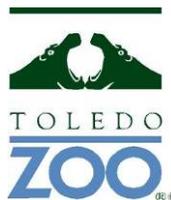


**Propagation Handbook for
the Karner Blue Butterfly,
Lycaeides melissa samuelis.
Fourth edition, October 2006**



**Department of Conservation and Research
The Toledo Zoo
P.O. Box 140130
Toledo, OH 43614-0801
(419) 385-5721 x 2112**



Introduction

The object of this propagation manual is the Endangered Karner blue butterfly (KBB), *Lycaeides melissa samuelis*, which has suffered catastrophic population declines in the U.S. over the past decade. The KBB has declined by 99% or greater over its range in the past 100 years (USFWS 1992), and an IUCN PHVA (IUCN 1992) estimated that 90% of this decline has occurred in the past 10-15 years. Within this period the KBB has been extirpated from Illinois, Massachusetts, Ohio, Ontario, and Pennsylvania. As our current KBB research season was nearing an end, we learned that the New Hampshire population was also extirpated. The KBB is a signature species of the extremely rare oak savanna ecosystem, a globally endangered habitat characterized by meadows of prairie plants dispersed among stands of widely spaced oak trees. Oak savanna in Ohio is limited to a small region of Northwest Ohio known as the Oak Openings. It was the last area in Ohio where the KBB was observed (Magdich 1989; Grigore and Windus 1994). The region, as well as the KBB, has national significance, and the dedication to restore the KBB to Ohio is shared by a powerful coalition of conservation partners in this project, which includes the U.S. Fish and Wildlife Service (USFWS), the Ohio Department of Natural Resources (ODNR), and The Nature Conservancy.

This handbook is not meant to be the last word in Karner blue butterfly propagation. Each season is a learning experience for us, and we constantly strive to apply results of our current research to KBB conservation breeding. We still have problems relating to low fertility in certain egg clutches, and questions remain about the factors contributing to fertility in captivity for this species. However, it is both possible and practical to mass-rear hundreds of KBBs for release or conservation breeding, housing pairs or groups of reproductive butterflies or larvae in individual pots containing living lupine and nectar plants. These techniques were developed in consultation with the USFWS and ODNR KBB Recovery Teams. There have been some excellent KBB rearing studies completed in the past and the reader is encouraged to consult them (See Lane and Welch 1993; Herms et al. 1996)

Propagation of host plant- *Lupinus perennis*

Local lupine seed is collected in July before dehiscence is complete. Seed is stored dry outdoors until the following spring. Immediately before planting seeds are scarified with medium grade sandpaper and are placed in hot water for 1 hour. Seeds are strained from the water and mixed with *Rhizobium* inoculate, type H (Prairie Moon Nursery, Winona, MN) and placed in a clear plastic box for 24 hr. in sunlight. The next day, seeds are planted in 2-gallon plastic pots in the following sand mix:

- 3-4 parts sand
- 1 part potting soil
- 1 part sphagnum peat
- 1 part small pine bark chips
- 1 part perlite



An alternate potting mix has been suggested by the Portage Valley Nursery in Holland, Ohio, consisting of one part sphagnum peat and one part small pine bark chips. Five seeds are planted per 2-gallon pot at a soil depth of 1/4 inch. Seeds are watered thoroughly every day in summer; once a week in the winter months.

Wild lupine is extremely susceptible to root rot, especially in the winter, and any soil mix must be well draining. We never water our plants thoroughly; instead we merely mist the plants and dampen the top 1 cm of soil. Plants are maintained under shaded poly in open air, and are fertilized every week throughout the growing season to increase protein content for larvae. Lupine quality was increased, e.g. lusher foliage, later senescence, and better survival, by growing them under DeWitt 55% horticultural shade cloth.

Pupae that metamorphosed from lupine plants that were fertilized once /week with Miracid® 30-10-30 fertilizer had statistically significantly higher body masses (Student's t test@ 0.001; $t = -6.364959$, $df = 96$) and significantly longer body lengths (Student's t test@ 0.001; $t = -3.650759$, $df = 98$) than pupae from unfertilized plants. This sample of 50 pupae raised on fertilized plants had mean body lengths of 9.2 mm and mean body masses of 64.1 mg. In contrast, a sample of 49 pupae raised on unfertilized plants had a mean body length of 8.9 mm and a mean body mass of 52.9 mg. Some recent research has also suggested that protein deficiency reduces lifespan in certain pollen and nectar feeding insects (Schmidt 1998) and that fecundity in some lepidoptera may be limited by protein obtained as larvae (Stamp et al. 1993) or adults (Labine, 1986; Pierce 1985; Dunlap-Pianka 1995).

Spider mites commonly infest cultivated lupine. In the event of spider mite or white fly infestation the foliage is cleansed with a solution of dish soap (1/4 tsp./cup) and water applied with a spray bottle. The treated plants should be set aside for several days and then thoroughly rinsed with tap water before allowing them to be used by larvae.

Collection

The Karner blue 1st brood flight period usually begins the second week in May at our latitude and lasts for three weeks. We consult with the Michigan Department of Natural Resources staff biologists at the Allegan State Game Area in early May to learn when the flight has commenced. Our first collection begins on a morning one-week after the first KBB sighting in the spring. Then a collection date is scheduled one morning each week after that until the adequate number of females has been obtained. A collection of wild caught females is assembled from several different locations to insure genetic diversity.



The females also collected on different dates, spaced approximately one week apart to insure that the majority of the females collected were recently emerged and gravid. Our greatest egg production has always been from females collected during the second week of the flight. The females are captured with a large butterfly net and transferred to a 10 cm x 10 cm x 18 cm transparent, plastic container (AMAC Plastics Products Corp., Sausalito, CA) marked with the date and capture locality. An artificial nectar source is fastened inside of each plastic container consisting of a rubber-capped florist tube with a cotton wick protruding through the cap filled with a 10% clover honey/water solution. This allows the butterflies to have access to some nourishment and prevents dehydration during the transport. This is especially important, as recently emerged females may not have had a chance to feed.

Transport

The plastic containers are placed within thick-walled 32 quart Styrofoam coolers that contain a single ice pack separated from the butterfly containers by bubble-pack insulation. The temperature inside the cooler was maintained at $<20^{\circ}\text{C}$ (because of the chill and darkness the butterflies are less active and therefore less likely to batter themselves en route). A data logger is placed inside of the cooler to record the temperature. A digital electronic thermometer with a probe is used to monitor the cooler temperature in real time. Readings are taken every 30 minutes. Following collection and storage the butterflies are immediately brought back to the holding area at the zoo. We have maintained a 100% survival rate for collection and transport using this method.

Holding and care of adults



Upon arrival at the Zoo the butterflies are placed into an enclosure consisting of a mesh-covered potted lupine plant in a 2-gallon plastic pot. This covering is a cylinder of white poly mesh netting # 65-50 (Jason Mills, Westwood, NJ) sewn together with the seams arranged on the outside of the enclosure to assure that none of the butterflies can become trapped in the seam and harm themselves. Lycaenids tend to walk into corners and crevices and may become trapped. The nets fit snugly over the pot rim, and are secured with a # 107 (7" x 5/8" x 1/16") rubber

band to prevent escape of butterflies and to deny entrance to predators. Each pot is numbered with a stamped metal tag identifying each butterfly with a studbook number. Each mesh-covered lupine plant contains a small 3" pot of *Lantana camara* or *Pentas sp.* as a nectar source. These plants were chosen because of their short flower tubules, for their long blooming period, and availability. We normally purchase the nectar plants from commercial nurseries. It is extremely important that the plants be pesticide-free before exposing butterflies to them. An additional nectar source is provided from a rubber-capped florist tube with a cotton wick protruding through the cap filled with a 10% clover honey/water solution (the same unit used as artificial nectar source in transport). This is placed in the soil of the potted plant. The artificial nectar source is refilled every day and replaced and disinfected in diluted common household bleach every other day. Disinfected tubes and wicks are rinsed 10x in tap water and 5x in distilled water before being reused. We recommend daily hand feeding of adults to maximize longevity. In this process, adults are encouraged to climb on the honey-moistened wicks of the feeding tubes. They are then gently placed with the tubes in one of the 10 cm x 10 cm x 18 cm transparent plastic containers use for transport. After the proboscis is withdrawn, the butterflies are placed back into their netted enclosures. For breeding males, a slurry of animal dung should be provided to help provide essential nutrients for the sperm packet.

Wild caught females are monitored every day for egg laying. An official egg count is performed every 2nd day. Eggs are counted by visually inspecting each leaflet and stem of the lupine plant. The undersides of the leaflets are viewed with a dentist mirror. One must make sure that the nectaring plants are adequately watered to insure that they are producing nectar. The condition of the host plant and



nectar plant is also monitored every other day. We have estimated the ideal carrying capacity of each healthy host plant to be between 10-12 larvae/lupine. When 10 eggs have been laid the female is moved to a new host plant. However, some females may lay over 50 eggs in a single night. Nectar plants that are replaced when the flower heads are spent should be also checked for eggs. We prefer to remove eggs by simply clipping off the area of foliage containing the egg/s and placing it in the host plant pot. In the event that a host plant should become infected with spider mites, the foliage supporting eggs can be gently washed, clipped from the infected plant, and transferred to a healthy host plant. Loose eggs, such as those oviposited on the substrate, can be gently transferred with a damp #2 camel hair artist brush.

It is important to maintain humidity in the enclosures. To prevent dehydration the mesh-covered enclosures are misted by hand or, during periods of heat and low humidity, by an Ecologic Technologies® Rainmaker misting system (Ecologic Technologies, Pasadena, MD). The Rainmaker was set up to mist for a 2-minute duration every 15 min. from 1100-1800 h. In extremely hot weather (> 30° C) a garden soaker hose is placed on the cement pad near the enclosures to provide added humidity through evaporation. Cool temperatures and low light intensity may prevent females from oviposition. The enclosures are arranged on shelves in an area with high light intensity and on overcast days quartz lighting was provided. In years when there are low temperatures in late May and early June a large propane heater is also employed to warm the polyhouse.

Inventory and holding of larvae/pupae

The wild-caught females are assigned a house studbook number maintained in an informal Microsoft Excel® studbook with such information as their capture location, capture date and fecundity. A running total is kept for all



eggs, larvae, pupae, and adults produced by a given female in the studbook. In addition to the studbook, a data sheet is produced for each



female each time the eggs, larvae, pupae, or 2nd flight adults are counted. Examples of a studbook page and the data sheets are provided in Appendix A. A numbered metal tag is attached to the pot of the first mesh-covered lupine plant used. When that lupine plant is replaced for a given female, a secondary number is assigned to the next pot to identify the founder of each individual set of eggs. For example, the first pot for female # 45 would have a numbered metal tag reading "45". After 10-12 eggs/lupine have been laid then the next pot used for that female would be affixed with a plastic tag reading "45A", then, for the next "45B", and so on. This enables us to easily keep individualized founder information on each set of eggs. This also allows us to look back and attempt to determine why a certain set of eggs did not hatch, or why certain pupae lived and others, in a different pot from the same female, did not. See Table I.

Studbook number	Capture date	Death date	Locality	Pot or Enclosure	Number of eggs	Number of larvae	Number of pupae	Number of adults	As of
116	22-May	13-Jun	Pipeline	116, A, B, C	6	0	0	0	25-May
				D	14	0	0	0	28-May
					15	0	0	0	30-May
					16	0	0	0	1-Jun
					19	1	0	0	4-Jun
					24	9	0	0	5-Jun
					27	10	0	0	6-Jun
					35	13	0	0	8-Jun
					41	15	0	0	11-Jun
					42	30	0	0	13-Jun
					57	36	0	0	15-Jun
					57	36	1	0	20-Jun
					57	43	5	0	22-Jun
					57	47	13	0	25-Jun
					57	47	17	0	27-Jun
					57	47	23	0	29-Jun
					57	47	27	8.1	2-Jul
					57	47	29	9.5	4-Jul
					57	47	30	13.7	6-Jul
					57	47	32	14.8	9-Jul
					57	47	32	15.14	11-Jul
					57	47	32	15.15	13-Jul
					57	47	32	15.16	16-Jul
					57	48	32	15.17	17-Jul

Table I. Informal studbook for 1st flight female Karner blue butterflies.



The larvae and pupae in each pot are counted and recorded three times a week on the data sheet and a running total is kept for each instar. We found that larval survival was enhanced when they were kept in Rubbermaid plastic box with a substrate of paper toweling. Larvae in the boxes were fed fresh lupine clippings placed in a rubber-capped florist tube. Condition of larvae and pupae are noted. For example, pupae are green as pupation occurs, transform to a brown color as they age, and finally become semi-transparent when exclosure is imminent. At this time, the adult coloration, particularly the purple hues of the male, are visible through the semi-transparent cuticle of the pupa.

Larval counts will often fluctuate from day to day, as larvae will disappear, then reappear. They often move in and out of the soil around the root system and between the pot wall and the soil. Larvae are transferred to new host plants as leaves are denuded. It is important not to overcrowd the larvae or to keep larvae of differing sizes on the same host plant. Larger larvae can and will cannibalize their smaller conspecifics. As the larvae approach the 3rd and 4th instars they tend to become more mobile and will often crawl off

the plant. It appears that in the fourth instar the innate behavior to find a perfect place to pupate will drive them to migrate more than usual. In captivity, they can often be found in dangerous places like the outside pot edge, under the pot, up in the net, or on the substrate near the pot. This makes it extremely easy to inadvertently smash the larvae or pupae. For this reason, extra care should be taken during the 4th instars when moving or handling the netted lupine enclosures. When pupation is imminent, pots are provided with several large pine bark chips. Fourth instar larvae often will pupate under these refugia. As soon as 2nd brood adults emerge they are sexed and recorded in the studbook. However, 2nd flight adults are not assigned their own studbook number- they are only identified by their mother's studbook number and locality. A running total of the 2nd flight adults is also kept under the studbook entry for the mother.

Larval synchronization

Oviposition by wild and captive KBB females typically spans a three-week period. Thus, individuals hatched from eggs oviposited early in the season metamorphose three weeks before individuals from the late hatches. In order to maximize opportunities for mating in captivity it would be advantageous to have metamorphosis synchronized so that maximum numbers of adults emerge within 1-3 days of each other. As cooling will retard metamorphosis, we tested whether cooling can be used as a tool to synchronize emergence. Fourteen 4th instar KBB larvae were cooled slowly to a regime of 5° night temperature and 10° C day temperatures for one week on potted lupine plants housed in Percival® E-36 plant growth chambers. As no mortality occurred, we extended the cooling period for an additional week. We were able to show that cooling indeed retarded emergence, although not without some risk. Although no larvae died during the experiment, six of the 14 died as pupae and failed to metamorphose. Table II shows that the mean number of days from larva to adult increased 8 days when compared to the control group.

Means of pots cooled at 5° C and 10° C	Means of pots not cooled
42.00 days egg to adult	32.75 days egg to adult
35.50 days larva to adult	26.25 days larva to adult
26.50 days larva to pupa	18.50 days larva to pupa
10.50 days pupa to adult	8.75 days pupa to adult



Table II. Number of days from life stage to adult in cooled vs. uncooled Karner blue butterfly larvae.

Predator control

Predators threaten KBBs in every stage of the life cycle. Spiders are the primary threats to larvae, pupae, and adults. Centipedes, which live in the soil, are a secondary threat. Ants, particularly the tiny *Monomorium emarginatum*, will predate KBB eggs. They may actually cut the egg almost in half, or cut a hole in the egg through which they

pull the larva. They are most active in the morning and in the evening. Although *Myrmica sp.* ants protect KBB larvae in the wild, they will also predate the eggs.

Trichogrammid wasps parasitize KBB eggs. Up to 7 wasp larvae may be deposited in each egg (Spor, pers. comm.).

No pesticides are ever used in the polyhouse or on the host or nectar plants. We depend on vigilance and exclusion tactics to protect our butterflies. Each lupine plant must be thoroughly checked (i.e. examination of each leaflet) for predators before placing a KBB of any life stage upon it. Polyester netting covering the plants excludes predators, but each plant is checked every 2-3 days to ensure that no predators have entered the pots nor have hatched from undetected eggs. Any detected predators are simply crushed with our fingers.

Adult care and breeding

Second brood adults are placed into breeding enclosures that are essentially individual camping tents with “no see-um” mesh. (Tropic Screen II®, Bioquip Products, Inc., Gardena, CA).

These mesh tents have a nylon floor and are placed approximately 4" off of the cement pad of the polyhouse by plastic shipping pallets to keep them dry and allow ventilation. This prevents the formation of dangerous pools of water (that will catch and drown small butterflies) or harmful mildew growth. The parabolic shape of the tent prevents the adults from getting caught in corners or on the peak as they would with a conventional tent. The breeding enclosure (mesh tent) is



equipped with six large, healthy lupine plants, six large *Lantana camara*, *Pentas sp.* or *Asclepias tuberosa* plants, and two large artificial nectar sources placed on pedestals. These are fashioned from disinfected 4" diameter white scrub pads cut to fit into 4" diameter Petri dishes and then saturated with the 10% clover honey/water solution. Elevating the artificial nectar source on a pedestal prevents the attraction of harmful pests like ants to the breeding enclosure and makes them more visible to the butterflies.

Males and females from the same locality (10.10) are placed within the breeding enclosures for mating and oviposition. Under this system females have the opportunity to serially mate. Serial mating may be important in this species, as we have seen egg fertility decline in subsequent clutches of wild-mated females (See Drummond 1984; Oberhauser 1974; Rutkowski et al 1997; Wannatabe 1988).

The adults are provided with a timed overhead misting system that was previously described under "holding and care of adults". Dead adults are removed from the floor of the tent on a daily basis and replaced by new butterflies. It is difficult to count eggs oviposited in the tents because of the potential harm to the breeding adults. Oviposition is estimated by viewing the host plants through the mesh and determining whether or not adequate numbers of eggs have been laid. After the target numbers of eggs are reached for each locality (usually 50 eggs), the remaining 2nd brood adults are released at the reintroduction site. Deaths and daily releases of 2nd brood adults are recorded on a daily basis and tracked on a separate chart and a tag on each tent.

In the 2001 field season we discovered that adult longevity was significantly increased when the adults were hand fed a 10% clover honey solution daily. Butterflies are encouraged, by gentle nudging, to walk on the saturated wick of one of our artificial nectar tubes. They usually will immediately commence feeding. Using this technique, we have increased adult survival to as much as four weeks in the breeding facility.

2nd flight egg over-wintering protocol



Data collected from the field at the Allegan State Game area indicates that over-wintering eggs in the duff are subjected to very high humidity in the winter, usually 98-100% Relative Humidity (RH) with infrequent spikes to lows of 80 % RH in a near-condensing atmosphere. Temperatures under snow cover are remarkably stable- 0° C to -5° C. Our best 2nd brood hatching success (ca. 40% hatching) has occurred under these conditions. We place our eggs in Mason jars containing a 2" diameter insert constructed

from Plexiglas tubing. The tubing has a support of chiffon fabric 1" below the lip of the tubing. To construct this support, a 9 " section of tubing is sawn into two pieces- one of 1" length, and one of 8" length. A circular patch of fabric is glued between these two pieces of tube using Silastic aquarium cement. Replacing the dome lid with a chiffon fabric insert glued into the band with silicone aquarium cement further alters the Mason jar and protects the eggs from marauding ants. Ten to 12 eggs are placed in each jar and the jar is labeled with the number of eggs and the collection locality of the parents. Eggs are whitish green when deposited but change color to a dirty gray as they over-winter.



This color change is normal. In the extremely hot and dry months of July and August, ca. 200 ml of distilled water is added to the bottom of each jar to keep humidity levels high for the eggs. Care must be taken to ensure that no water condenses around the eggs as the chiffon fabric insert in the band may inhibit evaporation, particularly under rainy conditions when ambient RH is high. Lids can be removed from jars with condensation to allow condensed water to evaporate. Jars should never be left without tops overnight, as the eggs may be predated or parasitized. Jars can be placed in a shaded location protected from rain. We prefer to bury the jars, protected by covering them with a sheet of polyfilm, beneath snow cover

when possible.

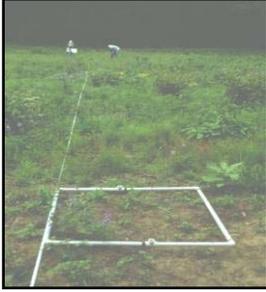
Release protocol

Second brood adults that have previously been in breeding enclosures or are freshly eclosed are transported to the reintroduction site in a mesh-covered pot containing lupine and a nectar plant. They are released in early afternoon in fair to good weather conditions in an area where there are adequate nectar and host plants.



Evaluation of release sites

Site selection-sites on KTP were chosen to represent a spectrum of lupine habitat, including mature oak savanna (Oak Dune, Julia's Savanna), lupine partially shaded by secondary forest (Bond Tract) and lupine in full sun on essentially treeless dunes (North Piels, South Piels). These sites included one which formerly supported populations of the



Karner blue (Bond Tract), two which were not known to have KBBs but which support populations of the frosted elfin, *Callophrys irus*, and the Persius dusky wing, *Erynnis persius* (Oak Dune, Julia's Savanna), and two which have extensive patches of lupine but which do not support populations of any lupine-dependent butterflies (North Piels, South Piels). Data was collected concurrently in the Allegan State Game Area, Allegan County, Michigan, at three sites that support reproducing populations of the KBB (42nd St., Gun Club, and Pipeline). Sites

were chosen in the Allegan State Game Area based on their similarity in vegetation structure and species composition to those sites on the Kitty Todd Preserve that once supported populations of the KBB.

Methodology- percent canopy cover and density, frequency, and phenology of lupine and nectar plants were quantified at the sites described above. These data were collected in randomly selected 0.5m² quadrats using the transect-quadrat method of Bonham (1989) at the density of one transect / 850m² (Papp 1996). Density of lupine was calculated as the number of lupine stems/ m² within each quadrat. Brood 1 and brood 2 nectar plant densities were calculated as the number of flowering stems of nectar plants / m² within each quadrat (see Tables II and III for species lists of first and second brood nectar plants at KTP and the ASGA). Canopy cover was calculated using a crown densiometer. The number of hits per transect (one reading for each quadrat) were divided by the total number of quadrats at the site to give the percentage of canopy cover (Tolson 1998).



Remote computerized data loggers were programmed to take data readings every 30 minutes for surface temperature, solar radiation, and relative humidity at the level of the lupine crowns (i.e. ca. 5.0 cm above soil surface). Equipment used was Onset Computer Corp.'s StowAway temperature logger model STIB-37+46, StowAway relative humidity logger model SRHA-32, and Hobo light intensity logger model HLI. Each site had one each of the listed data loggers. Data were downloaded weekly, using the Onset Computer Corp. Stowaway and Logbook software, to a laptop computer. Percent canopy cover of oak and invasive secondary forest was also quantified at each site. Canopy readings were taken weekly over the season of activity for the KBB (normally May-July) as the canopy emerged.

Analysis- Data on nectar plant resource availability were recorded and analyzed separately for first and second brood KBBs. Comparisons between locations (Michigan and Ohio) were made using an independent measures t-test and analysis of variance (ANOVA). Tests were performed to determine differences among sites within each location. ANOVA was followed up with the Tukey test to determine which sites within ASGS and KTP varied from each other. The two highest potential reintroduction sites on KTP, i.e. those with the highest mean densities of lupine and nectar plants (Bond Tract, Julia's Savanna), were compared with each of the ASGA sites independently using an independent measures t-test.

Species	Common name	Present at ASGA	Present at KTP	Exotic
<i>Amelanchier spicata</i>	Dwarf serviceberry	?	Yes	No
<i>Arabis lyrata</i>	Lyre-leaved rock cress	Yes	Yes	No
<i>Arenaria stricta</i>	Rock sandwort	Yes	?	No
<i>Berteroa incana</i>	Hoary alyssum	Yes	?	No
<i>Fragaria virginiana</i>	Wild strawberry	Yes	Yes	No
<i>Geranium maculatum</i>	Wild geranium	?	Yes	No
<i>Helianthemum canadense</i>	Frostweed	Yes	Yes	No
<i>Hieraceum pillosella</i>	Mouse-ear hawkweed	Yes	No	No
<i>Krigia virginica</i>	Dwarf dandelion	Yes	Yes	No
<i>Lithospermum canescens</i>	Hoary puccoon	?	Yes	No
<i>Lithospermum caroliniense</i>	Golden puccoon	Yes	Yes	No
<i>Lupinus perennis</i>	Wild lupine	Yes	Yes	No
<i>Potentilla sp.</i>	Cinquefoil sp.	Yes	Yes	No
<i>Rosa carolina</i>	Rose	Yes	Yes	Yes
<i>Rubus sp.</i>	Dewberry	Yes	Yes	No
<i>Tephrosia virginiana</i>	Goat's rue	Yes	Yes	No
<i>Viola pedata</i>	Birdfoot violet	Yes	Yes	No

Table III. Inventory of brood 1 nectar plants at ASGA and KTP utilized by the Karner blue butterfly.

Species	Common name	Present at ASGA	Present at KTP	Exotic
<i>Achillea millefolium</i>	Yarrow	Yes	Yes	Yes
<i>Asclepias tuberosa</i>	Butterfly milkweed	Yes	Yes	No
<i>Asclepias verticillata</i>	Whorled milkweed	Yes	Yes	No
<i>Baptisia tintoria</i>	Yellow indigo	Yes	Yes	No
<i>Ceanothus americanus</i>	New Jersey tea	Yes	Yes	No
<i>Centaurea maculosa</i>	Spotted knapweed	Yes	No	Yes
<i>Coreopsis lanceolata</i>	Lance-leaved coreopsis	Yes	Yes	No
<i>Dianthus armeria.</i>	Deptford pink	Yes	Yes	Yes
<i>Erigeron annuus</i>	Daisy fleabane	Yes	Yes	Yes
<i>Euphorbia corollata</i>	Flowering spurge	Yes	Yes	No
<i>Helianthus divaricatus</i>	Woodland sunflower	Yes	Yes	No
<i>Helianthus occidentalis</i>	Western sunflower	Yes	Yes	No
<i>Hieraceum aurantiacum</i>	Orange hawkweed	Yes	No	Yes
<i>Hieraceum pratense</i>	Yellow hawkweed	Yes	No	Yes
<i>Hypericum perforatum</i>	St. Johnswort	Yes	Yes	Yes
<i>Lespedeza sp.</i>	Bush clover	Yes	Yes	No
<i>Liatris aspera</i>	Blazing-star	No	Yes	No
<i>Liatris cylindracea</i>	Cylindric blazing-star	Yes	No	No
<i>Lotus corniculatus</i>	Birdsfoot trefoil	Yes	?	No
<i>Monarda fistulosa</i>	Wild bergemot	Yes	Yes	No
<i>Monarda punctata</i>	Dotted horsemint	Yes	Yes	Yes
<i>Polygala polygama</i>	Racemed milkwort	Yes	Yes	No
<i>Rudbeckia hirta</i>	Black-eyed Susan	Yes	Yes	No
<i>Solidago sp.</i>	Goldenrod	Yes	Yes	No
<i>Specularia perfoliata</i>	Venus looking-glass	Yes	?	Yes
<i>Vicia cracca</i>	Cow vetch	Yes	?	Yes

Table IV. Inventory of brood 2 nectar plants at ASGA and KTP utilized by the Karner blue butterfly.

Monitoring

Monitoring for 1st flight KBBs begins approximately the third week in May. The transect area is searched each day except in the rain until no butterflies have been sighted for at least three days. It is then suspended until the 2nd brood adults emerge, approximately 28 days after the 1st flight. Then monitoring resumes again in the same fashion until three days after the last 2nd flight KBB has been seen. Emigration from the release site is determined by carefully walking a transect route for each site in our reintroduction area with significant stands of lupine. The transect areas are always searched by two spotters. The primary release site is the major transect area. This area has a plot of 120m x 100m with transect widths of 10m. In this primary area a spotter walks every 5m in alternating directions with each other. Secondary transect sites consist of ca.20m wide strips. The route went through the sites, alternating direction from one strip to the next until the entire site was covered. Sightings of KBBs are plotted on a topographic map of the preserve.

Acknowledgements

The Toledo Zoo is grateful for the generous financial support from the following agencies that are allowing the Ohio KBB recovery effort to be accomplished: The Ohio Department of Natural Resources, Division of Wildlife; The Institute of Museum and Library Services; and The U.S. Fish and Wildlife Service. Without adequate habitat, no recovery would be possible, and we gratefully acknowledge the unceasing activities of the Nature Conservancy's Oak Openings Program Office staff and the Department of Natural Resources, Division of Natural Areas and Preserves, in securing a place for the KBB in Ohio. Several entities and individuals have provided invaluable counsel and advice as the recovery effort has progressed: The Ohio KBB Recovery Team, particularly Carolyn Caldwell and Bill Roshak; The USFWS Recovery Team, particularly Cathy Carnes; The Michigan Department of Natural Resources, particularly Maria Albright of the Allegan State Game Area and Mary Rabe and David Cuthrell of the Michigan Natural Features Inventory.

Literature Cited

- Baker, H.G. and I. Baker. 1983. A brief historical review of the chemistry of floral nectar. *The Biology of Nectaries* (eds. B.L. Bentley and T.S. Elias), pp. 126-152. Columbia University Press, New York.
- Bonham, C.D. 1989. Measurements for terrestrial vegetation. John Wiley & Sons, Inc., New York, New York. 338pp.
- Drummond, B.A. 1984. Multiple mating and sperm competition in the Lepidoptera. In: *Sperm Competition and the Evolution of Animal Mating Systems* (ed. R.L. Smith), pp. 291-371. New York: Academic Press.
- Dunlap-Pianka, H., C.L. Boggs and L.E. Gilbert. 1977. Ovarian dynamics in Heliconiine butterflies: programmed senescence versus eternal youth. *Science* 197:487-490.
- Goodpasture, C. 1974. Foodplant specificity in the *Plejebus* (*Icaricia acmon* group Lycaeninae) J. Lepid. Soc. 43:68-71.
- Grigore, M.T. and J.L. Windus. 1994. Decline of the Karner blue butterfly in the Oak Openings of Northwest Ohio. . Pp. 135-142. In: Andow, D., R. Baker, and C. Lane (eds.) Karner Blue Butterfly: a Symbol of a Vanishing Landscape. Misc. Publ. 84-1994, Minnesota Agr. Expt. Station

- Herms, C.P., D.G. McCullough, D.L. Miller, L.S. Bauer, and R.A. Haack 1996. Laboratory rearing of *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae), an endangered butterfly in Michigan. *Great Lakes Entomologist* 29(2)63-75.
- IUCN 1992. Karner Blue Butterfly Population Habitat & Viability Analysis. The Wilds and IUCN/SSC Captive Breeding Specialist Group, Zanesville, Ohio.
- Labine, P.A. 1968. The population biology of the butterfly *Euphydryas editha*. VIII. Oviposition and its relation to oviposition in other butterflies. *Evolution* 22:799-805.
- Lane, C.P. and R. Welch. 1993. Rearing Karner blue butterflies for experimentation, re-establishment and supplementation. Report to Wisconsin Department of Natural Resources. 18p.
- Magdich, M.L. 1989. A survey of Karner blue (*Lycaeides melissa samuelis* Nabokov) populations in the Oak Openings and Northwest Ohio. Final Report submitted to Division of Natural Areas and Preserves, Ohio Department of Natural Resources. 47 p.
- Oberhauser, K.S. 1974. Effects of spermatophores on male and female monarch butterfly reproductive success. *Behav. Ecol. Sociobiol.* 25:237-246.
- Papp, C. 1996. The endangered Karner blue butterfly (Lepidoptera: Lycaenidae) in Michigan: habitat suitability, potential impacts of gypsy moth (Lepidoptera: Lymantriidae) suppression, and laboratory rearing. MS Thesis, Michigan State University. 180pp
- Pierce, N.E. 1985. Lycaenid butterflies and ants: Selection for nitrogen-fixing and other protein-rich foodplants. *American Nat.* 125:888-895.
- Stamp, N.E.; Casey, T.M. (eds.). 1993. Caterpillars: ecological and evolutionary constraints on foraging. New York: Chapman and Hall; 587 p.
- Rutkowski, R.L., Gilchrist, G.W., and B. Terkanian. 1987. Female butterflies mated with recently mated males show reduced reproductive output. *Behav. Ecol. Sociobiol.* 20:319-322.
- Tolson, P.J. 1998. Microhabitat Analysis of Selected Sand Barren Areas in the Kitty Todd Preserve, Oak Openings, Lucas County, Ohio: Evaluation for Potential for Reintroduction of the Karner Blue Butterfly. Final Report submitted to Division of Wildlife, Ohio Department of Natural Resources. 9p.
- Tolson, P.J., M.L. Magdich, and C. L. Monak. 2001. Captive Breeding and Reintroduction of the Karner Blue Butterfly, *Lycaeides melissa samuelis*, to the Oak Openings of Lucas County, Ohio. Interim Report submitted to U.S. Fish and Wildlife Service. 10p. Research Service, USDA, Washington, DC.
- Wannatabe, M. 1988. Multiple matings increase the fecundity of the yellow swallowtail butterfly, *Papilio xuthus*, L. in summer generations. *J. Ins. Behav.* 1:17-30.
- White, J.W. Jr. and O.N. Rudyi. 1978. The protein content of honey. *J. Apicul. Res.* 17(4) 234-1617.
- USFWS 1992. Endangered and threatened wildlife and plants: determination of endangered status for the Karner blue butterfly. *Federal Register* 57(240): 59236-59244.
- Schmidt, J.O. 1998. All pollens are not created equal. *Agri. Res. Oct.* 1998. p23.

Bibliography

Andow, D.A., R.A. Baker, and C.P. Lane 1994. Karner blue butterfly- symbol of a vanishing landscape. Pp. 1-2. *In: Andow, D., R. Baker, and C. Lane (eds.) Karner Blue Butterfly: a Symbol of a Vanishing Landscape. Misc. Publ. 84-1994, Minnesota Agr. Expt. Station.*

Andow, D.A., R.J. Baker, and C.P. Lane. 1994. Research needs for management and recovery of Karner blue butterfly Pp. 209-218 *In: Andow, D., R. Baker, and C. Lane (eds.) Karner Blue Butterfly: a Symbol of a Vanishing Landscape. Misc. Publ. 84-1994, Minnesota Agr. Expt. Station.*

Atstatt, P.R. 1981. Lycaenid butterflies and ants: selection for enemy-free space. *Amer. Nat.* 118:638-654.

Bleser, C.A. 1994. Karner blue butterfly survey, management and monitoring activities in Wisconsin: 1990-Spring 1992. Pp: 153-162 *In: Andow, D., R.J. Baker, and C.P. Lane (eds.) Karner Blue Butterfly a Symbol of a Vanishing Landscape. Misc. Publ. 84-1994, Minnesota Agr. Expt. Station.*

Cushman, J.H. and D.D. Murphy 1993. Conservation of North American Lycaenids- an overview. Pp.37-44 *In: T.R. New (ed.) Conservation Biology of Lycaenidae (Butterflies). IUCN, Gland, Switzerland.*

Dethier, V.G. 1959. Egg-laying habits of Lepidoptera in relation to available food. *Canad. Entomol.* 9:554-561.

Dirig, R. 1994. Historical notes on wild lupine and the Karner blue butterfly at the Albany Pine Bush, New York. Pp. 23-36. *In: Andow, D., R. Baker, and C. Lane (eds.) Karner Blue Butterfly: a Symbol of a Vanishing Landscape. Misc. Publ. 84-1994, Minnesota Agr. Expt. Station.*

Dirig, R. and J.F. Ryan 1976. The Karner blue project: January 1973 to December 1976. *Atala* 4:22-26.

Goodpasture, C. 1974. Foodplant specificity in the *Plejebus (Icaricia acmon* group Lycaeninae) *J. Lepid. Soc.* 43:68-71.

Grigore, M.T. and J.L. Windus. Decline of the Karner blue butterfly in the Oak Openings of Northwest Ohio. . Pp. 135-142. *In: Andow, D., R. Baker, and C. Lane (eds.) Karner Blue Butterfly: a Symbol of a Vanishing Landscape. Misc. Publ. 84-1994, Minnesota Agr. Expt. Station.*

Grundel, R., N.B. Pavlovic and C.L. Sulzman 1998. The effect of canopy cover and seasonal change on host plant quality for the endangered Karner blue butterfly (*Lycaeides melissa samuelis*). *Oecologica* 114:243-250.

Grundel, R., N.B. Pavlovic and C.L. Sulzman 1998. Habitat use by the endangered Karner blue butterfly in oak woodlands: the influence of canopy cover. *Biol. Cons.* 85:47-53.

Herms, C.P. 1996. The endangered Karner blue butterfly (Lepidoptera: Lycaenidae) in Michigan: habitat suitability, potential impacts of gypsy moth (Lepidoptera: Lymantriidae) suppression and laboratory rearing. MSc. Thesis, Michigan State University, 180p.

Herms, C.P., D.G. McCullough, D.L. Miller, L.S. Bauer, and R.A. Haack 1996. Laboratory rearing of *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae), an endangered butterfly in Michigan. *Great Lakes Entomologist* 29(2):63-75.

IUCN 1992. Karner Blue Butterfly Population Habitat & Viability Analysis. The Wilds and IUCN/SSC Captive Breeding Specialist Group, Zanesville, Ohio.

Lane, C.P. and R. Welch 1993. Rearing Karner blue butterflies for experimentation, re-establishment and supplementation. Report to Wisconsin Department of Natural Resources. 18p.

Magdich, M.L. 1989. A survey of Karner blue (*Lycaeides melissa samuelis* Nabokov) populations in the Oak Openings and Northwest Ohio. Final Report submitted to Division of Natural Areas and Preserves, Ohio Department of Natural Resources. 47 p.

Martin, M.L. 1994. Karner blue butterfly in Indiana: 1990 status survey for the U.S. Fish and Wildlife Service. Pp: 97-106 *In*: Andow, D., R.J. Baker, and C.P. Lane (eds). Karner Blue Butterfly a Symbol of a Vanishing Landscape. Misc. Publ. No. 84.

New, T.R. 1993. Introduction to the Biology and Conservation of the Lycaeninae. Pp. 1-21 *In*: T.R. New (ed.) Conservation Biology of Lycaenidae (Butterflies). IUCN, Gland, Switzerland.

Opler, P.A. and G.O Grizek. 1984. Butterflies east of the Great Plains. The John Hopkins University Press, Baltimore. 194p.

Schweitzer, D.F. 1989. Fact sheet for the Karner blue butterfly with special reference to New York. Report to The Nature Conservancy, New York Field Office. 7pp.

Schweitzer, D.F. 1994. Recovery goals and methods for Karner blue butterfly populations. Pp. 185-194 *In*: Andow, D., R.J. Baker, and C.P. Lane (eds). Karner Blue Butterfly a Symbol of a Vanishing Landscape. Misc. Publ. No. 84.

Spoor, R.P. and E.P. Nickles. 1994. Fate of eggs of second brood Karner blue butterfly females, pp. 47-52 *In*: Andow, D., R. Baker, and C. Lane (eds.) Karner Blue Butterfly: a Symbol of a Vanishing Landscape. Misc. Publ. 84-1994, Minnesota Agr. Expt. Station.

Tolson, P.J. 1998. Microhabitat Analysis of Selected Sand Barren Areas in the Kitty Todd Preserve, Oak Openings, Lucas County, Ohio: Evaluation for Potential for Reintroduction of the Karner Blue Butterfly. Final Report submitted to Division of Wildlife, Ohio Department of Natural Resources. 9p.

Tolson, P.J., M.L. Magdich, and C.L. Monak. 1998 Captive Breeding and Reintroduction of the Karner Blue Butterfly, *Lycaeides melissa samuelis*, to the Oak Openings of Lucas County, Ohio. Interim Report submitted to U.S. Fish and Wildlife Service. 10p.

USFWS 1992. Endangered and threatened wildlife and plants: determination of endangered status for the Karner blue butterfly. Federal Register 57(240): 59236-59244.

Wilsmann, L.A. 1994. Status of the Karner blue butterfly in Michigan. Pp. 107-112 *In*: Andow, D., R.J. Baker, and C.P. Lane (eds). Karner Blue Butterfly a Symbol of a Vanishing Landscape. Misc. Publ. No. 84.

Appendix A

Amac Plastic Products
P.O. Box 750249
Petaluma, CA 94975-0249
(800) 852-7158
www.amacbox.com

BioQuip Products
17803 Lasalle Avenue
Gardena, CA 90248-3602
(302) 324-0620
www.biobook.com

Carolina Biological Supply
P.O. Box 6010
Burlington, NC 27216-6010
(800)-334-5551
www.carolina.com

Ecological Technologies
P.O. Box 1038
Pasadena, MD 2123-10338
(410) 431-7106
www.cloudtops.com

Forestry Suppliers, Inc
205 West Rankin Street
P.O. Box 8397
(800)-752-8460
www.forestry-suppliers.com

Jason Mills, Inc
349 Kinderkamack Road
Westwood, NJ 07675
(201) 358-6500
www.jasonmills.com

Onset Computer Corporation
536 Macarthur Blvd.
P.O. Box 3450
Pocasset, MA 02559-3450
(508) 759-9100
www.onsetcomp.com

Percival Scientific, Inc
505 Research Drive
Perry, IA 50220
(800) 695-2743
www.percival-scientific.com

Prairie Moon Nursery
Route 3, Box 163
Winona, WI 55987
(507) 452-1362

Waldo & Associates, Inc
28214 Glenwood Road
Perrysburg, OH 43551
1-800-468-4011