FINAL PERFORMANCE REPORT

Federal Aid Grant No. F22AP02970 (E-94-R-3)

Investigations into the life history and ecology of the Frosted Elfin subspecies, *Callophrys irus hadros*

Oklahoma Department of Wildlife Conservation

January 1, 2023 - December 31, 2023

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State: Oklahoma **Grant Number: F22AP02970 (E-94-R-3)**

Grant Program: Cooperative Endangered Species Conservation Fund, Traditional Conservation Grants Program

Grant Title: Investigations into the life history and ecology of the Frosted Elfin subspecies, *Callophrys irus hadros*

Grant Period: January 1, 2023 – December 31, 2023

Project Leader: Brenda D. Smith, Oklahoma Natural Heritage Inventory, Oklahoma Biological Survey, University of Oklahoma, Norman, Oklahoma

Executive Summary

Surveys in Oklahoma for the Frosted Elfin (*Callophrys irus hadros*) in 2023 were successful. Prior to the start of this project in 2021, we knew of only a few locations for this species in but three counties (Garvin, Murray, and McCurtain). Historical data suggested that there were only two populations in the state, separated by >140 mi (230 km). We bridged much of that gap by bringing the number of known locations of Frosted Elfins in Oklahoma to 52 sites (64 patches) in nine counties. We now have 188 records of the species in the state. During the course of the project (2021–2023), we drove many thousands of miles searching for host plant patches and we investigated approximately 325 sites. Our efforts expanded our knowledge of the Frosted Elfin's phenology, life history, behavior, and ecology.

Objectives:

1) Monitor select populations of *Callophrys irus hadros* in Oklahoma.
2) Further investigate habitat associations, including attempting to discover why documented occurrence gaps persist within the species' range.
3) Further investigate the life history, behavior, and ecology of *C. irus hadros*.

Summary of Progress:

<u>Oklahoma Frosted Elfin Project Background</u>

The Frosted Elfin, *Callophrys irus*, is a butterfly in the family Lycaenidae (gossamer-wing butterflies) and is a species of conservation concern in North America due to recent precipitous population decline (Shepherd 2005, USFWS-NY 2018; Fig. 1). Its geographic range includes the eastern half of the United States, but it has been considered rare or at best locally uncommon where found (Brock and Kauffman 2003). It is a small butterfly with a wingspan of 25 to 32 mm (1 to 1¼ inches). Larval host plants are various species in the pea family (Fabaceae), including wild indigo, *Baptisia* sp., and wild lupine, *Lupinus* sp. Major threats to this species are loss of

habitat due to urban development or agriculture, poor vegetation management that results in declines in host plant populations, and pesticide use (USFWS-NY 2018).

Callophrys irus hadros was first documented in Oklahoma in the early 1990s when Chuck Harp, entomologist and Lepidoptera specialist, encountered the species in Garvin and Murray Counties in 1991–1993 (note that precise locations of these encounters have not been fully discerned). In 2011, Bryan E. Reynolds, Lepidoptera expert and wildlife photographer, searched in Murray County at locations Harp had encountered the species in the 1990s. Many of these locations could not be accurately re-located or appeared to have been developed. Fortunately though, Reynolds was able to find the species elsewhere, a location now dubbed the "Sulphur Colony," given its proximity to the town of Sulphur. The Garvin County location had not been re-surveyed prior to this project.

Callophrys irus hadros were reported for the first time away from the Garvin and Murray County area in 2008, when Berlin A. Heck, formerly of the Little River National Wildlife Refuge, discovered the species on his property southeast of Idabel, McCurtain County, a location >140 mi (230 km) southeast of the "Sulphur Colony". Heck saw the species again the following year and in 2012, but it has not been reported from that locality since. In 2018, although unsuccessful at finding the species at Heck's property, Reynolds found an adult Frosted Elfin about 5 mi (8 km) to the east near the town of Haworth, dubbed the "Haworth Colony." A subsequent survey in 2021 found the right-of-way portion of the site disturbed with host plants still present but no Frosted Elfins detected.

The Frosted Elfin is single brooded (one generation annually). In Oklahoma, adults emerge early in the year, typically starting in mid to late March, and fly through late April. Early instar larvae (caterpillars) can then be observed in April, with late instars present into early summer (Fig. 2). In Oklahoma, prior to this project, the only documented larval food source was yellow wild indigo, *Baptisia sphaerocarpa*. In nearby Arkansas, it had been found primarily on Nuttall's wild indigo, *B. nuttalliana*, which is quite common in the Ouachita Mountains. In 2021, we determined that Frosted Elfins in Oklahoma also use *B. nuttalliana*. In Texas, the elfin is associated with these *Baptisia* sp. as well as blue false indigo, *B. australis* (Glassberg and Jue 2018). Possible associations of Frosted Elfins with *B. bracteata*, longbract wild indigo, have been reported but it appears that the species may not be particularly suitable host plant.

As with the nominate subspecies *Callophrys irus irus, C. i. hadros* is believed to occur in small colonies and to stay in very close association to their host plant. The adult flight is well before the emergence of flowers of their host plants and has even been reported before host plants have begun to grow (Laurie Sheppard, *pers. comm.*, Hagerman National Wildlife Refuge Frosted Elfin population, Texas; *pers. obs.*, Oklahoma Frosted Elfin team). Prior to this project, the Frosted Elfin locations known in Oklahoma were found on or near pastures, fence lines, roadside ditches, and other disturbed areas that contained *B. sphaerocarpa*.

Oklahoma is at the western periphery of the species' range in North America, where a disjunct population exists. This population is comprised of the subspecies *Callophrys irus hadros*, found only in Arkansas, Louisiana, Texas, and Oklahoma (Fig. 3). *Callophrys irus hadros* was recently assigned a NatureServe national subspecies rank of T3 (Vulnerable Subspecies). The subnational

ranks (SRank) have been: S1 (Critically Imperiled) for Oklahoma and Arkansas, unranked in Louisiana, and was recently downgraded to S4 (Apparently Secure) in Texas; https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.113674/Callophrys_irus_hadros.

The species itself is currently being considered by the US Fish and Wildlife Service (USFWS) for listing under the Endangered Species Act (ESA). The species' historical range in Oklahoma was thought to include the southcentral and southeastern portions of the state, encompassing an area approximately 19,500 km^2. It is reasonable to believe that the species occurs in suitable habitat throughout that area, but prior to this project it was only documented from three counties at nine localities, only two of which were known to have extant colonies. Because of the paucity of *C. i. hadros* data for the state, the Oklahoma Natural Heritage Inventory (ONHI) surveyed for the species throughout its range in 2018 (Reynolds 2018, Reynolds and Smith-Patten 2018). We attempted to do so in 2019 and 2020, but weather and health issues, especially the COVID-19 pandemic, hindered our research. The 2018 surveys produced positive results (Reynolds 2018, Reynolds and Smith-Patten 2018) and given the positive results in a similar timeframe in both Arkansas and Texas, we expanded our efforts in 2021–2023 with funding through the Oklahoma Department of Wildlife Conservation (ODWC).

Prior to our recent investigations, many gaps existed in the known distribution of *Callophrys irus hadros* and *Baptisia* spp. in Oklahoma. We also knew relatively little of the Frosted Elfin's life history and phenology in the state. Our surveys in 2021, at 228 sites within 20 counties plugged many gaps in the known distribution of the butterfly and its host plants. We also learned much about life history. These aspects were further investigated in 2022 (Stone, et al. 2023) and 2023 by visiting established project sites, finding new locations, and monitoring select populations of Frosted Elfins in line with USFWS protocol (USFWS 2020).

Methods

During the course of the project (2021–2023), we drove many thousands of miles searching for host plant patches and we investigated approximately 325 sites (Fig. 4). In 2023, the Oklahoma Frosted Elfin Project teams surveyed for *Callophrys irus hadros* in the southeastern quadrant of Oklahoma and in the Wichita Mountains area at sites with potential suitable habitat. Previously known locations for *C. i. hadros* were also monitored to determine if populations were still extant. Although not to the same extent as in previous years of the project, historical sites of host plants, which were identified via the Oklahoma Biodiversity Information System (OBIS; https://obis.ou.edu), https://www.oklahomaplantdatabase.org/and supplemented by others from the online museum specimen portal, iDigBio, https://www.idigbio.org/portal/search, and iNaturalist, https://inaturalist.org, were visited to determine if patches persisted and if *C. i. hadros* were present.

When a cluster of host plants were previously known about or were newly found, a site number was assigned to that location. Sites were divided into host plant patches once permission to survey the site was granted (Fig. 5). Site and patch designations were in line with USFWS monitoring protocol (USFWS 2020).

Timing of surveys for *C. i. hadros* was aimed at maximizing field activities during the regional adult flight season and larval activity season, i.e., between late-March and late May 2023 (Fig. 2, Table 1), and included only daytime surveys. Daytime surveys were standard area searches of suitable habitat in which adults, larvae, and eggs were sought out by examining host plants and adjacent habitat. The amount of time spent surveying at each site depended upon habitat patch size; times were noted both for patch survey and time spent at the overall sites. Searches for host plant patches continued into the summer until *Baptisia* species had died back.

Basic data collected during surveys and monitoring included but were not limited to: presence or absence of *C. i. hadros* during a given visit; presence of other lepidoptera species and other associated organisms when personnel were able to confidently identify them; individual counts by species and life stage; habitat associations, including notation of host plants of *C. i. hadros*; weather conditions; and geographical location data for each site and patch.

Monitoring of sites was done in two forms. The first was by simply returning to sites during a given field season and/or between years to monitor presence and persistence of *C. i. hadros*, as adults or larvae. The second was to fully or partially adhere to the USFWS monitoring protocol (USFWS 2020), which was aimed primarily at censusing adults. The USFWS monitoring protocol entailed visiting a location at least three times, preferably during the flight season of *C. i. hadros*, with a minimum separation of 4 and maximum of 7 days. Within each patch, a transect was established through the most densely populated portions of the host plants (Fig. 6). Walking the transect enabled the observer to visually examine the majority of host plants by zigzagging one's way through the patch without doubling back. Observers walked slowly but steadily and at the same rate at each visit and did not stop nor capture butterflies. Beginning and end times of transect were recorded at the set patch entry and exit points. Counts of adult *C. i. hadros* were tallied during the transect walk and then recorded at the exit point. Confirmation and documentation of identifications could then be made by returning to the patch to capture and/or photograph *C. i. hadros* adults. Due to limited person power as well as inclement weather, it was logistically difficult to adhere to the USFWS protocol. After transects, larval surveys, which were not part of the USFWS protocol, could then be commenced (details below). Habitat assessments were made during peak flowering, as per the USFWS protocol.

Table 1. Oklahoma Frosted Elfin Project Task Table.

TASKS TABLE

		Early Spring (March – early April)	Mid Spring (mid – late April)	Late Spring – Summer (May – early June)
Host plants		absent to emerging	growing to possibly peak condition	peak condition to fading
FE adults		emerging to present	present	not present
FE eggs		present	may be present	not present
FE larvae		not present	emerging to present	present to pupating
FE pupae		not present	likely not present	present
patch extent		unclear	visible	fully visible
activities		• **contact landowners** for access permission • **watch weather** for opportune time to start surveys • **start surveys by 20 March** (even if HPs not present) • **visit or survey sites** • **prepare for monitoring** • **monitor** • **life history & behavior** data collection	• look for **new sites** • **visit or survey existing sites** • **monitor** • **life history & behavior** data collection	• look for **new sites** • **visit or survey existing sites** • **monitor** • **life history & behavior** data collection • fully **map sites/patches** • collect **peak season habitat data** • ensure **landowner data** complete (full name, phone #, mailing & physical address)
key tasks by level of effort	**minimum**	**visit existing sites** to determine if 1) HPs present 2) FEs present	**visit existing sites** to determine if 1) HPs present 2) FEs present	**visit existing sites** to determine if 1) HPs present 2) FEs present
	better	**survey existing sites** 1) note **HP condition** 2) count # of **adult FEs** present	**survey existing sites** 1) note **HP condition** 2) count # of **adult FEs** or notes absence 3) watch for **FE larvae**; when present, count # (by instars; use scaled sampling strategy for plants to examine)	**survey existing sites** 1) note **HP condition** 2) count # of **adult FEs** or note absence 3) count # of **FE larvae**, ideally by instar (use scaled sampling strategy for plants to examine)
	best	in addition to the above, prepare for, then start **monitor**ing for **adult FEs** 1) delimit patches 2) follow USFWS protocol	**monitor sites** 1) walk transect, count **adult FEs** 2) after transect, record additional data (Form E) collect **life history & behavior** data	collect **additional data** to above (see data sheets) collect **life history & behavior** data
additional tasks *(optional)*		DNA sampling (personal time)	UV light surveys DNA sampling (personal time)	UV light surveys pupae sampling DNA sampling (personal time)

HP = host plants, FE = Frosted Elfins

Non-USFWS protocol life history and behavior data were collected both opportunistically and at specific study sites. Data collected were dependent upon life stage. Data were collected in a specific order, dependent upon *C. i. h.* phenology and whether we attempted to implement the USFWS adult monitoring protocol at a location. Order of data collection would proceed as:

If visit was within adult flight season
Complete USFWS adult monitoring protocol transect
Conduct 15-min adult observation period (Table 2)
Note adult behaviors observed outside of the 15-min adult observation period

If visit was within adult flight season and larval season
Complete USFWS adult monitoring protocol transect
Conduct 15-min adult observation period (Table 2)
Note adult behaviors observed outside of the 15-min adult observation period
Conduct larval survey (Table 3, 4)

If visit was within larval season
Conduct larval survey (Table 3, 4)

Table 2. Adult behaviors recorded during 15-minute adult observation period or afterwards.

Behavior definitions:
♂ *(male) displaying* = if a single adult is flying up into the sky, it is likely to be a male doing a mating display
pair(s) = a male and female coupling; typically this behavior is aerial at initiation, occurring at or near top of host plants or lower, with coupling occurring on vegetation, including at or close to the ground.
ovipositing = female(s) laying eggs
dogfighting = two or more males flying into the sky while being aggressive to each other

Table 3. Larval characteristics and plant data recorded during larval surveys.

Larval surveys
opportunistic data: count larvae by instar and note number of plants examined
study site data: count larvae on six randomly-selected host plants

Record
Life stage and length of larva
host plant species
plant height and width and number of ramets
level on plant that larva was on
plant structure larva was on
herbivory
species associations
myrmecophily

Once *Callophrys irus hadros* larvae were first noted for the season on *Baptisia*, we counted them on plants in each patch surveyed. We examined stems, flowers, buds, and both upper and lower surfaces of leaves for larvae as well as looking for them at or near the ground at the base of plants.

After consulting a statistician and study design colleague, in 2023 we implemented a scaled sampling strategy to tackle the disparity in host plant patch sizes (5–3000 plants) and difficulties presented by limited person power. This scaled approach saturates effort on plots with low host plant count but stratifies thereafter. We scaled using an asymptotic decay, such that when N is low a high percentage of plants are checked with the percentage decreasing to a plateau as N increases. We used a simple form of such a decay, $\exp(-aN)$ with $a = 0.01$. To streamline field application of this sampling strategy, we set thresholds that approximated the decay by quartile as well as 10%. Doing so simplified field implementation (Table 4). The number of plants examined and the number of plants total within the patch were noted as were the number of larvae encountered.

Table 4. Sampling strategy for larval surveys.

total # plants in patch	% to examine	# to examine
1–45	100	1–45
46–100	75	35–75
101–200	50	50–100
201–499	25	50–125
500+	10	50+

Photographs were taken to document the composition, size, and condition of survey sites. Photographs were also taken to identify and document species of plants and animals found on the site when specimens/vouchers were not taken. Photographs were archived by ONHI. Data are being archived with the ONHI's Oklahoma Biodiversity Information System (OBIS; https://obis.ou.edu), the official repository of biological data for Oklahoma.

Surveys and monitoring in 2023 were conducted by three teams lead by: 1) the Principal Investigator, Brenda D. Smith, 2) the on-the-ground project manager, Marie E. B. Stone, and 3) Dr. Leah Dudley and Jose Montalva of East Central University. Surveys were conducted within the projected historical range or possible range of this species, which includes all or portions of Atoka, Bryan, Carter, Choctaw, Cleveland, Coal, Garvin, Grady, Haskell, Hughes, Jefferson, Johnston, Latimer, Le Flore, Love, Marshall, McClain, McCurtain, McIntosh, Murray, Muskogee, Okfuskee, Okmulgee, Pittsburg, Pontotoc, Pottawatomie, Pushmataha, Seminole, Sequoyah, and Stephens Counties in Oklahoma. Additionally, we attempted to determine if the species occurs as far west as the Wichita Mountains region (Comanche, Kiowa, Caddo, and Cotton Counties), but we were only able to survey in Comanche County. We also sought data from border areas in Texas.

Teams sought out suitable habitat in Oklahoma for the Frosted Elfin within the survey zones assigned to them at the discretion of Smith and Stone. Many hundreds of miles were driven in 2023 searching for suitable habitat. Team leaders secured legal permission to survey or monitor sites, and they ensured that they had expressed permission to enter properties during each visit. Each team leader also ensured that she/he and their team members were allowed to collect specimens/vouchers/genetic samples on those properties by obtaining and having in their possession all necessary federal, state, tribal, and local permits. The Oklahoma Biological Survey/Oklahoma Natural Heritage Inventory (OBS/ONHI) helped to identify sites and facilitate access.

We analyzed locations of *C. i. hadros* in ArcGIS to examine spatial relationships to biogeographical regions (EPA Level III Ecoregions; https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states), public lands, and land cover classes, the latter as indicated by the National Land Cover Database (NLCD; US Geological Survey, 2016, https://www.usgs.gov/node/279743). We used ArcGIS Zonal functions to discern which cover classes were present and in what quantities within 1-km buffer zones of known *C. i. hadros* localities. Elfin locations were examined for Oklahoma alone and in combination with all known regional rangewide data. We also examined host plant sites to determine why some sites have elfins while others do not. Once results were obtained, we ran unpaired, two-tailed t-tests to determine statistical significance of differences found between datasets.

Activities outside the direct scope of the 2023 Section 6 funding but which were parts of previously funded activities and which continued to be beneficial to the project included DNA sampling and continued network building. The Dudley team, on their own time, participated in the range-wide collection of samples for analyses by a group of colleagues. That project includes colleagues from the US Fish and Wildlife Service, the Museum of Natural History at the University of Florida, Gainesville, the Florida Natural Areas Inventory, the Florida Resources and Environmental Analysis Center at Florida State University, Hendrix College, and the University of Massachusetts.

Numerous DNA samples from Oklahoma were taken by the Dudley team in 2022 and 2023 and by Matt Moran, of Hendrix College, in 2022, within five counties: Atoka, Garvin, McCurtain, Murray, and Pushmataha. They used noninvasive and nonlethal techniques of genetic sampling (e.g., removal of one adult leg or collection of larval frass). The DNA team, among other population genetic studies, is sequencing the Cytochrome c oxidase subunit 1 (COX1) gene, a commonly used sequence to detect different species, to determine if *C. irus hadros* is a separate species from *C. irus irus*, as has been suspected.

Network building activities during the project (2021–2023) included working with private landowners and community members to establish and foster good relations to facilitate the understanding of the importance of researching and protecting species of conservation concern such as *Callophrys irus hadros*. Various entities, including the US Forest Service, the US Fish and Wildlife Service, the National Park Service, the Oklahoma Department of Wildlife Conservation, Weyerhaeuser, and the Choctaw Nation, participated in and/or facilitated research. Teams also enlisted volunteers, e.g., David Arbour, Dr. Claire Curry, Dr. Bruce Hoagland, Vonceil Harmon, Kevin Stubbs, and Dan Winings, who helped find host plant patches and

Frosted Elfin populations. A Frosted Elfin finding guide and volunteer manual was developed to help these individuals and encourage others to participate in the project. Social media posts, television and print news stories, and printed flyers were utilized to draw attention to the importance of conserving the elfin and its host plants as well as seeking the public's help in finding populations of both.

Results

In 2023, project teams surveyed and monitored between 25 March and 31 May for 34 days (Table 5). Teams made 77 site visits to 34 unique sites, at which they spent at least 90 hours. There was one newly discovered site in 2023. Some sites contained multiple host plant patches that were individually surveyed and/or monitored, accounting for 42 patches and 102 patch visits. Investigations occurred in eight Oklahoma counties. Six counties produced records in 2023 of *C. i. hadros* at 15 sites and 22 patches. Additionally, Kevin Stubbs, of the USFWS, surveyed in Grayson County, Texas, on his private property during his personal time. Despite there being ample *Baptisia sphaerocarpa* (at least 100 plants) and *Baptisia bracteata* (perhaps hybrids, too) no elfins were detected.

During the project (2021–2023), we surveyed at or near 11 Oklahoma state and/or Federal properties and within numerous right-of-ways of the Oklahoma Department of Transportation. All *C. i. hadros* locations fall within either the Chickasaw Nation (26.9%) or the Choctaw Nation (73.1%).

Table 5. Surveys and monitoring for the Frosted Elfin (*Callophrys irus hadros*) in Oklahoma in 2023. Surveys and monitoring in 2023 occurred between 27 March and 31 May. Effort totals are by team as well as by overall effort combined into project totals.

team	survey dates	# counties	# days	# sites	# patches	site visits	patch visits
Dudley/Montalva	1 April – 10 May	2	16	6	10	27	38
Smith	12 Apr – 17 May	5	9	19	20	21	22
Stone	27 Mar – 21 May	2	9	9	12	29	42
project totals		**8***	**34**	**34**	**42**	**77**	**102**

*Atoka County was visited by Dudley/Montalva and Smith.

Search effort at patches ranged from 1 minute (patches where host plants had yet to emerge due to poor weather conditions or where plants were eradicated, e.g., from mowing or development) to 3.75 hours, with an average of 40 minutes/patch. There were 62 records of Frosted Elfins (adults or larvae) reported from 15 sites (22 patches) of the 34 sites surveyed (6.8%) for the year. The drop in effort from previous years (2022: 15.44%; 2021: 15.34%) is due to a concentration of the project on monitoring locations and collecting life history data as opposed to widespread presence/absence surveys.

The start of surveys was slightly delayed in 2023 due to precipitation, which was >2 inches above normal for March. That amount apparently did not deter *C. i. hadros* adults from emerging as usual because first detection was roughly the same as in previous years (2018: 24 March, 2021: 20 March, 2022 and 2023: 27 March). In previous years, precipitation was below normal (Table 6). There is no obvious signal in temperatures between years that could help predict time of emergence. In relation to first detection, number of days at or below freezing preceding emergence does not seem to matter. It may be that when average temperatures by day of month are 50°F or above for approximately 15 days preceding emergence (or actual maximum temperatures are 50°F or above for approximately 22 days) that adult elfins will begin to fly. Further analyses need to be conducted with weather variables, including examining soil temperature at varying depths, to discern what factors elfin pupae respond to in order to facilitate emergence.

Surveys and monitoring in 2023 provided more documentation in support of *Baptisia sphaerocarpa* and *B. nuttalliana* being the host plants for *Callophrys irus hadros* in Oklahoma. Of the elfin patches for which there are at least opportunistic data for *Baptisia* (1991–2023, species composition not consistently reported), 72.9% had only the two known host plants reported and 27.1% have those species as well as other species of *Baptisia* (*alba*, *australis*, *bracteata*, hybrids, or undetermined species). During the project (2021–2023), we investigated 21 patches with *B. bracteata*, a species hypothesized to be a host plant for *C. i. hadros*. Five of these patches, all also having *B. nuttalliana* and/or *sphaerocarpa*, had *C. i. hadros*. No elfins were detected at the five patches containing solely *B. bracteata* nor at the remaining 11 patches with *B. bracteata* as part of the *Baptisia* composition. Most (71.4%) *C. i. hadros*-positive patches had *B. sphaerocarpa* as the only *Baptisia* species. Fewer patches have solely *B. nuttalliana* (22.4%) with fewer still having both species (6.1%).

Table 6. Weather variables for south central Oklahoma (Healdton NOAA data), 2018 and 2021–2023. First detection of Frosted Elfins (FE) for each year is given.

		max temperatures				min temperatures			average temperatures		precipitation	
	1st FE	avg max	max diff	#days>49	#days>59	avg min	min diff	#day<33	#days>49	#days>59	precip total	precip diff
2018 Mar	24-Mar	68.3	3.2	23	20	39.7	1.0	11	13	8	2.7	-0.1
2018 Apr		69.5	-4.3	–	–	40.2	-6.8	–	–	–	2.4	-0.9
2018 May		85.6	4.8	–	–	62.4	5.1	–	–	–	3.3	-1.9
2021 Mar	20-Mar	68.4	3.3	19	17	42.5	3.8	4	14	7	1.7	-1.1
2021 Apr		69.4	-4.4	–	–	44.3	-2.7	–	–	–	5.3	2.1
2021 May		75.3	-5.5	–	–	58.0	0.7	–	–	–	6.5	1.3
2022 Mar	27-Mar	66.9	1.8	21	18	34.6	-4.1	14	13	4	1.4	-1.4
2022 Apr		75.8	2.0	–	–	49.0	2.0	–	–	–	2.7	-0.6
2022 May		83.2	2.4	–	–	59.2	1.9	–	–	–	4.9	-0.3
2023 Mar	27-Mar*	66.2	1.1	25	18	40.3	1.6	6	18	7	4.8	2.1
2023 Apr		71.0	-2.8	–	–	44.6	-2.4	–	–	–	1.9	-1.3
2023 May		80.3	-0.5	–	–	58.6	1.3	–	–	–	3.1	-2.1

*1st detection date is from Le Flore County. South central Oklahoma was first surveyed on 25 March 2023, but was not revisited until 2 April.

Of the 148 patches visited (1991–2023; Fig. 7) that had *Baptisia nuttalliana*, *B. sphaerocarpa*, or both, only 41.9% (n = 62) were positive for *C. i. hadros*. Some thoughts on why that percentage

is low are: survey effort (i.e., number of visits), timing of visits, differences in temperatures during visits, and/or landscape matrix differences or habitat degradation.

Visits to the 86 patches without *C. i. hadros* detections were between 1–6 times with mean visits of 1.87. Elfin-positive patches were visited between 1–24 times with the majority, 77.4%, being visited between one and five times (mean visits 4.27, median 3). The difference there is undoubtedly due to elfin-positive patches being visited more often because they were being monitored. In terms of first detection of elfins, all elfin-positive patches had detections within the first or second visit to a patch (80.6% 1st visit, 19.4% 2nd visit).

Timing of visits also did not provide an explanation for the low percentage of host plant patches being elfin-positive. There is nothing qualitatively different about seasonality of visits between those with detections of *C. i. hadros* and those without. Visits were made approximately during the same time period. For elfin detections, that was 12 March to 1 June with a mean date of 18 April. For negative patches, that was 21 March to 21 May with a mean date of 19 April. The mean dates are approximate to what preliminary analyses indicate is the switchover period from adult activity to larval presence.

Temperatures also did not offer insight. Temperatures during positive elfin (adults and larvae) visits ranged from 45–95.2°F (mean 73.3°F). When only adults were detected, temperatures were 45–95.2°F (mean 74°F), when only larvae were present temperatures were 49–89°F (mean 73°F), and when adults and larvae were detected together temperatures were 57.9–79.4°F (mean 71.1°F). During negative visits, temperatures were 36–95.5°F (mean 72.7°F).

Preliminary habitat associations, as indicated by the NLCD land cover classification system, suggest that *C. i. hadros* presence is associated with four cover classes (Table 7). To examine why some host plant sites (having *Baptisia nuttalliana* and/or *B. sphaerocarpa*) have *C. i. hadros* while other do not; we associated NLCD land cover classes to each of those datasets (Table 8). Some of the same cover classes ranked highly in both of the NLCD analyses (*C. i. hadros*-present data versus the HP +/- *C. i. hadros* data). There is a switchover in forest types when looking at host plant sites with and without elfins. Habitat degradation, as indicated by total development within 1-km of a host plant site, did not differ between sites with and without elfins.

Table 7. National Land Cover Database associations to known Frosted Elfin (*Callophrys irus hadros*) localities within Oklahoma and the region. Four cover classes have the highest percentages within 1-km buffer zones of localities regardless of whether data are only from Oklahoma or added to all known localities. Forest type differs between datasets.

	Oklahoma only	**ALL**
Developed, High Intensity	0.3%	0.3%
Developed, Medium Intensity	1.0%	1.0%
Developed, Low Intensity	2.1%	2.4%
Developed, Open Space	3.8%	3.8%
total development	7.2%	7.5%
Barren Land	0.0%	0.1%
Deciduous Forest	**21.4%**	**12.3%**
Evergreen Forest	**10.5%**	**28.8%**
Mixed Forest	7.3%	9.8%
Shrub/Scrub	2.4%	4.1%
Cultivated Crops	0.0%	0.0%
Woody Wetlands	1.0%	4.9%
Emergent Herbaceous Wetlands	0.1%	0.2%
Herbaceous	**19.9%**	**11.6%**
Hay/Pasture	**28.0%**	**18.4%**
Open Water	2.2%	2.3%

Table 8. National Land Cover Database associations to sites that have *Baptisia nuttalliana* and/or *B. sphaerocarpa*. Sites used for the analysis were those known to have Frosted Elfin (*Callophrys irus hadros*) populations (*n* = 52) and those without detected Frosted Elfins (*n* = 84). Percentages are within 1-km buffer zones of localities.

	no FE	**FE**	*p* value
Developed, High Intensity	0.08%	0.53%	
Developed, Low Intensity	1.40%	2.14%	
Developed, Medium Intensity	0.40%	1.27%	
Developed, Open Space	3.18%	3.73%	
total development	5.06%	7.68%	
Barren Land	0.03%	0.05%	
Deciduous Forest	**14.12%**	**20.55%**	0.04
Evergreen Forest	**20.32%**	**9.69%**	0.01
Mixed Forest	12.43%	6.90%	0.00
Shrub/Scrub	3.41%	2.12%	
Cultivated Crops	0.06%	0.10%	
Woody Wetlands	2.83%	1.26%	
Emergent Herbaceous Wetlands	0.56%	0.08%	
Herbaceous	**11.23%**	**20.50%**	0.02
Hay/Pasture	**28.40%**	**29.00%**	
Open Water	1.55%	2.08%	

statistically significant defined as *p* value = <0.05

Biogeographically speaking, *C. i. hadros* is known to fall within five ecoregions within Oklahoma: Cross Timbers, Ouachita Mountains, Arkansas Valley, South Central Plains, and the East Central Texas Plains. The latter ecoregion was previously only known from elfin sites in Texas, but with the addition of new site in Bryan County in 2023, *C. i. hadros* is now known from that ecoregion in Oklahoma as well.

In 2023, adult *Callophrys irus hadros* were observed between 27 March and 30 April during 14 field days at 11 sites and 17 patches. Larvae were observed between 6 April and 20 May during 18 days at 15 sites at 21 patches. Highest counts per host plant patch were 97 for adults and 582 for larvae. A minimum of 3642 individuals were reported, consisting of 647 adults and 2995 larvae. Mean adults (18.64) in 2023 were consistent with the mean for all reports across all years (1991–2023: 18.04). Mean larvae in 2023 were 83.19, which was greater than reports across all years (1991–2023: 60.58).

We measured (by scale or visual estimate) 2622 of the larvae encountered in 2023. Although instars are yet to be clearly defined, we implemented rough size classes as a proxy for instars using a similar methodology to McElveen, et al. (2020). We recorded 1486 as early instars (1–6 mm), 888 as mid-instars (>6–13mm), and 248 as late instars (235 were >13–19mm, 13 were >19). The largest larvae we measured were 22mm in length.

In 2022 and 2023, from 30 April to 1 June, we recorded exact measurements (using a scale as opposed to quantifying size classes) for 267 larvae. These data were taken at 20 patches in six counties (Atoka, Bryan, Le Flore, McCurtain, Pittsburg, and Pushmataha). It is important to note that getting accurate measurements of larvae in the field is difficult. Larvae can be quite active and they do not like to be handled so they will scrunch up, necessitating some time to allow them to relax before taking measurements. Although these measurements should not be considered extremely precise, they are sufficient to account for general lengths. Lengths ranged from 1.5 mm to 22 mm (mean 11.6 mm, median 10 mm). Early instars accounted for 8.2%, mid instars for 56.2%, and late instars for 35.6% of all larvae measured. Early instars appear to drop off in early May, with mid instars following within a week. Late instars are present from at least the end of April until early June.

Because larval length data were primarily collected opportunistically, associations with host plant species can be only broadly stated. The vast majority of lengths came from patches having *Baptisia sphaerocarpa* (92.1% of larval data, from 16 patches). Only three patches contained *B. nuttalliana*, which produced 7.1% of larval measurements. One patch contained both host plants. Not surprisingly, lengths of larvae between the host plant species did not differ.

Behaviors noted throughout the project include ovipositing, pairings, and "dogfighting", or two or more males aerially fighting, presumably over females and/or territories. Myrmecophily (ant association) is another behavior noted on multiple occasions. This behavior is common with Lycaenidae butterflies, and it has been studied to some extent with eastern populations of larval *Callophrys irus* (Albanese, et al. 2007, McElveen, et al. 2020). Generally, myrmecophily is considered advantageous to caterpillars but it is not always so. For example, in McCurtain County, at site MCCU012 on 28 April 2022, Brenda Smith and Bruce Hoagland observed a *C. i. hadros* mid instar larva, measuring 8 mm long, being attacked by ants. The larva appeared to have tried to protect itself by partially producing a silk encasing, but the larva was dying or perhaps fully dead. A video was taken. Another interesting behavior noted in 2022 was congregation of later instar larvae at the base of host plants (reported by Jose Montalva). Presumably congregating occurs when larvae are feeding on the main stem of the host plant in preparation for pupation. This behavior has been noted elsewhere with myrmecophily (McElveen 2018).

Numerous private landowners allowed access to their properties and we received numerous calls and emails from the public in Oklahoma and Texas reporting possible host plant patches and showing support for the Frosted Elfin Project. We continued to work with state and federal agencies, conservation organizations, and other institutions to further research of the Frosted Elfin. Some of the groups, or personnel of, that have cooperated with, facilitated, and/or promoted the project include the Oklahoma Department of Wildlife Conservation, the Oklahoma Department of Transportation, Oklahoma Gas & Electric, Oklahoma State University's Kiamichi Forestry Research Station, Okies for Monarchs, The Nature Conservancy, Bebb Herbarium, US Fish and Wildlife Service, US Forest Service, Weyerhaeuser, the Kerr Center for Sustainable Agriculture, and the Choctaw Nation.

Discussion

Frosted Elfins are indeed still present and seemingly stable within the projected historical range in Oklahoma and the region. In Oklahoma, there are now 188 records of *Callophrys irus hadros* at 52 sites (64 patches) within 9 counties (Fig. 4). Across the region (Arkansas, Louisiana, and Texas) where the *hadros* subspecies occurs, many dozens of additional records are known (Fig. 3).

Prior to our recent surveys (2021–2023), we knew of <20 records for the species in Oklahoma dating from the early 1990s to 2018 (Smith, et al. 2022). These records came from nine locations within only three counties (Garvin, Murray, and McCurtain). Historical data suggested that there were but two populations in the state, separated by >140 mi (230 km). Our 2021 surveys added an additional 45 records at 34 new localities within eight counties (Smith, et al. 2022). Five of those counties were previously unknown for the species. Our 2022 surveys not only added additional sites but also provided more in-depth information about specific sites due to repeated visits throughout the field season. Adult *C. i. hadros* were found at 12 sites; of these sites, five had high counts of 18–104 adults, while the other sites had fewer than 10 adults. We observed *C. i. hadros* larvae at 16 sites; nine sites had highest counts of more than 20 larvae (up to 337), while the others had 10 or fewer individuals. Our 2023 surveys had similar favorable results. For example, we added another *C. i. hadros*-positive site from an ecoregion (East Central Texas Plains) within which we had yet to find elfins. Our 2023 work also added much needed data on the life history, ecology, and behavior of the *hadros* subspecies.

Data indicate that *C. i. hadros*, as adults or larvae, may be detected with as few as 1–2 visits to a host plant patch. Some of the patches that we did not detect *C. i. hadros* at were visited up to six times. If we consider the proximity of some patches, for example MCCU004-01 and MCCU064-01, we could conduct visits to nine without detection. In this instance, it appears that elfins are not using the *Baptisia nuttalliana* along the northern road leading into the Little River National Wildlife Refuge Unit 2. Further analyses are needed to determine an optimal number of visits to a host plant patch to comfortably feel that it can be ruled out as harboring *C. i. hadros*. That said, if there are nearby populations of elfins, potential colonization must be considered in the future. Currently, it is thought that elfins do not disperse terribly far, but further research gauging their dispersal and colonization abilities are warranted.

Counts of both adults and larvae indicate that the species appears to have healthy populations in the state. Although censused with different techniques between years, thus limiting direct comparison of numbers, high counts of adults and larvae in Oklahoma indicate that the species can potentially, though hesitantly so, be considered locally common. For example, in 2022, the high count report was 104 individuals and in 2023, it was 97. For larvae, the high count in 2022 was 337 individuals and in 2023, it was 582. All high counts come from Murray County, with lower or much lower counts coming from the eastern Oklahoma populations. The highest counts out of the east come from Le Flore County where 23 adults and 133 larvae have been reported. This is in stark contrast to McCurtain County where high counts are only 9 adults and 10 larvae. Perhaps this attests to poorer site quality or suitability or perhaps it is just bad luck in finding larger patches of *Baptisia* like those found in the western portion of *C. i. hadros*' range in Oklahoma (Table 9). But certainly, there are differences in number of plants found within host

plant patches between surveyed areas in Oklahoma. Relationships of host plant number, patch extent, and population numbers of elfins should be further explored.

Table 9. Host plant patches appear to differ in number of plants they are comprised of depending on where in the state they are located (southeastern most: McCurtain County; east central: Le Flore County; westernmost: Murray County).

	McCurtain		**Le Flore**		**Murray**
	nutt	sphaero	nutt	sphaero	sphaero
min	4	3	5	35	12
max	1154	900	600	1850	5857
mean	122	143	150	504	1338
median	53.5	50	87	185	770

Our knowledge of the species' phenology in Oklahoma was also enhanced. Though flight season of adults (12 March–30 April) was not extended from previous years, during the project we learned that host plant emergence is not an indicator of elfin presence, as we once thought. Our working hypothesis was that elfin emergence in the spring likely followed plant emergence. We hypothesized that the cue plant emergence followed, presumably the preceding number of soil heating days above freezing, was also the cue elfin emergence followed, albeit by a few days or possibly weeks behind. As such, in previous years, we waited to commence surveys for the elfins until the host plants were present. In early spring 2021, we learned that in previous years in Texas, elfins emerged despite any flowering plants being readily or obviously available (Laurie Sheppard, *pers. comm.*, Hagerman National Wildlife Refuge [NWR] Frosted Elfin population, Texas). Consequently, we began surveys earlier in 2022, beginning on 16 March, despite host plants emerging many weeks later through much of the elfin's range in Oklahoma and nearby Texas. Due to bad weather, we were not able to start this early in 2023.

First detection of adult *C. i. hadros* in Oklahoma was on 27 March in 2022 and 2023. In 2022, new vegetative growth at one site consisted merely of a few grass sprigs. The other site, a recently burned patch, had much more grass sprouting and there were roughly a dozen *Baptisia sphaerocarpa* sprouts ≤ 7.5 cm tall. Tenpetal anemone (*Anemone berlandieri*, also known as tenpetal thimbleweed) was present, with elfins seemingly nectaring on it, as had been observed in Texas (Sheppard, *pers. comm.*). March 27 2022 was the same day that adult *C. i. hadros* were reported near the Oklahoma border at the Caddo National Grasslands in northeastern Texas where *Baptisia* was ≤ 28 cm tall (Mary Curry, *pers. comm.*). Adult elfins were first reported from Hagerman NWR, in 2022, also fairly close to the Oklahoma border, on 19 March despite host plants not being present; on 27 March 2022, Hagerman NWR host plants were still not present but tenpetal anemone was and adult elfins were nectaring on it (Sheppard, *pers. comm.*). In light of these findings, future surveys for adult *C. i. hadros* should begin earlier in the spring to better capture the beginning of the species' flight season. And, though the soil heating day concept may still hold, the alternate hypothesis of our prior expectations, i.e., elfin emergence can coincide with and even precede plant emergence, should be tested with future research.

Emergence may differ throughout the elfin's Oklahoma geographical range. We have wondered if surveys might be best started in southeastern Oklahoma earlier in March because it may be that adults emerge there earlier than we started surveys. If that is the case for McCurtain County that may explain why we have had so few adults and why, in 2023, no adults were encountered. Perhaps we conducted surveys too late in the flight season so we missed larger numbers. Alternatively, populations may just be smaller in McCurtain County. Further investigation is needed to discern what cues emergence of adult elfins. We investigated several weather variables but none appear to be useful in predicting timing of emergence. We did learn that temperatures for detecting adults were lower than we expected, down to at least 45°F. As such, future surveys may be appropriate in cooler temperatures than we did them.

During the project, larval phenology was found to be from early April to early June. In 2023, larvae were first recorded on 6 April, which is in contrast with 2022 when larvae were not reported until 23 April. Presumably this later date was due to the late host plant emergence throughout the elfin's northernmost regional range, because unlike adult elfins, larval elfins presumably do need to feed off their host plants. Future research of *C. i. hadros*'s phenology and life history ought to investigate if egg hatching is suspended when host plant conditions are not amenable to larval survival and if so, what mechanisms allow for that. Our last observance of *C. i. hadros* larvae in 2022 was on 1 June in Murray County (MURR029), which remains the latest known date for Oklahoma. We wondered if this late date was due to an overall shift in the activity season in 2022 due to late host plant emergence or if we were just better able to capture the species' real phenological persistence. Future research will aid in a better understanding of the bounding timeframe of larval activity.

Our measurements of over 2600 larvae indicate that the species grows to about 22 mm before pupation. Beyond that we were not able to discern instar stages. To do so successfully, a targeted field study will need to be initiated with a complementary lab study. Characteristics to examine include molting schedule and changes in body color and shape. We thought a couple of characters previously noted in the field, e.g., bronzing of overall coloration and development of the appearance of a side seam indicated instar development. However, we were unable to quantify those characters. We were able to confirm the ocelli and surrounding pigmentation pattern of *C. i. hadros* in Oklahoma are consistent with *C. irus. i.* larvae (Meyer and McElveen, n.d.). This confirmation was especially important given that, due to the difference in coloration of *C. irus i.* versus *C. i. hadros* larvae (green larvae versus yellow), there had been doubt cast upon whether larvae identified as *C. i. hadros* in Oklahoma were truly Frosted Elfins. Our diagnostic photos (Fig. 8) confirm that the larvae we have identified as *C. i. hadros* are indeed *C. i. hadros*.

It is still a mystery why gaps exist in the geographic range of *C. i. hadros* in Oklahoma. Granted, it is logistically difficult to get sufficient person power out across such a large area with cooperative weather conditions during the relatively short time frame *C. i. hadros* is active. Nonetheless, we continued to search within gaps and examine variables that might elucidate why *C. i. hadros* occurs where it does. Certainly landscape and weather are likely culprits.

Four land cover classes ranked in the top of habitat associations to *C. i. hadros* localities, regardless of whether only Oklahoma localities were examined alone or were combined with all

range wide data within the region (Table 7). Hay/pasture and herbaceous cover ranked fairly high in each of the datasets but forest type made a virtual switch with deciduous forest downplayed when all regional data were considered, and was replaced by evergreen forest. This is perplexing because if nearby forest type matters to elfins then there should not be a turnover in type between elfin-positive sites in Oklahoma versus elfin-positive sites across the region. But perhaps this is just a spatial sampling bias.

Equally puzzling is that this switchover between deciduous and evergreen forests shows in results of land cover associations to host plant sites with or without elfins. Those results indicate that elfin-positive host plant sites tend to be in landscapes with a good amount of deciduous forest and herbaceous cover whereas elfin-negative sites are more associated with evergreen forest and less herbaceous cover. Note that *p* values for these forest types indicate there is a statistically significant difference (Table 8), but interestingly, the number of sites found in or near deciduous forest between the two groups are similar (with elfins, $n = 18$; without elfins $n = 19$) but those found in or near evergreen forests are quite different (with elfins, $n = 5$; without elfins $n = 30$). So the question remains, does this switchover hold across the region and can these habitat differences explain why some host plant sites have elfins while others do not. Further investigations into habitat associations are warranted and would be useful for a better understanding of *C. i. hadros*'s ecological parameters.

Some unexpected and welcomed opportunities arose during the project. For example, the species was highlighted in an exhibit at the Chickasaw Cultural Center in Sulphur due to the work of the Dudley team. One of our team members, Jose Montalva, was also asked to write an article about the project for the Entomological Society of America's magazine. The project was also highlighted on multiple televised broadcasts at three regional television news stations as well as multiple print and online news outlets. We were also fortunate to be able to spur the protection of one of the project's elfin sites (PITT009) that lies along the Indian Nations Turnpike. The Oklahoma Turnpike Authority chose to protect the site and implement a conservation mowing schedule that will not conflict with *C. i. hadros*' phenology. The Oklahoma Department of Transportation implemented a similar mowing schedule in Pittsburg County and has this species on its radar for further protective measures. Such fortuitous highlights and accomplishments not only bring attention to the hard work of all our team members but more importantly brings needed attention to conservation and on-the-ground protection of this and other insect species.

Over the course of the project (2021–2023), we built upon the project's regional network of private landowners, agencies, and volunteers. While in the field, we met many landowners and drew support of the project and for conservation in general. Our continued work to create a network of landowners, agencies, and volunteers for this project undoubtedly will have much broader implications by continuing to foster good community relations for the Oklahoma Natural Heritage Inventory and the Oklahoma Department of Wildlife Conservation. These positive relations will further ford the unnecessary division between the lay public's understanding of science, the importance of protecting plant and animal species, and the role individuals can play in caring for our nationally shared natural resources.

Clearly, public interest in the species in recent years has grown. As a demonstration that efforts of ODWC and the USFWS have made a difference to this species, we draw attention to the rising

numbers of citizen scientist submissions of Frosted Elfin records to online portals such as iNaturalist (Table 10). It appears then that USFWS' Species Status Assessments are effective at getting the word out about conservation of organisms and as a means of encouraging citizen scientists to help fill data gaps.

Table 10. Public interest in the Frosted Elfin (*Callophrys irus*) as gauged by iNaturalist (https://www.inaturalist.org) submissions for the species in the region before (pre-2018) and after the USFWS Species Status Assessment process. Totals do not consider if submission was correctly identified.

	total	pre-2018	2018-2023
AR	28	2	26
LA	12	0	12
OK	5	0	5
TX	35	4	31

Re-evaluation of Conservation Rank

Taking into consideration data that we compiled from historical resources as well as those directly collected during this project, all of which range from 1991–2023, we can now better estimate the conservation status of this species. We used NatureServe's Conservation Rank Calculator (https://www.natureserve.org/products/conservation-rank-calculator) to re-evaluate the subnational rank (SRank) for *Callophrys irus hadros* in Oklahoma. The rank calculator takes into account the species' Extent of Occurrence (EOO, i.e., range extent) and its Area of Occupancy (AOO). We used GeoCat (https://geocat.iucnredlist.org/) to estimate these, which produced an EOO of 24,353.659 km2 and an AOO of 172 km2 based on a 2 x 2 km grid. We used 52 as the number of occurrences. A population size estimate was not calculated. Short-term and long-term trends were also not estimated because there is not sufficient data to do so. Overall threats were estimated to be Medium. With those factors, the SRank was calculated as S3.

Conclusions/Management Recommendations

A few key recommendations for the *Callophrys irus hadros* subspecies of the Frosted Elfin are:

- to continue surveying for new populations, especially within areas where there are gaps in our knowledge, for example along the border of Oklahoma and Texas and even into northern Texas where a relatively large area lacks records but likely harbors the species
- to determine the full geographical range extent of the species within Oklahoma and the region
- to continue monitoring known populations of the Frosted Elfin in Oklahoma and the region
- because so many Frosted Elfin localities are found within roadway right-of-ways, we strongly recommend that the Oklahoma Department of Transportation maintain their policy of pollinator conservation mowing, i.e., not mowing during the Spring and preferably not until early June or later, a policy that should be implemented throughout the species' range

- further investigations of the life history, behavior, and ecology of the Frosted Elfin are needed, including discerning instar stages, feeding behavior, and habitat associations
- further investigate why larvae of the *hadros* subspecies of the Frosted Elfin are yellow instead of green as with the nominate subspecies and why they vary in other characteristics from the nominate subspecies, including in larval setae length and limited fluorescence (Smith, et al. 2022; Stone, et al. 2023; Moskowitz 2019)
- further investigate ant associations with larval Frosted Elfins. Although myrmecophily is common with Lycaenidae butterflies, and it has been studied to some extent with eastern populations of *Callophrys irus* and we know little of this phenomenon with *Callophrys irus hadros*. Discovering what species of ants associate with *C. i. hadros* and determining if obligate or facultative mutualism is at play, or as in the apparent case with the 2022 observation, if some ant species prey on *C. i. hadros*.
- investigate where pupae overwinter and if ants play a role in their protection, e.g., might ants carry pupae to their colonies for overwintering?
- Because the known range of *C. i. hadros* in Oklahoma falls entirely within the Chickasaw and Choctaw Nations, the tribes must be involved with conservation of this species. State and Federal agencies need to work closely with the tribes to further conservation of the Frosted Elfin.

Significant Deviations:
All project objectives were met. However, the part-time Project Manager for this project took a full-time job after field activities for the project ended in the summer. As such, I was not able to re-hire her back in the fall to complete the job tasks that were budgeted. Additionally, the subcontractors invoiced for less than budgeted. Our need for materials and supplies was not as great as we expected. As a result, there were remaining funds in the budget that were not needed for completion of the project.

Equipment Purchased (Cumulative):
No equipment exceeding $5,000 in cost was purchased under this grant.

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Date Prepared: February 16, 2023

Approved by: Russ Horton, Assistant Chief of Wildlife
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Literature Cited:

Albanese, G, MW Nelson, PD Vickery, PR Sievert. 2007. Larval feeding behavior and ant association in Frosted Elfin, *Callophrys irus* (Lycaenidae). Journal of the Lepidopterists' Society 61(2):61–66.

Brock, JP, K Kauffman. 2003. *Kaufman Focus Guide to Butterflies of North America.* Houghton Mifflin Co., Boston, Massachusetts.

Glassberg, J, DK Jue. 2018. State status assessment of Frosted Elfins (*Callophrys irus hadros*) in Texas. Report submitted to the Texas Parks and Wildlife Department.

McElveen, D. 2018. *A Guide to Finding Frosted Elfins*. Tall Timbers Research Station, Tallahassee, Florida, Unpublished Copy on file with the Oklahoma Natural Heritage Inventory.

McElveen, D, D Jue, S Jue, V Dell Craig. 2020. Life history observations of *Callophrys irus* (Family: Lycaenidae) in north Florida, USA. Journal of the Lepidopterists' Society 74(1):51–56.

Moskowitz, D. 2019. Surveying for caterpillars of a rare butterfly using ultraviolet light: the Frosted Elfin butterfly (*Callophyrs irus*) as a test case. *Journal of Insect Conservation* 2019:1–6.

Meyer, RT, D McElveen. n.d. Identify Frosted Elfin (*Callophrys irus*) from Gray Hairstreak (*Strymon melinus*) Caterpillars. Unpublished, 2 pp.

Reynolds, BE. 2018. Frosted Elfin, *Callophrys irus*, Survey Report, Oklahoma, Spring 2018. Report on file with the Oklahoma Natural Heritage Inventory. 47 pp.

Reynolds, BE, BD Smith-Patten. 2018. The Frosted Elfin, *Callophrys irus,* in Oklahoma. Report on file with the Oklahoma Natural Heritage Inventory. 22 pp.

Shepherd, MD. 2005. Species Profile: *Callophrys irus*. In Shepherd, MD, DM Vaughan, SH Black (Editors). Red List of Pollinator Insects of North America. CD-ROM Version 1 (May 2005). Portland, OR: The Xerces Society for Invertebrate Conservation. Copy on file.

Smith, BD, L Dudley, J Montalva, M Moran. 2022. Surveys within Oklahoma's historical range of the Frosted Elfin (*Callophrys irus*) to determine current distribution and status. Final Report to the Oklahoma Department of Wildlife Conservation, Federal Aid Grant No. F21AP00052 (E-94-R-1). Dated 10 February 2022. 26 pp.

Stone, MEB, BD Smith, L Dudley, J Montalva. 2023. Surveys within Oklahoma's historical range of the Frosted Elfin (*Callophrys irus*) to determine current distribution and status. Final Report to the Oklahoma Department of Wildlife Conservation, Federal Aid Grant No. F21AP03585 (E-94-R-2). Dated 10 February 2023. 24 pp.

USFWS-NY [US Fish and Wildlife Service, New York Field Office]. 2018. Species status assessment report for the frosted elfin (*Callophrys irus*), Version 1.2. April 2018. Cortland, New York.

USFWS [US Fish and Wildlife Service]. 2020. Frosted Elfin Habitat and Butterfly Survey Protocol. December 2020.

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Figure 1. Top: Frosted Elfin, *Callophrys irus hadros*, adults in Oklahoma. Photos © Bryan E. Reynolds. **Below:** Size difference in Frosted Elfin, *Callophrys irus hadros*, larvae. Photo on left is an early-instar larva on a thumb and that in the middle is a mature larva (© Bryan E. Reynolds). Far right is of a late instar larva with a scale (© David Arbour).

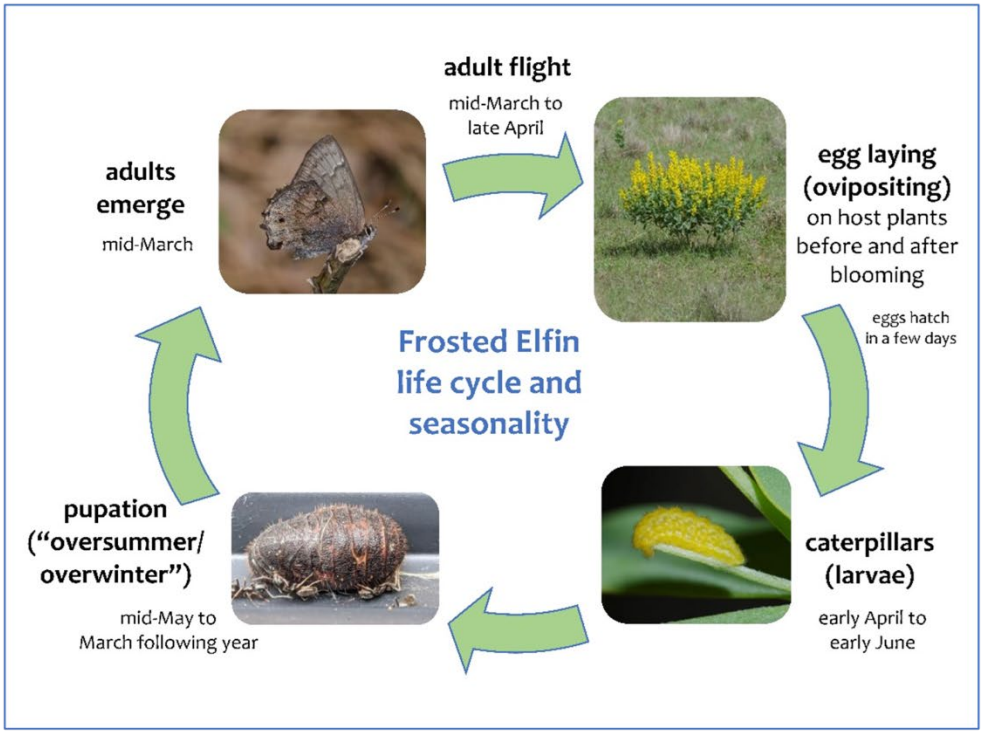

Figure 2. Life cycle and seasonality of the single brooding Frosted Elfin, *Callophrys irus hadros*. Photos © Bryan E. Reynolds or © Leah Dudley/Jose Montalva.

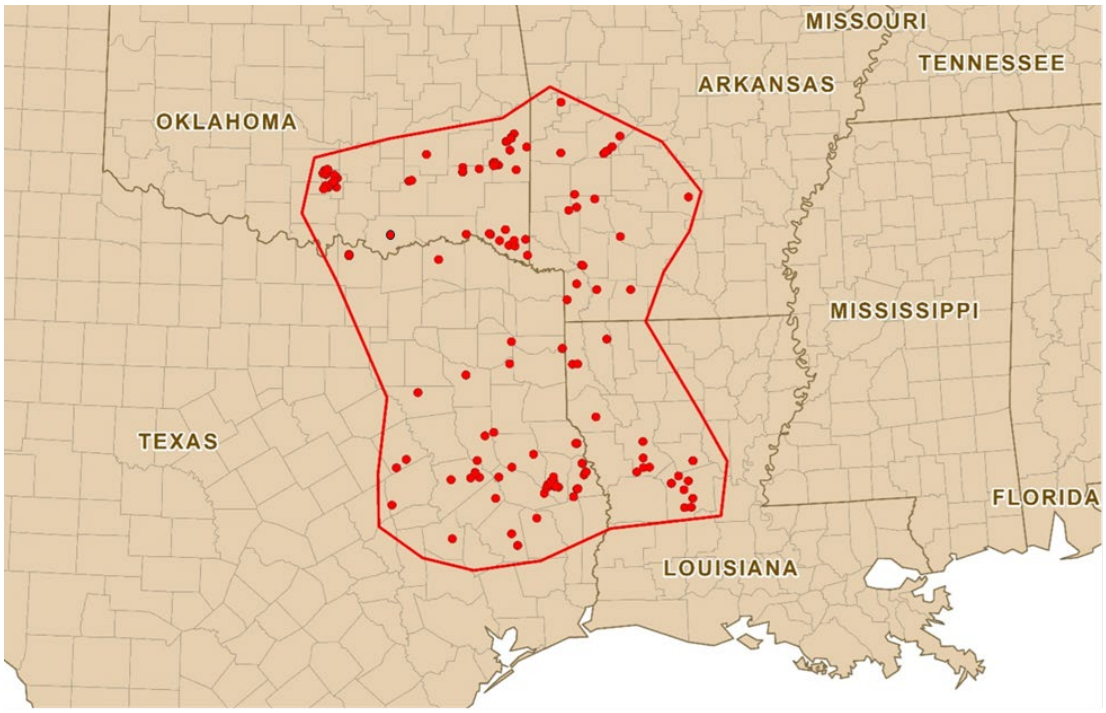

Figure 3. All known occurrences of the regional subspecies of the Frosted Elfin (*Callophrys irus hadros*).

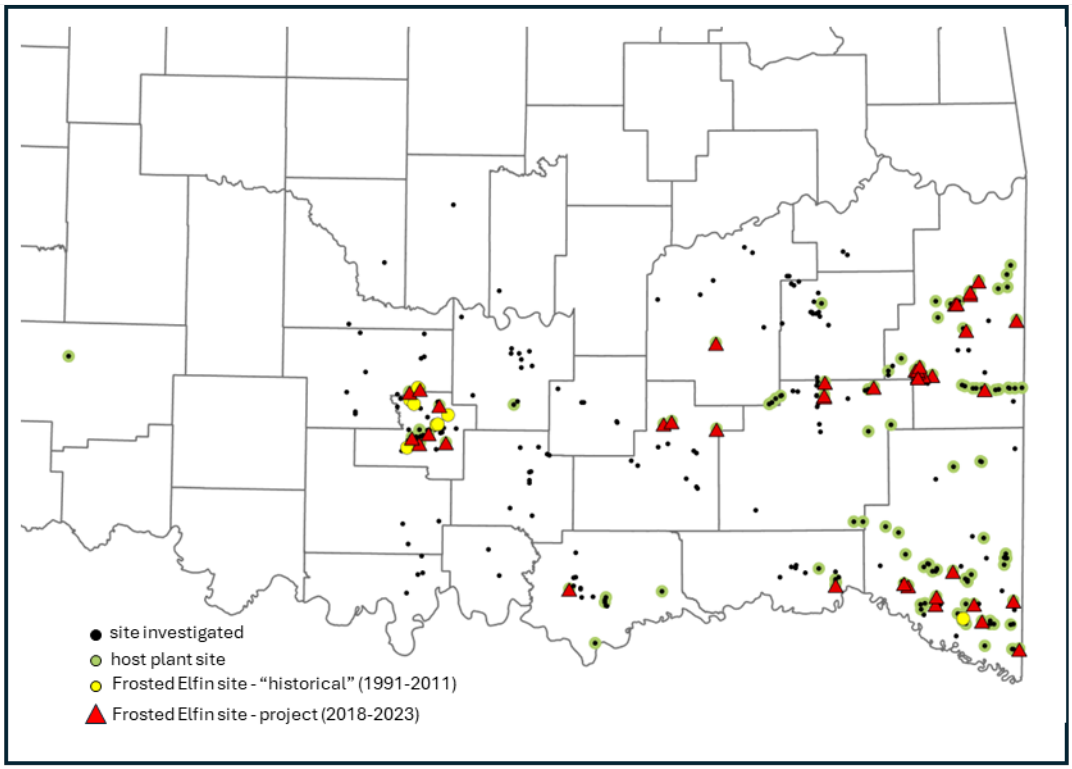

Figure 4. Distribution of sites investigated for the Oklahoma Frosted Elfin Project. Also shown are host plant patches and historical and contemporary locations for the Frosted Elfin.

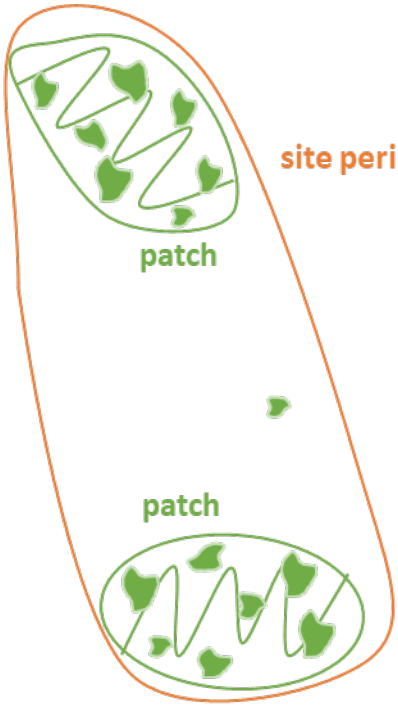

Figure 5. A "site" versus a "patch". Sites were assigned when a host plant patch or patches were found. If a patch was isolated from other host plants, the patch and site perimeters would be the same. If multiple patches were near one another but separated by breaks in the habitat, they were considered clustered enough to comprise a "site", with the site perimeter drawn to encompass all associated patches. As per the USFWS monitoring protocol (USFWS 2020), transects were established within each patch that was monitored (Fig. 5).

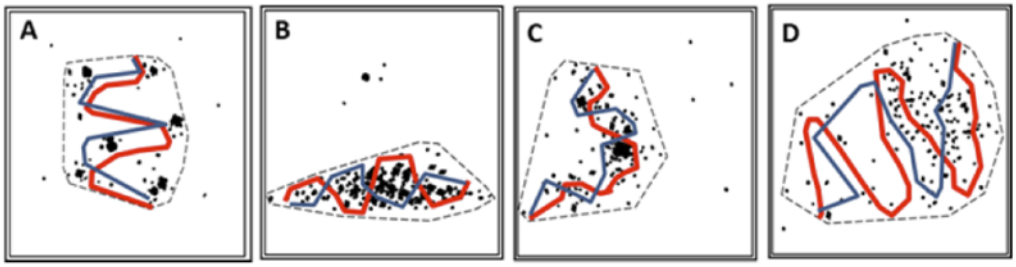

Figure 6. USFWS monitoring protocol transect scheme. Quoting their caption, "Walk-through count paths. Colored lines represent possible paths. Observers will mark a fixed beginning and end point, but the patch may vary somewhat each count. The path should weave through the patch allowing the observer to view the areas most densely populated by host plants, but without doubling back. Observers may need to step over plants to avoid trampling. Try to walk near most of the plants and observe closely—it will be easiest to visually confirm butterflies at rest on the plants. Do not attempt to net butterflies until the count is complete and only with necessary permits." (figure and caption, USFWS 2020)

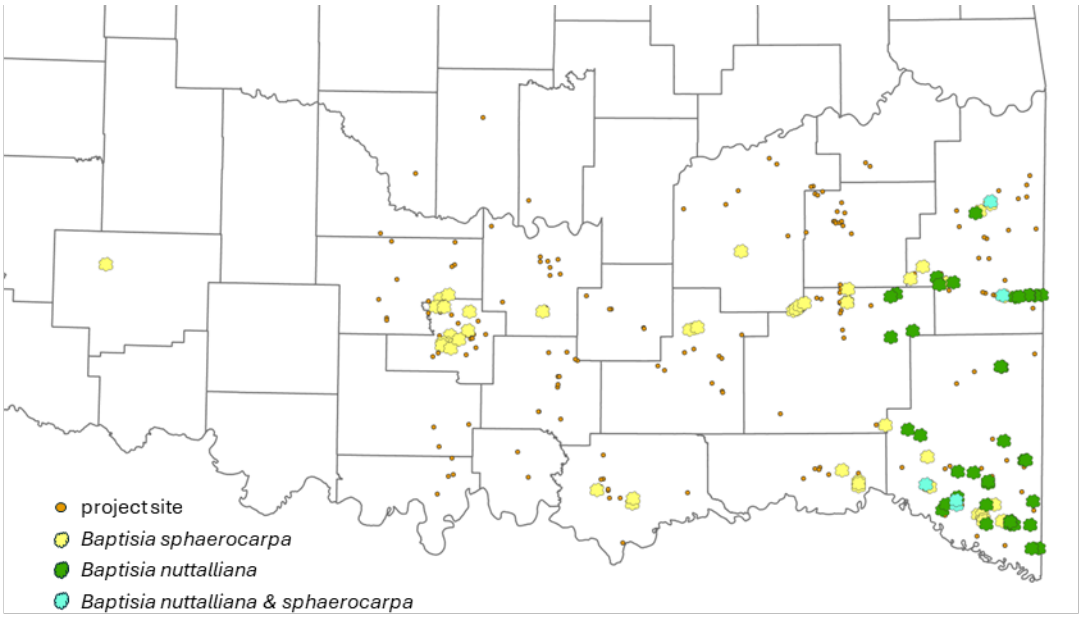

Figure 7. Distribution of host plant patches investigated for the Oklahoma Frosted Elfin Project. Those indicated by orange dots were either historical patches that were no longer present or could not be re-located or the *Baptisia* species was not reported or was not conclusively determined to species.

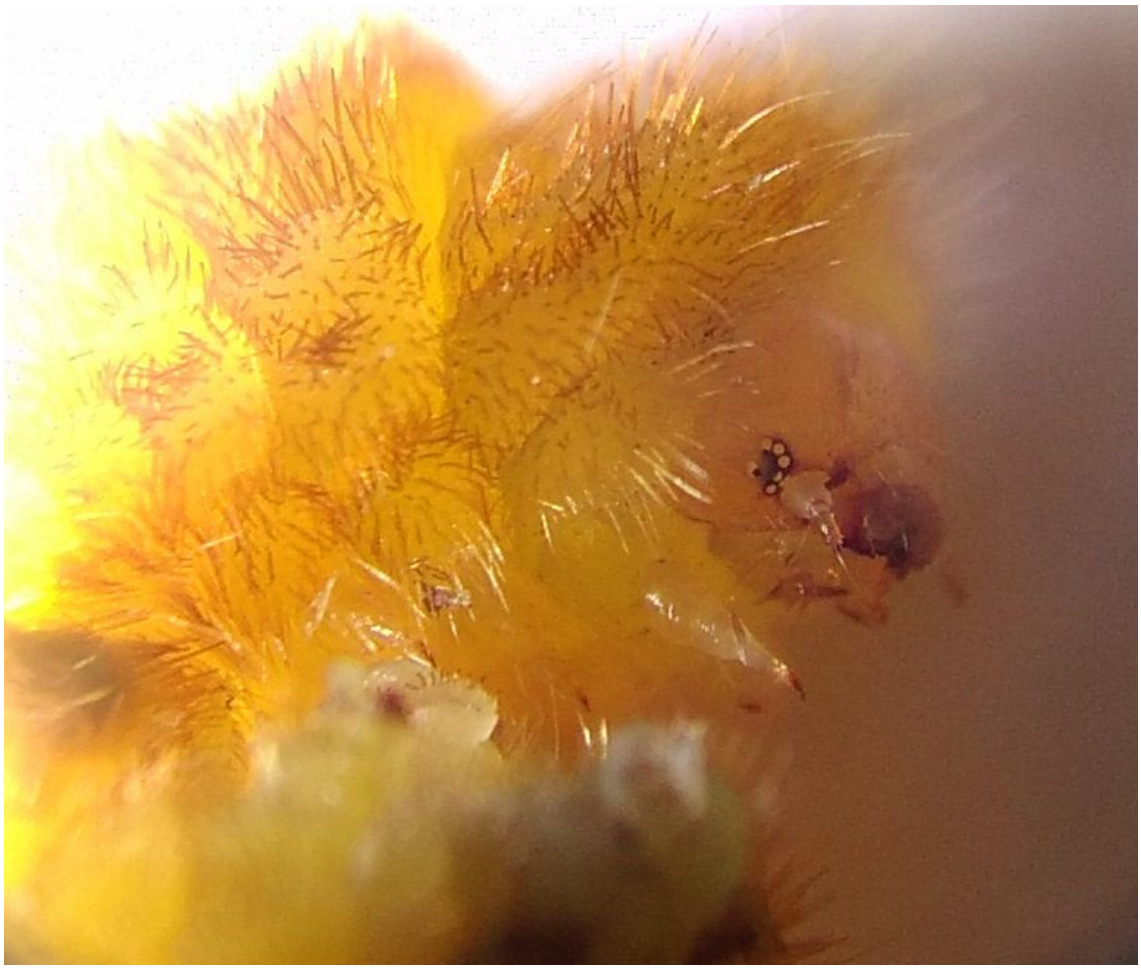

Figure 8. Ocelli and pigmentation of *Callophrys irus hadros* larva. The crescent shape to the pigmentation, not touching the sixth ocelli is diagnostic of the Frosted Elfin (Meyer and McElveen n.d.). Specimen collected on 4 May 2023 at PITT009-01, Pittsburg County, Oklahoma.