Evaluating the sensitivity of Quino Checkerspot Butterfly *(Euphydryas editha quino)* to two herbicides commonly used for habitat restoration and management.

Final Report
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EXECUTIVE SUMMARY

This project was designed to test for any effects of the commercially available taxon-specific herbicides Fusilade II®, Transline® and application surfactant on Quino checkerspot butterfly larval development, survival, and pupal weights. The experimental design tested for direct and indirect effects on the proportion of larvae that pupate as well as the weights of the pupa.

Test organisms were post-diapause larvae, obtained from Dr. Gordon Pratt (of UC Riverside) in two batches (referred to as group A and group B). Approximately 600 larvae were treated and measured between May and July 2011. Initially, larvae grew well but by July, they stopped feeding and re-entered another diapause (fuzzy) instar. Since the larvae did not pupate, we were unable to assess whether herbicide exposure, either direct or indirect, alters survivorship to the pupal stage or influences the size of pupa.

In order to assess whether there were any measurable treatment effects, we analyzed the larval weights from the earlier instars. We hoped that these analyses would provide some information on treatment effects, in the absence of a complete assessment of potential herbicide effects. While there were large differences in weights between the two larval groups, there was no significant difference in larval weights among treatments in either group.

Results from this experiment suggest that there are no direct or indirect effects on early growth of Quino Checkerspot butterfly larvae from exposure to Fusilade or Transline and/or surfactant. This is encouraging in that there is no initial indication of gross toxicity. We were unable to assess the effects of herbicide exposure on development through pupation. As a result, we cannot evaluate the potential magnitude of impacts of the herbicides on this species based on our *a priori* endpoints.

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BACKGROUND AND OBJECTIVES

The Quino checkerspot butterfly (*Euphydryas editha quino*) is endemic to grassland and coastal sage scrub natural communities in southern California. The species was listed as federally endangered in 2002. The recovery of the species is linked to improved habitat conditions. It has been extirpated from Orange, Los Angeles and San Bernardino counties and parts of Riverside and San Diego counties. Data suggest that grazing, recreational use and fire have increased cover of exotic plants in occupied and conserved habitat for the species. The exotic plant species compete with the native plants used as the principal larval food plants. The exotic plants reduce availability of food plants and thus threaten sustainability of the species' habitat.

This project is designed to accomplish 2 main objectives:

1. Test effects of the commercially available taxon-specific herbicides Fusilade II®, Transline® and application surfactant on Quino checkerspot butterfly larval development, survival, and pupal weights. (Note: both herbicides contain 24.5% of the active ingredient Fluazifop-p-butyl).

2. Preparation of a summary report of the results of the above testing. The report may subsequently be used or superseded by a manuscript submitted by the investigators for a peer-reviewed publication.

EXPERIMENTAL DESIGN

Initially, the experiment was designed to test for any effects of direct and indirect exposure to Fusilade and to the surfactant used when Fusilade is applied (Figure 1). The primary endpoints (response variables) were the proportion of larvae that pupate as well as the weights of the pupa. This would be able to detect changes in survivorship and growth into the pupal stage but would not detect changes in emergence or subsequent adult survivorship or fecundity.

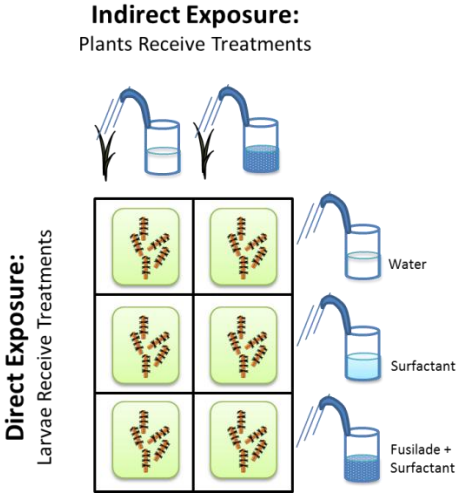

Figure 1: Initial experimental design. The initial experiment included 2 levels of indirect exposure (control and fusilade) and 3 levels of direct exposure (control, surfactant and fusilade).

The experiment was modified to include both Fusilade and Transline. In order to reduce the number of treatments, only certain combinations of indirect exposure (of the food plants) and direct exposure (of the larvae themselves) were used (Figure 2). These combinations were chosen to help estimate the effects of direct and indirect exposure to surfactant, Fusilade, and Transline. This mimics what might be expected in nature – with larvae being exposed to the same treatment as their food plants.

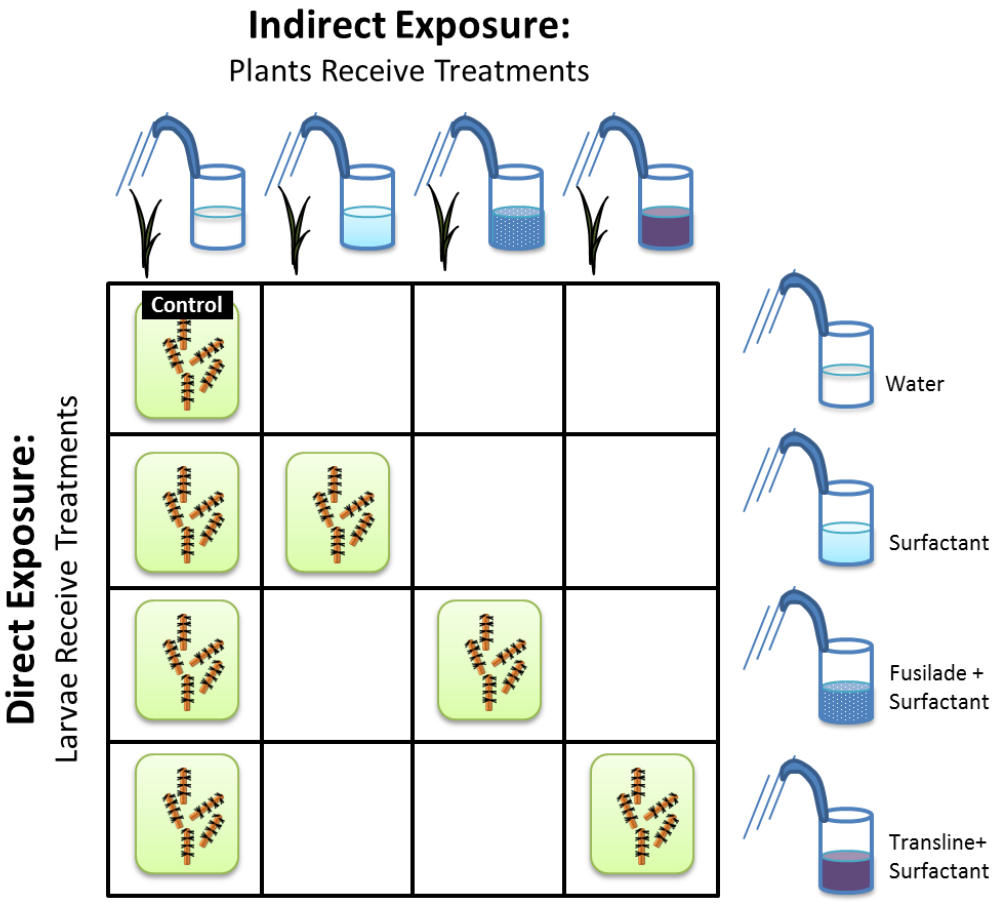

Figure 2: Final experimental design. The initial experiment included 4 levels of both direct and indirect exposure in a partial factorial design. There were three types of treatments, true controls (1 treatment) that received no exposure, direct exposure only (3 treatments) and double exposure (food plant and direct, 3 treatments)

Test organisms were post-diapause larvae, obtained from Dr. Gordon Pratt (of UC Riverside). Test herbicides and Pro Spreader/Activator surfactant were obtained from Mr. Mike Kelley (President, Kelly & Associates; President, San Diego Conservation Resources Network; and Conservation Chair, Friends of los Peñasquitos Canyon Preserve), and applied at field application rates for weed control that have been found to be effective in restoration of non-occupied habitat (for example, by Kelly & Associates in coastal sage habitat management, and by Recon Environmental in vernal pools on Otay Mesa).

Larvae

The first set of larvae (Group A) were obtained from Dr. Pratt in April, and when survival through diapause appeared to be low (35%), a second group (B) was obtained in May (Table 1). Survival of that group appeared to be even lower (27.5%). Originally we had planned to rear 10 larvae per terrarium. However, Dr. Pratt (who regularly rears these animals) suggested that Quino larvae grow much better when in larger groups. Thus the number per group was increased to 18 and 19 per cohort (Table 2).

Table 1: Quino larvae received for the experiment.

Group A		**Group B**	
ID	Count	ID	Count
1	187	A	12
2	200	B	26
3	178	C	6
4	250	D	18
Total	815	E	50
# Obtained	**285**	E2	5
Rate	*35.0%*	E3	45
		F	99
		G	112
		H	96
		I	163
		J	53
		K	116
		L	204
		M	55
		N	46
		Total	1106
		# Obtained	**304**
		Rate	*27.5%*

Table 2: Allocation of Quino larvae to treatments.
Sixteen terraria were used for each group of larvae.
Control treatment received additional replications.

Food Plant Treatment	**Larval Treatment**	*Group A* **Terraria**	**Cohort Size**	*Group B* **Terraria**	**Cohort Size**	**Total Larvae**
Water	Water	4	18	4	19	148
Water	Surfactant	2	18	2	19	74
Water	Fusilade	2	18	2	19	74
Water	Transline	2	18	2	19	74
Surfactant	Surfactant	2	18	2	19	74
Fusilade	Fusilade	2	18	2	19	74
Transline	Transline	2	18	2	19	74
					Total	**592**

Rearing

Larvae were reared on *Penstemon* until they had molted one time, as per methods used by Dr. Pratt and others to rear post diapause Quino (Mattoni et al. 1997, Quino Captive Breeding Manual, Pratt et al. 2001; U.S. Fish and Wildlife Service, 2003). Soon after they molted from their diapause instar, the larvae were sprayed lightly until just moistened with one of the herbicides, surfactant, or distilled water, depending on treatment. The liquids appeared as small droplets held by surface tension on the small spines covering their bodies.

Collensia concolor was grown from seeds both obtained from Dr. Pratt, collected from the Lake Skinner locale where the parents of the larvae used in the experiment were collected. Foodplants were grown in small pots in flats in a shade house at SDSU. Plants were sprayed the day prior to being fed to the larvae, so the herbicide was allowed to dry completely before the plants were introduced to the larvae. The growing, treated potted plants were placed in clear glass containers with larvae, and larvae could feed at will.

Larvae were reared in growth chambers on a controlled light cycle and at temperatures mimicking natural conditions for spring. Food was changed as needed. Only treated plants were offered to larvae. Larval censuses and weights were regularly assessed to document changes in larval sizes. However, since handling the larvae may be detrimental, larvae were weighed less often than censuses were conducted. This was done periodically rather than just measuring final weights, since these larvae are known to go back into diapause when they perceive conditions for pupation are not right. In fact, Dr. Pratt reports some returning to diapause as many as six times. This was a particular concern in the present study because the paperwork processing, larval delivery, and start of the experiment were seasonally delayed.

RESULTS

Larvae were weighed and/or counted on the following dates: 5/23/11 (start of feeding trials), 6/03/11, 6/15/11, 7/01/11, 7/17/11, 7/27/11 (by which time all were in diapause). Larvae grew well until the middle of July (some reaching their 5^{th} instar), with some rather typical mortality. But at that time, rather than molting into either the 4^{th} to the 5^{th} instar or into their final instar, many molted into another diapause (fuzzy) instar, and stopped feeding. Since the larvae did not pupate, we could not evaluate this experiment with our intended response variables of the proportion of larvae that pupate as well as the weights of the pupa . We are unable to assess whether herbicide exposure, either direct or indirect, alters survivorship to the pupal stage or influences the size of pupa. In order to assess whether there were any measurable treatment effects, we analyzed the larval weights from the earlier instars.

Analysis of larval weights

At the beginning of the trials, larvae were small with an average weight close to 10mg. As the experiment progressed, larval weights increased and became more variable (Figure 3). The coefficient of variation increased from ~40% at the beginning of the trials to ~90% at the end. In addition, the distribution became increasingly right-skewed. This results from the fact that many larvae remained small while a small number grew larger and larger. These changes in variability and skewness present a challenge in the analysis, since they violate the assumptions of parametric tests like the t-test and ANOVA.

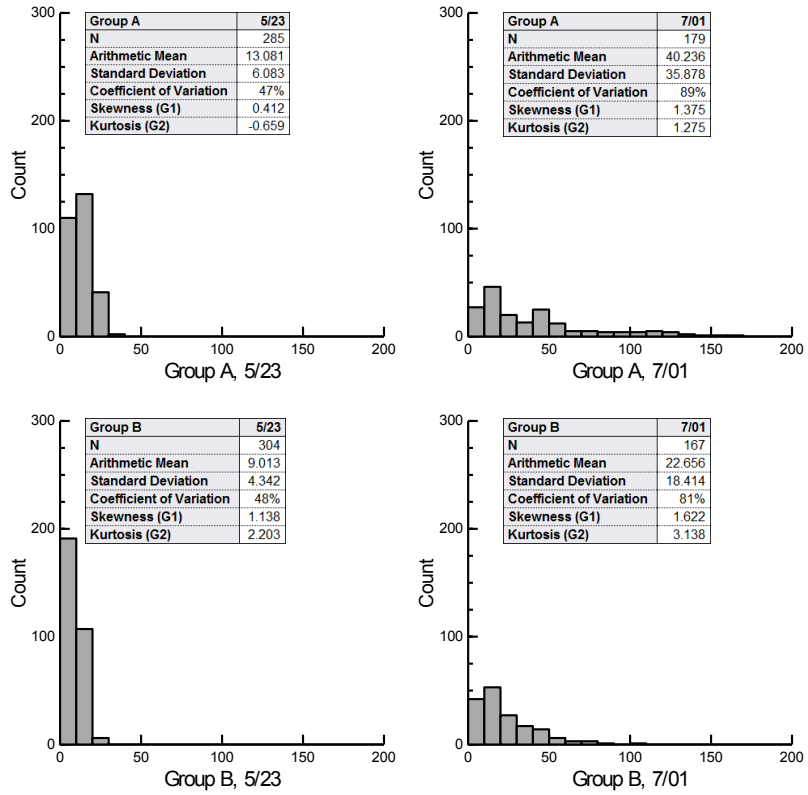

Figure 3. Larval weights through time. The distribution of the larval weights becomes more variable and right-skewed through time. This resulted from the fact that most larvae stayed small while a few grew larger and larger. On average, larval weights for group B were lower than those in group A.

Larval weights on July 01

Treatment effects were assessed on log-transformed larval weights throughout the experiment. For simplicity, we present the results from the July 01 measurements (~54 days into the experiment, See Table 3). The results are similar to those obtained on other dates.

Table 3: ANOVA on log-transformed larval weights on July 01, 2011.

n = 346, R^2 = 12.1%					
Source	Type III SS	df	Mean Squares	F-ratio	p-value
Group	24.408	1	24.408	31.85	<.001
Treatment	7.804	6	1.301	1.697	0.121
Interaction	6.641	6	1.107	1.444	0.197
Error	254.421	332	0.766		

While there were large differences in weights between the two larval groups, there was no significant difference in larval weights among treatments in either group. (Figure 4). The only exception was the apparent increase in larval weights in the Transline treated larvae from group A. This is likely a consequence of the small sample sizes since this effect was not seen for Fusilade in group A or for any herbicide in group B.

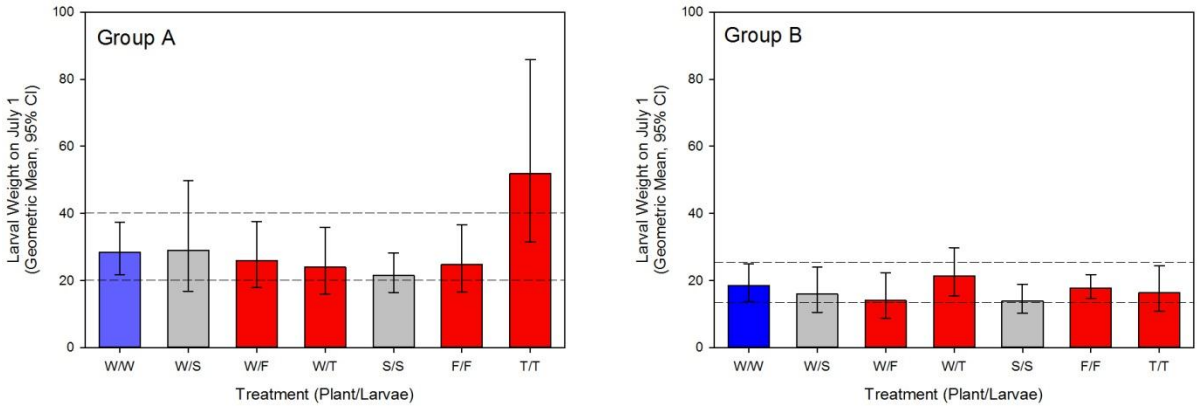

Figure 4. Larval weights across treatments. Larval weights for the controls (blue bars), larvae exposed to surfactant (gray bars), and larvae exposed to herbicides (red bars). Each treatment is specified by a 2-letter code specifying treatment of the food plant (first letter) and the larvae (second letter). W=water, S=Surfactant, F=Fusilade, T=Transline.

(Note: dashed lines represent the threshold for a treatment group that is significantly different from the control (W/W).

Larval weights on July 17

By July 17 (Day 70), more larvae had re-entered diapause and were removed from the analysis. As observed earlier, notable effects of herbicide and/or surfactant treatments on larval growth were still not detectable, even when the larval groups were pooled for analyses (Figure 5).

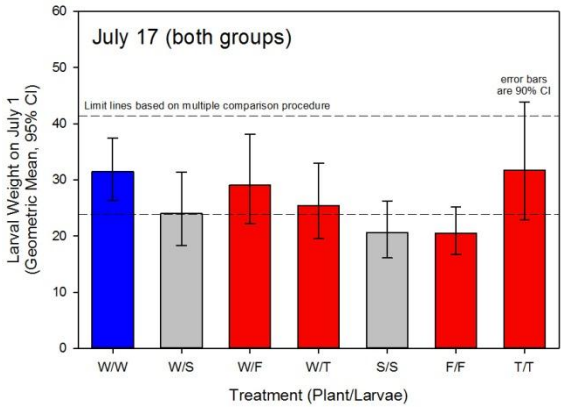

Figure 5. Larval weights on July 17. Larvae from both groups are pooled. Larval weights for the controls (blue bars), surfactant (gray bars), and herbicides (red bars).

DISCUSSION

Results from this experiment suggest that there are no direct or indirect effects on early growth of Quino Checkerspot butterfly larvae from exposure to Fusilade or Transline and/or surfactant. This is encouraging in that there is no initial indication of toxicity. No significant differences were detected from exposure of larvae to treated food plants or from direct exposure to herbicide. It is encouraging that we did not see any large effects on early growth. Unfortunately, because of the late start, the larvae were clearly not "tricked" into behaving like it was early spring (even with efforts to control day length), so the majority of larvae went back into diapause before pupating.

We were unable to assess the effects of herbicide exposure on development through pupation. As a result, we cannot evaluate the potential magnitude of impacts on this species pupation, i.e. the proportion and weights of larvae that pupate.

ACKNOWLEDGEMENTS

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