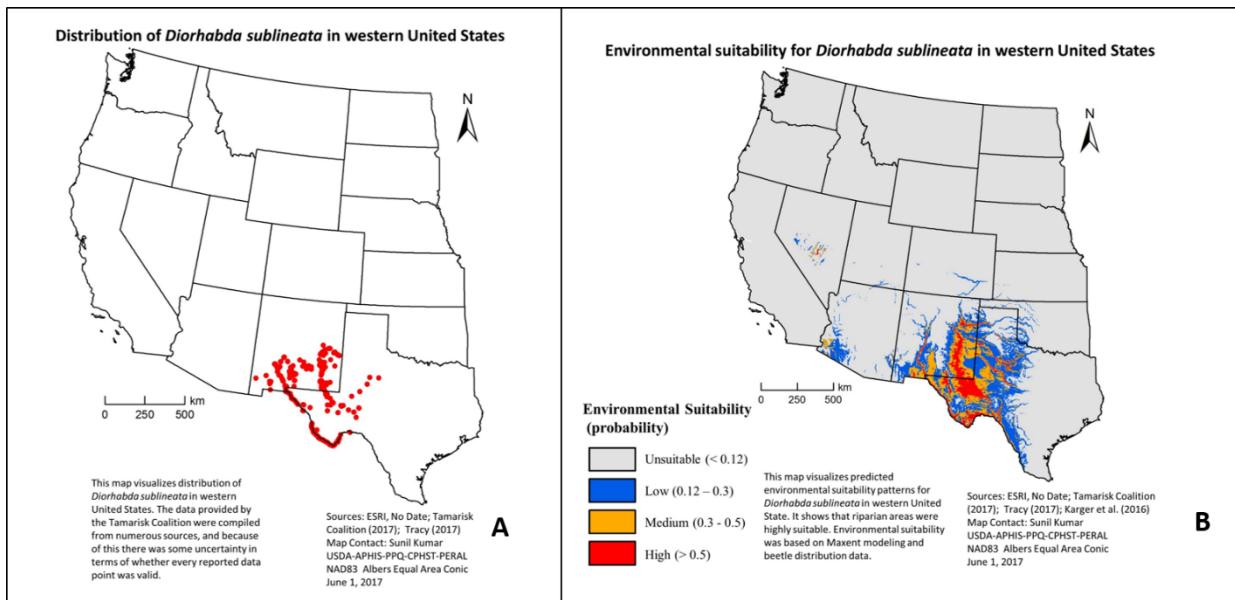


Figure 7: A) Occurrences of *Diorhabda sublineata* in the western United States. B) Predicted environmental suitability for *Diorhabda sublineata* in the western United States.

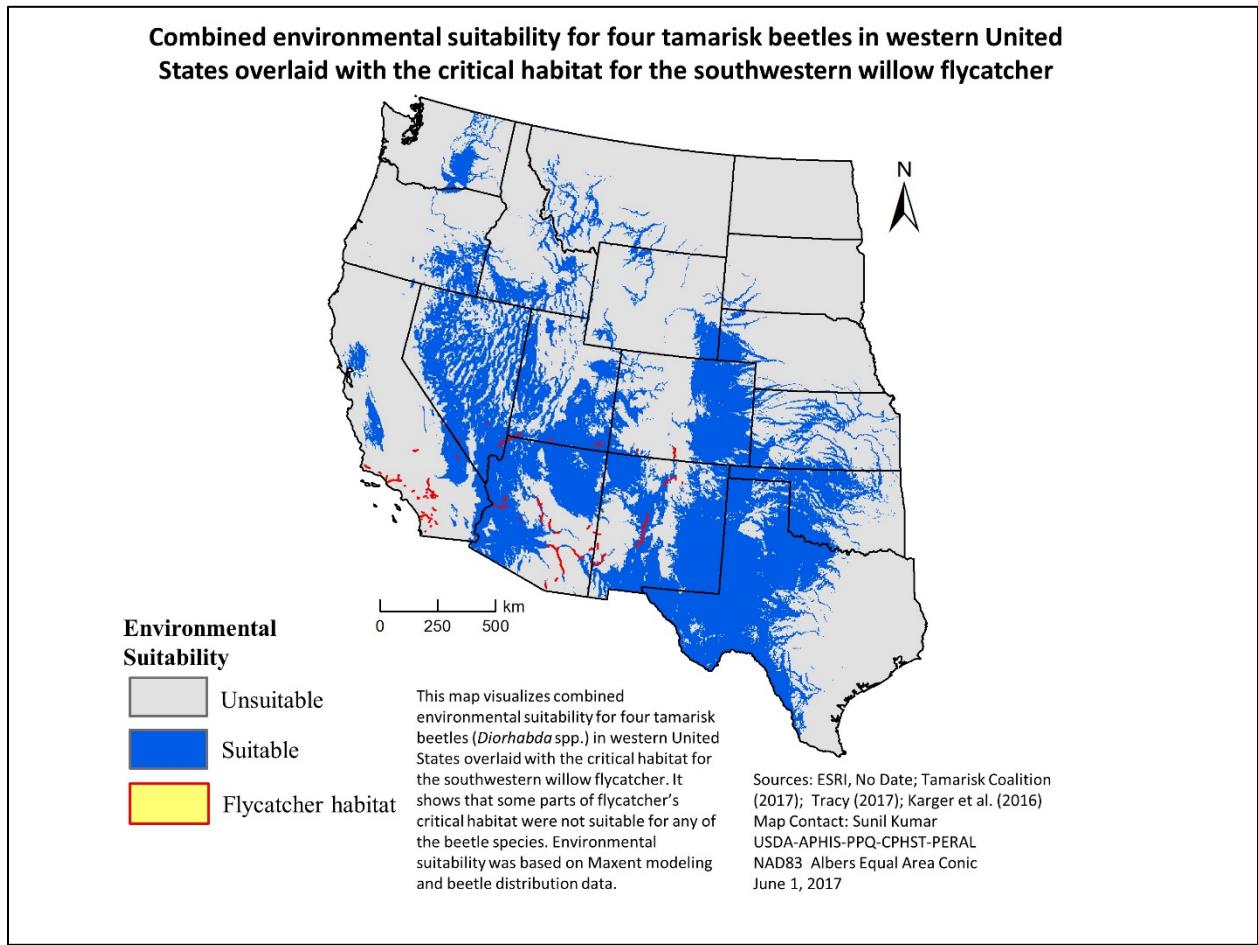


Figure 8: Combined environmental suitability for four tamarisk beetles in the western United States overlaid with the critical habitat of SWFL.

5. Mechanisms of spread

Diorhabda spp. have been spreading across the United States through several natural and human-mediated mechanisms.

5.1 Intentional releases

Populations of *Diorhabda* were first released in United States in 2001 (DeLoach et al., 2004) and have successfully established throughout much of the western United States (Carruthers et al., 2008). In 2010, APHIS-PPQ officially ended the saltcedar biological control program (PPQ, 2010). Ending the program means that: 1) PPQ discontinued issuing new permits for field cage or greenhouse studies using the tamarisk leaf beetle outside of a containment facility; 2) PPQ discontinued issuing new permits for interstate movement and environmental release of *Diorhabda*; 3) PPQ cancelled all issued (active) permits for interstate movement and environmental release of *Diorhabda*; and 4) PPQ indicated that it would no longer authorize the release of *Diorhabda* from containment or caged field study sites but would allow the continuation of existing *Diorhabda* activities in containment facilities. Furthermore, APHIS-PPQ

outlines penalties for improperly moving the beetles: “...any unauthorized human assisted movement of *Diorhabda* spp., particularly into the critical habitat of the southwestern willow flycatcher, is not authorized by APHIS, and may constitute a violation of the Endangered Species Act which could result in criminal punishment and/or fines. Additionally, the unauthorized collection, interstate transportation, and release of *Diorhabda* spp. in the U.S. may constitute a criminal and/or civil violation of the Plant Protection Act, with criminal penalties and/or fines assessed up to \$250,000 per violation.” (PPQ, 2010).

It is unclear whether the directive from PPQ has prevented further intentional spread of the beetles, particularly within individual states and outside of SWFL critical areas. *Diorhabda* beetles are not difficult to collect or to move to new locations. Newly emerged adults are collected with beat bucket traps, transferred to paper bags, and then moved to new tamarisk trees (Knutson, 2013). Within Texas, over one million beetles were relocated between 2006 and 2012 (Knutson, 2013). *Diorhabda* beetles are a very effective control for tamarisk trees, leading to the unregulated movement of beetle populations. In the USDA APHIS 2005 Environmental Assessment, the authors noted “There is also concern that because there is strong interest in the biocontrol program in western states, beetles may be ‘poached’ from current research sites and illegally redistributed to new locations. In Utah, beetles have been removed from a permitted site in Delta and distributed throughout the State.” (APHIS, 2005). It is likely that the unofficial but intentional redistribution of populations of *Diorhabda* spp. will continue as long as tamarisk continues to occur in areas where it is unwanted.

5.2 Unintentional human mediated spread

In addition to intentionally moving beetle populations, people are likely contributing to the spread of the beetle in unintended ways. We found no clear evidence in the scientific literature that humans are unintentionally moving the beetle into new areas, but anecdotal information indicates that this may occur. Private landowners have the responsibility to remove tamarisk from their land, while State and Federal agencies are responsible for removing it from their respective lands. Simple internet searches revealed numerous tree removal companies in tamarisk-infested areas that offer removal of tamarisk. Removing a beetle-infested tree could transport the beetles to a new area. This may explain how isolated populations of *Diorhabda* beetles have been detected in areas separated from stands of tamarisk and along highway corridors (Figure 9). As the beetles are highly mobile, it is also possible that individuals may hitchhike on people and vehicles moving through infested areas.



Figure 9: *Diorhabda* spp. detection at a truck stop more than 1,000 meters from a riparian area, indicating that it is probably not an established population due to limited tamarisk.

5.3 Natural dispersal

The literature on the natural dispersal capabilities of *Diorhabda* is not very robust, but it appears that *Diorhabda* beetles can spread at varying rates into new areas. For example, in one release study, beetles spread in an approximately 100-m radius from the initial site in two years and a 2.5-km radius in three years (DeLoach et al., 2003). Another study observed a spread rate of approximately 130 m per season following a beetle release the previous year (Sanabria and DeLoach, 2009). More recent field observations indicate that *Diorhabda* beetles are moving at a much faster rate than previously reported. For example, in a study along a 63-km stretch of the Virgin River between Littlefield, AZ and Lake Mead, NV, ground surveys, camera networks, and satellite imagery showed beetle damage progressing downstream at a rate of about 25 km per year (Nagler et al., 2014). The large discrepancy between the reported spread rates could be the result of several factors.

The early spread rates were primarily associated with newly introduced populations. The adaptation of the beetle to U.S. habitats has led to greater phenological synchrony with host plants and to larger populations in recent years (Bean et al., 2012). Larger, established populations appear to be able to spread faster due to dispersal events by swarms of reproductive adults (Nagler et al., 2014). Beetles appear to spread along riparian corridors throughout the summer and may disperse in a series of short-distance movements (Nagler et al., 2014). The aggregation pheromone allows large numbers of sexually mature beetles to move en masse and

establish in new areas with longer distance dispersal events being possible (Cossé et al., 2005a; Nagler et al., 2014). Also, abiotic factors like wind can affect the long-distance spread of *Diorhabda* beetles (Sanabria and DeLoach, 2009). Spread is also increased in areas with abundant continuous host material, as currently occurs along riparian corridors.

6. Predicted rate of *Diorhabda* spp. spread into SWFL critical habitat

We modeled the spread of tamarisk beetles into SWFL critical habitat via natural dispersal in tamarisk along riparian corridors. Since we were measuring linear distances associated with tamarisk beetle spread, we converted all data sets to an NAD83 USA Contiguous Equidistant Conic projection to minimize distortion of distance (Maher, 2010).

6.1 Critical habitats of SWFL

We used the final geospatial dataset for SWFL critical habitat that was produced by the U.S. Fish and Wild Life Service (USFWS, 2013) (Figure 10). This data set contains 130 SWFL critical habitats in six states: Arizona, California, Colorado, Nevada, New Mexico, and Utah. Critical habitats are considered essential for threatened or endangered species conservation and include foraging and breeding habitats for the flycatcher (USFWS, 2005).

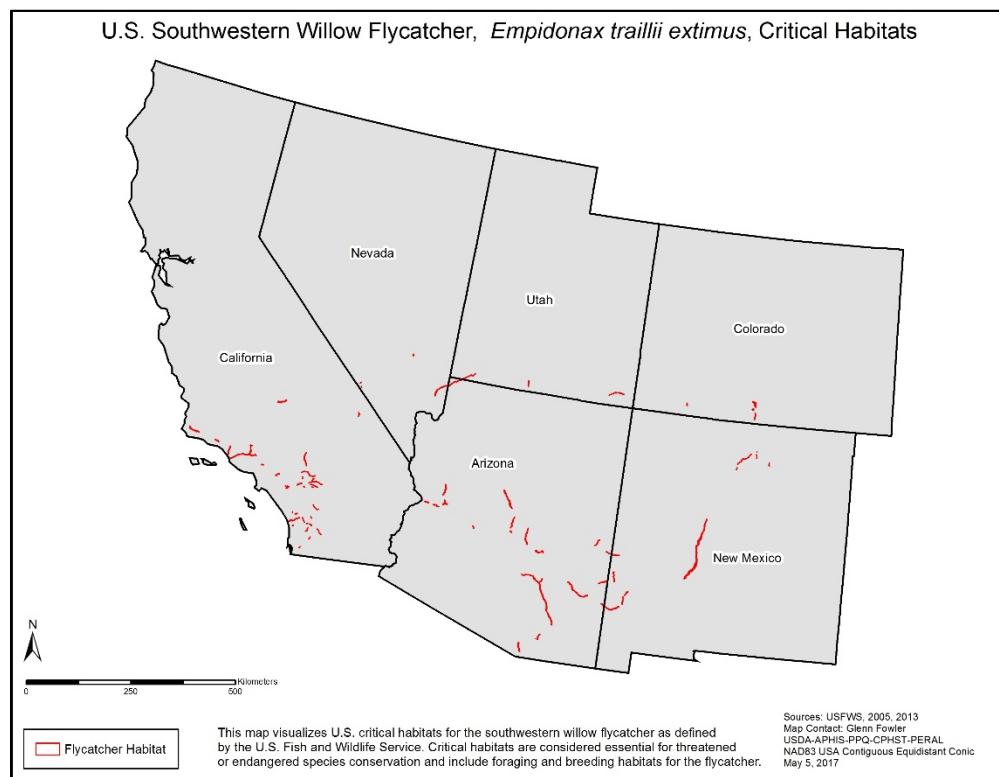


Figure 10: U.S. critical habitats for SWFL.

6.2 Tamarisk habitat

Tamarisk occurs in riparian areas (Hatten, 2016), so we used geospatially-referenced riparian data sets for 1) rivers and streams, and 2) bodies of water, such as lakes and reservoirs, to identify where tamarisk was likely to be in states with SWFL habitat (ESRI, No Date; USFWS, 2013) (Figure 11). We assumed tamarisk would only occur within 1,000 meters of a riparian area, based on the methodology used by Hatten (2016) for modeling tamarisk habitat. We therefore buffered this distance around the two riparian geospatial data sets to predict where tamarisk was likely to occur.

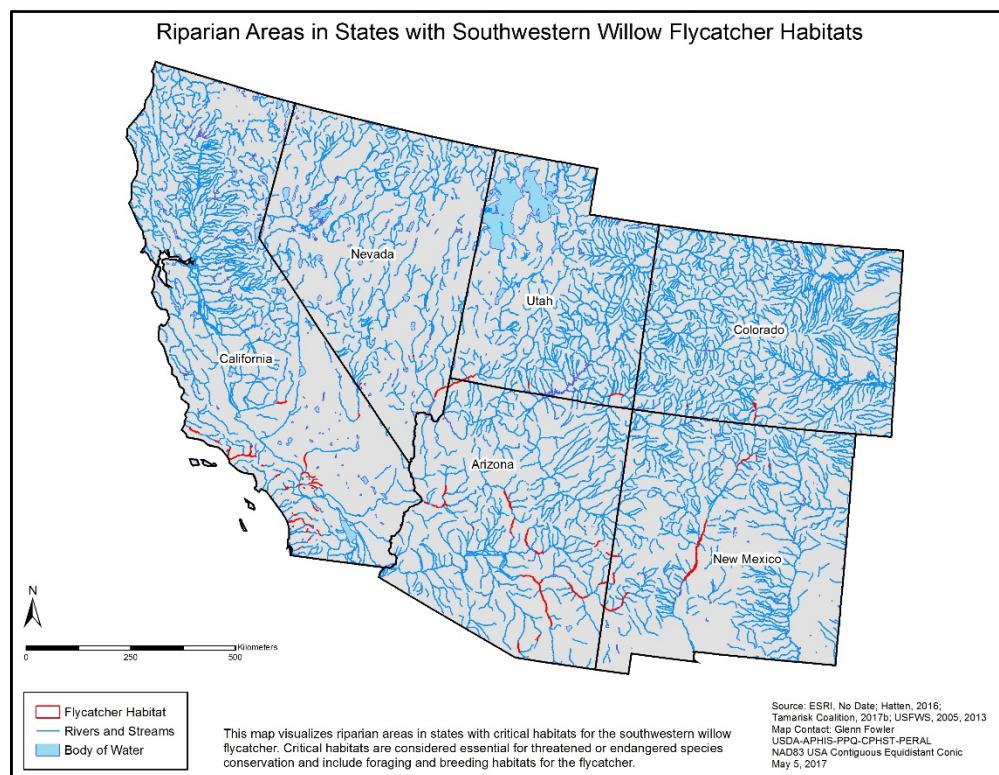


Figure 11: Riparian areas in states with critical habitats for SWFL.

6.3 Diorhabda occurrence

To update the risk analysis, we acquired tamarisk beetle detection points from 2017 (RiversEdge West, 2018) and overlaid them with the detections from 2007 to 2016 that occurred in states with SWFL critical habitat (Tamarisk Coalition, 2017; USFWS, 2013). We further refined the tamarisk beetle detection points to identify those that were likely established populations by selecting detections that occurred within 1,000 meters of a riparian area (i.e., areas where tamarisk likely occurs) (Figures 11 and 12). This step removed detections of individuals or small groups of beetles, such as beetles detected around truck stops (Figure 9), which are unlikely to successfully spread and colonize flycatcher habitat.

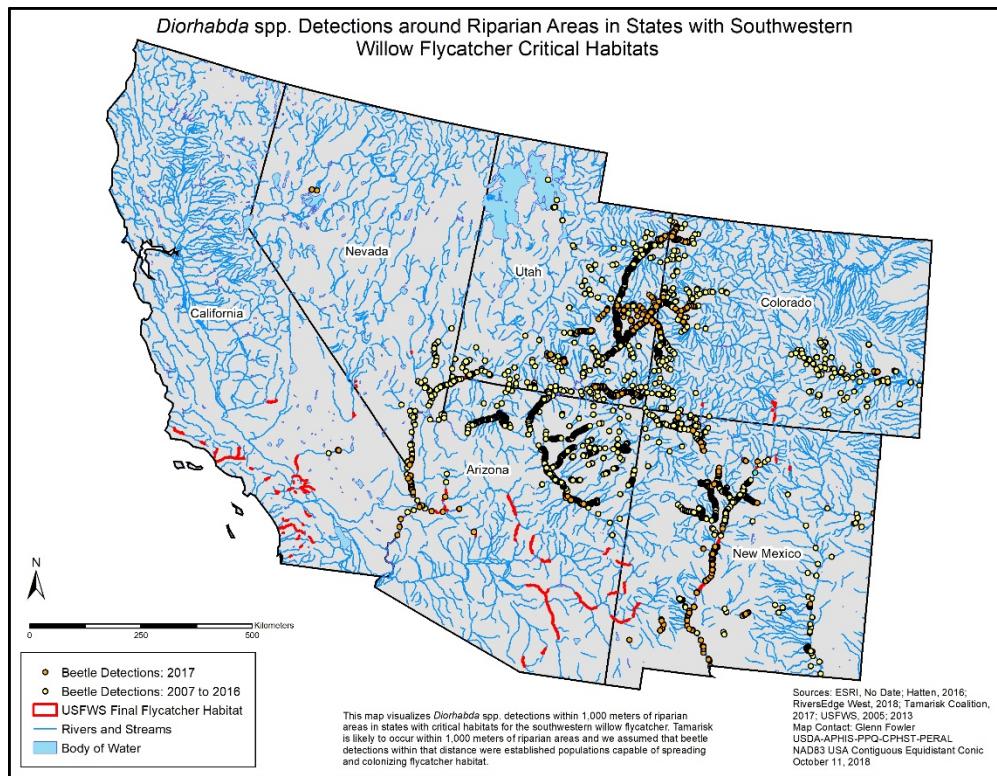


Figure 12: *Diorhabda* beetle detections within 1,000 meters of riparian areas where tamarisk is likely to occur in states with SWFL critical habitat.

6.4 *Diorhabda* spread rate to SWFL critical habitat

Diorhabda beetles are host-specific on tamarisk (Tracey and Robins, 2009), and we assumed they would follow riparian corridors, such as streams and rivers, in order to encounter SWFL critical habitat (Figure 13). We measured the distances from the nearest detection points to SWFL critical habitat along these riparian corridors.

Field observations demonstrated that *Diorhabda* can spread rapidly, and recent research estimates that they can disperse up to 25 km annually (Nagler et al., 2014). We recognize that this rate of spread may not be experienced in all areas where *Diorhabda* beetles may occur, but this value appears to be likely along riparian corridors like those in the SWFL critical habitat. Based on that evidence, we divided the measured distance along riparian corridors between the nearest *Diorhabda* detection and the SWFL critical habitat by 25 km to calculate the number of years until the beetle could gain entry into each SWFL critical habitat area. Flycatcher critical habitats containing *Diorhabda* detections were considered colonized, and we reported a zero for their years until entry. In one case, the Rio Grande between Pueblos in New Mexico, a flycatcher habitat was 256 meters from a detection point, and we considered it colonized due to the proximity.

Some gaps were present in the geospatial riparian data sets, such as breaks in a river or stream, and we assumed that *Diorhabda* would be able to fly directly across that gap if it was less than 25 km. Justifications for that assumption include 1) small streams and bodies of water between the gaps that were not visualized in the geospatial riparian datasets (ESRI, No Date; ESRI et al., 2017) but could contain tamarisk and sustain *Diorhabda* populations, 2) aggregation pheromones that allow for long-distance *Diorhabda* dispersal and establishment (Nagler et al., 2014), and 3) dispersion aided by wind (Nagler et al., 2014; Sanabria and DeLoach, 2009) that could facilitate movement across the gaps.

In nine cases, SWFL critical habitats occurred outside of the 1,000-m buffer around riparian areas. For those, we used satellite imagery to measure the distance from the riparian corridor along probable riparian routes to the flycatcher habitat (ESRI et al., 2017) (Appendices 2 to 4). This distance was then added to the distance from the nearest tamarisk beetle detection point and divided by 25 kilometers to estimate the number of years until entry.

The 25-km dispersal rate we used was based on a limited study area along the Virgin River and, consequently, this estimate has some uncertainty. Ninety-six percent of the *Diorhabda* detections in 2017, however, were within 25 km of the detections between 2007 and 2016, and 89 percent were within 25 km of the 2016 detections. This evidence indicates that 25 km is a reasonable upper estimate of *Diorhabda* natural annual dispersion potential: the beetles may move less than 25 km in a year but are unlikely to move further without human mediation.

The 2017 *Diorhabda* detections that occurred more than 25 km from the previous detections also provide useful information for informing future research and survey decisions. For example, two *Diorhabda* detections in 2017 along the Colorado River were 41 km and 60 km from the nearest 2016 detection. This indicates that 1) *Diorhabda* was naturally spreading faster than 25 km per year, 2) undetected populations in previous years caused the observed long-distance spread, or 3) the beetles were introduced at those locations via other means, such as human-mediated spread. Another 2017 detection in New Mexico near the base of the Burro Cienega Stream (Figures 12 and 13) was not near other *Diorhabda* populations but was 7.4 km from detections in 2016 and 2017 at a truck stop (Figure 9) and was alongside Interstate 10. This provides further anecdotal evidence that people are facilitating the spread of *Diorhabda* into new areas.

Additional research is needed to increase our understanding of *Diorhabda* spread rates and their fluctuation in different habitats. A source of uncertainty is the effect of elevation on the distribution of tamarisk and the associated effect on *Diorhabda* spread rates. Tamarisk can occur at elevations as high as 3,350 m (11,000 ft) (Brock, 1994; Zouhar, 2003). Tamarisk abundance, however, decreases with increasing elevation, and it spreads slowly above 1,220 m (4,000 ft) (Brock, 1994; Zouhar, 2003). Consequently, our 25-km *Diorhabda* dispersal rate may be a high estimate along riparian corridors at higher elevations where tamarisk is probably less abundant or even absent, such as portions of the predicted *Diorhabda* spread route into the San Francisco Northwest SWFL critical habitat in Arizona, which occurs at elevations above 2,400 m (7,784 ft)

(ESRI, No Date; USGS, 1999). Also, abiotic factors like wind can affect the long-distance spread of *Diorhabda* (Sanabria and DeLoach, 2009). Human-mediated movement of *Diorhabda* could also augment natural dispersal rates. For example, spread could be faster in areas with high concentrations of beetles, tamarisk, people, and commercial facilities. As more data becomes available, our spread estimates could be updated to reduce this uncertainty.

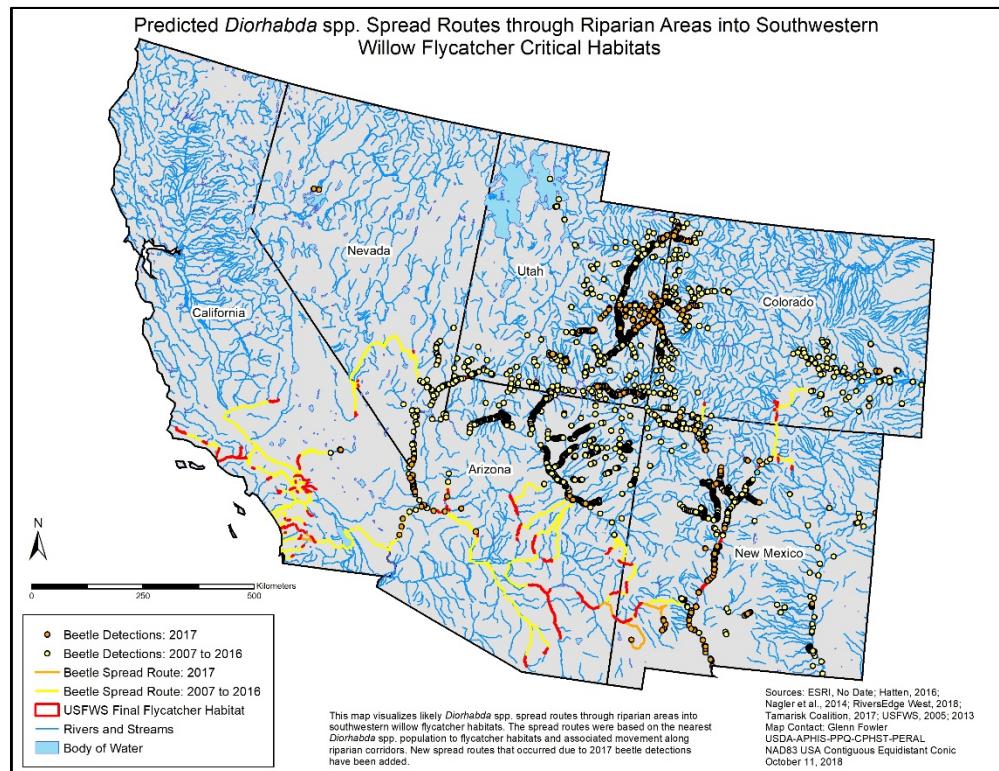


Figure 13: Predicted *Diorhabda* spp. beetle spread routes along riparian corridors into SWFL critical habitats.

6.5 Predicted number of years until *Diorhabda* enter SWFL critical habitat

Our spread analysis for *Diorhabda* provided estimates for the number of years until entry for each of the 130 flycatcher critical habitats by state. In Tables 3 and 4, we report the number of years predicted until *Diorhabda* entry into flycatcher habitat in Arizona and California. Table 5 reports the predicted number of years until beetle entry into flycatcher habitats in Colorado, Nevada, New Mexico, and Utah. The number of years we predict it will take for *Diorhabda* to enter the various flycatcher habitats based on our spread rate analysis varies between 0 for habitat that is already colonized and 20.2 for the flycatcher habitats farthest away from current *Diorhabda* populations.

For some of the SWFL critical habitats, the years expected until *Diorhabda* entry decreased due to the 2017 detections. We reported the new times in red text and the previous times in parentheses to denote this change. We also identified new spread routes due to 2017 *Diorhabda*

detections that were not along predicted spread routes in the previous risk analysis and resulted in faster entry times into SWFL critical habitats.

Table 3: Predicted number of years until beetle entry into SWFL critical habitats in Arizona.*

County	Flycatcher Habitat	Years until Beetle Entry	County	Flycatcher Habitat	Years until Beetle Entry
Apache	Little Colorado River West Fork	1.7	Mohave	Bill Williams River - Lincoln	0.1 (0.8)
Apache	San Francisco River - NW	3.3	Mohave	Virgin River	0.0
Apache	Little Colorado River	1.1	Mohave	Big Sandy River - north	0.0 (0.7)
Apache	San Francisco River - NE	3.2	Mohave	Bill Williams River - Alamo	0.1 (2.0)
Cochise	San Pedro River	18.8	Mohave	Santa Maria River	0.3 (2.3)
Gila	Tonto Creek	5.4	Mohave	Bill Williams River - NWR upper	0.0
Gila	Salt River	6.8	Pima	San Pedro River	18.4
Gila	Gila River - San Pedro	15.9	Pima	Cienega Creek	14.2 (19.7)
Gila	Verde River - middle N	6.0	Pima	Cienega Creek	14.0 (19.5)
Graham	Gila River - mid San Carlos ¹	8.3 (10.12)	Pima	Cienega Creek	14.4 (19.9)
Greenlee	Gila River - south ¹	5.8 (8.0)	Pima	Cienega Creek ²	13.8 (19.3)
Greenlee	San Francisco River - west	7.7	Pima	Cienega Creek	19.9
La Paz	Big Sandy River - Alamo	2.2	Pinal	San Pedro River	16.1
La Paz	Bill Williams River - Lincoln	0.1 (0.8)	Pinal	Gila River - San Pedro	13.9
La Paz	Bill Williams River - Alamo	0.1 (2.1)	Santa Cruz	Santa Cruz River	14.3 (19.8)
La Paz	Santa Maria River	0.3 (2.3)	Yavapai	Verde River - upper N	4.4
La Paz	Bill Williams River - NWR upper	0.0	Yavapai	Verde River - upper M	4.8
Maricopa	Hassayampa River	0.2 (5.7)	Yavapai	Verde River - upper S	5.3
Maricopa	Verde River - middle S	7.4	Yavapai	Verde River - middle N	6.0
Mohave	Big Sandy River - Alamo	0.3 (2.3)			

*Numbers in red are updated based on the 2017 *Diorhabda* detections. Numbers in parentheses are the values from the previous risk analysis.

¹Indicates a new beetle spread route to the SWFL critical habitat as a result of the 2017 *Diorhabda* detections.

²Indicates that satellite imagery was used to estimate the distance from a riparian corridor to SWFL critical habitat.

Table 4: Predicted number of years until beetle entry into SWFL critical habitats in California.*

County	Flycatcher Habitat	Years until Beetle Entry	County	Flycatcher Habitat	Years until Beetle Entry
Inyo	Amargosa River	15.2	San Bernardino	Willow Creek	15.5
Inyo	Willow Creek	15.5	San Diego	Agua Hedionda Creek - east	13.0
Kern	Canebrake Creek	20.0	San Diego	Agua Hedionda Creek - east	13.0
Kern	Kern River South Fork	19.1	San Diego	Agua Hedionda Creek - east	13.0
Kern	Kern River South Fork	19.0	San Diego	De Luz Creek	12.9
Los Angeles	Big Tujunga Canyon	10.4	San Diego	Pilgrim Creek	12.4
Los Angeles	Castaic Creek	15.3	San Diego	San Diego River - east	16.4
Los Angeles	Piru Creek	16.8	San Diego	San Diego River El Capitan - north	16.4
Los Angeles	Santa Clara River	9.2	San Diego	San Diego River El Capitan - south	16.3
Riverside	Bautista Creek - east	9.6	San Diego	San Felipe Creek	12.9 (15.3)
Riverside	Bautista Creek - middle	9.2	San Diego	San Luis Rey River - east 4	14.1
Riverside	Bautista Creek - west	9.0	San Diego	San Luis Rey River - east 5	14.5
Riverside	Santa Ana River - west	7.7	San Diego	San Luis Rey River - east 6	14.6
Riverside	Santa Ana River - west	7.9	San Diego	San Luis Rey River - east 7 ¹	14.3 (14.8)
San Bernardino	Amargosa River	15.5	San Diego	San Luis Rey River - middle 3	13.7
San Bernardino	Bear Creek	7.7	San Diego	San Luis Rey River - west 1	14.7
San Bernardino	Deep Creek	5.0	San Diego	San Luis Rey River - west 2	13.3
San Bernardino	Holcomb Creek	5.5	San Diego	Santa Margarita River	13.4
San Bernardino	Mill Creek	10.2	San Diego	Santa Ysabel Creek - east	14.8
San Bernardino	Mill Creek	10.2	San Diego	Sweetwater River	15.9
San Bernardino	Mojave River	3.1	San Diego	Temecula Creek	14.7
San Bernardino	Mojave River	5.0	San Diego	Temescal Creek ^{1,3}	15.0 (15.0)
San Bernardino	Oak Glen Creek	10.4	Santa Barbara	Mono Creek	18.1
San Bernardino	San Timoteo Creek	11.3	Santa Barbara	Santa Ynez River - east	18.1
San Bernardino	Santa Ana River - east	7.3	Santa Barbara	Santa Ynez River - middle	18.3
San Bernardino	Santa Ana River - middle	6.6	Santa Barbara	Santa Ynez River - west	20.2
San Bernardino	Santa Ana River - west	7.9	Ventura	Piru Creek	14.3
San Bernardino	Waterman Creek ²	6.2	Ventura	Piru Creek	16.0
San Bernardino	Waterman Creek ²	6.2	Ventura	Santa Clara River	13.3
San Bernardino	Waterman Creek ²	6.2	Ventura	Ventura River	16.0

*Numbers in red are updated based on the 2017 *Diorhabda* detections. Number in parentheses are the values from the previous risk analysis.

¹Indicates a new beetle spread route to the SWFL critical habitat as a result of the 2017 *Diorhabda* detections.

²Indicates that satellite imagery was used to estimate the distance from a riparian corridor to SWFL critical habitat.

³The predicted years until entry changed from 14.99 years to 14.97 years.

Table 5: Predicted number of years until beetle entry into SWFL critical habitats in Colorado, Nevada, New Mexico, and Utah.*

State	County	Flycatcher Habitat	Years until Beetle Entry
Colorado	Alamosa	Rio Grande SLV - NWR	4.50
Colorado	Conejos	Conejos River SLV	4.62
Colorado	Conejos	Rio Grande SLV - NWR	4.54
Colorado	Conejos	Rio Grande SLV - south	3.93
Colorado	Costilla	Rio Grande SLV - NWR	4.54
Colorado	Costilla	Rio Grande SLV - south	3.93
Colorado	La Plata	Los Pinos River	2.01
Nevada	Clark	Virgin River	0.00
Nevada	Lincoln	Pahranagat - NWR	2.78
Nevada	Nye	Ash Meadows NWR ²	14.74
Nevada	Nye	Ash Meadows NWR ²	14.61
Nevada	Nye	Ash Meadows NWR ²	14.30
Nevada	Nye	Ash Meadows NWR ²	14.31
Nevada	Nye	Ash Meadows NWR ²	14.34
New Mexico	Catron	San Francisco River - east	6.26
New Mexico	Catron	San Francisco River - NE	3.18
New Mexico	Grant	Gila River - east ¹	4.0 (4.7)
New Mexico	Grant	Gila River - south ¹	4.8 (6.5)
New Mexico	Hidalgo	Gila River - south ¹	5.5 (7.4)
New Mexico	Mora	Coyote Creek	2.97
New Mexico	Rio Arriba	Rio Grande - Between Pueblos ⁴	0.00
New Mexico	Rio Arriba	Rio Grande - upper	0.00
New Mexico	Socorro	Rio Grande - middle	0.00
New Mexico	Socorro	Rio Grande - middle	0.45
New Mexico	Taos	Rio Fernando de Taos	0.90
New Mexico	Taos	Rio Grande - upper	0.00
New Mexico	Taos	Rio Grande del Rancho	1.03
New Mexico	Valencia	Rio Grande - middle	0.00
Utah	Kane	Paria River	0.00
Utah	San Juan	San Juan River - west	0.00
Utah	Washington	Virgin River	0.00

*Numbers in red are updated based on the 2017 *Diorhabda* detections. Number in parentheses are the values from the previous risk analysis.

¹Indicates a new beetle spread route to the SWFL critical habitat as a result of the 2017 *Diorhabda* detections.

²Indicates that satellite imagery was used to estimate the distance from a riparian corridor to SWFL critical habitat.

⁴This SWFL critical habitat was 256 meters from a *Diorhabda* detection and was considered colonized.

We also conducted a statistical analysis of the mean years until *Diorhabda* spp. entry into flycatcher habitat by state. We did this to aid with policy and operational decisions, such as resource allocation for SWFL habitat replacement and survey funding. Specifically, we ran an analysis of variance (ANOVA³) to determine if states differed significantly with regard to time until *Diorhabda* spp. entry into flycatcher habitat, which they did ($p < 0.0001$) (PennState, 2017) (Appendix 5). We used a Welch ANOVA instead of a standard ANOVA because the variances in the data sets were not equal, which violates an assumption of the standard test (SAS Institute Inc., 2014) (Appendix 5). We then used the Tukey-Kramer HSD³ test for comparing means to determine which states were significantly different (PennState, 2017) (Appendix 6; Figure 14). This is a robust and commonly used test that is recommended for pair-wise comparisons (PennState, 2017).

For all states combined, the updated mean number of years until entry into SWFL critical habitats was 7.2 years, which was a reduction from 9.7 years in the previous analysis (Table 6). The mean number of years until entry into flycatcher habitats by state were 6.5 years for Arizona, 12.6 years for California, 4.0 years for Colorado, 2.3 years for New Mexico, and 10.7 years for Nevada. (Table 6, Figure 14). Arizona, California, and New Mexico exhibited lower estimates of the mean years until *Diorhabda* entry into SWFL critical habitats than in the previous risk analysis. New Mexico exhibited the greatest change with a reduction from 5.1 years to 2.3 years, which was due to the new detections near the base of the Burro Cienega Stream and along the Mimbres River.

The mean number of years that it would take for the *Diorhabda* to enter SWFL critical habitat was significantly higher in California than in Arizona, Colorado, and New Mexico (Tukey-Kramer HSD³) (Figure 14). In addition, the number of years until entry for Arizona and Nevada were significantly higher than for New Mexico. Because all of the SWFL critical habitat in Utah has been colonized, we excluded that state from the comparisons.

Table 6: Summary statistics for the mean number of years until *Diorhabda* entry into SWFL critical habitat by state.*

State	Number of Flycatcher Habitats (N)	Mean Years until Entry	Standard Deviation	Lower 95% Mean Confidence Limit	Upper 95% Mean Confidence Limit
Arizona	39	6.5 (7.8)	6.5 (7.2)	4.4 (5.5)	8.6 (10.1)
California	60	12.6 (12.7)	4.2 (4.3)	11.5 (11.6)	13.7 (13.8)
Colorado	7	4.0	0.9	3.2	4.9
Nevada	7	10.7	6.4	4.8	16.7
New Mexico	14	2.3 (5.1)	6.4 (6.3)	0.8 (1.4)	3.5 (8.7)
All States	127	7.2 (9.7)	6.2 (6.3)	7.9 (8.6)	10.1 (10.9)

*Numbers in red are updated based on the 2017 *Diorhabda* detections. Numbers in parentheses are the values from the previous risk analysis.

³ JMP® version 11.1.1 was used to conduct the statistical analysis at $\alpha = 0.05$ significance.

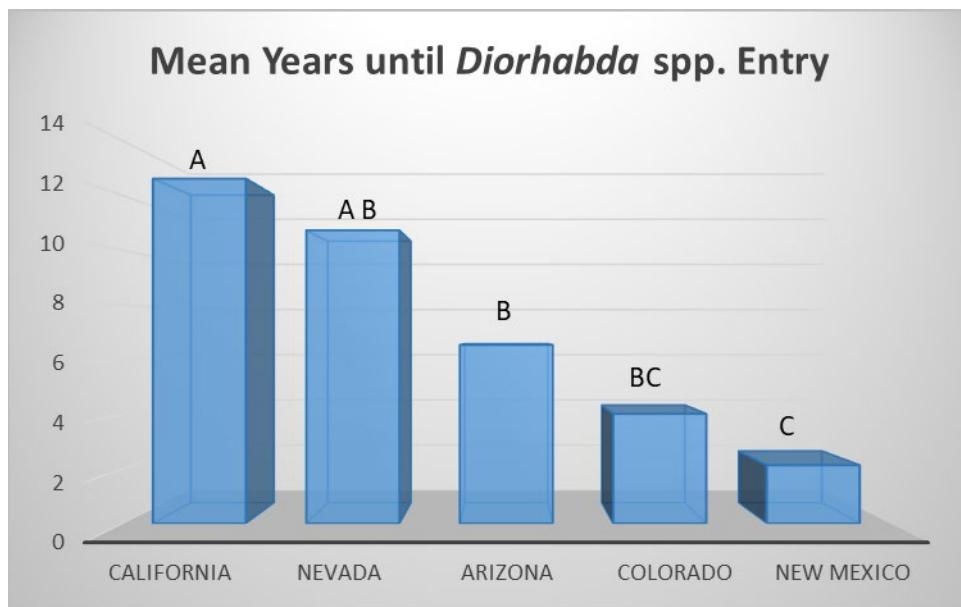


Figure 14: Mean number of years until *Diorhabda* entry into SWFL critical habitat by state. States with different letters are significantly different at $\alpha = 0.05$.

6.6 Combined analysis for *Diorhabda* establishment, spread, and years until entry into SWFL critical habitats.

The combined results of the suitable habitat model for *Diorhabda* establishment, the spread estimate, and the statistical analysis identified the SWFL critical habitats within states most at risk for *Diorhabda* introduction (Tables 3 to 6; Figure 14; Figure 15). The combined results also predict that 1) it will take significantly longer for *Diorhabda* to enter California SWFL critical habitats on average than those of Arizona, Colorado, and New Mexico and for it to enter Nevada SWFL critical habitats than those of New Mexico, 2) most of the SWFL habitats in California are not climatologically suitable for *Diorhabda* establishment, and 3) Arizona appears to have the most SWFL critical habitats at risk for *Diorhabda* establishment. This information could be useful for prioritizing funding decisions for conservation, policymaking, and survey activities within states.

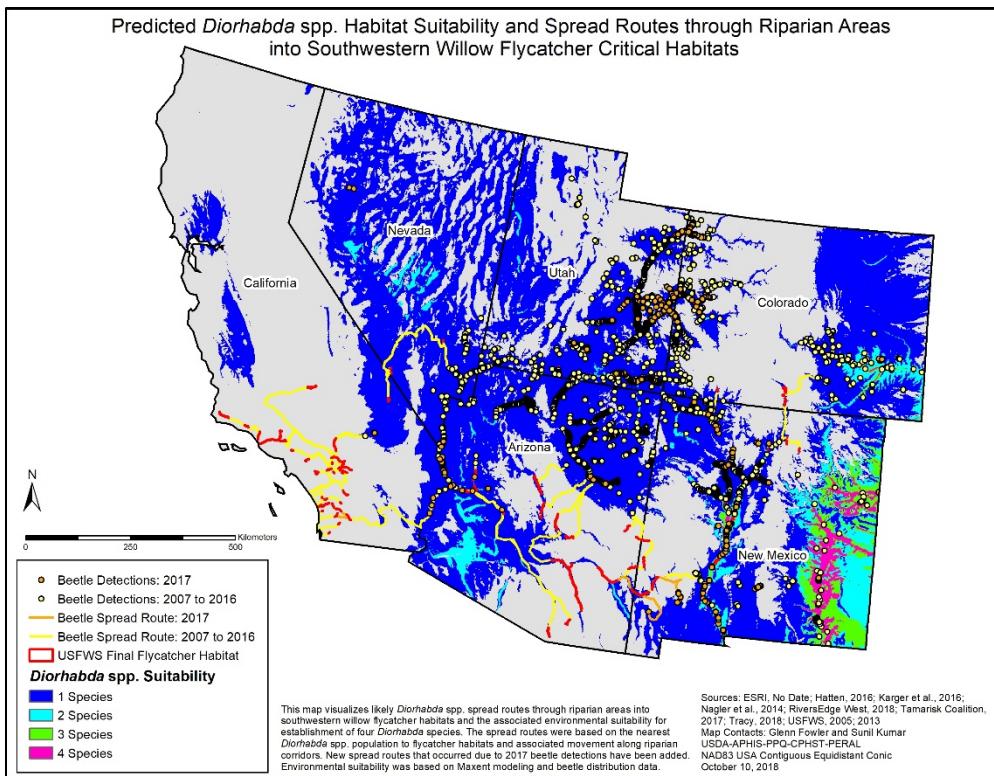


Figure 15: Suitable habitats for *Diorhabda* establishment and likely spread routes through riparian areas in SWFL critical habitat.

7. Evaluation of potential controls

Successful restoration efforts of tamarisk-infested areas have already been developed and implemented in many areas. These efforts typically focus on strategies involving replanting of native vegetation, removal of tamarisk, and flood control (Clements et al., 2015; Stevens et al., 2015). Using *Diorhabda* as part of the tamarisk control strategy is a common practice in many areas (Kennard et al., 2016; Sankey et al., 2016). Control strategies aimed at managing the beetle are far less common, and most of the literature specific to *Diorhabda* focuses on techniques to encourage beetle establishment (Jalalpour, 2002; DeLoach et al., 2003; Lewis et al., 2003). No controls are currently deployed to eradicate *Diorhabda* anywhere in the world. In light of this, we reviewed seven control methods for feasibility in slowing the spread of *Diorhabda*. We evaluate the potential of several control options for eradication or potentially slowing the spread of *Diorhabda*. Because *Diorhabda* is a biological control agent and not typically considered a pest, the use of these management methods is theoretical and is based on similar systems. If any of the options were to be implemented, considerable research would be necessary to prove their feasibility or effectiveness.

7.1 Thinning and intercropping

Thinning of forest stands can reduce risk from fire, insects, and disease and has been shown to prevent bark beetle outbreaks (Fettig et al., 2007; Nowak et al., 2008; Nowak et al., 2013).

Thinning has been shown to reduce chrysomelid beetle abundance in Japanese cedar (*Cryptomeria*) (Maleque et al., 2007) and in larch plantations (*Larix*) (Ohsawa and Nagaike, 2006). In these studies, thinning also improved understory biomass and species diversity of annual plant species (Maleque et al., 2007; Ohsawa and Nagaike, 2006); this acts like intercropping in masking host odors and disrupting pest populations. Because thinning limits host material, it is an effective way to manage insect populations (Fettig et al., 2007; Nowak et al., 2008; Nowak et al., 2013; Webster et al., 1978) and would also likely reduce *Diorhabda* populations. *Diorhabda* are reported to actively leave tamarisk stands after defoliation (Dudley and Kazmer, 2005; Jamison, 2016). In one study, the number of *D. elongata* adults and larvae was lower on defoliated plants, and the insects left the plants in large numbers in search of alternative forage (Dudley and Kazmer, 2005). The beetles do not appear to persist in areas without host material. In another study, *D. carinulata* populations abandoned tamarisk stands following defoliation, and populations in the following year were not new arrivals, but from the overwintering generation. The lack of food leads to evacuation of defoliated areas by the mobile adult beetles. In completely denuded areas, *D. carinulata* may be absent for at least one year (Jamison, 2016).

In larch stands, host density had an effect on the abundance of two Chrysomelidae species; *Batophila acutangula* Heikertinger and *Sphaeroderma tarsatum* Baly occurred at higher frequencies in areas where their host plants also occurred at higher rates (Ohsawa and Nagaike, 2006). Chrysomelidae abundance has also been studied in *Cryptomeria* plantations that were line thinned, and it was shown that Chrysomelidae abundance was lower in thinned stands than in stands that were not thinned (Maleque et al., 2007). Thinning of host material of southern pine beetle (SPB) (Curculionidae) has led to extremely reduced populations of SPB in thinned stands (Nowak et al., 2013). These studies show that thinning of host plants reduces the abundance of beetles in their respective systems.

Thinning has the added benefit of increasing biodiversity of plant species (Maleque et al., 2007; Ohsawa and Nagaike, 2006). Increased biodiversity is similar in effect to intercropping. Intercropping not only increases the space between host plants but also creates a diverse habitat to further mask or diffuse host odors (Hummel et al., 2009; Kimaru, 2013). Intercropping of agricultural crops has been implemented for the control of chrysomelid beetles in agricultural systems (Andow, 1991; Gold et al., 1989). For example, populations of flea beetles (Chrysomelidae), which are pests of several agricultural crops, are reduced by intercropping. In experimental plots of collards, flea beetle biomass was lower in experimental rows surrounded by diverse vegetation than in monoculture collard plots. Collards grown adjacent to meadow vegetation also had lower herbivore loads than those grown in pure stands (Root, 1973). *Diorhabda* beetles aggregate in large numbers and are attracted to male aggregation pheromones (Cossé et al., 2005a; Cossé et al., 2011) as well as to green leaf volatiles (Cossé et al., 2006b; Cossé et al., 2011; Weaver, 2011; Weaver, 2014). Intercropping native vegetation among

tamarisk could lead to the diffusion of pheromone plumes, leading to less dense populations of the beetle.

Removal or thinning of tamarisk trees and associated regrowth of desirable native species could effectively create an intercropping system which would also likely reduce *Diorhabda* populations based on the theory that insect herbivore populations are directly influenced by the abundance of and distance between their hosts (Root, 1973). Intercropping would not only reduce beetle populations but may also slow the spread of *Diorhabda* into new habitats (Andow, 1991). This approach will not eliminate *Diorhabda* populations unless all host material is removed, which is not feasible. Other limitations to thinning include the area that would need to be managed. The effectiveness of thinning has been uncertain in some instances with mountain pine beetle; it may be less effective if thinned plots are surrounded by unmanaged stands where beetle populations are high (Fettig et al., 2007). Thinning and intercropping are not always effective and may not work when beetle populations are high (Weiss et al., 1994).

7.2 Chemical control

One study investigated the use of pesticides to control *Diorhabda sublineata* on *Tamarix aphylla* in Mexico (Estrada-Muñoz and Sánchez-Peña, 2014). *Tamarix aphylla* is an exotic ornamental used for shade in northern Mexico and is a host for the tamarisk beetle (*Diorhabda sublineata*). Drench treatments using imidacloprid, a neonicotinoid insecticide, were tested in laboratory and field settings for efficacy against *Diorhabda sublineata* and for the longevity of the chemical in tamarisk foliage (Estrada-Muñoz and Sánchez-Peña, 2014). Drench treatments of 70 g imidacloprid caused significant reduction in feeding on *T. aphylla* and killed *Diorhabda sublineata* adults and larvae (Estrada-Muñoz and Sánchez-Peña, 2014). Drench soil treatments of imidacloprid are more targeted than broadcast or spray applications and minimize negative environmental impacts (Estrada-Muñoz and Sánchez-Peña, 2014). Imidacloprid is less toxic to aquatic organisms than other pollutants; however, it does persist in aquatic environments (Tišler et al., 2009). Tests of imidacloprid on non-target insects such as leaf-shredding aquatic insects showed various results; leaves from treated trees did not seem to have an impact, but direct treatments at similar levels and leaves from high-dose trees did cause significant mortality (Kreutzweiser et al., 2007).

Imidacloprid a systemic insecticide. It is most widely used in agricultural applications; however, it has been effectively used against the invasive emerald ash borer (Coleoptera: Buprestidae: *Agrilus planipennis*), Asian longhorned beetle (Coleoptera: Cerambycidae: *Anoplophora glabripennis*) (Kreutzweiser et al., 2007), and Japanese beetle (Coleoptera: Scarabaeidae: *Popillia japonica*) (Frank et al., 2007). Imidacloprid is the primary insecticide for defoliators in landscape and nursery tree production (Tenczar and Krischik, 2007). It has also been studied in containerized poplar clones (*Populus nigra x maximowiczii*) against a chrysomelid beetle (*Chrysomela scripta*) (Tenczar and Krischik, 2007). In the poplar study, granular, drench, and tablet treatments were effective against leaf beetles for 12 months (Tenczar and Krischik, 2007).

Ash trees have been shown to be protected from the emerald ash borer for one year following injection treatments with imidacloprid (Smitley et al., 2010). Insecticides are also primary tools in controlling Japanese beetle in ornamental street trees (linden, *Tilia* spp.). In a study of imidacloprid soil drenches on linden trees, the treatment provided protection from major defoliation for two seasons (Frank et al., 2007).

One significant limitation to the use of imidacloprid for the control of *Diorhabda* is the feasibility of application over large areas. While imidacloprid would likely be effective, the applications are labor intensive, as soil drenches and injections are applied to one tree at a time over multiple years. Additionally, the use of soil drenches may be restricted near riparian habitats. The applications are also costly. For example, ash tree injections of imidacloprid to control emerald ash borer were estimated to cost \$73 per tree per year (Smitley et al., 2010). Although it is likely that treatment of individual trees could protect the trees from defoliation by the tamarisk beetle, it could potentially be environmentally and economically costly.

Imidacloprid treatments could be used in conjunction with other management strategies. Other insecticides may be effective against *Diorhabda*; however, imidacloprid is the only one that has been studied. The impacts associated with leaching of imidacloprid into riparian streams present a potential hazard. Imidacloprid is safer for many aquatic organisms than other chemicals, but it does negatively impact aquatic insects (Kreutzweiser et al., 2007; Tišler et al., 2009). More research is needed to determine the impacts of imidacloprid from an economic and environmental standpoint.

7.3 Mating disruption

Mating disruption is a pest control practice typically used to disrupt the ability of males to find females by saturating the environment with synthetic female pheromones (Carde and Minks, 1995); it has primarily been used against Lepidoptera. The theory behind the use of this practice is that the males use the female pheromone to locate a single point source (i.e., the female), and thus by increasing the number of sources (i.e., through synthetic pheromone saturation of the environment), the ability of the males to find females is disrupted (Carde, 1990; Carde and Minks, 1995; El-Sayed et al., 2006). The goal of mating disruption is to reduce mating to a level that brings the population below the threshold for that pest to persist (Carde, 1990). Mating disruption is more effective when populations are low (Carde, 1990; El-Sayed et al., 2006), and it is highly dependent on population dynamics (e.g., density, migration, generations per year) as well as on the size of the area being treated (Carde and Minks, 1995; El-Sayed et al., 2006; Rechcigl and Rechcigl, 1999).

Isolated areas, such as geographically separated tamarisk stands, might be good areas for mating disruption if they are large enough and have sufficient buffer zones and if the correct number of dispensers is used to prevent immigration (Carde and Minks, 1995). Successful mating disruption programs typically require treatment of the entire area (Carde and Minks, 1995) and

typically occur over homogenous landscapes (i.e., orchards, crop systems). Tamarisk stands do not represent a typical mating disruption landscape. Tamarisk is estimated to occupy more than 800,000 ha of riparian areas spread across the United States and Mexico (Carruthers et al., 2008), making it very difficult, if not impossible, to treat the entire suitable area. Because the riparian corridors are connected, treatment in these areas may increase risk of immigration if they are not sufficiently isolated.

Another factor influencing mating disruption success is population size in the area being treated (Carde, 1990; Carde and Minks, 1995; El-Sayed et al., 2006; Rechcigl and Rechcigl, 1999). Mating disruption is only effective when population densities are low and populations are geographically isolated from each other (Carde, 1990; Carde and Minks, 1995; El-Sayed et al., 2006). Tamarisk beetle populations are high in the areas where it has become established (Dudley and Bean, 2012) and do not appear to be geographically isolated for the most part (Tracy, 2017) (Figures 1, 12, 13, and 15). Further, populations can grow very quickly, with a doubling time of 6.2 days according to Lewis et al. (2003). Therefore, mating disruption would have to be deployed quickly, in sufficiently isolated areas, before populations were able to reach a critical mass that would overcome the synthetic pheromone.

Female-produced sex pheromones have not yet been identified in *Diorhabda* and consequently no synthetic female pheromone is available for this beetle. It is likely that the tamarisk beetle uses female-produced sex pheromones, because the aggregation of most insects, including chrysomelids, for the purpose of reproduction is mediated by female-produced sex pheromones (Landolt, 1997; Wertheim et al., 2005). For example, in the closely related Chrysomelidae *Diabrotica* (Kim et al., 2003), female-produced sex pheromones have been identified for several species (Chuman et al., 1987; Krysan et al., 1989; Ventura et al., 2001). They have also been identified for other Chrysomelidae ([e.g. *Leptinotarsa decemlineata* (Jermy and Butt, 1991), *Aulacophora foveicollis* (Kumar and Nadarajan, 2008), and *Longitarsus jacobaeae* (Zhang and McEvoy, 1994)]. Given the commonality of this trait, it is likely that *Diorhabda* also uses female-produced sex pheromones. Female-produced sex pheromones have not yet been studied in *Diorhabda*, but male-produced aggregation pheromones have been identified. The use of male aggregation pheromones by the tamarisk beetle (Cossé et al., 2005a) and the likely use of female-produced sex pheromones indicate that *Diorhabda* employs dual mate recruitment. Dual mate recruitment is a major impediment to mating disruption because the presence of male aggregation pheromones would attract both sexes to an area, failing to prevent mating (Carde and Minks, 1995).

Mating disruption is not likely to be an effective option for controlling *Diorhabda* species in the United States primarily because of dual mate recruitment. Mating disruption is ineffective when both females and males produce attraction pheromones (Carde and Minks, 1995). Although no specific studies have been completed, this may be the case for *Diorhabda*. Several species of

Diorhabda are known to produce male attraction pheromones: six ecotypes of *D. elongata* as well as *D. carinulata* are all attracted to the same male aggregation pheromone (Cossé et al., 2006b; Cossé et al., 2005a). Male aggregation pheromones are different from female-produced pheromones because instead of attracting only one sex to a single point source, both sexes are attracted to an area in equal proportions and are further guided by host chemical and visual cues. Research would need to be conducted to confirm the presence of female-produced sex pheromones in *Diorhabda*, but the male aggregation pheromone likely precludes mating disruption from being a viable method of control because it attracts both sexes to an area in equal ratios, rather than attracting one sex to a single point source.

Although the male aggregation pheromone has been identified and several studies have examined its effectiveness for monitoring beetle populations (Cossé et al., 2003; Cossé et al., 2005a; Cossé et al., 2005b; Cossé et al., 2006a; Cossé et al. 2006b; Cossé et al. 2011), further research would be necessary to understand the pheromone complex employed by the beetles.

7.4 Mass trapping and lure or attract-and-kill

Mass trapping and attract-and-kill are similar methods that have successfully been used to control Lepidoptera, Coleoptera, and Diptera (Rechcigl and Rechcigl, 1999; El-Sayed et al., 2006). Mass trapping uses pheromones that attract insects to a trap where they cannot escape and eventually die. Similarly, attract-and-kill technologies use pheromones to attract insects to a point source where they come into contact with an insecticide (El-Sayed et al., 2006). The method of killing differs between the two techniques, which means there are operational differences as well as potential differences in effectiveness depending on the behavior of the insect (El-Sayed et al., 2006). Both methods require a good understanding of the pheromone release rate (dose) and the behavior of the insects in response to the pheromone (El-Sayed et al., 2006). Green leaf volatiles that are attractive to *Diorhabda elongata* (Cossé et al., 2006b), as well as male aggregation pheromones (Cossé et al., 2005a), have been identified and field tested. These have been formulated into sustained-release formulations (SPLATTM) and could be effective tools for trapping (Weaver, 2014), but more research is necessary.

Like mating disruption, however, mass trapping is most effective with isolated low-density populations and is not successful with moderate to high populations over large areas (Fisher et al., 1985; Potter and Held, 2002; Wawrzynski and Ascerno, 1998; El-Sayed et al., 2006; Rechcigl and Rechcigl, 1999). For this reason alone, mass trapping by itself is unlikely to be successful in significantly reducing *Diorhabda* populations in most settings. Mass trapping may, however, be effective if population reductions are achieved in some other way (El-Sayed et al., 2006). It may also be effective in areas where initial populations are low, but this may be unlikely for *Diorhabda* spp. due to fast population growth rates [population doubling time of 6.2 days (Lewis et al., 2003)]. Mass trapping is often most effective when combined with other treatments such as chemical control (Rechcigl and Rechcigl, 1999). If mass trapping is used in isolated areas where initial populations are low and buffers from other infested areas are

sufficient, they might be effective in reducing populations of *Diorhabda* in new areas. Mass trapping has had limited success in control of invasive species, but when combined with other methods, it may be effective (El-Sayed et al., 2006). The size of the buffer zones, the population dynamics, the beetle biology, the pheromone rates, the dispersal distances, and the trap types would need to be researched in order to determine the feasibility of mass trapping or attract-and-kill technologies for use on *Diorhabda* beetles.

7.5 Beetle herding

“Beetle herding” has been proposed as a possible way to direct the movement of *Diorhabda* beetles to or from tamarisk stands (Bloodworth et al., 2016). Beetle herding is in the early stages of development, and the effectiveness of this approach is still unknown. Research on beetle herding has, however, been conducted with small study sizes and limited replication since 2011.

Initial work appeared to be focus on determining the release rate of pheromone and host plant volatiles from different formulations and demonstrating proof of concept for directing *Diorhabda carinulata* (Weaver, 2011). A later proposal focused more on developing formulations for the male-produced aggregation pheromone of *Diorhabda carinulata* for field trials (Weaver, 2014). SPLAT™ technology is typically used for mating disruption or for attract-and-kill (Mafra-Neto et al., 2013). Preliminary results from field applications of the SPLAT™ formulation were reportedly promising to the researchers in their ability to attract *Diorhabda* (Weaver, 2014). It appears as if the pheromone system studied may aid in monitoring tamarisk beetle populations at least on small scales (Weaver, 2014) if combined with an effective trap.

Recently, Cossé et al. (2011) isolated a *Diorhabda* aggregation pheromone that might be used in an attempt to direct beetle movement (Caraher, 2018; Dudley, 2018; Gaffke et al., 2018). Gaffke et al. (2018) were able to use the pheromone to attract *D. carinulata* to tamarisk in greater numbers, resulting in greater dieback than in control plants. This pheromone could be used to detect new beetle populations when paired with a trap or to increase tamarisk dieback in areas with low beetle density. Additionally, a *Diorhabda* repellent pheromone has been isolated that could be used in conjunction with the aggregation pheromone as part of a “push/pull strategy” to direct beetle movement for desired purposes (Bean et al., 2018).

The study by Gaffke et al. (2018) highlights one limitation to “beetle herding” or use of a “push/pull method”: the movement of larvae away from defoliated plants. Their study shows faster defoliation rates and greater dieback in trees treated with the pheromone (Gaffke et al., 2018), but the authors also point out that the treated trees would likely become less attractive, causing the control plants to become more attractive based on the level of defoliation. This means a tamarisk tree chosen for protection might only be made less attractive for a short period of time in areas where *Diorhabda* densities are high.

Another limitation to “beetle herding” is the potential for hybridization with other *Diorhabda* spp. The aggregation pheromone is likely to attract all species of *Diorhabda*. The “beetle herding” projects have focused on *D. carinulata* (Weaver, 2011; Weaver, 2014), but the male aggregation pheromones have identical components between *Diorhabda* spp., although the component ratios differ (Cossé et al., 2006a). The use of these pheromones in the field may lead to increased encounters between *Diorhabda* spp. and increase the potential for hybridization. It is not known what the long term effects of hybridization in the field would be.

The ability to predict where beetles will migrate, with or without pheromones, is not well developed and might be another limitation. For example, it is not known whether the use of male aggregation pheromones can successfully direct tamarisk beetles to specific locations. In one of the preliminary studies, it appeared that the attractants worked so well, they not only attracted beetle populations to the targeted treatment trees, but caused “spill-over” effects in that the beetles migrated to nearby controls without pheromone lures (Weaver, 2014).

An objective evaluation of this method cannot be completed without published data that addresses the following questions: From what distance will a beetle in the field be able to orient to the pheromone? Can aggregation pheromones hold beetles in an area long enough to prevent them from spreading to new areas? What are the immigration and emigration rates in a tamarisk stand treated with aggregation pheromones using SPLATT™ and in one that is not? How do landscape features, weather patterns, and tamarisk populations affect beetle movement naturally and with pheromone augmentation? Can the SPLATT™ formulation containing the pheromone be combined with a chemical insecticide and still be effective? Answering these and other questions would take time and resources. A significant amount of research would be needed to evaluate the feasibility of this type of control for *Diorhabda*.

7.6 Sterile Insect Technique

Sterile insect technique (SIT) involves the mass release of reproductively sterile male insects to mate with the wild population of females, preventing the production of offspring for the following generation (Lance and McInnis, 2005). In order to be successful, an SIT treatment requires the ability to rear, sterilize, and distribute sterile males in sufficient numbers and of sufficient competitive ability to outcompete wild male populations (Lance and McInnis, 2005). Mass rearing facilities are not in place for *Diorhabda* beetles and are often very costly to develop. Furthermore, because *Diorhabda* populations are well established in the United States and because these insects are capable of rapid population increase (Lewis et al., 2003; Carruthers et al., 2006) it is unlikely that sterile beetles would be able to outcompete wild populations. For example, the feasibility of SIT was tested on Japanese beetles in small, isolated populations, but it was not successful enough to warrant future investment as a management strategy despite the release of 235,000 sterile males weekly for several months (Potter and Held, 2002). SIT does not appear to be a commonly employed to control Chrysomelidae, and more research is needed to determine how feasible this approach would be for the management of tamarisk beetles.

7.7 Biological control

Entomopathogenic nematodes (EPNs) are generalist insect predators that have been used in biological control for decades (Lessord, 2016). One study by Foye et al. (2016) identified several EPNs as generalist predators infesting *Diorhabda*. *Heterorhabditis georgiana* is a generalist EPN which was shown in the lab to kill 80 percent of *D. carinulata*. *Heterorhabditis zealandica* was demonstrated to kill up to 100 percent of the beetles. Other EPNs were identified that had lower efficacies (Foye et al., 2016). All EPNs tested could also infect *Galleria mellonella* (Lepidoptera) larvae (Foye et al., 2016), indicating that the EPNs identified do not have a narrow enough host range for use against *Diorhabda* beetles. Riparian corridors where invasive tamarisks are infested with *Diorhabda* beetles are sensitive ecological habitats; introduction of a generalist predator would not be possible due to likely impacts to non-target species. No other entomopathogens (fungi, viruses), parasitoids, or predators that specifically attack *Diorhabda* beetles were found in the literature.

7.8 Evaluation of control options for *Diorhabda* in the United States

Aside from complete removal of tamarisk hosts, no control strategies currently available would be able to successfully eradicate *Diorhabda* from an area. Based on our spread model, it is unlikely that any known control methods will prevent its spread into new areas; however, thinning or removal of hosts may limit the distribution of the beetle in some areas. Also, recent research regarding beetle herding with dispersion pheromones has shown promise in delaying tamarisk dieback in areas with low *Diorhabda* density. Consequently, beetle herding may provide an additional method for slowing *Diorhabda* damage in the future. Some of the other strategies could be used together to slow the spread of *Diorhabda* beetles into new areas. Some control methods could be deployed to reduce *Diorhabda* populations in undesirable locations, which may affect its subsequent spread to new areas. Our model cannot predict how much the time before entry into new SWFL habitats would increase by slowing the spread of *Diorhabda* because the effectiveness of the treatments requires more research.

Based on the spread model presented in this document, all tamarisk within 25 km of SWFL critical habitats, excluding the protected area itself, could be removed to reduce the likelihood of natural spread of the beetles into these areas. Human-mediated dispersal of the beetle, however, could negate the effectiveness of these buffer zones. If the beetles reach SWFL critical habitat, the focus should be turned to reducing populations. Hosts plants could be removed or reduced in number (thinned) so that they could not support large beetle populations within critical habitats. This will likely be more effective at reducing *Diorhabda* populations in areas where saltcedar is not dominant.

Here is one example of a hypothetical control method involving thinning and chemical control: Tamarisk trees that are nesting sites for SWFL could be selected for protection, and an appropriate proportion of all surrounding tamarisk trees could be removed during the winter when SWFL are not present. As described, thinning has been shown to reduce population

abundance and may disrupt aggregation. Selective thinning could be combined with imidacloprid treatments of nesting trees to suppress beetle populations long enough for alternative nesting vegetation to replace the removed tamarisk. It is unknown what the impact of using imidacloprid would be on SWFL. Although declines in insectivorous bird populations have been associated with imidacloprid use, the cause has not been identified and neonicotinoids are considered safe for vertebrates such as birds and mammals (Hallmann et al., 2014). Nesting trees would be protected only until suitable native tree species have emerged and are used by the flycatcher for nesting. Then chemical controls could be stopped, and *Diorhabda* could resume defoliating the tamarisk, giving way to native reforestation.

Mating disruption and mass trapping or lure-and-kill trapping should only be considered if the beetle populations are small and isolated, but even in these scenarios they may not be effective. Pheromone based control methods have several limitations (Carde, 1990; Carde and Minks, 1995; El-Sayed et al., 2006). They should only be applied when specific conditions are met; otherwise they could induce beetles to immigrate into protected areas. Considerable research is necessary to determine the feasibility and effectiveness of any control method for *Diorhabda*.

8. Need for new technology and knowledge

- It is assumed that the male aggregation pheromones identified for *Diorhabda elongata* will attract all species of *Diorhabda*. This assumption, however, could be validated with further laboratory and field tests.
- Evidence indicates that *D. carinata*, *D. elongata*, and *D. sublineata* are reproductively compatible and that their crosses produce viable offspring. Furthermore, hybrids have been detected in the field. It is unknown how common or fit hybrids are in the field. Further research could be done to fully understand the ways in which hybridization will impact population dynamics in nature.
- Much of the population data available for *Diorhabda* spp. is unverified. The largest data set provided by RiversEdge West (formerly the Tamarisk Coalition) was compiled from numerous sources. Therefore, it is uncertain whether every reported data point is valid and whether the data points represent populations or single beetle captures. The overall completeness of the data set is also uncertain. Verified survey data could improve the climate suitability predictive models as well as the spread model.
- The distribution of tamarisk species throughout the United States is generally understood. To successfully employ any control strategy, however, a more detailed understanding of host availability is needed. Additional detailed surveys of tamarisk presence would improve response activities.
 - Work being conducted by the U.S. Geological Survey may be of use for conducting more detailed surveys of tamarisk habitat, flycatcher presence, and likely beetle

damage (Hatten et al., 2017). Specifically, they are developing a raster database using a remote sensing model to characterize riparian vegetation into suitable and unsuitable SWFL habitats based on a 40 percent probability threshold (Figure 16). They can also differentiate the riparian vegetation into probability classes for SWFL habitat suitability. SWFL habitat varies over time depending on factors such as drought, so the researchers are creating an archive of SWFL habitat rasters from 1986 to the present. They also plan to develop a web-based mapping application that would allow users to download model outputs in raster format.

The final objective of their research is to develop an SWFL occupancy model that incorporates the likelihood of SWFL being in a given habitat with SWFL habitat suitability. This will allow them to conduct a variety of analyses to inform surveys and response activities. Examples include 1) estimating SWFL colonization and extinction rates, 2) modeling SWFL and *Diorhabda* dynamics, and 3) simulating SWFL abundance and distribution under different management scenarios and ecological events such as drought.

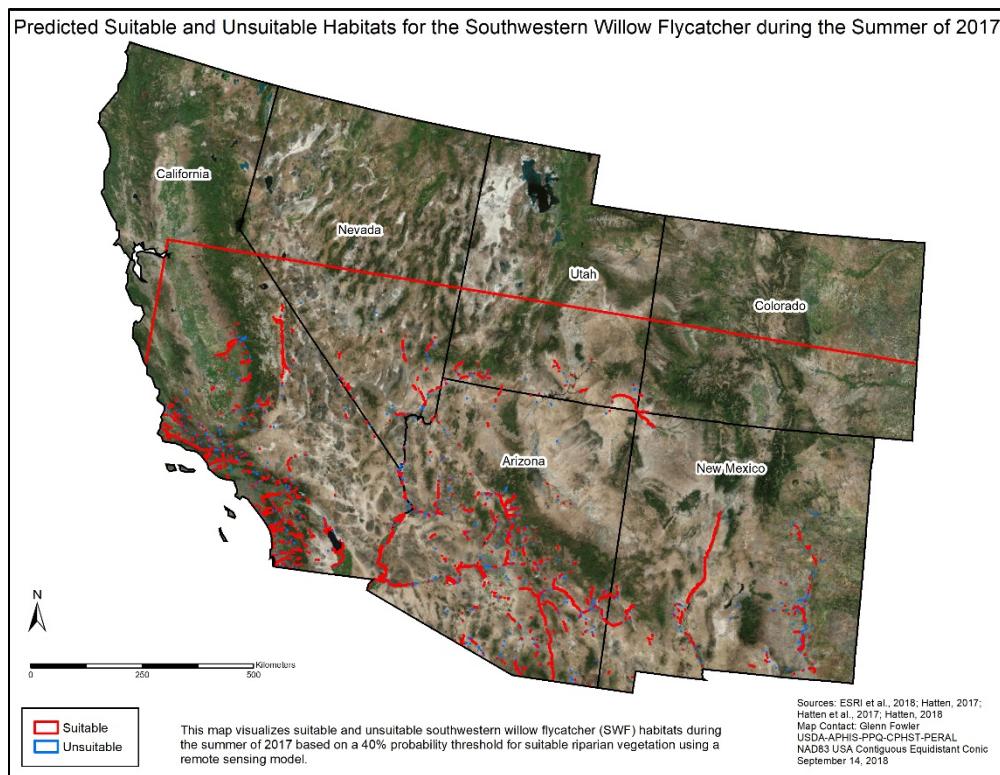


Figure 16. Predicted suitable and unsuitable SWFL habitats during the summer of 2017.

- Research is needed on how quickly SWFL habitats affected by *Diorhabda* spp. can be restored and on what factors, such as tendency of an area to flood, would affect the

possibility and speed of SWFL habitat restoration. The model being developed by Hatten et al. (2017) may help answer these questions.

- Robust data on the dispersal potential and spread rate of *Diorhabda* spp. is lacking. Studies focusing on the natural spread rate of *Diorhabda* spp. could provide more accurate data to improve our model and subsequent predictions on the spread of tamarisk beetles into SWFL habitat. Recent research has shown that yellow hot melt sticky board traps baited with 3Z-hexenol could detect low populations of *Diorhabda* spp. in the field (Cossé and Francese, No Date). This lure could be used to monitor *Diorhabda* spp. movement and characterize the natural spread rate of the beetle.
- If chemical control is employed to protect target trees or to reduce *Diorhabda* spp., further research should be undertaken to fully understand the potential environmental impacts.
- In the literature, imidacloprid is the only chemical that has been reported to be used against *Diorhabda*. Other chemical control formulas should be researched in order to understand the full suite of chemical control options available for *Diorhabda* spp.
- High populations and dual mate recruitment of *Diorhabda* spp. are the primary reasons why mating disruption may not be an effective control option for this beetle. This technique has never been tested for *Diorhabda* spp., and further research is needed to fully evaluate its effectiveness.
- More research is needed to test the efficacy of a mass trap or attract-and-kill method for reducing tamarisk beetle populations. It appears that the large populations of *Diorhabda* spp. would make this method ineffective; however, further research and field testing specific to *Diorhabda* spp. is needed to determine the efficacy of this method.
- The potential control strategy of beetle herding has several critical knowledge gaps that would need to be thoroughly researched before the effectiveness of this method can be determined.
 - From what distance will a beetle in the field be able to orient to the pheromone?
 - Can aggregation pheromones hold beetles in an area long enough to prevent them from spreading to new areas?
 - How do the immigration and emigration rates in a tamarisk stand treated with aggregation pheromones using SPLAT™ compare to those of an untreated stand?
 - Can aggregation and dispersal pheromones be used to herd *Diorhabda* spp. for desired purposes?
 - How do landscape features, weather patterns, and tamarisk populations affect beetle movement naturally and with pheromone augmentation?
 - Will the use of these pheromones in the field lead to increased encounters between *Diorhabda* spp. and increase the potential for hybridization?
- Sterile Insect Technique (SIT) appears unlikely to be effective for *Diorhabda* spp. control, but further research and tests are needed to determine its feasibility for this system.

- A further cost-benefit analysis on any management approach should be conducted before any management strategies are employed.

9. Conclusion

In this analysis we described tamarisk leaf beetles, *Diorhabda* spp., predicted the potential for them to establish in SWFL critical habitats, characterized the spread rate into this habitat, evaluated potential control options, and identified pertinent research and knowledge gaps.

Four *Diorhabda* spp., *D. elongata*, *D. carinata*, *D. sublineata*, and *D. carinulata*, have been introduced into the United States since 2001. These beetles can aggregate in large numbers and infest tamarisk trees, leading to complete defoliation. Multiple defoliations over time ultimately lead to tree death. In this analysis, we predicted the climatically suitable areas for establishment of all four *Diorhabda* spp. and confirmed that SWFL critical habitat is climatically suitable for *Diorhabda*.

Diorhabda are continuing to spread in the United States naturally and through human mediation. We used predictive mapping to model the spread of *Diorhabda* spp. along riparian corridors into U.S. SWFL critical habitats and to determine the expected number of years until *Diorhabda* entry.

The expected number of years until entry varied from 0 for habitats already colonized to 20 for the SWFL critical habitats farthest away from *Diorhabda* spp. populations. The mean years until entry into SWFL critical habitats by state were: 6.5 years for Arizona, 12.6 years for California, 4.0 years for Colorado, 2.3 years for New Mexico, and 10.7 years for Nevada. The mean time until entry was significantly higher for California than for Arizona, Colorado, and New Mexico and was significantly higher for Nevada than for New Mexico.

Finally, we evaluated several potential control options for *Diorhabda* spp. to prevent or slow the spread of the beetles into SWFL critical habitat. Due to the wide distribution of *Diorhabda* spp. and their ability to spread, it is unlikely that the beetles can be prevented from entering SWFL critical habitat. Of the options evaluated, host removal (thinning) and chemical control have been demonstrated to be effective in similar insect species. Although considerable research is needed, it appears as though a push-pull method involving repellants and pheromones could be used in areas with low *Diorhabda* density to delay dieback of tamarisk trees in SWFL critical habitats. *Diorhabda* spp. are not currently controlled anywhere in the world, so data on the efficacy of several treatment options is severely lacking.

The results of our analysis can be used to inform operational and policy decisions regarding the spread of *Diorhabda* spp. into SWFL critical habitats that are within authority of the agency to limit *Diorhabda* impacts to the SWFL and promote the recovery of the species.

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11. Appendices

Appendix 1. Description of Maxent Modeling.

List of 21 environmental variables considered in the Maxent Model:

Bio1 = Annual Mean Temperature (°C)

Bio2 = Mean Diurnal Range [Mean of monthly (max temp - min temp)]

Bio3 = Isothermality [(Bio2/Bio7) * 100]

Bio4 = Temperature Seasonality (standard deviation * 100)

Bio5 = Max Temperature of Warmest Month (°C)

Bio6 = Min Temperature of Coldest Month (°C)

Bio7 = Temperature Annual Range (Bio5 - Bio6) (°C)

Bio8 = Mean Temperature of Wettest Quarter (°C)

Bio9 = Mean Temperature of Driest Quarter (°C)

Bio10 = Mean Temperature of Warmest Quarter (°C)

Bio11 = Mean Temperature of Coldest Quarter (°C)

Bio12 = Annual Precipitation (mm)

Bio13 = Precipitation of Wettest Month (mm)

Bio14 = Precipitation of Driest Month (mm)

Bio15 = Precipitation Seasonality (Coefficient of Variation)

Bio16 = Precipitation of Wettest Quarter (mm)

Bio17 = Precipitation of Driest Quarter (mm)

Bio18 = Precipitation of Warmest Quarter (mm)

Bio19 = Precipitation of Coldest Quarter (mm)

Elev = Elevation (m)

WaterDist = Distance from streams and water bodies (m)

Maxent does not use environmental variables directly. Instead, it transforms them into a set of feature classes. It uses a regularization multiplier (RM) to reduce the number of parameters and thus automatically controls the model complexity (Phillips and Dudik, 2008; Elith et al., 2011). The default RM value is 1; a smaller value of RM may potentially overfit the model and produce more restricted distributions, whereas a higher value results in simpler models with less discriminating power and broader potential species distributions (Kumar et al., 2016). A number of models with different RM values and feature types were fitted for all four tamarisk beetles, and the model with the optimal level of complexity was selected. Performance of the Maxent model was evaluated using a Maxent-generated area under the receiver operating characteristic (ROC) curve (AUC) (Phillips et al., 2006). The AUC values vary from 0 to 1: 0.5 shows that the model performance is not better than random, values less than 0.5 are worse than random; values from 0.5 to 0.7 indicate poor performance; 0.7 to 0.9, moderate performance; and 0.9, high performance (Peterson et al., 2011). The 10-fold cross-validation procedure in Maxent was used for model validation, and averaged test AUC values across the 10 replicates were reported.

The best Maxent models for all four tamarisk beetles had high performance with test AUC values between 0.93 and 0.98 (Table 7). The number of variables in the best models varied from 7 to 11. Distance from streams and water bodies was one of the top three predictors in all four models. The predicted environmental suitability for all four beetles was highest close to the streams and water bodies.

Table 7: Model performance and top environmental predictors in the best Maxent models of four tamarisk beetles (TB).

Species	RM ⁴	Test AUC ⁵ (\pm SD ⁶)	No. of variables	Top predictor variables (% contribution to Maxent model)
<i>D. carinulata</i>	1.5	0.93 (\pm 0.006)	11	- Mean annual precipitation (29%) - Distance from streams/water (23%) - Minimum temp. of coldest month (16%)
<i>D. sublineata</i>	1.0	0.98 (\pm 0.005)	11	- Distance from streams/water (27%) - Mean temp. of wettest quarter (19%) - Mean temp. of coldest quarter (16%)
<i>D. elongata</i>	1.0	0.98 (\pm 0.006)	7	- Mean temp. of coldest quarter (43%) - Mean temp. of driest quarter (20%) - Distance from streams/water (17%)
<i>D. carinata</i>	1.0	0.98 (\pm 0.008)	10	- Distance from streams/water (29%) - Precipitation of warmest quarter (18%) - Mean temp. of driest quarter (18%)

Predicted environmental suitability for all four beetle species conformed well to their current known distribution in the study area (Figures 8 & 9). Maxent-predicted suitability was categorized into four classes: High (covered 60 percent of the species occurrences), medium (20 percent of occurrences), low (18 percent of occurrences), and unsuitable (covered approximately 2 percent of the species occurrences). The best Maxent model for *D. carinulata* predicted highly suitable areas primarily in the central part of the study area (Figure 4). The model predicted suitable areas for *D. elongata* in western Texas along the Rio Grande and Pecos Rivers and in parts of the central valley of California (Figure 5). The best model for *D. carinata* predicted suitable areas in northern Texas; western Oklahoma; and parts of Colorado, Kansas, and eastern New Mexico (Figure 6). For *D. sublineata*, highly suitable areas were predicted in western Texas, southern and southeastern New Mexico, and parts of Idaho and Arizona (Figure 7). The models also predicted suitability in areas where none of the beetles currently occur, such as Idaho, central Washington, southwestern Montana, northeastern Colorado, southeastern Wyoming, and southwestern Arizona (Figure 8). The environmental habitat suitability for the four beetles in and around SWFL critical habitat is shown in Figure 9. Our results indicate that many of the SWFL critical habitats in California are unsuitable for *Diorhabda* spp.

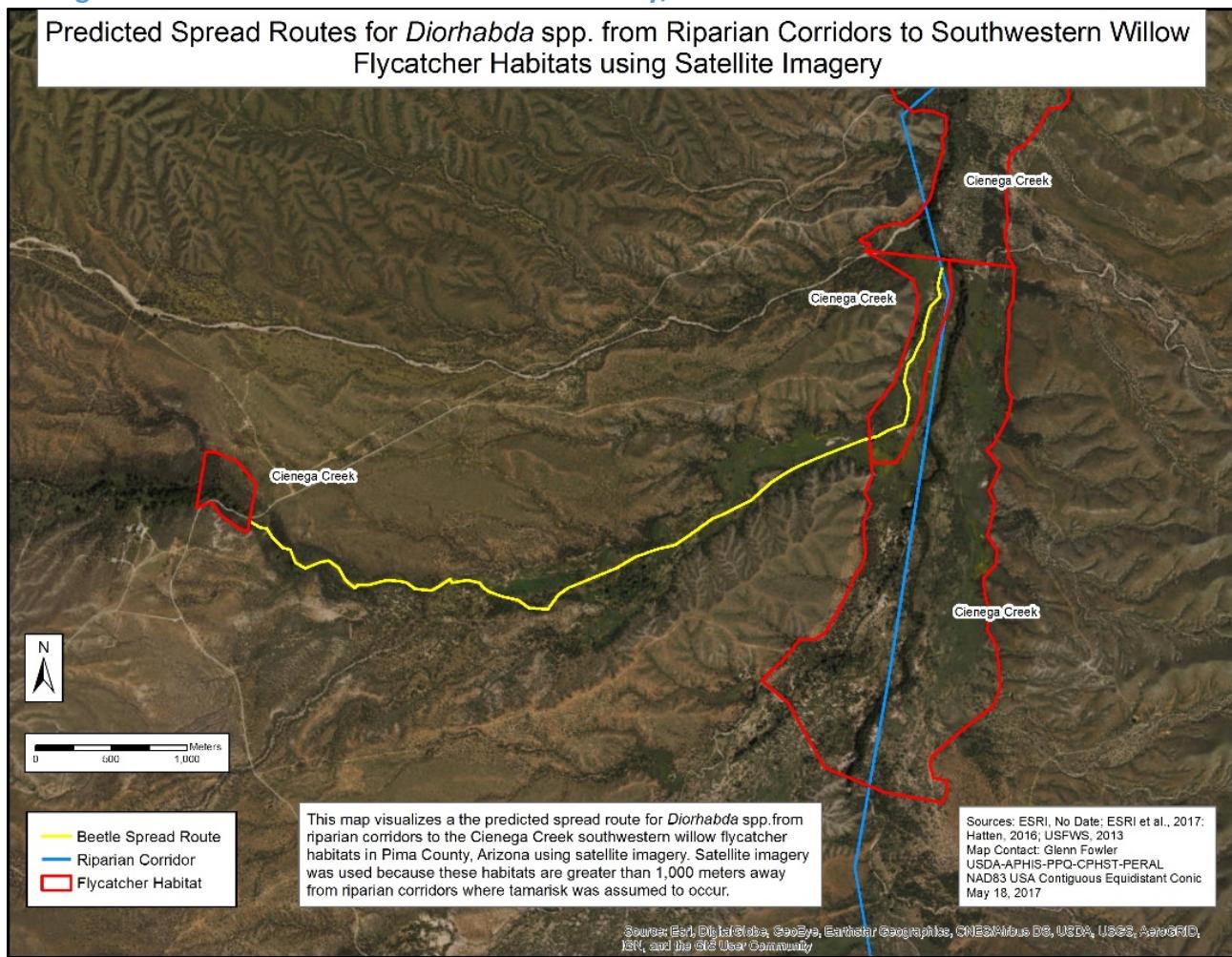
⁴ RM = “regularization multiplier” in Maxent

⁵ “AUC” is the area under the receiver operating characteristic curves (a measure of goodness-of-fit), features- L-linear, Q-quadratic, P-product, and H-hinge

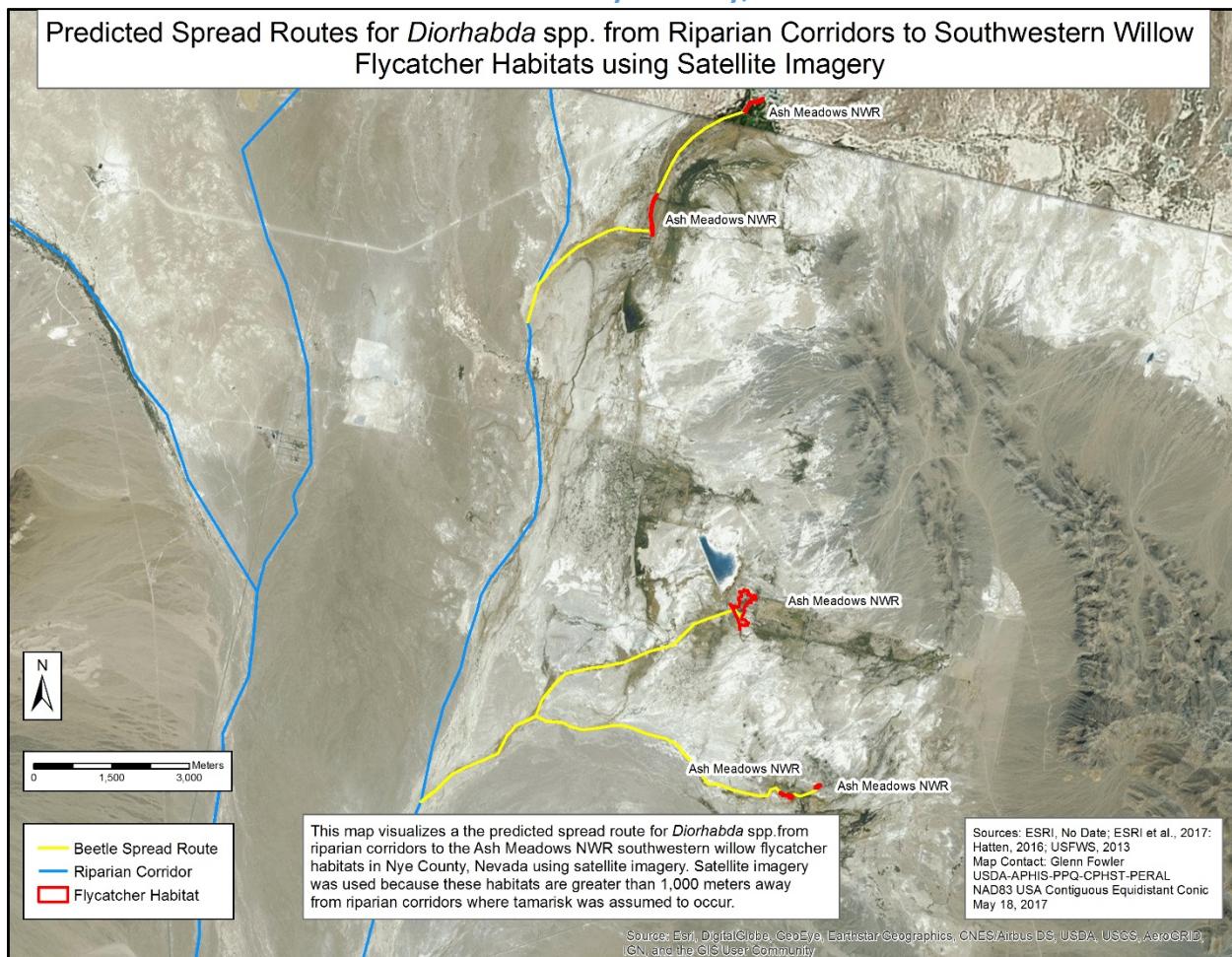
⁶ SD = standard deviation

Results from ecological niche models or species distribution models may be affected by a number of decisions made during model calibration, such as selection of predictor variables, multicollinearity among predictor variables, spatial accuracy of species occurrences, and spatial autocorrelation in occurrence data (Jarnevich et al., 2015). We were aware of these uncertainties and performed additional analyses such as spatial filtering and 10-fold cross-validation (see Methods) to mitigate any problems due to these issues.

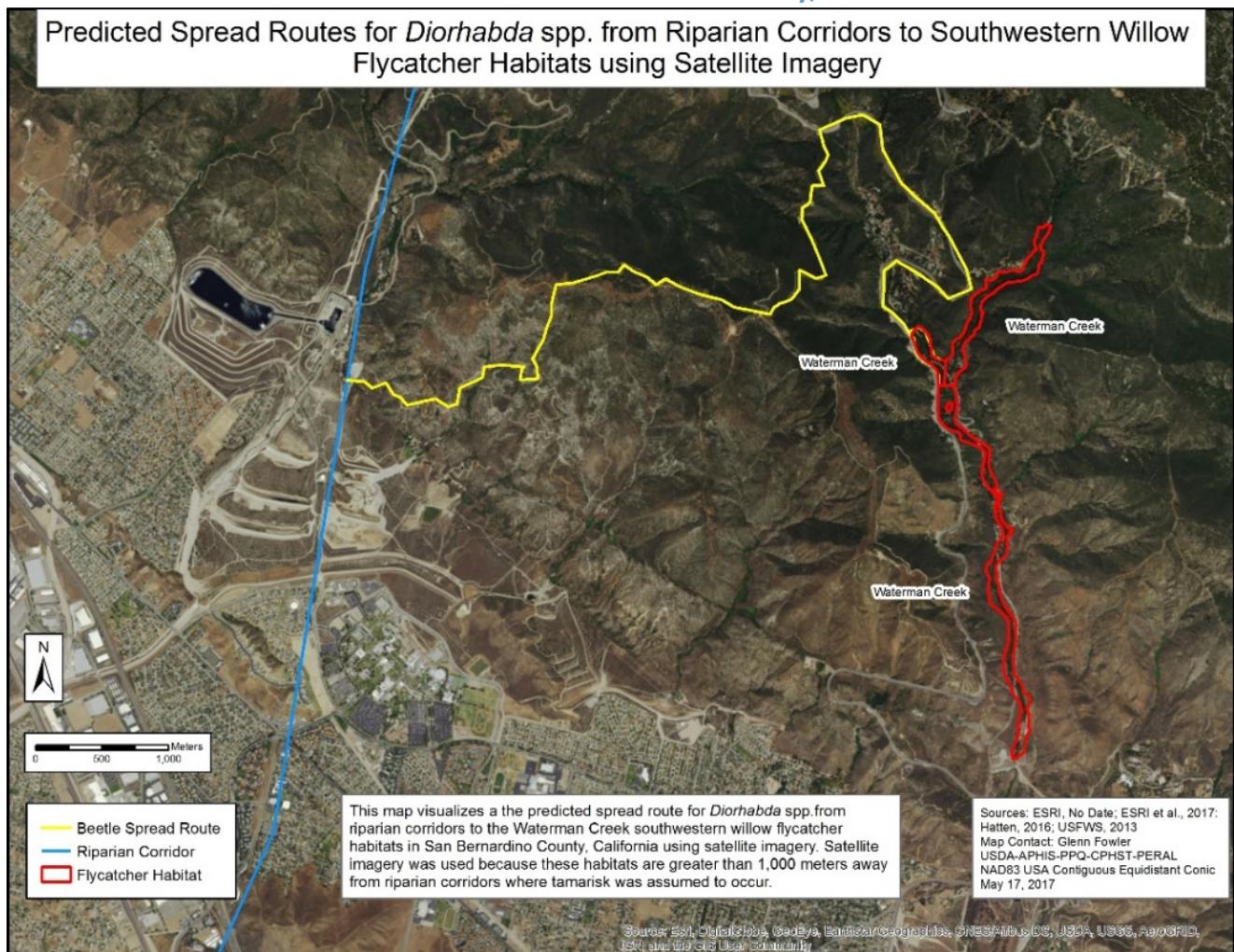
Appendix 2. Use of satellite imagery to model likely spread routes of *Diorhabda* spp. into the Cienega Creek SWFL critical habitats in Pima County, AZ.



Appendix 3. Use of satellite imagery to model likely spread routes of *Diorhabda* spp. into the Ash Meadows NWR SWFL critical habitats in Nye County, NV.

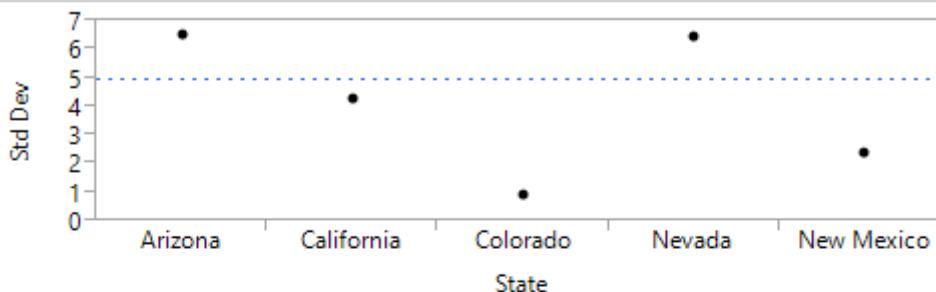


Appendix 4. Use of satellite imagery to model likely spread routes of *Diorhabda* spp. into the Waterman Creek SWFL critical habitats in San Bernardino County, CA.



Appendix 5. Tests for equal variances and Welch Analysis of Variance (ANOVA) results for years until *Diorhabda* spp. entry into SWFL critical habitat by state.

Tests that the Variances are Equal



Level	Count	Std Dev	MeanAbsDif	MeanAbsDif
			to Mean	to Median
Arizona	39	6.478880	5.429704	5.250000
California	60	4.246840	3.538333	3.435667
Colorado	7	0.929767	0.617143	0.547143
Nevada	7	6.429919	5.334694	3.801429
New Mexico	14	2.358484	2.094286	1.937143

Test	F Ratio	DFNum	DDF Den	Prob > F
O'Brien[.5]	6.8254	4	122	0.0001
Brown-Forsythe	5.1574	4	122	0.0007
Levene	8.7209	4	122	0.0000
Bartlett	8.1091	4	.	0.0000

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DDF Den	Prob > F
51.1457	4	28.048	<.0001*

Appendix 6. Tukey-Kramer HSD ordered differences report for pairwise comparisons between states for the mean number of years until *Diorhabda* spp. entry into SWFL critical habitats.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
California	New Mexico	10.49000	1.468082	6.42488	14.55512	<.0001*
California	Colorado	8.61000	1.975544	3.13972	14.08028	0.0003*
Nevada	New Mexico	8.59571	2.289658	2.25565	14.93578	0.0024*
Nevada	Colorado	6.71571	2.643869	-0.60516	14.03659	0.0886
California	Arizona	6.08795	1.017381	3.27082	8.90508	<.0001*
Arizona	New Mexico	4.40205	1.541046	0.13490	8.66921	0.0396*
Nevada	Arizona	4.19366	2.030353	-1.42838	9.81571	0.2419
Arizona	Colorado	2.52205	2.030353	-3.10000	8.14410	0.7267
California	Nevada	1.89429	1.975544	-3.57600	7.36457	0.8729
Colorado	New Mexico	1.88000	2.289658	-4.46006	8.22006	0.9237