Journal of the Lepidopterists' Society
73(2), 2019, 87–92

PUPATION SITES AND CONSERVATION OF FROSTED ELFINS (LYCAENIDAE) IN MARYLAND, USA

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ABSTRACT. The frosted elfin (*Callophrys irus* Godart) is a globally rare butterfly that inhabits disturbance-dependent habitats often managed by prescribed fire. Natural history observations and published data are equivocal on whether frosted elfin caterpillars pupate below or above the soil surface, and some evidence suggests that pupation sites differ for caterpillars that feed on lupine (*Lupinus* spp.) versus wild indigo (*Baptisia* spp.). Pupation site has important implications for management because pupae located beneath the soil surface will likely be afforded greater protection from fire than those above the soil surface. Our study of both lupine- and indigo-feeding larvae at a single site in Worcester County, Maryland, found that pupation occurs above the soil surface in the leaf litter 92% of the time, and does not differ significantly with food plant preference. We recommend that land managers using prescribed fire as a habitat management tool for frosted elfin habitat assume some level of pupal mortality in burned areas and utilize a rotational burn schedule that maintains areas of unburned refugia in high-density elfin areas. We also recommend that managers try to establish a metapopulation structure for frosted elfin to address the possibility of natural occurring fires.

Additional key words: *Callophrys irus*; disturbance-dependent; prescribed fire; *Lupinus perennis*, *Baptisia tinctoria*

The frosted elfin (*Callophrys irus* Godart, Family: Lycaenidae) is a globally rare butterfly currently being reviewed as a potential candidate for federal listing (USFWS 2018). It is listed as Endangered in the state of Maryland (MNHP 2016). The status of this species can be largely attributed to loss of habitat and food plant (*Lupinus* and *Baptisia*, Family: Fabaceae), as the frosted elfin occurs in early-successional, disturbance-dependent habitats that typically succeed to mature woodland in the absence of periodic, active management. Frosted elfins spend the majority of the year as pupae, occupying this life stage from about mid-summer until early spring of the following year, depending on region. Knowledge of where frosted elfins pupate has important implications for management, as prescribed fire is often used to maintain these early-successional habitats and food plants (Grigore & Tramer 1996, Kwilosz & Knutson 1999). It is generally assumed that pupae that occur below the soil surface are likely to be afforded some protection from fire, while those in the leaf litter above the soil surface will be more vulnerable. This has important implications for Maryland as prescribed fire in frosted elfin habitats would occur during the pupation stage.

Limited previous research and observation suggests that frosted elfin pupation sites are variable. Captive lupine-feeding (*Lupinus*) caterpillars in New Hampshire and New York pupate 1 cm beneath sandy substrates when both sand and leaf litter are offered (Schweitzer et al. 2011). Alternately, captive indigo-feeding (*Baptisia*) caterpillars in New Jersey pupate in the leaf litter above the soil surface when offered a choice of sand or litter (Schweitzer et al. 2011). Lupine-feeding larvae in the field in Florida pupate at (or above) the soil surface or up to 3 cm beneath the soil surface (Thom 2013, McElveen & Craig 2017). These results for lupine-feeders and limited data for indigo-feeders preclude any broad inferences.

To our knowledge, there are no data comparing pupation sites of lupine- and indigo-feeding frosted elfin larvae at the same site, or even within the same state. Frosted elfin caterpillars feed on both lupine and indigo at a site in Worcester County, Maryland (Frye & Tangren 2013, Frye & Robbins 2015), allowing for such a comparison. The purpose of this study was to determine whether frosted elfin caterpillars pupate beneath the sandy soil or on top of it in the leaf litter, and more specifically, to determine whether pupation site was dependent on the type of plant used.

Study Site

The study was conducted in Worcester County, Maryland, United States, on the Atlantic Coastal Plain east of the Chesapeake Bay. The habitat is xeric woodland dominated by oak (*Quercus* spp.) and pine (*Pinus* spp.) and was formerly managed for commercial

FIG. 1. Wild indigo treatment showing host plant surrounded by areas of sand and leaf litter collected from the field site.

timber production. Dominant shrubs and herbaceous vegetation include blueberry (*Vaccinium* spp.), huckleberry (*Gaylussacia* spp.), Virginia tephrosia (*Tephrosia virginiana*) and various sedges (*Carex* spp.). A 2.1 ha portion of the site harbors a population of sundial lupine (*Lupinus perennis*) that has been managed as a semi-open, mixed hardwood clearing, with pine removal and tree thinning occurring every few years. Much of the area around the lupine clearing has been recently burned using prescribed fire. Only a small portion of the 2.1 ha was burned because there are no other known frosted elfin populations in the immediate vicinity. Wild indigo (*Baptisia tinctoria*) is present in the lupine area but is most common along the sandy roads that traverse the habitat. The exact locality of this population is not given, in accordance with a request from the Maryland Department of Natural Resources (DNR).

MATERIALS AND METHODS

In early June of 2018, we collected 17 lupine-feeding and 14 indigo-feeding frosted elfin caterpillars. All were collected on or at the base of lupine or indigo plants. Using size and appearance as a rough guide, the lupine sample included six 4th (ultimate) instar, seven 3rd instar, and four 2nd instar larvae. Similarly, the indigo sample included ten 4th instar and four 3rd instar larvae. Caterpillars were carefully removed from the food plants and placed in Petri dishes with leaflets (and flowers or seed pods in the case of lupine) from their respective food plant. The Petri dishes were then placed in a cooler and transported to the Salisbury Zoo to an outdoor enclosure constructed specifically for this research.

We collected sandy soil from the study site and placed it into circular containers of three different sizes: small (30.5 cm diameter x 9 cm depth), medium (43.2 cm diameter x 9 cm depth) and large (66.0 cm diameter x 15.5 cm depth); we used multiple sizes solely to maximize space in the outdoor enclosure. Soil was collected from multiple areas and always within 3 m of a given food plant. We took photographs of the vegetation and leaf litter at the collection site and collected the leaf litter in a separate bag. We then dug up soil in large sections (approximating the size of the container it was going into) using shovels to maintain the natural soil layers. We attempted to recreate litter conditions on the soil surface after it was transferred to the respective container using the photographs. Any small shrubs and grasses that were present were maintained. All containers offered caterpillars areas of open sand and areas of leaf litter (Fig. 1). The litter in the lupine containers consisted mostly of oak leaves and vegetative debris while the litter in the indigo containers contained abundant pine needles in addition to oak leaves and vegetative debris.

The large containers offered larvae more space and therefore more pupation options. The addition of small

FIG. 2. BugDorm tents housing the three sizes of treatment containers. Tents consisted of a fine polyester screen mesh and vinyl sides and provided some protection from potential predators and parasitoids.

Fig. 3. Pupa of a lupine-feeding frosted elfin in oak litter. The left photo shows the observer's finger indicating the curled up leaf containing the pupa; the right photo shows the observer opening the leaf to expose the pupa.

and medium containers allowed for a greater number of lupine and indigo treatments. Each plant treatment had two large containers, two medium containers, and one small container. To maximize the number of caterpillars we were able to observe, we placed 4–6 larvae in each of the large containers, 2–3 larvae in each of the medium containers, and one larva in each of the small containers. This is generally consistent with field observation, as we have observed up to three larvae on a single lupine plant and up to ten larvae on a single indigo plant.

Indigo plants were purchased from a local nursery, were pesticide-free and grown from seed collected locally. They were planted directly into each indigo-treatment container and watered as needed. Lupine grown from locally-collected seed the previous year could not be used for this study as the plants were too small to sustain larval feeding and did not offer flowers or seed pods which may be preferred over foliage (NatureServe 2018). Transplanting mature lupine plants from the site was not possible as the tap roots were deep and extensive. Instead, we fed larvae with lupine clippings kept moist with florist water picks that were placed directly into the containers. This method is

Fig. 4. The tip of the pen indicates the position of the pupa attached to the white flashing where an artificially created gap existed between compacted soil and the flashing itself.

FIG. 5. "Shelter" of indigo-treatment frosted elfin pupa between two small grass clumps. Photo on the left shows the small piece of wood (indicated by the yellow arrow) that the pupa was underneath; photo on the right shows the shelter when lifted from the surface.

FIG. 6. Example of an indigo-treatment frosted elfin pupa found beneath leaf litter at the surface of the soil. The left photo indicates the litter that covered the pupa; the right photo shows the exposed pupa (indicated by yellow arrow) when some of the litter is removed.

not recommended as lupine leaves wilted quickly after being cut and had to be replaced at a minimum every other day.

All containers were kept in BugDorm insect rearing tents with clear plastic and white polyester screen mesh siding for easy observation and proper ventilation (Fig. 2). The tents prevented caterpillars from escaping and provided some protection from predators and parasitoids. We lined the inner perimeter of each container with white plastic flashing coated with a thin layer of Vaseline along the upper edge to prevent caterpillars from climbing out of the containers.

We waited a minimum of 3–4 days after caterpillars were no longer seen feeding to carefully examine the leaf litter and top 3 cm of soil for pupae. The remaining soil and debris were run through a sieve to ensure that intact pupae were not discarded accidentally. Recovered pupae were then released at the original site.

To determine the effect of food plant on pupation site, we tabulated a 2 x 2 contingency table with the variables being pupation site (below versus above ground) and food plant (lupine versus wild indigo). A two-tailed Fisher exact test was used to test whether pupation site was independent of food plant.

Results

Lupine Treatment: We recovered 13 of 17 pupae from the lupine treatments. The individuals that were not recovered may have been predated or may not have survived on the foliar clippings. Of the 13 intact pupae recovered from the lupine treatments, none were found beneath the soil surface. Twelve were found beneath leaf litter at the soil surface or within litter slightly above the soil surface; six of the 12 were found in curled up oak leaves (Fig. 3). The remaining pupa was found attached to the white flashing in an artificially created gap between the flashing and compacted soil (Fig. 4).

Indigo Treatment: All 14 pupae were recovered from the indigo treatments, although one had been predated and only fragments were recovered. Of the 13 pupae that were intact, two were found below the soil surface. One was 15 mm below the soil surface, and the other was found between two clumps of grass separated by about 2 cm under a small piece of wood and surrounded on all sides by strands of sandy soil (Fig. 5). Ten pupae were found beneath leaf litter at the soil surface or within litter slightly above the soil surface (Fig. 6). One pupa was found attached to the white flashing in an artificially created gap between the flashing and compacted soil.

Table 1. Contingency table for pupation site (above ground or below ground) versus foot plant (lupine or wild indigo). A hypothesis of independence is not rejected by a Fisher exact test (p=0.48).

	Above Ground	Below Ground	
Lupine	12	0	**12**
Wild Indigo	10	2	**12**
	22	**2**	**24**

Statistics: Pupation in leaf litter was more common than pupation beneath the soil surface, as 22 of 24 of the pupae (92%) used in the analysis were found in the leaf litter (the two pupae found in artificially created gaps were excluded from the analysis). The data also indicate that pupation site does not vary with food plant preference (Table 1, p=0.48 by Fisher's exact test).

Discussion

Frosted elfin larvae were observed to pupate in leaf litter above the soil surface regardless of host plant. We did observe variation amongst pupation sites within the leaf litter. For example, pines have been removed from the densest lupine areas at our study site for over a decade making oak leaves the dominant component of the leaf litter in these areas. By contrast, indigo was most abundant along roadsides where pines were dominant, and so pine needle litter was more abundant in these treatments. Nearly half of the lupine-treatment pupae were found in curled oak leaves while this was not observed in any of the indigo-treatment pupae. Soil conditions may also affect pupation although at our site there were no appreciable differences in the top layers of soil, which were sandy throughout.

Caterpillar behavior may have been altered in our experimental setting due to limited space in the containers. Caterpillars can travel several meters from the food plant in search of a pupation site (see Schweitzer et al. 2011). In 2018, we tracked an indigo-feeding caterpillar for 80 minutes; it moved approximately two meters from the food plant to pupate within a clump of pine and oak litter.

When assessing the results of all the small-scale frosted elfin pupation studies (Schweitzer et al. 2011), including this one, we conclude that there are probably

no definitive rules determining where frosted elfin caterpillars will pupate, regardless of food plant. It is our impression that pupation sites are most likely determined by available soil and litter conditions. Certainly, our results are consistent with this view.

Frosted elfin pupae will almost certainly experience some level of direct mortality when their habitat is managed by prescribed fire if most pupae are in the leaf litter. This statement is consistent with previous studies (e.g., Swengel & Swengel 2001). Despite this, rotational prescribed fire should remain a preferred method for maintaining the mixed hardwood and semi-open canopy that benefits lupine, indigo and frosted elfins (Schweitzer 1992, Kwilosz & Knutson 1999, Wagner et al. 2003, Albany Pine Bush Preserve Commission 2010, USFWS 2018). We recommend that land managers utilizing prescribed fire as a management tool assume that frosted elfin mortality will be incurred to some degree and utilize a rotational burn schedule that maintains areas of unburned refugia in high-density elfin areas. Similar precautions were also advised for Karner blue butterflies by Kwilosz & Knutson (1999).

Finally, we ask why frosted elfins pupate most frequently above the ground in a habitat that is maintained by occasional fire. This behavior, especially when caterpillars are capable of pupating underground, would seem to be maladaptive, albeit we do not yet know how pupation site affects mortality due to predators. A possible factor is that frosted elfins normally inhabit metapopulations (Albanese et al. 2007, NatureServe 2018). In the event of a fire, dispersal from a non-burned population can recolonize the burned area. This line of reasoning is speculative, but creating a metapopulation structure of habitats might also be a high priority goal for land managers, especially for safeguarding single populations, such as the one that we studied, from naturally occurring fires.

ACKNOWLEDGEMENTS

We offer our sincere thanks to the following people: Ian Shelley, Collections Manager at the Salisbury Zoo, for initiating a collaboration between the Maryland Department of Natural Resources, Salisbury University and the Zoo, for his interest in local species in need of conservation, and for his assistance with multiple aspects of this project both in the field and at the Zoo; Zoo Director Ralph Piland and the staff and volunteers at the Salisbury Zoo for accommodating this work on Zoo grounds; Clifton P. Griffin, Dean of Graduate Studies and Research, for providing funding and equipment; Mike Schofield, DNR Forest Service; Shane Hernandez. A portion of this work was funded by a State Wildlife Grant administered by the US Fish and Wildlife Service.

LITERATURE CITED

ALBANESE, G., P.D. VICKERY & P.R. SIEVERT. 2007. Habitat characteristics of adult frosted elfins (*Callophrys irus*) in sandplain communities of southeastern Massachusetts, USA. Biol. Conserv. 136: 53-64.

ALBANY PINE BUSH PRESERVE COMMISSION. 2010. Management plan and final environmental impact statement for the Albany Pine Bush Preserve. 147pp + Appendices

FRYE, J.A. & S. TANGREN. 2013. Dual host plant use by *Callophrys irus* (Godart) (Lycaenidae) larvae at a single site on the Maryland Coastal Plain. News Lepid. Soc. 55: 156-157.

FRYE, J.A. & R.K. ROBBINS. 2015. Is the globally rare frosted elfin butterfly (Lycaenidae) two genetically distinct host plant races in Maryland? DNA evidence from cast larval skins provides an answer. J. Insect Conserv. 19: 607-615.

GRIGORE, M.T. & E.J. TRAMER. 1996. The short-term effect of fire on *Lupinus perennis* (L.). Nat. Areas J. 16: 41-48.

KWILOSZ, J.R. & R.L. KNUTSON. 1999. Prescribed fire management of Karner blue butterfly habitat at Indiana Dunes National Lakeshore. Natural Areas Journal 19: 98-108.

MARYLAND NATURAL HERITAGE PROGRAM. 2016. List of Rare, Threatened and Endangered Animals of Maryland. Maryland Department of Natural Resources, 580 Taylor Avenue, Annapolis, MD 21401. 23pp.

MCELVEEN, D. & V.D. CRAIG. 2017. A successful search for *Callophrys irus* pupae. News Lepid. Soc. 59: 176-177.

NATURESERVE EXPLORER. 2018. An online encyclopedia of life [web application]. Version 4.6. Arlington, Virginia, USA: NatureServe. Available online at http://www.natureserve.org/explorer. Accessed September 2018.

SCHWEITZER, D.F., M.C. MINNO & D.L. WAGNER. 2011. Rare, Declining and Poorly Known Butterflies and Moths (Lepidoptera) of Forests and Woodlands in the Eastern United States. United States Department of Agriculture, Washington, DC. 517pp.

SCHWEITZER, D.F. 1992. Comments regarding *Erynnis persius* and Incisalia irus. Ohio Lepid. 14: 21–23.

SWENGEL, A.B. & S.R. SWENGEL. 2001. Effects of prairie and barrens management on butterfly faunal composition. Biodiv. Conserv. 10: 1757-1785.

THOM, M.D. 2013. The Ecology and Conservation of *Callophrys irus* Godart: the Role of Fire and Microhabitat. Doctoral Dissertation, University of Florida 189pp.

U.S. FISH AND WILDLIFE SERVICE. 2018. Species status assessment report for the frosted elfin (*Callophrys irus*), Version 1.2. March 2018. Cortland, NY.

WAGNER, D.L., M.W. NELSON, AND D.F. SCHWEITZER. 2003. Shrubland Lepidoptera of southern New England and southeastern New York: Ecology, conservation, and management. For. Ecol. Manage. 185: 95–112.

Submitted for publication 1 November 2018; revised and accepted 10 December 2018.