

EGG LAYING BEHAVIOUR AND LARVAL SHELTER-CONSTRUCTION PATTERNS
OF THE ENDANGERED MOTTLED DUSKYWING (*ERYNNIS MARTIALIS*) BUTTERFLY'S
WESTERN POPULATION IN CANADAJUSTIS HENAULT^{1*}, D. RYAN NORRIS², JESSICA E. LINTON³, AND RICHARD WESTWOOD¹¹ Department of Biology, University of Winnipeg, 515 Portage Avenue, Winnipeg, MB, Canada, R3B 2E9² Department of Integrative Biology, University of Guelph, 50 Stone Rd. E, Guelph, ON N1G 2W1³ Natural Resource Solutions Inc., 415 Phillip St., Unit C, Waterloo, ON N2L 3X2

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ABSTRACT. The Mottled duskywing (*Erynnis martialis*) butterfly is endangered, living in pine forests and oak barrens in Canada and the eastern United States of America. While host plants and larval behaviour is documented in Mottled duskywing's eastern range, these life components are poorly known in Manitoba. We observed adult behaviour, host plant species used and larval foraging to better understand these biological aspects of *E. martialis*. We observed eggs laid exclusively on *Ceanothus herbaceus*, and larvae consuming *C. herbaceus* in leaf shelters near the periphery of plants. Early instar larvae tied leaves together with cells of silk creating partly open shelters while later instar larvae completely sealed shelters. Shelters constructed out of young leaves at the edge of plants are likely easier to digest. Later-instar larvae may nocturnally harvest food to consume in shelters during the day to reduce predation risk. Larvae were found in clearings adjacent to *Pinus banksiana* dominated forests, with these openings likely providing suitable microhabitats for egg development and larval feeding. We observed newly emerged adults during weeks 1 to 5 of the flight period; eggs, larvae and adults overlapped. We recommend direct observations of larval foraging—during the day and night, as well as transitions into and out of diapause—to more accurately describe their behaviour and physiology. We started to characterise microhabitats, however further research is needed. Our research may help to guide critical habitat designations, leading to successful Mottled duskywing recovery in Manitoba.

Additional key words: endangered, microhabitat, larval feeding, flight period

The Mottled duskywing (*Erynnis martialis* (Scudder, [1870]): Hesperiiidae, Pyrginae) is an endangered butterfly in Canada (Committee on the Status of Endangered Wildlife in Canada 2012, Fig. 1). It predominantly occurs in pine forests and oak barrens (Committee on the Status of Endangered Wildlife in Canada 2012). The Mottled duskywing population in Manitoba is referred to as the Boreal Population Designatable Unit, separated from eastern populations in Ontario and the United States (Committee on the Status of Endangered Wildlife in Canada 2012). This butterfly occurs in several areas in southern Ontario and reintroduction efforts are underway to support viable populations in the long-term (Committee on the Status of Endangered Wildlife in Canada 2012, Committee on the Status of Species at Risk in Ontario 2013, Linton & Otis 2018). Mottled duskywing occurs as one generation per year in most of Canada however warmer areas in Canada and the United States may support two generations (Schweitzer et al. 2011, Committee on the Status of Endangered Wildlife in Canada 2012, Committee on the Status of Species at Risk in Ontario 2013).

Mottled duskywing has been historically recorded in south-eastern Manitoba in areas that include several provincial forests and parks. Past records are geographically scattered and sporadic over the last 60 years (Committee on the Status of Endangered Wildlife

in Canada 2012, data provided by M. Curteanu [Canadian Wildlife Service, Government of Canada] pers. com. 2019). Therefore, details about distribution in Manitoba, size of the population and individual behaviours are scant.

Mottled duskywing larvae have been observed feeding on *Ceanothus herbaceus* Raf. (Rhamnaceae) (Narrow-leaved New Jersey tea, Prairie Redroot) and



FIG. 1. Adult *E. martialis* nectar feeding on *Ceanothus herbaceus* (J. Henault). Its wingspan is approximately 5 cm (Layberry et al. 1998).

Ceanothus americanus L. (New Jersey tea) in eastern Canada and the United States (Olson 2002, Schweitzer et al. 2011, Committee on the Status of Endangered Wildlife in Canada 2012, Committee on the Status of Species at Risk in Ontario 2013). Larvae have been reported to use shelters during their development at some point (Committee on the Status of Endangered Wildlife in Canada 2012), and Olson (2002) reported that larvae feed during the night in the United States. In areas where an annual generation is more likely, larvae feed during the summer, initiate diapause in the fall and pupate in the spring in dead leaves (Schweitzer et al. 2011). *Ceanothus herbaceus* has been reported in Manitoba, however *C. americanus* has not (*C. Murray* [Manitoba Conservation Data Centre, Government of Manitoba, Canada] pers. comm. 2019). *Ceanothus herbaceus* and *C. americanus* prefer sandy, well drained soils in central states (including Minnesota) and eastern Canada and the United States (Coladonato 1993, Shuey 2005, COSEWIC 2012, McClain & Ebinger 2014). To us this suggests both species could be well adapted to sandy pine parkland ecosystems in southeastern Manitoba. Researchers have not reported adult oviposition details, larval host plant species used, pupal substrate or the time of day larvae feed in Manitoba (Committee on the Status of Endangered Wildlife in Canada 2012, Committee on the Status of Species at Risk in Ontario 2013). Observations of Mottled duskywing in eastern Canada indicates that suitable habitat likely requires *Ceanothus* spp., potentially nectar species and a lack of grazing pressure (Schweitzer et al. 2011, Committee on the Status of Endangered Wildlife in Canada 2012). Adults have been observed feeding on *C. herbaceus* in Manitoba (Dodgson 2020) but the identities of other nectar plants in Manitoba have not been reported.

Butterflies lay eggs in locations with suitable host food plants and microclimates to support immature development (Wiklund & Åhrberg 1978, Kopper et al. 2000). These locations may contain specific degrees of sun exposure, generated from the host plant itself (between the edge and the centre of vegetation) or from nearby plants (sun or shade) to provide suitable microclimates for immature stages (Albanese et al. 2008, Jugovic et al. 2017).

Most Mottled duskywing records in Manitoba are located in forests that are managed by the Government of Manitoba facilitating commercial forestry operations or at the sites of historical wildfires (Agriculture and Resource Development Manitoba 2021). Although commercial forestry protocols currently promote suitable habitat for at risk species of many taxa in Manitoba, strategies specific to endangered Mottled

duskywing have not been developed (Agriculture and Resource Development Manitoba 2021). A comprehensive understanding of *E. martialis* biology may guide the development of strategies that create suitable habitat while also ensuring forest products can be harvested.

We assumed that *E. martialis* laid eggs on specific host plants and larval feeding patterns may be specific to this species. Our goal was to observe what these traits were in the *E. martialis* population in Manitoba. The objectives of this research were to 1) observe adult behaviour, including population-level emergence timing, 2) identify egg host species and suitable plant attributes and 3) observe larval feeding behaviour. We chose to research these life components of *E. martialis* to create a biological framework during designs of future recovery strategies in Manitoba.

MATERIALS & METHODS

We identified locations where Mottled duskywing and *Ceanothus* spp. had been historically observed in southeastern Manitoba. Historic records of Mottled duskywing were acquired from the Canadian Wildlife Service, Environment and Climate Change Canada (Canadian Wildlife Service M. Curteanu pers. com. 2019) and records of *Ceanothus* spp. from the Canadian Wildlife Service, Environment and Climate Change Canada and the Manitoba Conservation Data Centre, Government of Manitoba (Canadian Wildlife Service M. Curteanu pers. com. 2019, Manitoba Conservation Data Centre C. Murray pers. com. 2019). The only recorded *Ceanothus* spp. in Manitoba were *C. herbaceus*. We plotted Mottled duskywing and *Ceanothus* spp. locations in ArcGIS Pro (ESRI Inc. 2021). We visually assessed the proximity of skipper and potential host plant locations to each other, evaluated satellite imagery and incorporated our experience of vegetation and skippers found in forested habitats in Manitoba to choose areas that may contain suitable habitat for both *Ceanothus* spp. and Mottled duskywing to survey. All historic locations and research sites we chose, were in Agassiz, Bélair, Northwest Angle, Sandilands and Wampum Provincial Forests as well as Nopiming and Whiteshell Provincial Parks. We used a diameter of 500 m to circularly delineate each candidate site.

A degree day emergence prediction model was used to time adult surveys with *E. martialis* emergence in the field (Dearborn & Westwood 2014). Adult surveys for Mottled duskywing spanned May 13 to June 24, 2019, June 1 to July 6, 2020 and June 2 to 30, 2021. We surveyed sites using a meandering transect walk technique (Royer et al. 1998) and noted adult

behaviours. In 2019, sites were visited between 1000 h and 1745 h with an average temperature of 25.6 °C, in 2020 between 1030 h and 1715 h with an average of 22.5 °C and between 1000h and 1600 h with an average of 23.8 °C in 2021. We surveyed on days without precipitation and with light to moderate winds in all years. All sites were surveyed for 10 min (2 surveyors; 2019 and 2020) or 15 min (1 surveyor; 2021) at least two times during the flight period. We surveyed 64 locations in 2019, 49 in 2020 and 20 in 2021. In 2020 and 2021, we adjusted survey locations by revisiting, adding or removing sites based on Mottled duskywing observations in 2019. Six locations were surveyed in all three years, and 13 sites overlapped between adjacent years (2019–2020 and 2020–2021). Since we explore behaviour and population level wing wear we think the small overlap amongst sites permits analysing these aspects.

Research sites contained vegetated areas that bordered retired fire patrol or commercial forestry operation roads and trails. The vegetation in these areas is dominated by *Pinus banksiana* Lamb. (Pinaceae) (Jack pine) -exclusive, or mixed-coniferous and deciduous, forests adjacent to natural or artificially (forestry harvest) created clearings. Vegetated openings contained grasses such as *Andropogon gerardii* Vitman (Poaceae) and *Hesperostipa spartea* (Trin.) Barkworth (Poaceae) forbs *Lithospermum canescens* (Michx.) Lehm. (Boraginaceae) and *Campanula rotundifolia* L. (Campanulaceae) and shrubs *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem. (Rosaceae) and *Prunus virginiana* L. (Rosaceae). Our research site was in the Lake of the Woods Ecoregion, Boreal Shield Ecozone (Manitoba's Protected Areas Initiative 2013).

Mottled duskywing were identified using colour pattern descriptions and reference picture specimens reported in Klassen et al. (1989) and Layberry et al. (1998). Species names and authorities were updated to Pohl et al. (2018). We occasionally caught specimens to confirm identification and determine wing wear. Mottled duskywing wing wear was evaluated when caught or when adults were stationary on a habitat substrate. Condition rankings assigned included the following categories: 1. excellent - scales not worn and wings intact, 2. good - little wear of scales and infrequent wing tear, 3. moderate - scales worn and frequent wing tears and 4. poor - scales extensively worn and/or missing and wings damaged. In 2020, we focused wear measurements on 2 sites where we thought adult abundance would be the most consistent. We held individuals in iced coolers (approximately 15 min; methods as in Otis and Linton (2016)) while completing the site survey to count abundance. Cooling

didn't induce behavioural changes in Otis and Linton (2017), so we think these methods minimised negative fitness consequences (such as health or reproduction) to individuals. In 2021 we measured wing wear in all sites, but cooled individuals only occasionally because different degrees of wear of individuals enabled us to distinguish between the few individuals we found on any given day. We think these methods minimized the likelihood that we double-counted during wing wear enumeration. When identifying and evaluating Lepidoptera, we briefly handled them to reduce any potential for damage. Lepidoptera were released as soon as we determined their identity. In 2021, travel restrictions to reduce wildfire risk limited surveys during weeks 1, 3 and 4 (no surveys in week 4). Several voucher specimens representing their physical distribution were deposited in the Lepidoptera collection housed at the University of Winnipeg (515 Portage Avenue, Winnipeg, Manitoba, Canada, R3B 2E9).

We followed individual adult Mottled duskywing to directly observe oviposition activity on host plants and feeding on nectar sources. Adults flew throughout sites occasionally doubling back in the same areas. As a result, the same skipper's egg laying or other activity preferences may have been measured more than once during observations. We recorded the coordinates where adults were observed using a Garmin GPS, and marked plants where eggs were laid with a metal pigtail stake. The time, date and weather conditions during each Mottled duskywing observation were recorded.

We identified host plants and nectar feeding species using dichotomous keys and images in Looman & Best (1987) and Johnson et al. (1995), and updated nomenclature to Tropicos.org (Missouri Botanical Garden 2021). A host plant voucher specimen was collected to confirm identification in the laboratory and was deposited in the vegetation collection at the University of Winnipeg.

We recorded the height of an egg or larva on the host plant, the distance to the tip of the newest leaf and the side of leaf surfaces occupied. The depth of duff (large and decomposing loose dead plant material) was measured at the base of host plants. Duff traps heat (Stuhldreher & Fartmann 2014) and specific winter temperatures may be needed by larvae during diapause (Ewing et al. 2020), so we hoped to record the optimal depth of leaves for *E. martialis* survival. *Callophrys irus* (Godart, [1824]) (Lycaenidae) and *Phengaris alcon* (Denis and Schiffermüller, 1775) (Lycaenidae) larvae were found in microhabitats exposed by vegetation to different degrees of sunlight and likely subsequent microclimates (Albanese et al. 2008, Vilbas et al. 2016).

We recorded the height of vegetation within a 0.5 m radius of host plants to generally assess sun exposure, without recording temperature and humidity using dataloggers.

We checked eggs for development, recording the colour and shape, until they hatched in June (2019 and 2020). We observed larvae every few days from egg hatch until late July to document the feeding, resting and other behaviours.

Ceanothus spp. plants were examined for the presence of *E. martialis* larvae during formal surveys. We surveyed on July 26, 2019 where Mottled duskywing was observed during adult surveys in 2019 ($n = 3$). In each site we searched all *Ceanothus* spp. plants along a 50 m transect for larvae or shelters. When we found shelters, we opened them to observe larvae then immediately closed them with small twigs to try to maintain any microclimate or anti-predation benefits to larvae until they could be resealed by caterpillars. We counted the number of larvae found (all were in shelters) and recorded the location of larvae on plants (distance to the tip along the plant stem and vertical distance to the ground). We estimated the size of plants (based on the area they covered (m^2)) we searched, recording whether they hosted an egg(s) or not. The number of larvae we observed were summed in each site. We completely opened three shelters to observe larval behaviour and how they constructed each shelter, but only opened the remaining shelters enough to confirm they were *E. martialis*. Our damage may have reduced the fitness of these endangered larvae. In our opinion, three shelters was a reasonable balance to establish behaviour patterns, but limit the risk to the population.

In spring of 2020, we attempted to locate immature stages in sites where larvae were observed in 2019 but were unable to find either larvae or pupae. We searched leaf litter at the base of *Ceanothus* spp. plants and stems. Leaf litter is the most likely location that larvae overwinter because it likely provides insulation to diapausing larvae or pupae (Schweitzer et al. 2011). Formal larval surveys were not conducted in 2020 and 2021, however we did record incidental observations of adult and larval behaviour in both years as well.

Data manipulation. We observed and took pictures of newly-hatched larvae, to create a first instar control (approximate size, lack of defined body stripes and poorly visible red spots on the head). We estimated that during available development dates above-freezing temperatures (June egg deposition to the fall in Manitoba) larvae would likely complete 3 to 5 instars. Using our notes and pictures, we compared larvae found later in the year to estimate their developmental

stage. We assumed that larvae did not move between plants, thus that plants with larvae were the same plants where eggs were deposited earlier and therefore assessed them accordingly (applies to duff depth and surrounding vegetation height measurements). During larval and host plant transect surveys, the total number of observed larvae was divided by the estimated area of *Ceanothus* spp. searched, to calculate the density of larvae per area of *Ceanothus* spp..

We calculated summary statistics in RStudio (RStudio Team 2021) with R (R Development Core Team 2020) as the base. Adobe Illustrator (Adobe Inc. 2021) was used to illustrate figures (copyright J. Henault).

RESULTS

Egg oviposition and host species. Egg laying was observed at four sites in Sandilands provincial forest between June 13 and 21, 2019. A total of five oviposition events were observed and 22 eggs were found during this period. All eggs were laid on *Ceanothus herbaceus* plants (Fig. 2); no other plant species were found with Mottled duskywing eggs or larvae during systematic surveys in 2019. Eggs were also laid and larvae observed on *C. herbaceus* exclusively during observations in 2020 and 2021.

Prior to oviposition, females reduced their flying speed and approached a potential host plant (*C. herbaceus* in all events). At the periphery of the host plant, females hovered for a few seconds at multiple locations. Females then moved to briefly contact the plant with their abdomen or tarsi, and subsequently either resumed hovering behaviour or laid one egg (we didn't observe multiple egg depositions on one plant). After either interaction, females continued to hover at multiple peripheral locations of the plant or fly to another *C. herbaceus* plant. We occasionally observed females laying an egg on multiple host plants sequentially. After laying eggs, females increased their flight speed to fly amongst their habitat, sometimes rapidly flying several metres away and we lost track of them.

Eggs were laid a mean 17.1 mm (range: 4.0 to 36 mm ($n = 21$)) from the tip, and 20.2 cm (range: 4.1 to 35.9 cm ($n = 19$)) above the ground, on the youngest leaves developing at a stem tip, a flower bud or living plant stem. Eggs were pale yellow-green initially, and changed to orange after approximately one week (Fig. 3). Eggs had ribbed chorions. Females laid eggs 0.5-0.9 cm away from an egg already on the plant (leaf, flower bud, or stem) occasionally ($n = 3$). However individual females were not observed to lay an egg, then revisit the plant to oviposit next to the same egg. Five of



FIG. 2. *Ceanothus herbaceus* host plant adjacent to a sand-gravel path.



FIG. 3. Fresh *E. martialis* eggs are pale yellow-green (left) and week-old eggs turn orange (right).



FIG. 4. Mottled duskywing larva in *C. herbaceus* leaf shelter.

twenty-seven eggs hatched. In all circumstances when eggs hatched, the top of the egg shell was eaten (presumably by larvae from the egg) leaving the sides and egg base attached to leaf. We did not find the 22 remaining eggs again or observe evidence of larvae. However no evidence of ceased egg development indicated by transparent egg embryos or parasites hatching from eggs was observed.

Eggs were laid on plants growing where the depth of duff was 3.7 cm mean (range: 1.4 to 6.5 cm ($n = 17$)). The height of surrounding vegetation where eggs were laid was 23.7 cm (range: 12.8 to 35.6 cm ($n = 18$)). The height above the ground eggs were laid (20.2 cm as above) does not appear to be substantially different than the height of surrounding vegetation, although our limited replicates prevented formal statistical comparisons.

Larval foraging behaviour. Estimated third to fourth instar larvae (observed in July) had faint white lateral lines along their body, white coloured bumps scattered over the body and fine hairs (approximately 0.1 mm wide and 1 mm long) sparsely covering the cuticle. Eggs and larvae during the developmental stages we observed were otherwise similar in colour and shape as described in Scott (1986), Layberry et al. (1998), Olson (2002) and Committee on the Status of Endangered Wildlife in Canada (2012).

We found larvae consuming *C. herbaceus* in grassy open areas adjacent to *P. banksiana* forests (Fig. 4). A total of 16 caterpillars were found between June 18 and July 24, 2019. Most larvae were observed in leaf shelters at periphery of plants ($n = 16$), including only one on a single-stemmed shrub close to the ground but still at the edge of the plant. In 2021, we observed larvae in shelters 1.5–2.1 mm (range) from the leaf tip at the plant periphery, and 20.5 - 31.0 cm above the ground. Newly hatched and estimated second instar larvae made



FIG. 5. Young larval shelter constructed with silk cells. Note larva underneath the top fold of the leaf.

shelters by curling a single leaf along its stem axis, holding the leaf edges together with silk constructed into cells (approximately 1–2 mm wide; 1 to 2 cells across expanse between leaves); this pattern was unique to *E. martialis* larvae (Fig. 5). Other Lepidoptera feeding on *C. herbaceus* (micromoths, likely Tortricidae Latreille, 1802 spp.) used silk to completely seal their shelters. *Erynnis martialis* larvae fed by creating small circular holes in their shelters, near the petiole of the shelter leaf. Larvae in 2019 fed 11.8 mm (range: 7 to 17 mm (n = 4)) from the tip of the leaf and 9.9 cm (range: 9.7 to 10.2 cm (n = 3)) above the ground. Most shelters had no feeding damage, but occasionally occupied shelters had feeding damage with discoloured edges (instead of moist leaf tissue) indicating they were not fresh. Therefore, larvae ate the newest leaves sprouting from stems during observations (n = 4). All larvae feeding we observed occurred during the day.

We observed larvae of all instars resting within shelters when not feeding. At the end of July we found larger larvae (not first instar) resting within leaf shelters with the sides completely attached by silk (compared to silk cells by first instars), and at the top of *C. herbaceus* plants. There was no evidence larvae ate their occupied shelter or consumed leaves nearby. We did not track individual larvae over the season, therefore were unable to observe larvae starting diapause and determine

whether they completed development to an adult. In sites containing larvae, 0.5 larvae were found per 1 m² *C. herbaceus* (or 1 larva/2 m² *C. herbaceus*).

During larval transect surveys, the estimated area of individual *C. herbaceus* with an egg was 0.6 m² mean (range: 0.1 to 2.0 m² (n = 11)) and without was 0.7 m² (range: 0.1 to 2.0 m² (n = 236)). The height of surrounding vegetation where caterpillars were observed was 17.7 cm, range 12.8 – 22.5 cm (n = 2).

The host oviposition species, position of eggs and larvae on *C. herbaceus*, shelter use, feeding behaviours and the earliest month of larval feeding we observed in 2019 were also observed in 2020 and 2021. We observed a new behaviour during two instances in 2020 where two larvae were occupying one shelter; during both observations we estimated larvae to be first or second instars. In 2021, we observed one larva in a shelter, 1.5 cm away from an empty shelter on the same shoot; the larva occupied the most distal plant shelter.

We observed larvae feeding, during the same dates that adults were observed laying eggs. Three stages were observed simultaneously.

Adult life history and behaviour. Adults most often flew nearby *C. herbaceus* in areas adjacent to both forests and sand-gravel paths (graminoids and bare ground cover) and uncommonly but consistently in all years flying across the paths. No *E. martialis* adults were

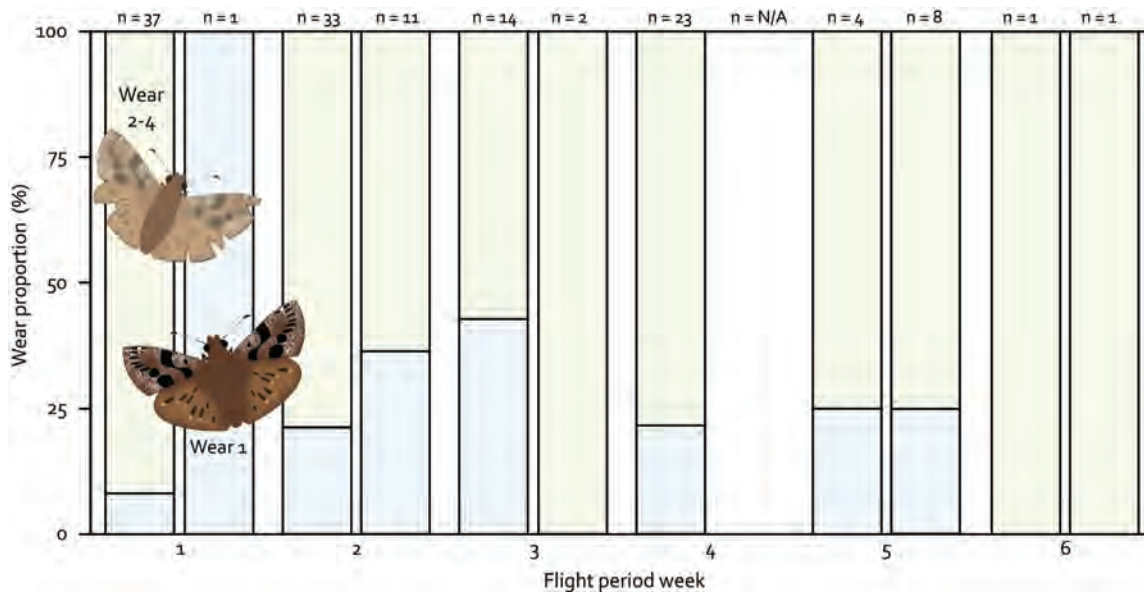


FIG. 6. Wing wear proportion (blue = 1, green = 2-4) during the flight periods in 2020 (left columns) and 2021 (right). Refer to methods for wear descriptions. In 2020: 2 sites only, 2021: all sites. During 2021, fire travel restrictions limited our surveys during weeks 1 and 3, and we did not conduct surveys during week 4. We include the number of total observations to contextualise percentages, however not to encourage comparisons of adult abundance between years.

observed flying into or within the forest stands during surveys. Mottled duskywing were observed nectar feeding on *L. canescens*, *Apocynum* L. spp. (Apocynaceae) and *Lathyrus* L. spp. (Fabaceae) in addition to *C. herbaceus* (reported in Dodgson 2020) in 2019. *Potentilla norvegica* L. (Rosaceae), *Sibbaldia tridentata* (Aiton) Paule & Soják (Rosaceae), *C. herbaceus*, *L. canescens* and *Apocynum* spp. were observed as nectar sources in 2020. In 2021, adults were observed feeding on *C. herbaceus*.

Using the degree day model, we predicted emergence on June 3, 2020 and first observed adults June 1 (double-sine model; calculated in mid-May). In 2021 we predicted May 26 (double-sine; mid-May) and Kirstyn Eckhardt first observed an adult on May 26 (K. Eckhardt pers. com. 2021; deposited at iNaturalist (Eckhardt 2021)). In 2021, we consider May 26 – June 1 the first week of the flight period. We assessed adults as wear category 1 (most fresh wear category) during weeks 1 to 5 of the flight period in 2020 and 2 to 5 in 2021 (Fig. 6). Thus adult emergence appeared to be staggered across the flight period, with fresh adults observed during several weeks. Also, the abundance of fresh adults appeared to peak during weeks 2 and 3, trending as a bell-shaped emergence period. We didn't compare abundance between years because we used some different sites in 2021 and wildfire travel restrictions influenced the number of survey visits in 2021.

DISCUSSION

Egg oviposition and host species in Manitoba.

We observed *E. martialis* adults laying eggs on and larvae eating exclusively *C. herbaceus* — the first reports for the population in Manitoba. We didn't observe *C. americanus* during our study, the host species used by Mottled duskywing in eastern Canada and the United States (Olson 2002, Schweitzer et al. 2011, Committee on the Status of Endangered Wildlife in Canada 2012, Committee on the Status of Species at Risk in Ontario 2013). Butterflies use specific host plant species in different portions of their range (Thomas et al. 2001), and in Manitoba the absence of *C. americanus* appears to result in an exclusive *C. herbaceus* diet. Thin prairie, oak barren, alvar and pine forest soils support *C. americanus* (Coladonato 1993, Shuey 2005, Committee on the Status of Endangered Wildlife in Canada 2012, McClain & Ebinger 2014) and sandy soils dominated our research locations supporting *C. herbaceus*. We assume that these soil types promote water drainage, apparently preferred by *Ceanothus* spp.. Curiously, given the almost contiguous *E. martialis* and *Ceanothus* spp. habitat types from the eastern and southern range

to Manitoba, we did not observe *Ceanothus* spp. in alvars during 2021 in Manitoba (JH unpublished). We are interested to know if *E. martialis* in Manitoba would add newly colonized *C. americanus* to their diet (as larval host or adult nectar plants) if this species happened to colonize at our research locations (possibly caused by humans transporting seeds or climate change). Although outside our focus, we are intrigued why *C. americanus* does not live in Manitoba.

We observed females consistently reducing their flying speed near *C. herbaceus* hosts, hovering at the periphery of host plants, probing the candidate plants and either laying an egg or departing. Ovipositing Lepidoptera are reported to be attracted sequentially by cues while flying, then to different cues while contacting the host plant (Wiklund 1984, Dempster 1997, Singer & McBride 2010, Lund et al. 2019). We think accrued cues during flight and probing motivated females to lay eggs, while in other instances initially attractive plant cues may have been outweighed by undesirable traits upon probing resulting in deferred egg laying and resumed host plant searching (motivated as defined by Singer et al. (1992)). Although reduced flight pace, hovering and probing behaviours followed by oviposition or departing have also been observed to describe Lepidopteran oviposition behaviour in other studies (Wiklund & Åhrberg 1978, Singer 1983, Wiklund 1984, Scott 1986, Thomas et al. 1986, Doak et al. 2006) to the best of our knowledge it has never been defined. We propose “microhabitat location” to term this collective set of Lepidopteran oviposition behaviours, but think refinements of this definition require focused research.

Female Mottled duskywing laid eggs singly in consecutive suitable microhabitats, as described in other butterfly species (Roy & Thomas 2003, Čelik & Vreš 2018). Conceivably, after females have laid several eggs in a series, they may nectar feed to replenish internal nutrients or regulate their temperature by basking. After oviposition *Parnassius mnemosyne* (Linnaeus, 1758) (Papilionidae) were reported basking, and then feeding on nectar sources or laying more eggs (Konvička & Kuras 1999). The causes initiating and terminating distinct activity shifts (between egg laying, nectar feeding and potentially basking) of *E. martialis* in natural habitats requires additional research.

We observed oviposition near the periphery of respective host plants, as observed in *Aporia crataegi* (Linnaeus, 1758) (Pieridae) (Jugovic et al. 2017) and *Anthocharis cardamines* (Linnaeus, 1758) (Pieridae) (Dempster 1997). These authors theorised that these sunnier locations likely provided warmer microclimates. There is evidence that cues relaying information on host

quality, sun exposure and temperature of microclimates motivate butterflies to lay eggs (Wiklund & Åhrberg 1978, Kelber 1999, Kopper et al. 2000, Eilers et al. 2013). We hypothesize adult *E. martialis* locate microhabitats with specific microclimates produced by the degree of sun exposure and appropriate forage health (age of leaves, plants and lack of senescence). Sparse outer portions of *C. herbaceus* plants likely provide suitable microclimates and food for immature development. However, additional research to identify *E. martialis* cues and the attributes (host and microclimate) required by developing immature stages is needed.

We observed eggs change from yellow-green to orange after approximately one week, and faint lateral lines, cuticle bumps and sparse hairs on larvae. While egg-colour changes have been reported for *E. juvenalis* in this subfamily (Scott 1986, Layberry et al. 1998), our observations of larvae and eggs, including images of chorion-structure, contribute additional details to the western Boreal Population Designatable Unit and *E. martialis* as a species (Scott 1986, Layberry et al. 1998, Olson 2002, Committee on the Status of Endangered Wildlife in Canada 2012).

Larval feeding behaviour. We observed *E. martialis* in shelters consuming *C. herbaceus* in Manitoba. Since feeding damage on shelter edges

wasn't fresh (unlike petiole holes), we don't know if *E. martialis* or another species' larvae created these marks. We observed larvae using shelters, thus generated evidence that shelter use is consistent in *E. martialis* of any given population (Olson 2002, Schweitzer et al. 2011). However, the detail that shelters are at the periphery of plants appears to be unreported. Younger leaves of many species have fewer physical barriers and chemical defenses compared to older (Bowers & Stamp 1993, Howlett et al. 2001, Hellmann 2002); since we observed the youngest *C. herbaceus* leaves also at the periphery of plants, these edge locations are likely easier for larvae to digest. Interestingly, the *E. martialis* larva near two shelters in 2021 occupied the most distal one, perhaps moving out of a shelter initially constructed at a shoot tip, to construct a shelter using fresh leaves as the plant continued to grow. Peripheral locations are likely more exposed to the sun and potentially provide warmer microclimates to feeding *E. martialis* larvae (as observed *Coenonympha oedippus* in Čelik et al. (2015)).

We observed young larvae feeding during the day but no evidence of nocturnal feeding or leaf harvesting by older larvae as described in the eastern range (Olson 2002). We postulate that diurnal feeding and nocturnal leaf harvesting occur in both regions, however the small larvae and lack of nocturnal research limited direct observations in the eastern range and our study



FIG. 7. Larval foraging behaviour during young and later instars. Left, young larvae eat holes in shelters during the day and use silk cells to hold leaf-edges together. We hypothesise that older larvae harvest leaf parts during the night and completely seal shelters. During the day, larvae may then consume leaf parts within a shelter (black circle removes a leaf section to see into the shelter).

respectively. *Hemileuca lucina* H. Edwards, 1887 (Saturniidae) larvae developed at specific temperatures (Stamp & Bowers 1990), leaf shelters buffered Coleoptera larvae from temperature and humidity (Larsson et al. 1997) and butterfly larvae in leaf shelters survived predation better and were less frequently parasitized than exposed larvae on plants (Morse 2017, Baer & Marquis 2020). We propose first that shelters provide suitable digestion microclimates for young larvae, and larval shelter-feeding damage (small holes at petiole) does not attract predators enough to reduce fitness. Then, shelter-feeding by older larvae would likely leave larger and more conspicuous feeding marks, so nocturnal leaf harvesting accompanied by diurnal feeding within buffered and protective shelters may be more adaptive (Fig. 7). We observed an empty shelter (1.5 cm away from an occupied shelter) only once (in 2021) but not any other year, suggesting larvae likely consume old shelters before making a new one and likely reduce conspicuousness in the process. The optimal development temperatures for *E. martialis* are unknown, however any heat retained within shelters overnight may also provide a temporary reprieve for nocturnally foraging larvae on wind-exposed twigs. Clearly, creating holes in a shelter would seem maladaptive. However, potentially the risk of leaving shelters to feed is greater than the risk of parasites entering in openings. We recommend direct observations of larval foraging during the day and night, throughout *E. martialis*' range to more accurately describe their feeding behaviour.

The estimated area of host plants with eggs did not appear to be different than plants without. Multiple Lepidopterans have been reported laying eggs on larger host plants (Robakiewicz & Robbins 2001, Lund et al. 2019), possibly to support immediate aspects of their biology (for example, specific microclimates for egg development) while relying on larvae to locate suitable development locations on the host plant (Wiklund 1984, Kopper et al. 2000, Henault 2021). We also observed multiple eggs deposited close to one other, perhaps due to multiple females similarly recognizing optimal characteristics for development (*P. alcon* in Osváth-Ferencz et al. 2016 and Vilbas et al. 2016). *Chlosyne harrisii* (Scudder, 1863) (Nymphalidae) larvae moved to different leaves on the same plant when competing for food with conspecifics (Dethier 1959), and we observed *E. martialis* larvae during young instars individually or paired in shelters and older instars individually in shelters. Therefore, *E. martialis* appear to lay eggs at locations where ova density does not influence their development (such as with microclimates), hatched larvae then create a

shelter and feed in the vicinity, sometimes with another occupant, and older instars move to another area of the plant to feed individually. If large plants were required to reduce competition by providing spaces with lower densities of conspecifics, then we expect plants with eggs to be larger than those without. However since this prediction is not consistent with our observations it appears that for the most part a limited forage quantity does not limit larval survival. The eggs we observed were at different maturities (yellow-green, and orange) and adult lifespan is unknown, therefore we cannot determine whether females laid eggs next to their own eggs (deposited at some point earlier) or next to other female's eggs.

We counted 0.5 larvae for every 1 m² of *C. herbaceus* plants, and thus generated an indication of the host plant-based carrying capacity (*Polyommatus bellargus* (Rottemburg, 1775) (Lycaenidae) population was limited by *Hippocrepis comosa* L. (Fabaceae) host plant availability in Roy & Thomas (2003)). However, we did not observe larvae for their entire life cycle so don't know how much *C. herbaceus* area is needed to support one larva. Studies determining the ratio of larvae to adults, the total volume of *C. herbaceus* eaten during immature stages and additional unknown factors are required to estimate field capacity.

We measured two aspects of microhabitats — duff depth and surrounding vegetation height — however more detailed microhabitat descriptions (including microclimates) are needed to generate robust inferences. Larvae in Manitoba likely overwinter at the base of *C. herbaceus* amongst dead leaves (as in eastern range Olson (2002) and Schweitzer (2011)), a location we think may be easily accessed by dropping off the plant. Microhabitats that were partly shaded hosted *C. irus* larvae more often than sun-exposed (Albanese et al. 2008), while taller and exposed host plants hosted *P. alcon* (Vilbas et al. 2016). In addition to microhabitat characterisation, direct spring observations of larvae and exploring overwintering development physiology (and throughout their life cycle) may better describe microhabitat requirements and spring feeding as well as pupation ecology in Manitoba. An understanding of where immature stages live during different times of the year may help to time conservation or commercial habitat disturbances to ensure the least risk to immature stages.

Young larvae created partially open shelters while shelters were completely closed by older larvae. Expensive shelter construction (*Depressaria pastinacella* Duponchel, 1838: Elachistidae) silk and time resources in Berenbaum et al. (1993)) may cause *E. martialis* early instar larvae to create open cells, and

multiple occupants may reduce construction costs. By the time they are older, larvae can afford to create sealed shelters. Shelters of other Lepidoptera (micromoths) on *C. herbaceus* were completely sealed with silk, likely inhibiting small-body parasite access (although possibly accessed by ovipositors through leaf tissue). Cell sizes may be used to identify parasites of *E. martialis* early instar larvae, by filtering species with bodies that could fit between the cells or ovipositors long enough to access larvae. Given that *E. martialis* evolved with parasite-accessible shelters, we wonder if parasitism may control populations (as observed in some forest Lepidoptera (Royama (1984)) by ensuring adequate *C. herbaceus* leaves for larvae. We encourage research into the adaptive consequences of shelter style with parasites in *E. martialis*.

Mottled duskywing life cycle biology. We observed adults flying in clearings adjacent to but not within forests, in vegetated or sand-gravel path areas. This is an initial description of how adults in Manitoba use habitats. Adults consumed nectar of several species (most new records in Manitoba), including the larval host plant. *Oarisma poweshiek* (Parker, 1870) (Hesperiidae, Hesperinae) nectar feed opportunistically and conduct activities (nectar feed, lay eggs, rest) within prairie areas but not adjacent wetlands or forests (Henault 2021). Similarly, *E. martialis* may also take advantage of available nectar sources nearby *C. herbaceus* but not within forest stands. Within forest habitats, areas containing the reported *E. martialis* nectar species may be designated by policy specialists as critical habitat to support long-term success of this species (assuming the area also meets any other required criteria such as larval host species).

The degree day model helped us predict emergence within a few days in 2020, and apparently the exact date in 2021. We started to record emergence dates in 2019, so only hoped for somewhat of a useful tool to help time our surveys. We are glad our predictions were accurate without a reasonable sample size, appearing to time our surveys well with adult emergence. We anticipate we can generate even better predictions with models matured with additional annual emergence dates.

Adults with “excellent” wing wear were observed during several weeks in this study, indicating that adult emergence is staggered in Manitoba. In 2020, adult abundance appeared to be similar over the first few weeks of the flight period and then noticeably dropped later (instead of slowly increasing, peaking, then dropping), however the emergence of fresh adults appeared to bell-shaped throughout the six weeks. In all years, we observed the egg, larval and adult stages

during the same dates. To the best of our knowledge, no researchers have reported dates that freshly emerged adults were observed. Therefore, we are not sure whether *E. martialis* emerge as a staggered population in other parts of their range, or only in Manitoba. *Pararge aegeria* (Linnaeus, 1758) (Nymphalidae) are also active as multiple development stages simultaneously (Wiklund & Friberg 2011). Adults are active when nectar is accessible (Warren 1992) and larvae when host plant quality is high and have enough time to develop before the overwintering stage (Posledovich et al. 2015). *Ceanothus herbaceus* was observed producing new shoots throughout the summer (unpublished). We found that nectar species bloom throughout the flight period, supporting adults. It appears consistently produced *C. herbaceus* shoots may enable larvae that hatch between mid-June and mid-July to feed on high quality host plant material. This continuous new growth trait may also explain how multiple generations are supported in areas with warmer temperatures described in Schweitzer et al. (2011), Committee on the Status of Endangered Wildlife in Canada (2012) and Committee on the Status of Species at Risk in Ontario (2013). Also, although we assume synchronous emergence would make it easier to find mates, *E. martialis* eclose asynchronously therefore this population trait is likely not maladaptive enough to be selected against. If *E. martialis* is territorial, then a staggered emergence of males that use the same territory multiple times during a given year, may support a larger overall population; however we need to further research this concept. Historical Mottled duskywing records do not report wing wear, therefore the degree of synchrony in other years cannot be determined.

The staggered adult emergence and egg laying as well as continuous *C. herbaceus* growth that we observed suggests larvae may develop along two routes depending on when they were deposited as eggs. Assuming larvae develop at the same rate whether they were laid as eggs in mid-June (route 1) or mid-July (route 2), route 1 larvae would enter diapause as more mature larvae than those in route 2. Multiple stages of *Chilo suppressalis* (Walker, 1863) (Pyralidae) larvae were reported to be physiologically equipped to survive diapause (Xu et al. 2011), so maybe *E. martialis* is similar. *Ceanothus herbaceus* does not leaf out until approximately the date that adults first emerge in early June in Manitoba (JH and RW 2019 & 2020 unpublished), providing no opportunity for larvae to feed in spring. In the spring, route 1 larvae may exit diapause and enter pupation without feeding on fresh *C. herbaceus*. At the same time, route 2 larvae may exit

cold-diapause and enter warm-aestivation until *C. herbaceus* leaves flush. *Celastrina neglecta* (Edwards, 1862) (Lycaenidae) flies in the summer, feeds as larvae until diapause on *C. americanus* (with presumably similar enough leafing phenology), extends diapause past when the first leaves emerge the following spring to resume feeding in the summer and then pupates (Layberry et al. 1998). Route 2 *E. martialis* development may be similar to *C. neglecta*. For *E. martialis*, once new leaves emerge we postulate that route 2 larvae crawl up from the base along the stems to leaves, continue feeding and pupate to hatch later in July. Individuals of each route may start and end their development during date blocks, with the boundary between individuals of the end of route 1 (June) and beginning of route 2 (July) difficult to identify, therefore not appearing as distinct cohorts. When we investigated in the spring, we did not find larval shelters or route 2 larvae so we recommend additional research to locate and observe behaviour of *E. martialis* immatures in natural habitats in the spring to determine post-diapause and complete development biology in Manitoba.

We did not track individuals daily to observe behavioural patterns, however we encourage future research to track *E. martialis* individuals of all life stages. Tracking individuals would have helped determine sequential female behaviours to explore the mechanisms to start and stop adult activities and to study daily activities of larvae.

Suitable microhabitats. We observed adult oviposition and nectar feeding behaviour, larval host food species and shelter use to generate biological knowledge of the *E. martialis* population in Manitoba. Most butterflies require specific vegetative, physical and microclimatic attributes to fly, bask or nectar feed as adults and develop as immatures (Grundel et al. 1998, Dennis et al. 2004, Jugovic et al. 2017, Ewing et al. 2020). *E. martialis* appear to require microhabitats on host plants that are 0.1 to 2.0 m² area, growing in substrates with approximately 2 to 6 cm of duff. We will continue to research habitat attributes that support *E. martialis* across their distribution in Manitoba.

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