# The Effects of Highways and Highway Construction Activities on Valley Elderberry Longhorn Beetle Habitat

Final Report FHWA/CA09-0925 Submitted to the California Department of Transportation Contract Number 65A0222



Prepared by: Theresa S. Talley and Marcel Holyoak Department of Environmental Science & Policy University of California Davis, CA 95616

Prepared for: State of California Dept of Transportation Division of Research and Innovation Office of Materials and Infrastructure Research 1101 R Street Sacramento, CA 95811

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16. ABSTRACT

By the time most species are classified as "of concern", they are too rare to be adequately studied. Recovery Plans, which follow listing on the United States Endangered Species List, include threats to the species derived from best guesses based on minimal existing data. This is the case with the Valley elderberry longhorn beetle (*Desmocerus californicus dimorphus* Fisher, Cerambycidae). Endemic to the highly urbanized and cultivated Central Valley of California, many necessary management activities and infrastructural elements are considered threats to this beetle. Until now, the effects of these factors on the presence and abundance of the beetle have not been tested. We tested the effects of proximity to highways and highway construction on the beetle and its host plant, blue elderberry (*Sambucus mexicana*). Additional studies were conducted to test the effects of trimming elderberry on the beetle. While both types of activities influenced elderberry characteristics and beetle presence or abundances in various ways, it is clear that efforts to recover the beetle and these necessary elements of society may be compatible.

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# DISCLAIMER

The contents of this report reflect the views of the authors who are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views or policies of the State of California or the Federal Highway Administration. This report does not constitute a standard, specification or regulation.

# EFFECTS OF PROXIMITY TO HIGHWAYS AND HIGHWAY CONSTRUCTION ON THE THREATENED VALLEY ELDERBERRY LONGHORN BEETLE

# ABSTRACT

Roads not only divide remnant habitats, they also contribute particulates, mineral nutrients, and noise from traffic and construction activities. Particularly susceptible to these effects of roads are species like the threatened Valley elderberry longhorn beetle (Desmocerus californicus dimorphus) whose habitat has been bisected by roads, and whose relatively short movements may limit its use of this network of isolated habitat fragments. Until now, the effects of proximity to highway and highway construction on the beetle, a subspecies endemic to California's Central Valley, and its host shrub, elderberry (Sambucus mexicana), had not been tested. Field studies revealed that dust accumulated faster with greater distances from highways. Deposits within 200 m of roads, however, had increasing concentrations of elements commonly found in vehicle exhaust as evidenced by increases in concentrations of foliar sulfate and manganese. Shrubs near roads also tended to be more stressed (higher water potentials) and lower nutritive quality (higher C:N) than those farther away. Both site and shrub occupancy rates of the beetle were comparable to those found in non-highway sites across the northern Central Valley. Furthermore, beetle emergence occurred following construction activities in two of three construction sites despite simultaneous increases in foliar sulfate and shrub stress. This illustrates that the beetle's life cycle could be completed in the presence of nearby construction. While the availability of sites and occupancy of the beetle were low, power analysis revealed that at least large effects could be detected by these surveys of elderberry. Studies of rare species in urban regions are often hampered by difficulties in obtaining data but are especially important for optimizing remaining impacted habitats. Consideration of using roadside areas as habitat connecting existing populations is discussed along with suggested management actions based on this information.

**Keywords**. Blue elderberry, Central Valley of California, dust, exhaust, highway construction, endangered species, noise, roads, Valley elderberry longhorn beetle, vibration

# **INTRODUCTION**

#### Background

By the time most species are classified as endangered or "of concern", they are too rare to be adequately studied. Recovery Plans, which follow listing on the United States Endangered Species List, include threats to the species derived from best guesses based on minimal existing data, and information from closely related species. This is the case with the Valley elderberry longhorn beetle (*Desmocerus californicus dimorphus* Fisher, Cerambycidae; hereafter "the beetle"). This species was listed as Federally threatened in 1980, primarily due to a loss of riparian habitat. Threats to its survival and recovery include management activities that potentially harm the beetle indirectly, through effects on its host shrub, blue elderberry (*Sambucus mexicana*), or directly, through death or disturbance of feeding or reproductive activities. Until recently (Talley et al. 2007), there has been little or no scientific evidence of the effects of these activities specifically on the beetle or its host shrub. Of particular concern in the urbanized and cultivated Central Valley are the effects of roads on the beetle (USFWS 1984).

Roads can fragment and isolate natural areas (Bhattacharya et al. 2003) limiting the movement in and out of these isolated habitat fragments by the beetle, and other species with short-distance movements. Further, highways and highway-associated activities, such as road construction and vehicular traffic, can affect soils, plants and animals in a number of ways. The effects of road materials and exhaust may include increases in moisture from exhaust and runoff (Forman et al. 2003), particulates (Farmer 1993, Boudet et al. 2000), heavy metals (Udevitz et al. 1980, Naqvi and Khattak 1996, Pagotto et al. 2001, Bocca et al. 2003) and mineral nutrients (e.g., nitrogen, phosphorus, sulfur; Angold 1997, Bell and Ashenden 1997). The chemical inputs of high traffic roads include metals such as lead, copper, cadmium and zinc (Fakayode and Olu-Owolabi 2003, BEST 2005), which are often found associated with roadside plant leaves, roadside soil and on the surface of highways (e.g., Naqvi and Khattak 1996, Pagotto et al. 2001). Roads may also lead to increases in light, noise or vibrations (Forman and Alexander 1998, Longcore and Rich 2004).

These various road effects can influence the quality and quantity of elderberry (e.g., Fisher 1993, Sharifi et al. 1999), which would in turn affect the presence and abundance of the beetle (e.g., Haack and Slansky 1987, Koch-Munz and Holyoak 2008). These road effects may also have direct influences on the beetle through smothering or interference of behaviors, such as mate detection (e.g., Goulson et al. 1994, Boiteau and Misener 1996), feeding, mating, and ovipositing (e.g., Boiteau and Misener 1996, Rothschild and Bergstrom 1997).

Talley et al. (2006) revealed that non-toxic dust from low-traffic access roads slightly affected elderberry condition but not beetle occupancy over river-wide scales (20-30 km). Localized ( $\leq 100$  m) variations in dust near unsurfaced compared to surfaced roads, however, had negligible effects on elderberry density and condition, or on beetle presence (Talley et al. 2006). Still uncertain, however, have been the effects of high traffic roadways where higher dust levels and pollutant inputs from exhaust may influence roadside elderberry and beetle populations, as with other plant and animal communities (e.g., Przybylski 1979, Port and Thompson 1980, Wong et al. 1984, Angold 1997). While the rarity of the beetle makes it unreasonable to directly test the effects of these factors on the beetle, responses can be inferred from incidence of exit holes, as well as the abundance and quality of elderberry host shrubs (Barr 1991, USFWS 1980, 1984).

# **Objectives**

We used field surveys in existing highway and construction sites to answer the following question: How does proximity to (a) highways and (b) highway construction activities affect beetle occupancy and characteristics of its elderberry host shrubs? We emphasize the effects of particulates, pollutants (e.g., mineral nutrients and heavy metals) and noise. Study sites consisted of highway sites, or those containing elderberry shrubs located along busy highways, and construction sites, or those where construction took place previous to the start of this project (old construction) or in the year following the start of this project (new construction) in order to get a range of post construction ages. Construction projects included lane-widening and straightening efforts, which entailed establishing staging areas, scraping or planing asphalt surfaces, grading roadside soils and re-surfacing the new roads. As per the Conservation Guidelines for the beetle, shrubs located within 100 ft (30 m) of a project site were removed

and transplanted elsewhere (USFWS 1999); such shrubs have been considered elsewhere (Koch-Munz and Holyoak 2008) and are not investigated here. Furthermore, the vegetated area and/or rights of way of most highways are relatively narrow (often <100 m). These factors limited both the number of study sites, because all shrubs were often removed from project sites, and the number of elderberry shrubs at any one site. Nonetheless, several significant trends emerged, informing management decisions and indicating future avenues of investigation.

# **MATERIALS AND METHODS**

#### **Study sites**

Study sites were located along highways throughout the northern Central Valley (Figure 1, Table 1). If construction had not yet begun or if there were no effects of proximity to the old construction area on elderberry variables, then sites were also used as highway sites (Table 1). Criteria for inclusion of sites in the study included the presence of naturally occurring (non-planted) shrubs, the absence of other major sources of dust nearby (e.g., adjacent agricultural fields), absence of other obvious sources of toxins (e.g., pesticide or herbicide drift) and no irrigation. In cases where we could not find shrubs >50 m from a source (HWY-1, HWY-6), the nearest non-highway control sites, which were 1-2 km away, were used.

### Field surveys of highway and construction sites

Field surveys of highway sites, old construction sites and initial sampling of new construction sites were conducted between May and August 2006, with additional surveys of highway sites during July 2008. Post construction sampling of new construction sites took place in June and July 2007, 2-3 months following the completion of construction. To ensure measurable levels of dust had accumulated, we waited at least 2 weeks after rain events before sampling. At every shrub within each site we recorded the following: geographic location, shrub size (number and basal diameter of main stems, height), shrub fecundity (number and width of inflorescences), shrub condition (% dead stems, bark damage, past burning), shrub water potential, evidence of the beetle (number and age of exit holes), associated vegetation (cover of ground, shrub and canopy species), and presence of lichens. A Trimble Geo XM handheld GPS unit was used to record shrub locations. Counts of recent (new and 1 yr old exit holes) and old holes ( $\geq$ 1 yr old) were made. Mid-day (between 10 am- 2pm) shrub water potentials were measured using a portable nitrogen gas pressure bomb (PMS Pressure Chamber Model 600). Three branches per shrub were shaded for at least 15 min using a foil-covered Ziploc bag and then cut immediately before placement in the pressure bomb.

Background sediment deposition rates were measured using aluminum cake pans (33 cm diameter, 2.5 cm depth) that were set on the ground, in the open by each shrub and left for at least 3-4 weeks. Pans were set at varying distances from the dust sources if shrubs did not provide enough of a range of distances. Additionally, 100 leaflets were collected from each shrub to be used for further analyses (see "Laboratory analyses").

Noise levels at sites were measured by CalTrans during May 2007 using a Bruel and Kjaer portable sound level meter, type 2230. For all measurements, the meter was placed about 34 cm off the ground (on the carrying case) within 2 m of each highway. Measurements were

made after 15 and 30 min (only 30 min readings are presented since values overlapped). A windscreen was used during all measurements. Sound measurements are Leq, the equivalent sound pressure level that is the non-fluctuating sound level over a specific time period that has equal energy to the fluctuating sound level that occurs. Because noise levels are similar within sites, statistical analyses could not be performed on sound level measures, nonetheless the raw data provide useful information.

# Laboratory analyses of highway and construction samples

Leaves were analyzed for (a) leaf surface dust levels, (b) leaf area, (c) carbon to nitrogen ratios and (d) exhaust-element (sulfate and metal) concentrations. Leaves were refrigerated for less than 48 hrs after collection and rinsed of dust with distilled water. The dust solution was preserved in 70% ethanol until filtration and calculation of leaf dust levels. Leaf area was immediately calculated using a subset of 15-20 leaves, which were blotted dry with paper towels and imaged using a desktop scanner with resolution of 4600 x 9600 dpi. Area of leaf images was calculated using Image J software (NIH 2007) and average leaf area was multiplied by the number of leaves in the sample (100) for an estimate of total leaf area of the sample.

The fresh leaves were then dried in a 60°C oven, weighed, ground to a fine powder using coffee grinders and analyzed at the ANR Analytical Lab (2007) for C to N ratios and elements common in vehicular exhaust, including sulfate-sulfur, zinc, manganese, cadmium, cobalt, lead and nickel. Concentrations of cadmium, cobalt, nickel and lead were beneath detection limits for all samples so are not discussed further.

The preserved leaf dust samples were sequentially filtered through pre-weighed filter papers of 2 mesh sizes- an initial filtration through a Whatman no. 1 filter to capture larger particles (>11  $\mu$ m; e.g., sediments) and a subsequent filtration of the supernatant through a Whatman no. 5 filter to capture small particles (>2.5  $\mu$ m; e.g., clay, exhaust particles). Dust particles and filters were dried at 60° C and then weighed on an analytical balance and standardized using leaf area and dry weight of the samples to determine dust particle dry weight per leaf area, or per leaf biomass, per unit time.

The aluminum pans used to measure ambient dust levels were collected and placed in airtight plastic bags until their contents could be rinsed with distilled water and filtered in a similar manner as the leaf dust samples. Filter papers and particles were dried at  $60^{\circ}$  C and weighed to determine dust accumulation per area per unit time. Most of the sediment pans that were deployed were not retrieved; loss occurred because of roadside trash pick up efforts, destruction by vehicles or brush fires on roadsides, or unknown causes. Of the roughly 130 pans set out, 39 were recovered.

#### Statistical analyses

Relationships between the distance from source, either construction site or highway edge, and both dust accumulation rates and elderberry characteristics were tested within dates with nested ANOVAs (distance nested by site). Two analyses testing effects of distance from highways were run for each variable- one using data from all distances (0-2 km) and the second using shorter distances (0-200 m) from highways. *A-posteriori* multiple comparisons were made using Student's t-test. Site differences were greater than variation due to age since construction, so analyses of new construction sites (before and after comparisons) and of old

constructions sites were kept separate. The effects of new construction activities on dust accumulation rates and elderberry variables were tested with paired t-tests nested by site. Sequential Bonferroni-adjusted alpha values were used for all of the t-tests to give an overall alpha of 0.05, thereby controlling for Type I error associated with multiple comparisons (Sokal and Rohlf 1995).

The effects of roadside environmental variables (corridor width, habitat type, elderberry size and composition, associated plant cover, and dust accumulations) on beetle presence and abundance were tested using forward stepwise logistic and linear regressions respectively. Variables were included in the model if  $r^2 \ge 0.03$  and p was less than or equal to a sequential Bonferroni adjusted alpha (with overall alpha of 0.05).

Linear relationships were tested prior to analyses with simple regressions and  $log_{10}$  transformations were used if necessary. All statistical tests were performed using JMP Statistical software. To combine data across sites, the effects of sampling date were removed from relevant variables (e.g., shrub water potential) or variables were standardized for date, or time since rain when dust would have been rinsed from leaves (e.g., amount of dust per leaf area per day that dust accumulation could occur).

Power analysis was used to test the statistical power of each comparison to reveal the probability that non-significant results were real and not due to a lack of sufficient replication (we used G-Power 2.1.2; Erdfelder et al. 1996). The effect size, or the magnitude of the differences detected by the power analysis, was defined as "large", "medium" or "small" (*sensu* Cohen 1992). This is a standard convention for describing the degree to which the populations or distributions being compared differ from each other (e.g., the amount of difference between H<sub>0</sub> and H<sub>1</sub> distributions or between  $\mu_1$  and  $\mu_2$ ; Erdfelder et al. (1996)). Cohen (1992) describes a "medium" effect size as "an average size of observed effects in various fields." In reference to this study, that medium effect size represents an effect likely to be obvious the careful observer. For example, a medium effect size of differences in maximum trunk diameter would be visible and obvious to the careful observer. Small effect sizes would be noticeably smaller than medium but not so small as to be trivial (i.e., would still have biological significance), and large effect sizes to be the same amount above medium as small was below it. All analyses in this study were capable of detecting large effects unless noted otherwise.

# RESULTS

# **Ambient dust levels**

Deposition rates of background dust did not differ with distance from highway (HWY), old construction (OC) or new construction (NC) sources. Nor were there differences in background dust levels among sites (Table 2) (Two way analyses- HWY: P=0.23,  $F_{15,23}$ =1.39; OC: P=0.63,  $F_{3,1}$ =0.91; NC: P=0.32,  $F_{7,11}$ =1.35). The lack of significance may be due in part, however, to the low number of replicates (this was confirmed with a power analysis which indicated that at least 80-90 samples would be needed to detect medium sized effects).

# **Proximity to highways**

# *Elderberry and associated plants*

Elderberry stem densities ranged from <0.1 to 1.0 stem per 625 m<sup>2</sup> in non-riparian sites adjacent to highways and 6-8 stems per 625 m<sup>2</sup> in riparian sites and/or control sites located 1.5-2 km from highways (Table 3). The effects of proximity to highways on elderberry and environmental variables often varied with spatial scale. Average leaf biomass (i.e., thickness) decreased over 2 km scales but increased within the first 200 m of highway edges (Figure 2A, Table 4). Over 2 km scales, there were decreases in leaf manganese concentrations, shrub water potential (i.e., shrub stress) and percent ground cover, and increases in leaf dust accumulation rates and percent canopy cover, while none of these varied within 200 m of highway edges (Figures 2A-C, Table 4). Conversely, leaf sulfate concentrations increased immediately adjacent to highways but did not vary over larger scales (Figure 2B). For several variables, however, the effects of proximity to highways were similar with spatial scale. Abundances of elderberry inflorescences, leaf quality (higher proportions of nitrogen) and shrub cover increased over both 200 m and 2 km distances from highways (Figures 2A-C, Table 4).

Power analysis revealed that was generally enough statistical power (e.g., number of replicates) to detect medium to large differences among sites and distances, so that a lack of significance was not necessarily due to small number of sites.

#### Noise levels

Noise levels decreased with distance of the site from the highway ( $R^2=0.34$ , P=0.01,  $F_{1,16}=8.2$ ). Reductions of about 11 db occurred at ~100 m from SR 113 and 20 db at 500-1000 m from I-80 in the Sacramento metropolitan area (Table 2). Noise levels were, however, similar in sites located immediately adjacent to highways (Leq=65-72 db), despite the difference in size and traffic volume of the highways (Table 2). The comparable noise level along the smallest highway, the 2 lane SR-29, may have been due to motorcycle traffic, which was responsible for a maximum reading of 99 db. Truck traffic accounted for the loudest noise along the other highways. It is likely that high noise levels along SR-29 are more sporadic than along the other highways.

#### *Beetle presence and abundance*

About 40% of highway sites contained at least one new exit hole and 70% had a hole of any age. Within occupied sites, 1 to 50% of shrubs contained recent exit holes, although the high proportions resulted from two sites (HWY 4 and 8) that contained only 4-5 shrubs. Excluding these sites, occupancy rates ranged from 1-17%.

Habitat type and shrub availability primarily influenced the likelihood of recent and total (recent + old) exit hole presence, with no relationships found between distance from highways and beetle distributions (Table 5). About 17% of riparian shrubs contained recent exit holes and 54% had a hole of any age compared with 2.5% and 13% of non-riparian shrubs, respectively. This corresponds with *site* occupancy rates where 44% of the non-riparian sites contained old holes only (locally extirpated), 28% contained no holes (unoccupied) and 28% had old and new holes (continued occupancy) while 100% of the riparian sites contained old and new holes (continued occupancy). Larger and/or older shrubs, reflected by maximum basal diameter, also increased the chance of beetle occupancy, with a mean ( $\pm$ 1SE) maximum stem diameter of 18 $\pm$ 2 cm for occupied shrubs and 14 $\pm$ 0.5 cm for vacant shrubs (Table 5). The likelihood of any-aged hole being present was also increased with corridor width (Table 5).

abundance within occupied sites, illustrative of habitat quality, was also positively influenced by riparian habitat and by more stems per shrub, but both of these variables only explained 8% of the variance in beetle abundance (Table 5). The noise levels of each site were not correlated with site occupancy by the beetle (logistic regression,  $p \ge 0.81$ , Chi square  $\le 0.06$ , n = 9).

# **Distance from construction source**

# New construction

Initial sampling of areas slated for construction revealed that there were no effects of proximity to proposed construction site on any of the variables measured. The only differences were site differences in shrub water potential, and leaf dust accumulation, C:N and zinc concentrations (P $\leq$ 0.007, F<sub>9,22</sub>>=5.3, n=32, site p $\leq$ =0.05, distance p >=0.45). In only 3 of 5 "new construction" sites was proposed construction completed; projects in the other 2 sites were delayed or cancelled. Post-construction measures revealed that SO<sub>4</sub>-S and Mn concentrations increased within the  $\leq$ 400 m distances from construction source in two sites of the three sites (Figure 3, Table 6A). Additionally, SO<sub>4</sub>-S concentration and leaf dust accumulation varied between sites (Table 6A).

The only variable to change before vs. after construction was shrub water potential (stress), which increased following construction in one site (NC 4; Figure 4, Table 6B). Although not significant, SO<sub>4</sub>-S also increased over two-fold in this site after construction (Figure 4). Increased shrub stress and sulfate concentrations may have been due to a change in composition of particulates and not higher dust levels since the amount of dust accumulated on leaves was similar in 2006 and 2007 (1.5 and 1.0 mg dust/mm<sup>2</sup>/day, respectively; p=0.87). Shrub stress may have also been influenced by drought conditions with only half of the precipitation in the rainy season of 2007 than 2006, leaving precipitation levels 25% below average (CA-DWR 2007). Finally, while there were no detectable changes in leaf chemical content or dust accumulation after construction in 2007, leaves on shrubs immediately adjacent to highway 50 (NC1) were apparently coated in asphalt particles (black, sticky particles), likely making them unpalatable to adult beetles (and other herbivores).

#### Old construction

Most of the variables tested did not vary with distance from construction sites. The exception was that the average elderberry inflorescence width and the cover of elderberry canopy both increased with distance from construction source (Table 7), both patterns observed along non-construction highway sites.

#### *Beetle presence*

Old exit holes (>1 yr old), reflecting previous occupancy by the beetle, were found in all but 1 construction site (NC3). In each of two sites (NC2 and OC2), recent exit holes were found in two shrubs in 2006 and in one shrub in 2007 illustrating continued occupancy. One shrub in NC4 also had a recent exit hole in 2007, while none were found in 2006, illustrating post construction recolonization. While too few holes were found to perform statistical analyses, the continued- and re-occupancy of two of the three new construction sites indicated that the construction activities did not lead to local extirpations. The local extirpation of beetles from one of the old construction sites (OC-2) could have corresponded with construction activities (old holes are  $\geq$ 2 yrs old and construction took place between one to three years

ago). However local extirpations of the beetle, like other rare patchily distributed species, can be common (Collinge et al. 2001) so conclusions as to cause cannot be made from one site.

# DISCUSSION

# The habitat suitability of roadsides

The beetle occurred along roadsides and in highway post-construction sites at occupancy rates similar to non-highways sites in the northern Central Valley illustrating that these areas can serve as habitat for this threatened species. The two factors most strongly affecting beetle presence and abundance were roadside habitat type (riparian or non-riparian) and availability of host shrubs.

#### Roadside habitat types

Both site occupancy rates and rates of shrub occupancy within occupied sites were higher in riparian (100% of sites, 17% of shrubs) compared with non-riparian (28% of sites, 2.5% of shrubs) areas, a trend seen in non-highway sites (Talley et al. 2007). Further, all of the riparian sites had continued occupancy by the beetle while the non-riparian sites experienced 28% local extirpation, 28% continued vacancy and only 44% continued occupancy. The lack of continued occupancy in non-riparian areas may reflect a lack of habitat quality for the beetle, elderberry or both (Talley et al. 2007, Fremier and Talley 2009) and/or raises the possibility that nonriparian areas may act as population sinks (Pulliam 1988).

#### Elderberry host availability.

The suitability of roadsides as beetle habitat begins with the mere presence of host shrubs, giving many Central Valley roadsides the potential for supporting beetle populations since elderberry favors these sorts of edge environments (USFWS 1984, Crane 1989a,b). Beetle presence and abundance depend, however, on a range of shrub quantity and quality characteristics, leaving room for improvement along the state's highways. Host shrub characteristics associated with increased likelihood of beetle occupancy and greater abundances along roadsides included larger and/or older shrubs and higher stem densities, some of the same variables found to be important in non-highway sites (Talley 2007, Talley et al. 2007).

Elderberry stem densities, however, were six or more times lower in sites immediately adjacent to highways than in riparian and/or control sites located 1.5-2 km away from highways, and over an order of magnitude lower than in remnant "natural" riparian and non-riparian scrub areas (20-40 stems per  $625m^2$ ; Talley et al. 2007). Basal diameter of roadside shrubs ( $6\pm0.7$  to  $28\pm8$ ) were comparable to or larger than those of roadside riparian shrubs ( $11\pm1$  to  $17\pm3$ ) and shrubs in remnant natural riparian areas ( $10\pm0.3-12\pm0.3$ ; Talley et al. 2007). In only one roadside site (NC-5/HWY-10) were all stems smaller than 12-cm diameter. Shrub stress and carbon to nitrogen ratios tended to increase with proximity to highways despite water and nitrogen inputs from vehicles and road runoff (Farmer 1993). The lack of an effect of shrub stress and nitrogen levels on beetle presence may have been due to the affinity or tolerance of the beetle for stressed plants and not enough variability in nitrogen content over these scales. Percent nitrogen values, averaged within sites ( $1.6\pm0.1$  to  $3.8\pm0.4\%$  N), were comparable to those of shrubs growing in riparian areas across the northern Central Valley (e.g., American River  $2.3\pm0.1\%$  (n=252); Putah Creek  $2.8\pm0.1\%$  (n=42); Cache Creek

 $2.8\pm0.1\%$  (n=43), Cosumnes River  $3.1\pm0.2\%$  (n=7)) (Talley 2007, Talley and Holyoak unpublished data from spring 2003).

Host suitability is also influenced by the chemical and physical effects of road-generated particulates. There was, however, no effect of proximity to highways on dust accumulations and few effects on potentially toxic elements in leaves (e.g., sulfate, manganese) despite predicted accumulations of large dust particles (>50  $\mu$ m) within the first 8 m of roads, finer dust particles (>20  $\mu$ m) within 30 m (Farmer 1993), and 50% of submicrometer particles within 100 m of roads (Hitchins et al. 2000). Roadside ambient dust levels (90-359 mg/m<sup>2</sup>/d) were similar to those produced by unsurfaced access roads (75-367 mg/m<sup>2</sup>/d), which had no effect on elderberry density or beetle presence (Talley et al. 2006a). Even when road construction occurred, leaf dust levels and leaf elemental content remained similar to pre-construction levels. Increased concentrations of leaf sulfate with distance from construction, and with distance from highways, may have been due to the use of diesel-powered machinery and vehicles (Geller et al. 2005) and local conditions such as wind patterns and weather (e.g., Wåhlin et al. 2001) since this was not a consistent trend across sites. Effects of increases in these potentially toxic foliar compounds on the beetle are unclear but for now appear to be weak or none as evidenced by the lack of significant relationships.

#### *Limits to beetle use of roadsides*

Although rates of occupancy of sites and shrubs by the beetle were comparable to those in non-highway sites, amounts of available elderberry were much less. Based on the beetle's limited movements and higher occupancy rates of areas with high elderberry density (Collinge et al. 2001, Talley et al. 2007, Talley 2007), it is likely that beetle occupancy and persistent use of roadside habitats is limited by the isolation of sites and low density of shrubs. The beetle's low local densities make it susceptible to local extirpation and its limited dispersal distances make recolonization of particular areas unlikely, especially if isolated (Collinge et al. 2001).

The relatively infrequent occurrence of shrubs and low stem densities observed in this study are less likely due to the direct stresses of the highways than right-of-way management activities, such as mowing, pruning and, in the case of construction, off-site transplanting of large shrubs. Similar trends of low elderberry occurrences with increased development have been observed throughout the range of the beetle (Lang et al. 1989). Furthermore, despite similar site occupancy rates between 1991 and 1999, Collinge et al. (2001) reported declines in the beetle associated with the loss of elderberry-occupied sites due to agriculture and infrastructure development. This trend should not be a surprise since planting and encouragement of seedlings is often avoided in high-maintenance corridors (e.g., along levees, powerline access routes, roadsides) because pruning is currently treated as a threat to the beetle and/or its habitat (USFWS 1984) and requires mitigation (USFWS 1999). Recent studies have revealed, however, that trimming of shrubs is a relatively minor risk resulting in only temporary habitat loss for the beetle (Talley et al. 2005; this report), with the benefits of planting outweighing potential impacts of trimming.

# **Management recommendations**

Beetle presence and elderberry size and quality in this study appeared to be more affected by site-specific factors (e.g., site location, habitat type) than proximity to highways or construction sites since the beetle emerged from new construction sites and showed continued occupancy in many roadside habitats illustrating that conditions associated with highways and construction are not necessarily detrimental to the completion of the beetle's life cycle.

Based on our results, the largest risk of highways and highway-associated activities to existing roadside beetle populations is likely the direct effects of construction activities (noise, dust, movement) on adults or exposed larvae and eggs, so such projects should continue to be performed outside of beetle emergence season (March to June). Weather conditions can interact with effects of highways or construction activities. For example, dust accumulations during times of drought may further stress shrubs, leading to reduced host quality or death (Sharifi et al. 1999). Weather such as humidity and wind may also affect the amount and direction of airborne particulates (Wåhlin et al. 2001). Mitigation actions should be considered during dry and/or windy periods such as occasional rinsing of elderberry to remove dust and toxins, and wetting or covering of loose sediments and/or limited ground cover removal.

Management actions can also be undertaken to increase the role of highway rights of way in providing both habitat and corridors not only for the beetle, but other dispersal-limited species whose historic ranges have been bisected by highways and roads.

#### Habitat.

The creation of beetle habitat should begin, of course, with sites that can support elderberry including sufficiently moist, organic, well-drained soils (Fremier and Talley 2009, Koch-Munz and Holyoak 2008, Crane 1989a,b). Next, a high priority should be placed on riparian sites and/or those that are relatively wide and that abut, or are at least nearby, remnant natural areas since these areas were most likely to be occupied and hold the most promise of providing habitat for a diversity of other species. Wide roadsides, such as broad rights of way, contiguous remnant habitat, and the landscaped areas of interchanges (e.g., cloverleaves), have relatively low perimeter to area ratios (less edge). Lower proportions of edge mean lower accumulations of highway-associated dust and pollutants across the entire area, greater muffling of noise, and fewer edge effects, such as invasions of introduced or weedy species and desiccation that encourage brush fires (Saunders et al. 1991). Restoration or enhancement goals for these areas could include attaining similar communities to those found on nearby riparian, grassland or scrub ecosystems, whichever is appropriate for local environmental conditions.

# Corridors

Stretches of narrow highway rights of way have a potentially important role as corridors or stepping-stones between habitat areas (e.g., Saunders and Hobbs 1991). The beetle is dispersal-limited making unlikely the colonization of new, but isolated habitat areas (Holyoak et al. in press) and recolonization following extirpation from drainages or watersheds (Collinge et al. 2001). Since highways and roads bisect remaining habitat, their conversion to corridors may increase likelihood of (re)colonization (e.g., BEST 2005) of otherwise unoccupied areas. Increased connectivity provided by corridors is, however, controversial (Mann and Plummer 1995, Rosenberg et al. 1997). These same passageways may allow movement of disease and predators to established populations, or act as sinks due to low habitat quality (e.g., pollutants, physical stresses or presence of natural enemies) (e.g., Mann and Plummer 1995, Forman and Alexander 1998, NRC 2002). Establishing a mosaic of discrete patches of vegetation communities along these stretches of highway might increase regional diversity and complexity, reduce connectivity enough to limit disturbances such as brush fires or species invasions, and provide refuge from natural enemies and disturbance (e.g., Boughton 1999).

# **Future research directions**

The presence of exit holes in most sites, the lack of detectable increases in dust and most foliar toxins with proximity to roads, and the similar noise levels at the highway sites in this study suggest that these elements may not occur at high enough levels in the roadside habitats examined to exclude the beetle. Often, however, only large effects were observed in this study due to low replication. Larger-scale and/or controlled experimental studies are needed to test specific effects on the fitness and survival of beetle and elderberry individuals of elements that were identified in this study as being potentially abundant in one or more of our study areas (e.g., sulfate, manganese) and those that were not examined in this study (e.g., other trace metals, ozone).

Further study should also determine whether roadside patches act as sinks, luring individuals into an area that cannot sustain populations (Pulliam 1988). For example, potential benefits to the beetle of higher nitrogen content in shrubs near highways may be offset over the long term by potentially toxic effects of nitrogen dioxide or other emissions (Angold 1997, Bell and Ashenden 1997). The effects of highways on connectivity of patches (i.e., the landscape supporting the patches) should include tests of whether highways and/or traffic inhibit movement of adults either through behavioral modification (e.g., avoidance) or mortality (BEST 2005). The use of newly enhanced, narrow rights of way as corridors would also need to be tested to ensure there is successful movement with minimal mortality and limited use as longer-term habitat (Saunders and Hobbs 1991, Rosenberg et al. 1997).

Finally, tests of specific effects of roadside management practices on populations of elderberry and the beetle are needed. In particular, needed are comparisons of the effects of mowing and/or herbicide use vs. the planting of native, drought tolerant plants (including elderberry) on such variables as beetle presence and abundance, elderberry growth and size, overall diversity of naturally recruited native plants and animals, fire suppression, rates of species invasions. Subsequent studies examining levels of these variables in nearby natural areas are also recommended to determine the role of roadside habitats in contributing to regional ecosystem functioning. Furthermore, studies of the public's perceptions of enhanced areas, including ranking of aesthetic value and trash accumulation rates would reveal the social values of roadside habitats. In summary, the study of a rare species in urbanized areas comes with a number of challenges but such information, even while limited, is needed for developing conservation strategies that can optimize remaining urban-influenced habitats.

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Figure 1. Map showing location of sites used to test the effects of proximity to highways and highway construction on elderberry and the Valley elderberry longhorn beetle within California's Central Valley. HWY=highway site, NC=new construction site, OC=old construction site.



Distance from highway (m)

Figure 2a. Changes in elderberry shrub characteristics [(A.) leaf biomass, (B.) inflorescence abundance and (C.) shrub water potential] with distance from highways in the Central Valley. Data are from May- July 2006 and July 2008.



Distance from highway (m)

Figure 2b. Changes in elderberry leaf characteristics [(A.), leaf dust accumulation (B.) leaf manganese concentration (C.) leaf sulfate concentration and (D.) leaf carbon to nitrogen ratios] with distance from highways in the Central Valley. Data are from May-July 2006 and July 2008.

c. Associated plants



Figure 2c. Changes in associated vegetation [(A.), canopy cover, (B.) shrub cover and (C.) understory cover] with distance from highways in the Central Valley. Data are from May-July 2006 and July 2008.



Figure 3. Concentrations of sulfate (A.) and manganese (B.) in leaves of elderberry occurring at varying distances from road construction in each of three sites in the northern Central Valley. Data were collected June-July 2007, 1-2 mos. following the completion of construction. Curve fits are linear; log scale of distance is used for clarity of graphics. NC=new construction.



Figure 4. Percent change in elderberry shrub water potential (A.), leaf sulfate concentration (B.) and leaf dust accumulation (C.) from before and after road construction in three sites within the northern Central Valley. Data are from June-July 2006 and 2007.

Table 1. Location, site code, age of construction site if applicable and habitat type of site used to examine effects of proximity of highways and highway construction on the Valley elderberry longhorn beetle and its elderberry host shrub within California's Central Valley.

Location	Site Code(s)	Age	Habitat
SR-113 between Woodland & Davis	HWY-1	n/a	Non-riparian, highway
Covell Blvd, Davis	HWY-1	n/a	Non-riparian, surface street
I-5 near Williams	HWY-2	n/a	Non-riparian, highway
I-5 south of Sacramento	HWY-9	n/a	Riparian, highway
U.S. 50 W x Hazel Av, Rancho Cordova	HWY-3, NC-1	<1 yr	Non-riparian, highway
U.S. 50 W x Hazel Av, Folsom	HWY-4, NC-2	<1 yr	Riparian, highway
I-80 E x Richards Av, Davis	HWY-5, NC-3	n.c.	Non-riparian, highway
I-80, Two Rivers Trail, Am. River Parkway	HWY-6, NC-4	<1 yr	Riparian, highway
SR-29 near Kelseyville	HWY-10, NC-5	n.c.	Non-riparian, highway
U.S. 50 E x Hazel Av, Rancho Cordova	HWY-7, OC-1	5-7 yrs	Non-riparian, highway
SR-29 x Red Hills Rd, Kelseyville	HWY-8, OC-2	1-3 yrs	Non-riparian, highway
I-80 x Am. River Parkway, Sacramento	HWY-6, OC-3	2-3 yrs	Riparian, access road

HWY=highway, OC=old construction, NC=new construction, n.c.=construction not completed.

Table 2. Highway size, sound level and dust accumulation rates at each study site within California's Central Valley. Leq is the equivalent sound pressure level that is the non-fluctuating sound level over 30 min that has equal energy to the fluctuating sound level that occurs. Min and Max indicate the minimum and maximum noises over a 30 min period. Hwy 1 also shows dust accumulation along SR113 and more-distant (2 km) control site, respectively. Noise data are from May 2007, dust accumulation data are from June-July 2006.

Site Code(s)	No.	Noise level (db)		Ambient	Leaf dust	
	lanes				sedimentation	accumulation
					rate	rate
		Leq	Min	Max	Avg±1SE	Avg±1SE
					$(mg m^{-2} d^{-1})$	$(mg mm^{-2} d^{-1})$
HWY-1	4	56-67	39	89	240±101	$0.9{\pm}0.7$
HWY-1 (control)	4	No data		250±96	$2.2{\pm}0.5$	
HWY-2	4	68	45	85	359±87	1.3±0.3
HWY-9	8	N	o data		186±32	2.0±0.3
HWY-3, NC-1	6	56-65	44	81	106±8	$1.2{\pm}0.8$
HWY-4, NC-2	6	65	51	81	193±6	2.3±0.8
HWY-5, NC-3	8	67	56	79	281±51	1.8±0.5
HWY-6 (control), NC-4	8	52	47	67	176±56	1.5±0.4
HWY-10, NC-5	2	69	37	99	No data	$0.04{\pm}0.02$
HWY-7, OC-1	6	65	52	81	90±0	$0.7{\pm}0$
HWY-8, OC-2	2	69	37	99	153±47	0.8±0.1
HWY-6, OC-3	8	72	65	81	139±76	1.2±0.9

HWY=highway, OC=old construction, NC=new construction

Table 3. Characteristics of elderberry shrubs at each study site within California's Central Valley. Elderberry stem density is standardized to number of main stems in a 625m<sup>2</sup> area; Hwy 1 also shows density along SR113 and more-distant (2 km) control site, respectively. Data are from May-August 2006, July 2008.

Site Code(s)	Elderberry	Leaf chemical content				
	stem density	nitrogen sulfate		zinc	manganese	
	for site					
	(no. 625m <sup>-2</sup> )	(%)	(ppm)	(ppm)	(ppm)	
HWY-1	0.7	$2.8{\pm}0.1$	370±89	13±2	76±16	
HWY-1 (control)	8.2	$1.9{\pm}0.1$	96±18	14±2	52±4	
HWY-2	1.9	2.3±0.1	206±40	22±3	87±9	
HWY-9	6.8	3.2±0.2	183±40	22±1	70±5	
HWY-3, NC-1	1.0	3.2±0.1	148±42	18±1	58±6	
HWY-4, NC-2	0.4	2.3±0.1	262±83	34±5	73±5	
HWY-5, NC-3	0.2	2.9±0.1	192±61	12±1	62±5	
HWY-6 (control), NC-4	6.0	3.5±0.3	352±77	33±3	63±6	
HWY-10, NC-5	0.6	1.6±0.2	103±38	23±2	96±16	
HWY-7, OC-1	< 0.1	3.8±0	130±0	19±0	64±0	
HWY-8, OC-2	0.3	3.2±0.2	205±53	18±2	84±18	
HWY-6, OC-3	0.6	2.8±0.2	529±113	33±2	53±4	

HWY=highway, OC=old construction, NC=new construction

Table 4. Results of the nested ANOVAs showing effects of proximity to highways on elderberry characteristics, associated plant cover and dust levels. Top lines show results of analyses using data from all distances from highways (0-2 km), bottom lines show results using data from distances of 0-200 m from highway. Significant results only are shown. N.S.=not significant. Significance based on sequential Bonferroni adjusted alpha value of 0.05. (+ or -) indicates direction of relationship. ≥= not significantly different from adjacent group of sites, but greater than non-adjacent groups >=significantly greater than all other groups of sites.

Explanatory variable	V	Vhole model		p values		a-posteriori
Elderberry characteristics Stem	Р	F (df)	n	distance (+ or -)	site	multiple comparison of hwy sites
max basal diameter	N.S <0.001	3.4 (19,157)	177	0.46	<0.001	8≥1,5,7,10≥2,3,4≥9
% dead	N.S. <0.001	5.1(19,158)	177	0.18	<0.001	8≥1,5,7,10≥4≥3,6≥2,9
Inflorescence	es cromes a	Alter and a second second			2 - HOY 1419-1212	
no. per 2º stem	<0.001 <0.001	12.8 (17,175) 18.9(19,158)	192 177	<0.001 (+) 0.01 (+)	<0.001 <0.001	1≥5,8,10≥2,3,7,9≥6≥4 5≥7≥3,4,8>1,2,6,9,10
avg width	N.S. N.S.					
Leaf	1					
Area (avg)	N.S. N.S.	-	-			
Biomass (avg)	<0.001 <0.001	4.3 (17,175) 4.3(19,158)	193 177	<0.001 (-) 0.004(+)	<0.001 0.001	1,2,3≥4,5,7,8,9≥6 1≥7≥2,3≥4,5,6,9,10≥8
C:N ratio	<0.001 <0.001	10.1 (17,175) 7.6(19,158)	193 177	0.004 (-)	0.06	N.S. 10≥1.7≥4≥2.3.5≥8.9
SO4-S conc.	N.S. <0.001	10.6(19,158)	177	<0.001 (+)	<0.001	 1≥6.7≥4≥2.3.8.9.10≥5
Zn conc.	<0.001 N.S.	6.7 (17,175)	193	0.38	<0.001	4≥5,10≥6≥2,7,8,9≥1,3
Mn conc.	<0.001 N.S.	6.4(17,175)	193	<0.001 (-)	<0.001	2≥1,4,5,8≥3,6,7≥9>10
dust accumulation	<0.001 N.S.	39.7(17,175)	193	<0.001 (+)	<0.001	4≥10≥1,2,3,5,6,7,8,9
Shrub						
max height	N.S. 0.005	2.2(19,158)	177	0.67	0.007	 8≥1,4,5,7,10>3,6≥2,9
water potential	<0.001 <0.001	17.5 (17,175) 15.5(19,158)	193 177	<0.001 (-) 0.91	0.01 0.003	3,6≥2,4,5,8,9,10≥1 10≥1,6,7≥3,4,5≥2≥9≥8
Associated plants						
19 (A) (g)	<0.001	8.9 (17,175)	193	<0.001 (+)	0.73	N.S.
Canopy cover (%)	<0.001	8.2(19,158)	177	0.73	<0.001	9≥1,5,7,10>2,3,4,6,8
Shrub cover (%)	<0.001 <0.001	6.5(17,175) 6.6(19,158)	193 177	0.05 (+) 0.03 (+)	0.02 <0.001	6≥2,4,5,8,9≥1,3≥5,10 8≥1,5,7≥6≥2,3,4,9≥10
Ground cover (%)	<0.001 N.S	15.4(17,175)	193	<0.001 (-)	0.005	4≥9,10≥5,7≥1,2,3,6>8
Lichen presence	N.S. <0.001	96.0(19)	 177	0.74	<0.001	 2≥9≥1,3,4,5,6,7,8,10

Table 5. Results of analyses testing relationships between roadside environmental variables and the (A.) presence of recent (new + 1 yr-old holes) valley elderberry longhorn beetle exit holes, (B.) presence of any aged exit holes (recent + old holes), and (C.) abundance of recent exit holes in occupied sites along highways in the Central Valley of California. Forward, stepwise logistic (A and B) and linear (C) regressions were used. Data from May-July 2006 and July 2008.

			the hole preser		
Explanatory					direction of
variable	$\underline{\mathbf{R}^2 \text{ or } \mathbf{r}^2}$	<u>Chi Square</u>	<u>P or p</u>	<u>n</u>	<u>relationship</u>
Whole model	0.24	26	<0.001	192	
Habitat type	0.13	14	< 0.001		rip>non
Max diam	0.11	12	< 0.001		+

A. Recent exit hole presence

B. Any-aged exit hole presence

Explanatory	$\mathbf{p}^2$				direction of
variable	$\underline{R^2 \text{ or } r^2}$	<u>Chi Square</u>	<u>P or p</u>	<u>n</u>	<u>relationship</u>
Whole model	0.27	64	<0.001	192	
Habitat type	0.16	33	< 0.001		rip>non
Max diam	0.05	12	< 0.001		+
Corridor width	0.06	16	< 0.002		+

C. Recent exit hole abundance

Explanatory					direction of
variable	$\underline{\mathbf{R}^2 \text{ or } \mathbf{r}^2}$	<u>F or t</u>	<u>P or p</u>	<u>df (n)</u>	<u>relationship</u>
Whole model	0.08	7.3	<0.001	2,161(163)	
Habitat type	0.06	3.3	0.001		rip>non
Total no. stems					
per shrub	0.03	2.2	0.030		+

Habitat type= riparian (rip) or non-riparian (non), Max diam= maximum basil diameter, direction of relationships are indicated by + (positive) or – (negative).

Table 6. Results of (A.) nested ANOVA testing effects of proximity to recent construction within sites, and (B.) pre- vs. post-construction paired comparisons of elderberry characteristics, associated plant cover and dust levels. Significant results only are shown. N.S.=not significant. Significance based on sequential Bonferroni adjusted alpha value of 0.05. (+ or -) indicates direction of relationship.  $\geq$ = not significantly different from adjacent group of sites, but greater than non-adjacent groups >=significantly greater than all other groups of sites. Data are from May-August 2006 (pre-construction) and June-July 2007 (post construction.)

	A. Post-construction analyses						B. Pre vs. post construction comparisons						
Explanatory variable	Whole model			p values		a-posteriori	Whole model			p values		a-posteriori	
Elderberry				distance		site				pre v		site or date	
characteristics	Р	F (df)	n	(+ or -)	site	comparisons	Р	t (df)	n	post	site	comparisons	
Stem											-		
max basal diameter	N.S.						N.S.						
avg basal diameter	N.S.						N.S.						
max shrub height	N.S.						N.S.						
% dead	N.S.						N.S.						
Inflorescence													
no. per 2° stem	N.S.						N.S.						
avg width	N.S.						N.S.						
Leaf													
Area (avg)	N.S						N.S.						
Biomass (avg)	N.S						N.S.						
												2007>2006	
C:N ratio	N.S						< 0.001	5.7 (18)	19	0.030	0.003	1>2,4	
SO <sub>4</sub> -S conc.	0.006	9.4 (5,13)	19	0.002 (+)	< 0.001	4>1,2	N.S.						
Zn conc.	N.S						N.S.						
Mn conc.	0.003	6.7 (5,13)	19	0.006 (+)	0.11	N.S.	N.S.						
dust accumulation	0.017	4.2 (5,13)	19	0.78	0.02	2≥4≥1	0.0002	4.7 (18)	19	0.87	< 0.001	2>4>1	
Shrub													
max height	N.S.						N.S.						
water potential	N.S.						0.019	2.6 (18)	19	< 0.001	0.79	2007>2006	
Associated plants	-	1	1	1	r	Г		1	1	r	1	1	
Canopy cover (%)	N.S.						N.S.						
Shrub cover (%)	N.S.						N.S.		L				
Lichen presence	N.S.		1				N.S.						

Table 7. Results of the nested ANOVA showing effects of proximity to old construction sites on elderberry characteristics, associated plant cover and dust levels. Significant results only are shown. N.S.=not significant. Significance based on sequential Bonferroni adjusted alpha value of 0.05. (+ or -) indicates direction of relationship.  $\geq$ = not significantly different from adjacent group of sites, but greater than non-adjacent groups >=significantly greater than all other groups of sites.

Explanatory						
variable	W	hole model	-	p valu	a-posteriori	
						multiple
Elderberry				distance		comparison of
characteristics	Р	F (df)	n	(+ or -)	site	sites
Stem						
max basal diameter	N.S.					
avg basal diameter	N.S.					
% dead	N.S.					
Inflorescence		•				·
no. per 2° stem	N.S.					
avg width	< 0.0001	42.5 (3,8)	12	< 0.001 (+)	0.57	N.S.
Leaf		· · · · · ·		· · · ·		•
Area (avg)	N.S					
Biomass (avg)	N.S					
C:N ratio	N.S					
SO <sub>4</sub> -S conc.	N.S					
Zn conc.	0.008	8.3 (3,8)	12	0.74	0.005	3>2,1
Mn conc.	N.S					
dust accumulation	N.S					
Shrub				•		•
max height	N.S.					
water potential	N.S					
•						
Associated plants						
Canopy cover (%)	< 0.0001	48.0 (3,8)	12	< 0.001 (+)	0.36	N.S.
Shrub cover (%)	N.S.			, <i>č</i>		
Lichen presence	N.S.					

# THE EFFECTS OF PRUNING ON ELDERBERRY AND THE VALLEY ELDERBERRY LONGHORN BEETLE

# ABSTRACT

This project measured the impacts of pruning of elderberry shrubs on the federally threatened valley elderberry longhorn beetle (VELB, *Desmocerus californicus dimorphus*) and its habitat, blue elderberry (*Sambucus mexicana*), by conducting measurements on two experiments with different forms of shrub trimming. The results indicated that trimming, in the forms of pruning and topping (removal of the top 1 m of shrubs), did not directly impact numbers of VELB in elderberry shrubs and clumps of shrubs. We were also unable to detect any indirect changes in the condition of shrubs through plant chemistry or measurement of shrubs. The only negative effect of trimming observed was a temporary loss of habitat in the form of the cut stems. Direct removal of stems represented a loss of a small amount of habitat but these stems regrew within 3-4 years on average. We conclude that trimming of shrubs has only minimal impacts on the VELB. These data should aid both CALTRANS and USFWS in setting appropriate levels of mitigation for the threatened VELB in response to projects where trimming of elderberry shrubs is unavoidable.

# INTRODUCTION

The purpose of this project was to define the impacts of pruning of elderberry shrubs on the federally threatened valley elderberry longhorn beetle (VELB, *Desmocerus californicus dimorphus*). This task order contributed to a larger project that is measuring the degree to which pruning of shrubs impacts the beetle. The information collected will aid California Department of Transportation in Section 7 negotiations with US Fish and Wildlife Service (FWS) personnel by improving the accuracy of estimates of the degree to which pruning impacts the VELB. The project evaluated the biological information necessary to assess the impact of a common activity where incidental take of habitat and beetles comes about because of highway maintenance—specifically through the pruning of shrubs.

During summer 2006, we sampled two experiments to determine the effects of two forms of pruning on the federally threatened Valley Elderberry Longhorn Beetle ("VELB", *Desmocerus californicus dimorphus*) and its habitat, blue elderberry *Sambucus mexicana*. Both experiments took place along the American River Parkway in Sacramento, California, at the heart of the range of the threatened beetle. The pruning experiment, located near Glen Hall Park, consists of 120 individual shrubs and was established in late spring 2002, while the "topping" experiment, conducted in the Cal Expo-Woodlake area consists of 40 elderberry clusters and was established during summer 2003. Topping consists of the form of pruning that is conducted beneath powerlines or other overhead features (bridges, etc.) where only the tops of shrubs are removed. Because most beetles occur close to the ground, topping is expected to have a smaller impact on the VELB than pruning lower on shrubs.

# METHODS

#### The pruning experiment

The pruning experiment mimicked the trimming of shrubs that overhang roads or trails. Pruning involved the removal of 50% of all 2.5-cm or less diameter branches from each shrub. The experiment was established on 16 July 2002 over a 5.7 ha area of the Parkway. A total of 120 shrubs were selected; 60 of which contained new VELB exit holes in surveys conducted the year before and 60 of which had been recently uninhabited. Half of each of the VELB-occupied and unoccupied shrubs were haphazardly assigned to be pruned. An attempt was also made to select shrubs of similar size and condition. Each shrub consisted of one large (12-20 cm basal diameter) to 3 smaller (2-7 cm diameter) main stems. Shrubs were sampled before pruning, 2 weeks after pruning, and then annually during the following four springs (15-16 June 2003, 13-14 May 2004, 20 May 2005, 5 June 2006).

#### The topping experiment

The topping experiment investigated the form of pruning that occurs beneath power lines and overhead obstructions (e.g., bridges). Topping removed the top 1 m of a shrub or clump of shrubs. Most branches at this height are 2.5 cm or less in diameter. On 23 July 2003, branches were cut from across the top 1 m of the shrubs. The experimental design was similar to the pruning experiment, however, 40 large shrub clumps were selected, with 10 shrub clumps per treatment combination of VELB-no VELB and topped-not topped. Shrub clumps in this topping experiment varied from one to several large stems (30-40 cm) to a maximum of 47 smaller stems (20-cm or less diameter). The area covered by each shrub or clump ranged from 33 to 205 m<sup>2</sup>. Shrubs were sampled before topping on 8-10 July 2003, one month after topping (19 August 2003), during 20-21 May 2004, 30 June 2005, 8 July 2006. Field measures and collections, and the lab procedures were the same for both experiments.

#### **Field measures**

For each experiment measures of elderberry survival, growth and condition were made by measuring the maximum basal diameter of the shrub, the number main stems per shrub, shrub height, and the number of branches sprouting from each cut branch. Elderberry condition was estimated for each shrub by noting the percent of leaf, bark and stem damage caused by herbivores or fire, the percent of yellow leaves (representative of senescence due to stress), and the proportion of all stems and branches that are dead. The recovery of VELB habitat (elderberry branches) was assessed by searching each shrub for cut branches, and recording the number and maximum diameter of replacement branches. Finally, in order to assess the effects of trimming (pruning and topping) on the VELB occupancy, each shrub was searched for the presence and abundance of new VELB exit holes.

#### Laboratory analyses

In the lab in 2003 and 2004, the effects of pruning on relative plant nutrition levels were estimated using carbon to nitrogen ratios (C:N) for leaves and proportion of N content for pith. Pith material is very low density and because of this it could only be analyzed for % N. The leaf C:N data included a break-down of % C and % N so that data could be compared across plant material type . Leaves were rinsed with distilled water, while the pith was extracted from the sections of collected stems. All plant material was dried at 60 degrees C until a constant weight was achieved (at least 24 hrs), finely ground into a powder, and submitted to the UC-Division of Agriculture and Natural Resources Analytical Laboratory for C: N analysis.

The effects of pruning on the presence and amount of plant defense chemicals was tested using Fiegl-Anger test papers which turn blue in the presence of hydrogen cyanide gas such as would be emitted from plant material when cell damage occurs (Seigler 1991). The release of this toxic gas is thought to be an herbivore deterrent mechanism and is documented for other species of elderberry (*Sambucus racemosa, S. nigra*) (Buhrmester et al. 2000). Samples were tested in a standardized way following procedures described by Seigler (1991).

#### Statistical analyses

The effects of trimming (topping or pruning) and the initial occupancy status of the shrub (VELB, no VELB) on both VELB colonization (change from unoccupied to occupied) and extinction (change from occupied to unoccupied in a shrub), and the number of years a shrub was occupied (occupied for 0, 1, 2 3 and/or 4 yrs) were analyzed using G-tests. Effects of trimming and initial occupancy status on elderberry growth and condition variables were analyzed using 2-way ANOVAs. Elderberry growth was estimated by calculating differences in elderberry stem diameter and height across years. All statistics were run using Statistica or JMP Statistical Software.

# RESULTS

#### Effects of shrub trimming on VELB

Neither colonization nor loss of VELB from shrubs was affected by trimming in any of the years tested (G-tests, p>0.05). This is illustrated by the frequency of occupied shrubs within each treatment across all years (Figure 1); both declines and increases in occupied shrubs were independent of trimming and depended mostly upon whether VELB had been initially present if anything (G-tests, p $\leq$ 0.01). This trend was strongest at the start of each experiment and disappeared by year 3 (topping) or 4 (pruning) once the beetles initially present had apparently dispersed. The length of time that a shrub was either occupied or unoccupied by VELB was also unaffected by trimming. Length of occupancy was related to the occupancy status at the start of the experiment, with initially occupied shrubs more likely to remain occupied than by chance, while those without holes were more likely to remain vacant (G tests, p $\leq$ 0.001).

# Effects of trimming on elderberry

# Nutrient and defense chemical content

Neither pruning nor topping had any detectable effects on elderberry nutrition (Figure 2). Proportions of leaf nitrogen, leaf carbon, leaf C:N, and pith N did not differ initially in either experiment (2 Way ANOVA, P $\geq$ 0.12) and remained similar one month (P $\geq$ 0.64) and one year (P $\geq$ 0.53) after topping, and 2 weeks (P $\geq$ 0.58), one year (P $\geq$ 0.35) and two years after pruning (P $\geq$ 0.85). Similarly, hydrogen cyanide was absent or negligible at the start of both experiments (no test paper color change), and remained so in both experiments during all sample dates.

#### Shrub survival, growth and condition

There were no short-term changes (2-4 wks) in shrub survival, growth or condition in response to pruning or topping. Shrub mortality occurred only in the pruning experiment but did not differ between VELB-no VELB or pruned-not pruned shrubs for any year (G-test, p>0.24). Neither pruning nor topping affected yearly changes in the number of main stems per shrub (p $\ge$ 0.17), maximum basal stem diameter (p $\ge$ 0.34), or shrub height (p $\ge$ 0.31)(2-Way ANOVA). Elderberry growth across the whole experiment (between 2002 or 2003 to 2006) was also not affected by pruning or initial VELB presence (p $\ge$ 0.31, 2-way ANOVA). Elderberry condition was not affected by pruning or topping. The proportion of dead stems per shrub, yellow leaves, leaf herbivore damage, and bark herbivore damage were the same, and generally remained low (0-25% category class), for pruned or topped and uncut shrubs for all dates tested dates (G tests, p $\ge$ 0.29).

The only negative effect of trimming elderberry observed was a temporary loss of habitat in the form of the cut stems (Figure 3). After one year, an average of 2.3 new branches emerged from each pruned shoot and 2.0 new branches from each topped shoot. The new branches, which were thin ( $\leq 1$  cm diameter) and herbaceous so not usable by the VELB, emerged from the first node beneath the cut. After 2 years, there was an average of 1.8 new branches for each pruned branch and 1.2 new branches for each topped branch suggesting some mortality of these new shoots. In this second year, shoot diameters were 1.5 to <2 cm and had become fairly woody although they still appeared unsuitable for use by the VELB. By the third year, there was an average of 1 new branch per each pruned branch and 1.2 new branches for each topped branch. These new branches had an average diameter of 2.1 cm (pruned) and 2.5 cm (topped), and resembled the smallest of stems that are used by the VELB. In the fourth year (pruned only), there was an average of 0.8 new stems for each one pruned, and they were 2.6 cm diameter. By this fourth year, however, it was difficult to identify the original pruned stems because of canopy growth and natural stem mortality, especially in the larger and/or healthier shrubs (i.e., measures are becoming biased since cut stems are easier to see in smaller, poorer quality shrubs). Additionally, shrub mortality due to fire and natural causes contributed to a lower number of measurable, pruned shrubs than were present initially (i.e., lower replication with each year, n=46 shrubs in yr 4 vs. 60 in yr 1). These experiments suggest that, on average, each 2-2.5 cm diameter branch that is cut will be replaced in about 3 to 4 years (Figure 3).

# CONCLUSIONS

Our analyses were conducted with reasonable sample sizes for detecting the effects of pruning and topping on both VELB and elderberry. While it is possible that small effects would have not been detected, any more important effects would likely have been found in our analyses. The results indicated that trimming (pruning and topping) did not impact numbers of VELB in elderberry shrubs and clumps of shrubs. We were also unable to detect any changes in the condition of shrubs through plant chemistry or measurement of shrubs. The only negative effect of trimming observed was a temporary loss of habitat in the form of the cut stems. Direct removal of stems represented a loss of a small amount of habitat but these stems regrew within 3-4 years on average.

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Figure 1. The percent of elderberry shrubs occupied each year of the A.) pruning and B.) topping experiment. OO= no velb initially inhabiting shrub, not pruned or topped; OP= no velb, pruned or topped; VO= velb initially inhabiting shrub, no pruned or topped; VP= velb, pruned or topped. N=30 shrubs per treatment (pruning experiment) or =10 shrubs or clumps (topping experiment).



Figure 2. The ratio of carbon to nitrogen in elderberry leaves each year of the A.) pruning and B.) topping experiment. OO= no velb initially inhabiting shrub, not pruned or topped; OP= no velb, pruned or topped; VO= velb initially inhabiting shrub, no pruned or topped; VP= velb, pruned or topped. N=30 shrubs per treatment (pruning experiment) or =10 shrubs or clumps (topping experiment).





Figure 3. The average number of stems to replace each cut stem and the average diameter of those stems in the A.) pruning and B.) topping experiment. Pruning experiment was established in 2002, topping experiment in 2003. Mean  $\pm 1SE$  are shown. Horizontal lines in the graphs represent a 0 net gain of stems since one stem was lost to cutting. Red bars in the diameter graphs represent sizes that can be successfully used by the Valley elderberry longhorn beetle.