FINAL PERFORMANCE REPORT



Federal Aid Grant No. F21AP03585 (E-94-R-2)

Surveys within Oklahoma's historical range of the Frosted Elfin (*Callophrys irus*) to determine current distribution and status

Oklahoma Department of Wildlife Conservation

January 1, 2022 - December 31, 2022

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State: Oklahoma

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

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Principal Investigator: 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 2022 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). In 2021–2022, we bridged much of that gap by bringing the number of known locations of Frosted Elfins in Oklahoma to almost 50 in eight counties. We now have 130 records of the species in the state. During our 191 site visits in 2022, we recorded a minimum of 1522 individual elfins (adults and larvae), which has helped to assess relative abundance of the regionally endemic subspecies of Frosted Elfins. We monitored 12 sites in four counties using US Fish and Wildlife Service (USFWS) protocol for evaluating the conservation status of Frosted Elfins. Finally, we grew our network of private landowners, volunteers, and citizens interested in the project and conservation, in general.

Objectives:

- 1) Assess the continued presence of the Frosted Elfin in the projected historical range.
- 2) Assess relative abundance at known and new colonies.
- 3) Sample known and new colonies for genetic studies to determine the specific/subspecific status in Oklahoma and regionally.
- 4) Continue to build a network of private landowners, agencies, and volunteers willing to look for and monitor populations of the Frosted Elfin in successive years.

Summary of Progress:

Project Background

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 1991–1992 (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 appeared to have been developed but 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 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). In 2021, the Oklahoma Frosted Elfin Project's (OFEP) team lead by Matt Moran (Hendrix College) reported adult Frosted Elfins in association with *B. bracteata*, longbract wild indigo, and the OFEP's Leah Dudley (East Central University) team found two larvae on *B. bracteata* that appeared to be Frosted Elfins. We are hesitant about the larval record because the larvae were green instead of yellow as previously documented with *C. i. hadros*. Unfortunately, the

larvae did not pupate during lab rearing, so documentation of Frosted Elfins at that site is still lacking. One possibility still exists for confirmation. Frass was taken for DNA sampling, so it could indicate *C. irus hadros*, but there is a possibility that the larvae were instead *Strymon melinus*, the Gray Hairstreak, which are green and can be confused with *C. i. hadros*. Results are still pending.

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). 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* has been assigned a NatureServe national subspecies rank of T2 (Imperiled Subspecies); and a subnational rank (SRank) of 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 km². 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 and 2022 with funding through the Oklahoma Department of Wildlife Conservation (ODWC). This research will also be opportune for the consideration of federal listing of the species by September 30, 2023 (USFWS-NY 2018).

Prior to our recent investigations, many gaps existed in 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 by visiting established project sites, finding new locations, and monitoring select populations of Frosted Elfins in line with USFWS protocol (USFWS 2020).

Methods

Field Surveys and monitoring

In 2022, surveys were conducted 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 2021, historical sites of host plants, which were identified via the Oklahoma Vascular Plants Database, <u>https://www.oklahomaplantdatabase.org/</u>, and supplemented by others from the online museum specimen portal, iDigBio, <u>https://www.idigbio.org/portal/search</u>, and iNaturalist, <u>https://inaturalist.org</u>, 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. 4). 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 mid-March and early June 2022 (Fig. 2), 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* sp. had died back.

Once *Callophrys irus hadros* larvae were first noted for the season on *Baptisia* sp. plants, 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. Over the course of the field season, we developed a scaled sampling strategy to tackle the disparity in patch sizes (5–3000 plants) and difficulties presented by limited personpower. For patches with 20 or fewer plants, we checked every plant in the patch. For patches with >20 plants, we either noted the number of plants examined or examined approximately 10% of the total plants in the patch. We examined every fifth plant in a patch transect and recorded the number of larvae on the plant. Body characteristics (e.g., color, length, width, injuries) were noted as a first attempt at discerning instar stages. Behaviors, including myrmecophily (ant associations), were recorded. A sample of larvae were photographed and/or videoed. Pupal cases were searched for in late May and June by digging into soil and/or sifting through leaf litter at the base of the host plants.

A subset of sites was monitored fully or partially, as per the USFWS monitoring protocol (USFWS 2020). Monitored sites were in Atoka, LeFlore, Murray, and Pushmataha counties. Sites were monitored at least three times during the field season 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. 5). 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. Larval surveys, which were not part of the USFWS protocol, could then be commenced. Habitat assessments were made during peak flowering.

Data collected for 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. Stone measured all larvae encountered at two sites in Le Flore County. Others collected length data opportunistically at sites in McCurtain, Murray, and Pushmataha Counties.

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; <u>http://obis.ou.edu</u>), the official repository of biological data for Oklahoma.

Surveys and monitoring were conducted by four teams lead by: 1) the Principal Investigator, Brenda D. Smith, 2) the on-the-ground project manager, Marie E. B. Stone, 3) Dr. Leah Dudley and Jose Montalva of East Central University, and 4) Dr. Matt Moran of Hendrix College. Surveys were conducted within the projected historical 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, Pittsburg, Pontotoc, Pottawatomie, Pushmataha, Seminole, Sequoyah, and Stephens Counties. 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.

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 thousands of miles were driven 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; <u>https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states</u>), public lands, and land cover classes, the latter as

indicated by the National Land Cover Database (NLCD; US Geological Survey, 2016, <u>https://www.usgs.gov/node/279743</u>). 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.

DNA sampling and analyses

During 2021, the Moran team collected directly or acquired through donations, several hundred specimens of *Callophrys irus hadros* representing 15 states and 41 counties within the US. Samples (n = 53) from Oklahoma were taken by Dudley and Moran (Table 1) 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). At the start of the 2022 field season, Moran determined that further sampling was not necessary for his genetic study so additional samples were not taken. Moran's team 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 they suspect it to be.

Network building

Outreach activities 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*. Teams also enlisted volunteers, e.g., David Arbour, Dr. Claire Curry, Dr. Bruce Hoagland, Vonceil Harmon, 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

Field Surveys and Monitoring

Project teams surveyed and monitored between 16 March and 1 June 2022 for 35 days (or 49 person days, as defined by a combination of the survey day, location, and specific surveyor; Table 1, Appendix A). Teams made 191 site visits to investigate 136 unique sites, of which 42 sites were newly discovered in 2022 (Fig. 6). Some of the 136 sites could not be surveyed due to lack of landowner permission or other accessibility issues (e.g., flooding), so only their locations and/or basic data were recorded. We were able to survey and/or monitor at 101 sites, some of which contained multiple host plant patches that were individually surveyed and/or monitored,

accounting for 121 patches and 188 patch visits. Investigations occurred in 12 counties, including one new (Comanche) county different from previous project years (Fig. 6). Host plants were found in Comanche County but no elfins were detected. New host plant sites were discovered elsewhere, including in counties with little previous investigations, such as Bryan and Choctaw Counties. Six counties produced records of *C. i. hadros* (Fig. 7).

We visited 12 sites (20 patches) at least three times in 2022: in Atoka (2 sites), LeFlore (5 sites), Murray (3 sites), and Pushmataha (2 sites) counties. Eleven sites (19 patches) were monitored fully or partially as per the USFWS monitoring protocol (USFWS 2020).

team	survey dates	# counties	# days	# sites	# patches	site visits	patch visits
Dudley/Montalva	16 Mar – 1 Jun	5	16	12	19	27	41
Moran	25 Mar – 10 Apr	1	5	16	18	16	18
Smith	16 Mar – 1 Jun	9	18	95	77	94	94
Stone	3 Apr – 7 May	5	10	36	31	46	45
project totals		12	35	136	121	191	188

Table 1. Surveys and monitoring for the Frosted Elfin (Callophrys irus hadros) inOklahoma in 2022. Surveys and monitoring in 2022 occurred between 16 March and 1 June.Effort totals are by team as well as by overall effort combined into project totals.

Prior to observations of *Callophrys irus hadros* 2022 emergence, we visited 10 sites in three counties (Atoka, Murray, and McCurtain) between 16–26 March 2022. 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 5.75 hours, with an average of 37.77 minutes/patch. Frosted Elfins (adults or larvae) were recorded at 21 of the 136 sites surveyed (15.44%), which is similar to our findings in 2021 (15.34%). We surveyed at or near 11 Oklahoma state and/or Federal properties and within numerous right-of-ways of the Oklahoma Department of Transportation.

Adult *Callophrys irus hadros* were observed between 27 March and 23 April at 12 sites, and larvae were observed between 23 April and 1 June at 16 sites. A minimum of 1522 individuals were reported, consisting of 598 adults and 924 larvae, with at least one individual recorded at each positive location (25.4 mean individuals, 18.1 mean adults, 34.2 mean larvae). Highest counts per host plant patch were 104 for adults and 337 for larvae. Mean length of *C. irus hadros* larvae at two sites in LeFlore County were 10.87 mm (\pm 4.00 SD, range 4–19 mm, n = 102). Opportunistically collected larval length data at sites in McCurtain, Murray, and Pushmataha Counties ranged 8–22 mm (n = 36).

Surveys and monitoring provided more documentation in support of *Callophrys irus hadros* use of *B. sphaerocarpa* and *B. nuttalliana* in Oklahoma. *Callophrys irus hadros* were found in varying levels at sites with different *Baptisia* sp.: 11 of 29 sites with *B. sphaerocarpa*; six of 34 sites with *B. nuttalliana*; and two of three sites with *B. australis*. However, *C. i. hadros* were absent from all five sites with *Baptisia bracteata*. Two of ten sites with unidentified *Baptisia* sp. (likely *B. sphaerocarpa* or possible hybrids) had *C. i. hadros*.

Myrmecophily (ant association) 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), but we have only a small handful of observations in Oklahoma with *C. i. hadros* larvae. In 2022, we added one observation. In McCurtain County, at site MCCU012 on 28 April 2022, Brenda Smith and Bruce Hoagland observed a *C. i. hadros* early-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. Other ant associations were not reported for the field season. 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).

Biogeographically speaking, *C. i. hadros* is known to fall within four ecoregions within Oklahoma: Cross Timbers, Ouachita Mountains, Arkansas Valley, and South Central Plains. A fourth ecoregion, the East Central Texas Plains, is added when also including known localities in Texas. 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 2).

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

Table 2. National Land Cover Database associations to known Frosted Elfin (Callophrysirus hadros) localities within Oklahoma and the region. Four cover classes have the highestpercentages within 1-km buffer zones of localities regardless of whether data are only fromOklahoma or added to all known localities. Forest type differs between datasets.

DNA sampling and analyses

As of the writing of this report, the Moran team has successfully amplified DNA from multiple specimens and have a procedure that appears to be working well. They also sequenced a small number of specimens. They are currently in the process of amplifying and sequencing the remaining specimens. Research is on-going with the Florida State University Frosted Elfin DNA project. Results are pending.

Network building

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, and the Kerr Center for Sustainable Agriculture.

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. Such fortuitous highlights not only bring attention to the hard work of all our team members but more importantly it brings needed attention to the conservation of this and other insect species.

Discussion

Frosted Elfins are indeed still present and seemingly doing well within the projected historical range. Prior to our recent surveys (2021–2022), we knew of <20 records for the species in Oklahoma (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 (Fig. 7) 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. We now have almost 130 records of *C. i. hadros* in Oklahoma.

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. Previous high counts for adults were 30 (in 2021, for this project; Smith, et al. 2022) and 40 (in 2018, ONHI project). For larvae, the high count in 2022 was 337 individuals. The next highest report, of 25 larvae, is from 2021 (Smith, et al. 2022).

Our knowledge of the species' phenology in Oklahoma was also enhanced. Though flight season of adults (12 March–1 May) was not extended from previous years, 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 2022, 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.

The first sightings of adult C. i. hadros in Oklahoma were on 27 March, when they were seen at two sites in Murray County (MURR001, MURR029). 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 27th 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, also fairly close to the Oklahoma border, on 19 March despite host plants not being present; on 27 March, 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.

Further insight into *C. i. hadros* phenology was gained by our observations of larvae. The previously established early date of 1 April still stands, even though in 2022 our earliest sighting was 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). Thus, the documented end of the larval season was extended by 18 days (previous late date was 14 May). It is currently unclear if this late date was due to an overall shift in the activity season due to late host plant emergence or if we were just better able this year to capture the species' real phenological persistence. Future research will aid in a better understanding of the bounding timeframe of larval activity.

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 rangewide data within the region (Table 2). Hay/pasture and herbaceous cover ranked fairly high to high in each of the datasets but forest type made a virtual switch, with deciduous forest downplayed when all regional data were considered, replaced by evergreen forest. Further investigations into habitat associations are warranted and would be useful for a better understanding of *C. i. hadros*'s ecological parameters.

We continued to build upon the 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 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. We also hope that such relationship building will allow for the project to continue into subsequent years and be a useful tool to assist in the conservation of other species and habitat.

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, pigmentation near the ocelli, and limited fluorescence

- 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* (Albanese, et al. 2007, McElveen, et al. 2020), 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*.

Significant Deviations:

All project objectives were met except for that of sampling additional known and new colonies for genetic studies (objective #3). At the start of the field season, Dr. Moran's team determined that additional samples were not necessary for their study. Their pending genetic analyses may reveal that the *hadros* subspecies is actually a species unto itself, which may explain why *C. i. hadros* differs in many ways from its eastern counterpart.

Equipment Purchased (Cumulative):

No equipment exceeding \$5,000 in cost was purchased under this grant.

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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. 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 5. 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 6. All Oklahoma sites that have been identified, surveyed, and/or monitored for the Frosted Elfin (*Callophrys irus hadros*), 1991–2022 (top). A closer look at sites investigated in 2022 (bottom), 42 of which were new. Counties sites fall within are highlighted.



Figure 7. Known locations (n = 49) for the Frosted Elfin, *Callophrys irus hadros*, in Oklahoma. Dots represent locations found 1991–2021. Triangles are sites with the species in 2022.

APPENDIX A

Site Name	County	Surveyor Team	Site Visit Date	FE Lifestage
ATOK004	Atoka	Dudley/Montalva	3/16/2022	
ATOK004	Atoka	Dudley/Montalva	4/8/2022	
ATOK004	Atoka	Dudley/Montalva	4/19/2022	
ATOK004	Atoka	Dudley/Montalva	5/6/2022	Larvae
ATOK005	Atoka	Dudley/Montalva	4/8/2022	Adults
ATOK005	Atoka	Dudley/Montalva	4/19/2022	Adults
ATOK005	Atoka	Dudley/Montalva	5/6/2022	Larvae
ATOK006	Atoka	Stone	4/3/2022	
BRYA002	Bryan	Smith	4/21/2022	
BRYA003	Bryan	Smith	4/21/2022	
BRYA004	Bryan	Smith	5/6/2022	
BRYA005	Bryan	Smith	5/6/2022	
BRYA006	Bryan	Smith	5/6/2022	
BRYA007	Bryan	Smith	5/6/2022	
BRYA008	Bryan	Smith	5/6/2022	
BRYA009	Bryan	Smith	5/6/2022	
BRYA010	Bryan	Smith	5/6/2022	
BRYA011	Bryan	Smith	5/6/2022	
BRYA012	Bryan	Smith	5/6/2022	
BRYA013	Bryan	Smith	5/6/2022	
BRYA014	Bryan	Smith	5/6/2022	
CHOC001	Choctaw	Smith	4/21/2022	
CHOC008	Choctaw	Smith	4/21/2022	
CHOC009	Choctaw	Smith	4/21/2022	
CHOC010	Choctaw	Smith	4/21/2022	
COMA001	Comanche	Dudley/Montalva	5/14/2022	
GARV003	Garvin	Smith	3/27/2022	
GARV003	Garvin	Dudley/Montalva	4/3/2022	
GARV005	Garvin	Dudley/Montalva	4/3/2022	
GARV012	Garvin	Dudley/Montalva	4/3/2022	
GARV012	Garvin	Dudley/Montalva	5/13/2022	Larvae
LATI002	Latimer	Stone	4/22/2022	
LATI005	Latimer	Stone	4/22/2022	
LATI007	Latimer	Stone	4/22/2022	
LATI010	Latimer	Stone	4/22/2022	
LATI011	Latimer	Stone	4/22/2022	
LATI013	Latimer	Stone	4/22/2022	

Survey effort in 2022 for *Callophrys irus hadros*, the Frosted Elfin, in Oklahoma. Lifestage (larvae or adults) is indicated for the site visits that elfins were found.

LATI014	Latimer	Stone	4/22/2022	
LATI015	Latimer	Stone	4/22/2022	
LATI016	Latimer	Stone	4/22/2022	
LEFL002	Le Flore	Stone	4/6/2022	Adults
LEFL002	Le Flore	Stone	4/15/2022	Adults
LEFL002	Le Flore	Stone	5/6/2022	Larvae
LEFL002	Le Flore	Stone	5/7/2022	Larvae
LEFL004	Le Flore	Stone	4/10/2022	Adults
LEFL004	Le Flore	Smith	5/18/2022	
LEFL007	Le Flore	Stone	4/26/2022	
LEFL008	Le Flore	Stone	4/3/2022	Adults
LEFL008	Le Flore	Stone	4/14/2022	
LEFL009	Le Flore	Stone	4/3/2022	Adults
LEFL009	Le Flore	Stone	4/14/2022	Adults
LEFL009	Le Flore	Stone	5/7/2022	Larvae
LEFL010	Le Flore	Smith	4/29/2022	
LEFL011	Le Flore	Smith	5/11/2022	
LEFL013	Le Flore	Stone	4/27/2022	
LEFL013	Le Flore	Smith	5/11/2022	
LEFL014	Le Flore	Smith	5/11/2022	
LEFL017	Le Flore	Stone	4/26/2022	
LEFL018	Le Flore	Stone	4/26/2022	
LEFL020	Le Flore	Stone	4/3/2022	
LEFL020	Le Flore	Stone	4/14/2022	
LEFL020	Le Flore	Smith	5/19/2022	
LEFL021	Le Flore	Stone	4/3/2022	
LEFL021	Le Flore	Stone	4/14/2022	Adults
LEFL021	Le Flore	Stone	4/27/2022	Larvae
LEFL023	Le Flore	Stone	4/27/2022	
LEFL024	Le Flore	Smith	5/10/2022	
LEFL025	Le Flore	Smith	5/10/2022	
LEFL031	Le Flore	Stone	4/10/2022	
LEFL032	Le Flore	Stone	4/10/2022	
LEFL033	Le Flore	Stone	4/10/2022	
LEFL034	Le Flore	Smith	5/19/2022	
LEFL035	Le Flore	Stone	4/10/2022	
LEFL035	Le Flore	Smith	5/18/2022	
LEFL036	Le Flore	Stone	4/10/2022	Adults
LEFL037	Le Flore	Stone	4/10/2022	
LEFL038	Le Flore	Stone	4/10/2022	
LEFL045	Le Flore	Stone	4/27/2022	
LEFL045	Le Flore	Smith	5/11/2022	

LEFL046	Le Flore	Stone	4/10/2022	
LEFL046	Le Flore	Smith	4/29/2022	
LEFL046	Le Flore	Smith	5/11/2022	
LEFL048	Le Flore	Smith	5/1/2022	
MCCU001	McCurtain	Smith	4/28/2022	
MCCU002	McCurtain	Smith	5/13/2022	
MCCU004	McCurtain	Smith	4/22/2022	
MCCU005	McCurtain	Moran	3/27/2022	
MCCU007	McCurtain	Smith	5/13/2022	
MCCU008	McCurtain	Smith	5/13/2022	
MCCU012	McCurtain	Smith	4/28/2022	Larvae
MCCU013	McCurtain	Smith	4/28/2022	
MCCU014	McCurtain	Smith	5/13/2022	
MCCU015	McCurtain	Smith	4/28/2022	
MCCU016	McCurtain	Moran	3/26/2022	
MCCU016	McCurtain	Smith	5/13/2022	
MCCU017	McCurtain	Smith	4/22/2022	
MCCU018	McCurtain	Smith	5/13/2022	
MCCU019	McCurtain	Smith	4/22/2022	
MCCU020	McCurtain	Smith	5/13/2022	
MCCU022	McCurtain	Moran	3/27/2022	
MCCU022	McCurtain	Smith	4/22/2022	
MCCU024	McCurtain	Moran	3/26/2022	
MCCU024	McCurtain	Smith	4/23/2022	
MCCU025	McCurtain	Moran	3/26/2022	
MCCU026	McCurtain	Moran	3/26/2022	
MCCU026	McCurtain	Smith	4/23/2022	
MCCU028	McCurtain	Smith	4/23/2022	
MCCU031	McCurtain	Smith	4/23/2022	
MCCU031	McCurtain	Smith	5/13/2022	
MCCU032	McCurtain	Smith	4/28/2022	
MCCU033	McCurtain	Smith	4/23/2022	
MCCU035	McCurtain	Smith	4/21/2022	
MCCU036	McCurtain	Smith	4/21/2022	
MCCU038	McCurtain	Smith	4/29/2022	
MCCU039	McCurtain	Smith	4/29/2022	
MCCU043	McCurtain	Smith	4/28/2022	
MCCU046	McCurtain	Smith	4/22/2022	
MCCU046	McCurtain	Smith	5/12/2022	
MCCU049	McCurtain	Moran	3/27/2022	
MCCU050	McCurtain	Moran	3/25/2022	
MCCU051	McCurtain	Moran	3/26/2022	

MCCU052	McCurtain	Moran	3/26/2022	
MCCU053	McCurtain	Moran	4/3/2022	
MCCU054	McCurtain	Moran	4/3/2022	
MCCU055	McCurtain	Moran	4/3/2022	Adults
MCCU056	McCurtain	Moran	4/10/2022	
MCCU057	McCurtain	Moran	4/10/2022	Adults
MCCU058	McCurtain	Moran	4/10/2022	
MCCU059	McCurtain	Smith	5/13/2022	
MCCU060	McCurtain	Smith	5/13/2022	
MCCU061	McCurtain	Smith	5/13/2022	
MCCU062	McCurtain	Smith	5/13/2022	
MCCU063	McCurtain	Smith	4/22/2022	
MCCU063	McCurtain	Smith	5/12/2022	Larvae
MCCU064	McCurtain	Smith	4/22/2022	
MCCU064	McCurtain	Smith	5/12/2022	
MCCU065	McCurtain	Smith	4/28/2022	
MURR001	Murray	Smith	3/16/2022	
MURR001	Murray	Smith	3/27/2022	Adults
MURR001	Murray	Dudley/Montalva	4/2/2022	Adults
MURR001	Murray	Dudley/Montalva	4/10/2022	
MURR001	Murray	Dudley/Montalva	4/17/2022	Adults
MURR001	Murray	Dudley/Montalva	4/30/2022	Larvae
MURR012	Murray	Smith	5/20/2022	Larvae
MURR028	Murray	Dudley/Montalva	4/9/2022	Adults
MURR028	Murray	Dudley/Montalva	4/23/2022	Adults, Larvae
MURR028	Murray	Dudley/Montalva	4/30/2022	Larvae
MURR029	Murray	Smith	3/16/2022	
MURR029	Murray	Smith	3/27/2022	Adults
MURR029	Murray	Dudley/Montalva	4/2/2022	
MURR029	Murray	Dudley/Montalva	4/10/2022	Adults
MURR029	Murray	Dudley/Montalva	4/17/2022	Adults
MURR029	Murray	Dudley/Montalva	5/16/2022	Larvae
MURR029	Murray	Smith	5/20/2022	Larvae
MURR029	Murray	Smith	6/1/2022	Larvae
MURR032	Murray	Smith	3/27/2022	
MURR032	Murray	Dudley/Montalva	5/13/2022	Larvae
MURR033	Murray	Smith	3/27/2022	
MURR033	Murray	Dudley/Montalva	4/3/2022	
MURR038	Murray	Smith	5/20/2022	
PITT007	Pittsburg	Stone	4/22/2022	
PITT008	Pittsburg	Stone	4/22/2022	
PITT009	Pittsburg	Smith	5/10/2022	

PITT010	Pittsburg	Smith	5/10/2022	
PITT011	Pittsburg	Smith	5/10/2022	
PITT012	Pittsburg	Smith	5/10/2022	
PONT001	Pontotoc	Dudley/Montalva	5/12/2022	
PUSH002	Pushmataha	Smith	5/10/2022	
PUSH003	Pushmataha	Smith	5/10/2022	
PUSH004	Pushmataha	Smith	5/10/2022	
PUSH005	Pushmataha	Smith	5/10/2022	
PUSH006	Pushmataha	Smith	5/10/2022	
PUSH007	Pushmataha	Smith	5/10/2022	Larvae
PUSH007	Pushmataha	Smith	5/19/2022	
PUSH011	Pushmataha	Stone	4/3/2022	
PUSH011	Pushmataha	Stone	4/14/2022	
PUSH016	Pushmataha	Stone	4/3/2022	
PUSH016	Pushmataha	Smith	5/10/2022	Larvae
PUSH016	Pushmataha	Smith	5/19/2022	
PUSH017	Pushmataha	Stone	4/3/2022	
PUSH017	Pushmataha	Smith	5/10/2022	
PUSH020	Pushmataha	Smith	5/10/2022	
PUSH021	Pushmataha	Stone	4/3/2022	
PUSH021	Pushmataha	Smith	5/10/2022	Larvae
PUSH021	Pushmataha	Smith	5/19/2022	
PUSH024	Pushmataha	Smith	4/23/2022	
PUSH024	Pushmataha	Smith	4/28/2022	
PUSH026	Pushmataha	Smith	5/10/2022	
PUSH027	Pushmataha	Smith	5/10/2022	
PUSH028	Pushmataha	Smith	5/10/2022	
PUSH029	Pushmataha	Smith	5/10/2022	