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FINAL REPORT
FOR
PALOS VERDES BLUE BUTTERFLY YEAR 2002 CAPTIVE REARING
ON
DEFENSE FUEL SUPPORT POINT
SAN PEDRO, CALIFORNIA

COOPERATIVE AGREEMENT NUMBER:
N68711-02-LT-00010

October 1, 2002

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Photo: Daniel Soyka Beran © 2002

1. Introduction

After the rediscovery of the Palos Verdes blue butterfly ("PVB") in 1994 at the Defense Fuel Support Point, San Pedro, a captive propagation effort was begun. Immediate attention was justified because of the apparently population size (Mattoni 1994). The results of the captive rearing effort from 1995 to 1998 were poor, but increased effort yielded 627 pupae in 1999, 968 in 2000, and 299 in 2001. These results were comparable with other recorded efforts to rear lycaenids (Herms and others 1996). This report outlines the methods and results for captive rearing for the 2002 season. The rearing project meets in part the conditions of the U.S. Fish and Wildlife Service's Biological Opinion on the Formal Section 7 Consultation for the Chevron 1-8" Pipeline and Associated Government Pipelines Project, Defense Fuel Support Point, San Pedro, Los Angeles County, California (1-6-96-F-09). As such it utilizes methods developed by Mattoni and recommended by the U.S. Fish and Wildlife Service (Mattoni 1988).

Rearing was conducted under authority of Dr. Rudi Mattoni's permit (TE-807303-4). Dr. Mattoni is currently the only person permitted to rear Palos Verdes blue butterfly, and meets the criteria of the Biological Opinion 1-6-96-F-09 that "personnel involving in the breeding program shall possess at least two (2) years of direct experience with the captive propagation of the Palos Verdes blue butterfly."

2. Methods and Results

2.1. Facilities

Rearing activities were conducted at two locations. The first was a laboratory at the Defense Fuel Support Point in San Pedro. Improvements were made at the facility to provide hot water, a refrigerator with back-up power, bench space, and storage shelves. Because the lab facility was not ready at the start of the season, eclosion, and small cage mating occurred at Dr. Mattoni's residence in Santa Monica.

2.2. Pupae → Eclosion

All pupae from the combined stocks of 2000 and 2001 were removed from refrigeration on February 24, 2002. Each pupa was weighed in order to determine which were alive or dead on the basis of weight. Pupae less than 50 mg were assumed to be not viable, while pupae greater than 50 mg were assumed to be potentially viable. A frequency distribution of all pupal weights showed a distinct bimodal distribution, supporting this hypothesis (Figure 1). Weights of pupae less than 50 mg formed a normal distribution (skewness = 0.19; mean weight = 27.2 ± 10.0 S.D.), and weights of pupae greater than 50 mg formed a normal distribution (skewness = –0.10; mean weight = 86.1 ± 17.2 S.D.). Based on this assumption, the pupae from 2000 contained 143 live and 44 dead specimens. From 2001 there were 199 live and 24 dead pupae. Because all pupae have a certain likelihood of remaining in diapause, all will be set out for rearing during the 2002 season. Multiple year diapause is a common strategy of insects in unpredictable climates (Scott 1986), and PVB is no exception.

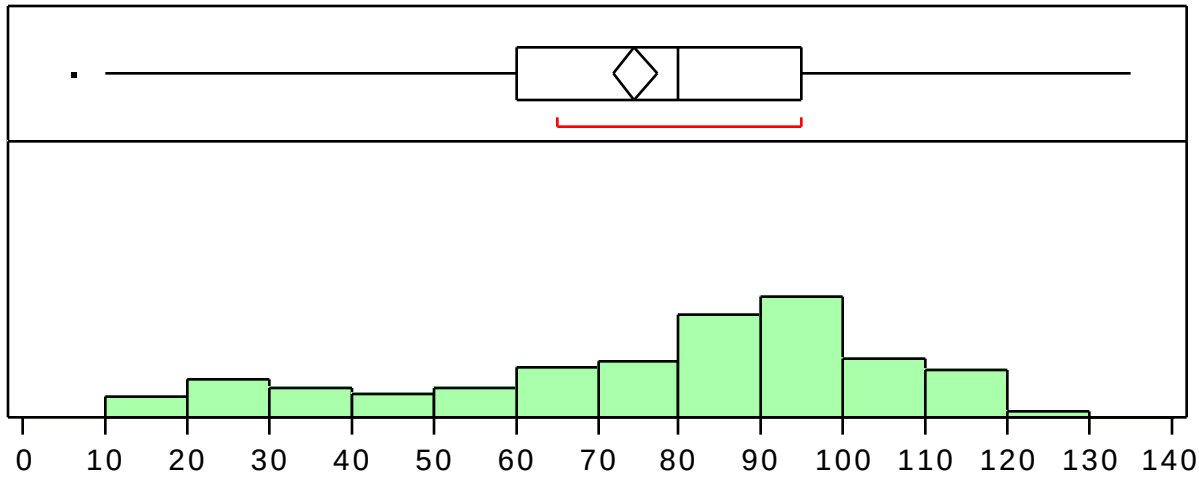

Figure 1. Distribution of Palos Verdes blue butterfly pupae weights in mg when removed from refrigeration to break diapause. Note bimodal distribution with peaks at 90–100 mg and 20–30 mg (skewness = –0.64).

Imagoes emerged from pupae during the second week of March. Of the 143 pupae considered viable from 2000, 17 eclosed (12%), while from the 199 pupae from 2001, 121 eclosed (61%). Three (18%) of the adults from 2000 were deformed, while 10 (8%) of the 2001 stock were

visibly malformed. None of the deformed individuals was used for rearing, and those pupae previously judged to be dead were mounted on cards (curated) for archival preservation at the Natural History Museum of Los Angeles County. Other imagoes had developed within the pupae sufficiently for wing venation and color pattern to be visible, but did not eclose. As observed in the field, the peak of male eclosion preceded that for females (Figure 2). This phenomenon of earlier male than female eclosion is referred to as protandry, which is "widespread but not ubiquitous" among butterflies (Zonneveld 1996).

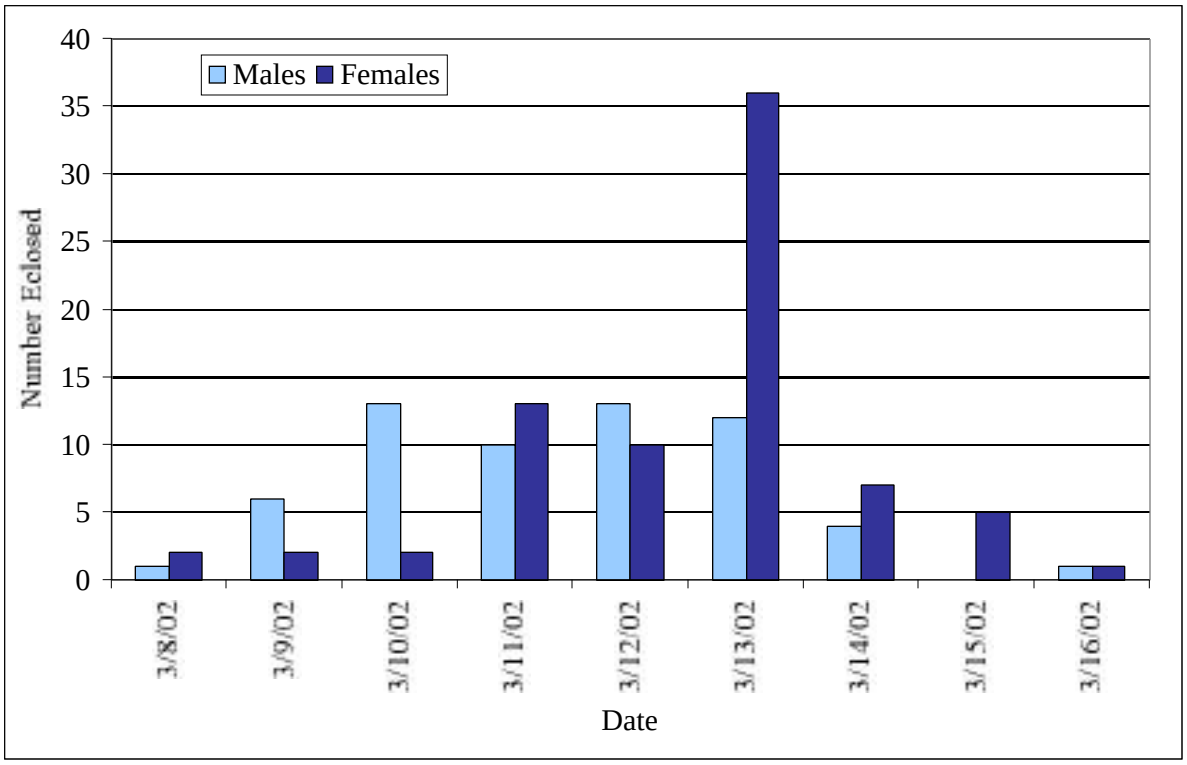

Figure 2. Eclosion of adult Palos Verdes blue butterflies under laboratory conditions. Pupae were removed from refrigeration on February 24, 2002.

There were four classes of aberrant individuals among the eclosed adults that were not used for mating: 1) grossly deformed by wings not properly expanding following eclosion from the pupal case or unable to escape the pupal case, 2) individuals with misshapened or missing prolegs, 3) underside wing pattern without any macules, and 4) one individual with underside wing pattern with macules enormously enlarged (Figure 3). To what extent any of these are genetic variants or developmental anomalies is unknown. However, it is noteworthy that all but the first class occurred in adults that eclosed in the first few days of the eclosion period. This would suggest these are developmental anomalies resulting from some shock associated with removal from refrigeration in relationship to the embryonic processes associated with their physiological status that occur during this crucial time.

Figure 3. PVB with large macules on underside wing pattern.

Photo: Daniel Soyka Beran © 2002

The grossly deformed individuals included several which actually failed to escape the pupal shell (Figure 4). Since the breeding stock of 2001 was largely offspring of the prior years' cohort, and given that the natural population has an effective breeding population size of far less than 400 (see Mattoni and Longcore 2002), it is possible that inbreeding effects could be in play. Our captive rearing protocol was based on mass selection — that is having mating occur at random with all offspring recovered for subsequent mating, also by groups — to avoid these inbreeding effects (Villanueva and others 2000). It has been shown elsewhere that substantial hidden genetic variation is present in natural populations of butterflies (see Dimock and Mattoni 1986).

Figure 4. Adult PVB that failed to escape pupal shell.

Photo: Daniel Soyka Beran © 2002

2.3. Mating Behavior

Two modal types of cages have been used in the mating and growing efforts, large "tent" enclosures designed to support populations on the order of one thousand larvae, and small cages for confinement of one or two mating pairs or a few gravid females. The large cages are suitable for production of large numbers of larvae from a number of random matings and provide conditions approximating those of the field. The small cages provide the opportunity for close observation under laboratory conditions and are presumed useful for careful propagation when population numbers are low and every individual is critically important.

2.3.1. Large Cages

Cages were constructed of 3/4-inch PVC piping to which screening material was affixed. Two sides of each top were closed with Velcro to allow access to cages. The cages varied from three feet square to more than four feet on a side (Table 1).

Table 1. Size of large cages used for rearing.

Cage #	Width (in)	Depth (in)	Height (in)	Volume (ft^3)
1	50	48	42	58.3
2	56	48	36	56.0
3	37	36	36	27.8
4	54	48	39	58.5
5	49	47	45	60.0
6	50	48	44	61.1
7	48	61	49	83.0
8	46	48	42	53.7
9	48	50	49	68.1
10	48	45	28	35.0

The cages were staked into the ground with the bottom edge of the screening buried both to prevent the PVB from escape and to preclude entry by larger predators and parasitoids. Lizards and large insects were removed from the cages prior to closure. Rainproof tops were constructed of corrugated plastic and secured with rope and stakes when rain was predicted, which occurred once during the rearing period.

2.3.1.1. Cage Placement

Cages were placed over large, mature *Lotus scoparius* plants in the field. Choice of plants was based on the following criteria:

a) Robust plant(s) with full, healthy foliage,

b) Either buds or flowers evident in abundance, and

c) Clustered in walking distance of laboratory operations.

These criteria resulted in the majority of cages being located around Building 108 and the nursery, enclosing plants that could receive supplementary irrigation. One cage was placed in the meadow (Polygon 6A) and two slightly farther way. The latter could not be irrigated.

2.3.1.2. Rearing (Adult → Egg → Third instar larvae)

Seventy-seven adults were placed in 10 cages in the field, positioned over robust adult *Lotus scoparius* plants. The number of adults was noted each day, and all behaviors were photodocumented. When females were placed in cages they quickly paired with males. Paired butterflies were observed in 9 of 10 cages. This behavior was noted in prior years' trials which utilized tent cages and is a powerful indicator that the butterflies exhibit "natural" behavior when enclosed in larger versus smaller spaces. Jeremy Thomas (pers. comm.) experienced difficulty in rearing the rare, ant-tended European large blues in small cages, but when finally transferring the effort to large outdoor cages found the same success under the more natural scale conditions.

Figure 5. Mating PVB in large cage.

Photo: Daniel Soyka Beran © 2002

Results from the 10 tent cages follow (Table 2). In all cases adults died or disappeared within seven days of introduction, in contrast with greater longevity the small cages (see below). No supplementary feeders were provided and the adults depended on deerweed flowers for nectar. Larvae were removed starting April 20, all in second instar or later. Many were ant tended (Argentine ants, *Linepithema humile*) and when removed, the tending ants were collected with the larvae. Locating larvae in the cages was facilitated by observing ant movements. Although eggs were noted in all cages (except 6), they were difficult to see and no attempt was made to quantify them. Larvae were only recovered from five cages, although mating and eggs were observed in all cages except number 6 (Figure 5). The problem with cage 6 was that the enclosed plant started drying out a few days after the adults were introduced, losing all flowers.

Predators were removed from cages as observed. These included wasps and spiders. In one instance an adult butterfly was captured by a spider web and killed (Figure 6). The web was constructed overnight in one of the tent cages and the spider and web were removed immediately upon observation.

Figure 6. Adult PVB captured in spider web.

Photo: Daniel Soyka Beran © 2002

Field observation early in the season indicated that productivity was going to be low. Consequently the initial planned objective of removing last instar larvae was abandoned and searches of all instars were started in late April. Second to fourth instar larvae were removed from cages and taken to the laboratory for rearing in individual containers. Following the end of the season the cages were removed and their circumscribed area thoroughly searched for pupae. None was found, increasing our confidence that all larvae were located and recovered to the laboratory for rearing.

The numbers recovered were disappointing given prior results. One of the two large cages used in year 2000 produced nearly 1,000 larvae from a total of 18 pairs of adults. The 2001 final yields were drastically reduced because the large cage populations were allowed to go to pupation in the cages. Although hundreds of larvae were noted, few pupae were recovered as a result of predation of the pupae by the common earwig. In contrast, the problem in 2002 was simply too few larvae from too few eggs, not losses from predation.

We found no evidence of loss to parasitoids. None of the recovered larvae was parasitized and inspection of the eggs found on the plants provided no sign of loss to the common egg-parasitic

wasp *Trichogramma.* The role of other predators is not clear. Spiders, predatory hemiptera, and earwigs could not be excluded and may well have resulted in loss of larvae.

Table 2. Number of PVB larvae recovered from large cages and subsequent number of pupae obtained.

Cage	Adults	Larvae Recovered	Pupae Obtained	Cage Volume (ft^3)
1	5 m 8 f	34	34	58.3
2.	6 m 15 f	88	80	56.0
3	8 m 17 f	4	4	27.8
4	6 m 13 f	1	0	58.5
5	6 m 10 f	0		60.0
6	5 m 6 f	0		61.1
7	4 m 8 f	0		83.0
8	4 m 8 f	0		53.7
9	4 m 8 f	4	4	68.1
10	4 m 8 f	16	14	35.0
Total	153	147	140	

2.4. Small Cage Rearing (Adult → Egg)

2.4.1. Cage Design

Small cages were constructed using cylinders of either thin vinyl plastic or rolled standard window screen of a diameter that exactly fit within the rim of one-gallon containers of foodplant. Each container had either a *Lotus scoparius* or an *Astragalus trichopodus* plant and the cage was tall enough to enclose the plant, approximately 18 inches. The top of each cylinder was fitted with a nylon mesh screen held in place with either rubber bands or a plastic double ring. Adults in these cages were provided with a 10% honey solution *ad libidum*.

Twelve cages were set up for individual breeding, and two pairs placed in each cage. The cages were kept outside in sunlight, and mating was observed in eight of the cages. An inventory of eggs was taken on March 28, and approximately 300 eggs were noted. In cages where no mating had been observed only a few eggs were observed, many of which had collapsed and were therefore judged infertile.

The adults were placed in the cages serially immediately after they had eclosed. The cages were brought into the laboratory at DFSP starting about April 5, at which time most adults had died, the remaining 8 females were then distributed into two new cages (13 and 14) until their demise. The results from the cages are shown in Table 3.

After relocation to the lab, the earlier noted irruption of aphis populations in the small cages occurred. This phenomenon was the cause of large losses in prior years. We believe that this is a result of an indoor environment, as control plants (without larvae) left outdoors did not have rapid aphis contamination. The effect was most pronounced in the plastic cylinder set as

opposed to screen top set. This may be attributed to air-flow. Control foodplants without any top in the lab became contaminated as well, however.

Table 3. Number of eggs, larvae, and pupae obtained from each small (gallon) rearing cage.

Cage	Foodplant	Number Eggs	Larvae Recovered	Pupae
1	*Astragalus*	none		
2	*Astragalus*	none		
3	*Astragalus*	none		
4	*Astragalus*	40+	15	none
5	*Astragalus*	20+	10	none
6	*Lotus*	50+	25	3
7	*Astragalus*	60+	20	6
8	*Lotus*	40+	25	none
9	*Lotus*	50+	15	none
10	*Lotus*	50+	30	19
11	*Lotus*	none		
12	*Lotus*	none		
13	*Lotus*	3	2	1
14	*Astragalus*	4	4	2
	Estimated total	317+	146	28

The effect of the rapid aphis development resulted in most leaves of both *Lotus* and *Astragalus* being destroyed and concentrations of aphis honeydew deposited over most plant surfaces. The PVB larvae reacted by leaving the plants — regardless of instar — and were collected or desiccated.

In order to minimize loss at this point, we decided to remove all larvae from the plants and maintain them using defined synthetic diet in one ounce creamers (Figure 7). This procedure follows prior work in order to maximize yields. However, if pest infestation can be avoided, greater success can be achieved with minimum handling of larvae, as shown with control green hairstreak effort described below.

2.5. Larvae → Pupae

All larvae recovered from both the large and small cages were placed in one ounce styrene creamers that contained 5 cc. of synthetic diet. The formula and preparation of the synthetic diet is given below. In our prior work this diet was usually accepted as a food source. However, this year the diet was for the most part rejected, with only three larvae accepting the diet. We therefore provided buds, flowers, and young seedpods of *Lotus scoparius* to all larvae through to pupation. The moisture of the synthetic diet helped to keep the plant material fresh. In this manner the creamers could be left for two days rather than one day between feedings. It was essential to maintain fresh diet for successful rearing. Oddly, a few of the green hairstreak

controls were placed on the diet and, contrary to past experience wherein they universally rejected the diet, at least five larvae fed to pupation on the medium.

Figure 7. PVB larva on deerweed in one ounce creamer with synthetic diet.

Photo: Daniel Soyka Beran © 2002

Figure 8. PVB larva on *Lotus scoparius*.

Photo: Daniel Soyka Beran © 2002

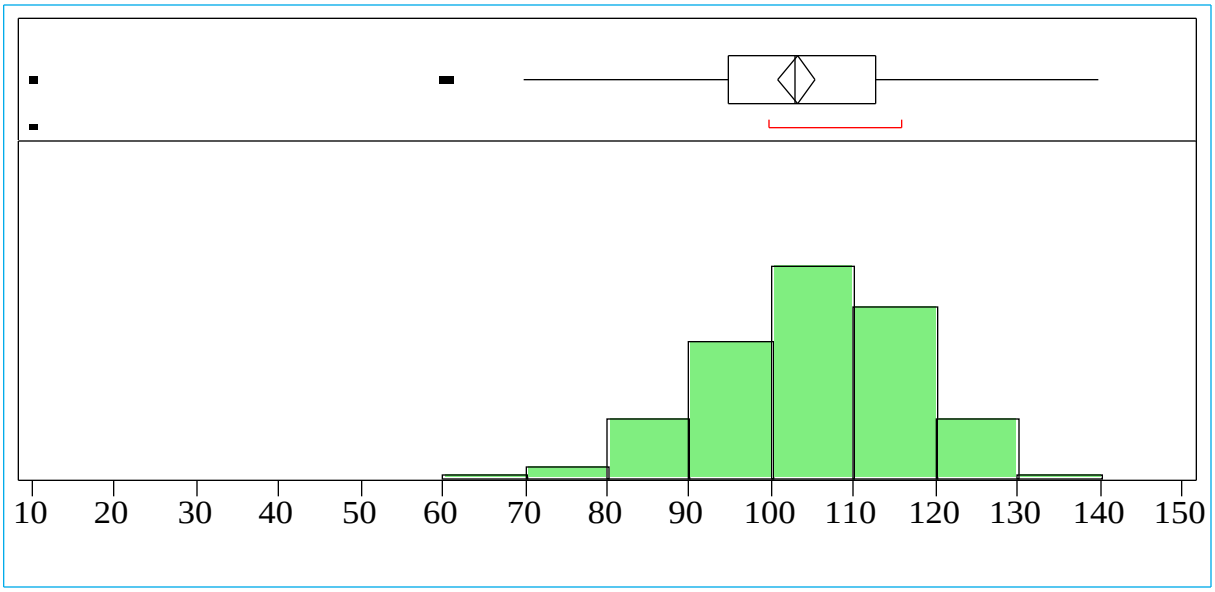

Figure 9. Distribution of Palos Verdes blue butterfly pupae weights in mg immediately following pupation and prior to refrigeration.

The cohort of pupae immediately following pupation displayed a normal distribution of weights (Figure 9), as compared to the pupae following nine months of storage. Indeed the pupae were significantly heavier than those pupae presumed viable from the 2000 and 2001 cohorts. Two explanations seem possible: 1) the 2002 cohort is heavier because of favorable conditions during rearing, or 2) the pupae lose water during diapause and are consequently on average lighter following storage. Of these, it seems much more likely that slight desiccation during storage explains the difference. We suggest that pupal weights be monitored during storage to investigate this possibility, and, when stock is sufficient, that investigators maintain some pupae without refrigeration as a control.

2.6. Pupae Storage

One month after pupation, larvae were each weighed and placed under refrigerated at constant temperature (39ºF) and high humidity. The storage refrigerator is a small unit equipped with a backup power system to circumvent failure in case of power outage and is located at in the rearing laboratory the Defense Fuel Support Point. Each pupa is in a creamer that is labeled with its cage origin. All pupae from 2000 and 2001 were curated except the few that still maintained weights over 50 mg.

2.7. Diseases Experienced During Rearing

There was some loss of larvae to disease in later instars from at least two infective agency causes. In past years, nearly every larva recovered from the field at second to third instar progressed to pupation. This year, however, a number of larvae in third and fourth instars showed signs of illness with most of these dying. The infections were observed both in the laboratory and in the field. Dead larvae have been provided to the Entomological Research Museum at the University of California, Riverside for diagnosis, which we await.

The etiology of the infections indicate that the commonly reported caterpillar death from BT (*Bacillus thuringensis*) is not causal. BT, either by infection or ingestion of the toxin, is indicated by eversion of the gut from the anus. It is always fatal. The symptom we found was an extreme attenuation of the prothoracic segment with attendant malaise. About half the larvae with this condition recovered, however. The second symptom of disease (and always death) was malaise followed by desiccation. Retained individuals were submitted to UCR. The symptoms are likely viral, and if so, likely manageable by environmental manipulation.

Figure 10. Green hairstreak eggs on *Lotus scoparius*.

Photo: Daniel Soyka Beran © 2002

2.8. Controls

As partial control for the protocols followed, we confined two gravid female green hairstreaks (*Callophyrs affinis perplexa*) with potted deerweed in gallon containers. The females were collected at Manhattan Beach and confined separately. One container was maintained outside at Dr. Mattoni's residence in Santa Monica, the other was taken to DFSP after the initial oviposition. The females were collected on March 10. The females survived until April 3 and 10 respectively.

Although not closely related to PVB, the hairstreak is nevertheless in the same family, uses the same foodplant (deerweed), and has the same life history phenology. The species is not ant tended and is not cannibalistic. It is sympatric and synchronic with the PVB. There is no likely displacement competition in nature because of the extremely large foodplant base relative to density of either butterfly and the difference in food preference (leaves versus reproductive parts of the foodplant). However, both species are likely subject to the same predators and parasitoids although no data are available.

We chose the species as the simplest material available to compare efficacy and efficiency of our protocols for the PVB.

2.8.1. Oviposition

Hairstreak eggs are about half the size of the PVB and are generally laid deep within terminal buds of the foodplant. Thus the estimate of yield is inexact and likely an underestimate, confounded by the long adult life and extended laying period. We approximated counts after about ten days of start and after female death. We estimated about 120 at Santa Monica and 100 at DFSP (see Figure 10).

2.8.2. Larvae

The Santa Monica foodplant was confined to its screen cylinder top until April 15 when the top was removed. Extensive larval foraging was evident. Four additional one gallon deerweed plants were positioned around the original plant. A total of 26 fourth instar larvae were removed between April 30 and May 12 and placed, 2–3 each, in creamers with diet. Cuttings were placed in the creamers daily with frass removed. Three larvae accepted the diet and used it until pupation, burrowing deep into the medium. The larvae became a very light yellow-green color indicating lack of pigment compounds in the diet.

By May 14 seven pupated and an additional nine mature larvae were in cups. No parasitoids were recovered from any larvae or pupae to date even though tachinid flies are present in the area. Sixteen additional last instar larvae were counted on the remaining foodplant mass, now substantially depleted.

The DFSP foodplant was left in the laboratory by the north-facing window. A total of at least 50 young larvae were noted, but only 6 were recovered to permit pupation in creamers. The remainder wandered off because the foodplant condition in the laboratory was poor in absence of adequate light. Had the plant been kept outdoors, the results would likely have been as the Santa Monica group.

2.8.3. Conclusions

By comparison with the PVB rearing on container plants, the green hairstreaks were very successful. The difference, however, is likely a function of foodplant quality. The hairstreak plant was left outside and remained robust. As biomass was eaten, the four plants added around the original became occupied, with larvae remaining *in situ*. There was no confinement, although the larvae would have doubtless wandered away to pupate.

The use of individual container plants thus is a viable approach. To optimize the method, which should be used in part as an alternate to the tent cages, a small outdoor screen/plastic chamber should be used.

3. Discussion and conclusions

The results clearly establish the tent cage approach as most rewarding, although not without problems. The recovery of over half (80 of 140) of the large cage pupae from a single cage is troubling in terms of genetic variation. This breeding cycle has been a bottleneck that will require addition of wild individuals into the system in the future.

In terms of yield the tent protocol is more productive, but limitations on maintaining viable development of larvae in the gallon cages may be due only to constrains in laboratory conditions. If deerweed could be kept aphis-free and healthy in the laboratory, greater success would be possible. However, the use of a yet larger, walk-in, outdoor cage with either container or on-the-ground foodplants should be tested. Large cages provide not only a less costly, but mass selection more closely approximating natural conditions. Their limitations parallel those of any monoculture system with potential devastation by predation and disease.

Both materials and methods are illustrated as are both normal and aberrant adult specimens and views of larvae that were either diseased or otherwise distressed. The sick individuals have been preserved and an attempt to investigate viral contamination is being attempted through the Insect Pathology lab at the University of California, Riverside.

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5. Inventory of Equipment

Avanti Refrigerator
Model: 320YW/321YB
Serial Number: 4ACN201521

Backup Power UX612
Serial Number: AY00082

GE Water Heater
Model: GE6P6A
Serial Number: GE060126374

Davis Weather Station
Model: Monitor II

6. Appendix: Lycaenid Synthetic Diet (R. Mattoni, March 1999)

200 g dried green lentils
800 ml distilled water
Place in one liter stainless beaker.
Bring to boil and leave one hour.

Add in order:
9 g bacto-agar
25 g wheat germ
5 g bacto yeast extract
5 g Wesson salt mix
10 g cellulose flour (Solka floc or equiv.)
5 g sucrose
2 g ascorbic acid
2 g potassium sorbate
2 g methyl paraben
0.8 choline chloride
0.25 g B-sitosterol
0.25 g. chlortetracycline
0.25 g. 50% procaine penicillin
0.4 ml linseed oil

Heat mixture in boiling water bath (or double boiler on hotplate) until temperature reaches 85–90^{o} C, stirring occasionally.

Then place mixture directly over low heat (flame or electric element) stirring constantly until mixture just comes to boil (this is necessary to dissolve the agar or the diet will not solidify).

Cool to about 80º C.

Then CAREFULLY pour about 1/3 into (Waring) blender, blend for ten seconds, after initial splashing, continue pouring remainder into blender until all well blended (about 30 seconds).

Dispense into containers (use automatic pipette to dispense 5 ml aliquots into 1 oz creamers set in trays for easy handling), immediately cover with clean paper towels.

Refrigerate when set (about 15 minutes), enclosing trays in clean plastic bags.
Can be stored under refrigeration for 30-45 days.

ALTERNATIVES
May substitute baby lima beans or other beans for lentils.
May substitute 400 g. frozen peas with 400 ml distilled water for lentils and 800 ml water (the frozen peas are about 40% dry matter)
May substitute complete defined vitamin mixes for yeast extract.