

Parasitism of Monarch Butterflies (*Danaus plexippus*) by *Lespesia archippivora* (Diptera: Tachinidae)

KAREN OBERHAUSER¹

University of Minnesota, Department of Fisheries, Wildlife and Conservation Biology,
1980 Folwell Ave., St. Paul 55108

ILSE GEBHARD

6450 N. 2nd St., Kalamazoo, Michigan 49009

CHARLES CAMERON

2818 Sherwood St., Greensboro, North Carolina 27403

AND

SUZANNE OBERHAUSER

N4174 Hemlock Lane, Caroline WI 54928

ABSTRACT.—*Lespesia archippivora* is a widespread generalist parasitoid whose hosts include monarch butterfly larvae. We report parasitism rates by this tachinid fly in wild captured monarchs, using data collected over 7 y by 77 volunteers in the Monarch Larva Monitoring Project, a North American Citizen Science Program. Data were collected in 21 U.S. states and one Canadian province, with focus sites in Wisconsin, Michigan and North Carolina. Overall, approximately 13% of monarch larvae contained tachinid flies. Parasitism rates varied by year, and this variation was, to a large extent, region-wide. For example, larvae collected in 2000–2002 throughout the Upper Midwestern U.S. suffered significantly more parasitism than those collected in 1999 or 2003–2005. There were no consistent patterns with respect to date of collection within years. The number of flies per parasitized monarch ranged from one to 10 and comparison to a Poisson distribution indicated that flies were more aggregated within hosts than expected by chance. Larvae collected during later stadia were both more likely to be parasitized and contained more flies per larva. Whereas the first pattern is likely to be the result of longer exposure to parasitoids, the latter suggests that females either respond to larger hosts by laying more eggs or that superparasitism (parasitism by more than one individual) occurs. When more than four flies emerged from a single host, their mean pupa mass was smaller, suggesting a cost to superparasitism. We report six cases of hyperparasitism by a wasp in the family Perilampidae. When three milkweed species with varying levels of cardenolides occurred in one area, monarchs found on a species with low cardenolide levels (*Asclepias syriaca*) were most likely to be parasitized, but those found on a species with very high levels of cardenolides (*A. curassavica*) showed higher levels of parasitism than those on found on the less toxic *A. incarnata*. Potential impacts of *L. archippivora* on monarch butterflies are discussed in light of these findings.

INTRODUCTION

Parasitoid larvae live on or in their hosts' bodies, generally killing the host; about 10% of all insects are parasitoids (Eggleton and Belshaw, 1992). Whereas about 25% of these are Diptera, most research has focused on hymenopteran parasitoids. Feener and Brown (1997) suggest that increased focus on dipteran parasitoids would be valuable for several reasons. Parasitoidism has probably evolved over 100 times in flies (Eggleton and

¹Corresponding author: oberh001@umn.edu

Belshaw, 1992), and only once in the Hymenoptera. Additionally, because Hymenoptera are haplodiploid and produce venom, both of which are important in their parasitoid lifestyles, they do not represent the biology of other parasitoids. Because of the lack of basic understanding of parasitoid biology, Eggleton and Belshaw (1992) recommended more observational work to clarify aspects of parasitoid natural history, especially of the Diptera.

The Tachinidae represent the largest taxon of dipteran parasitoids, with approximately 8000 species. All Tachinidae are parasitoids (Feener and Brown, 1997). Most of their hosts are Lepidoptera, although they also parasitize Hymenoptera, Heteroptera, Coleoptera, Diptera, Dermaptera, Orthoptera and Chilopoda, as well as scorpions and spiders (Eggleton and Belshaw, 1992). The most primitive tachinids lay "macro-type" (0.2–0.9 mm long) eggs on or in the host and have lifetime fecundities ranging from 100 to 400, whereas other species lay from 1000 to over 6000 "micro-type" (0.02–0.2 mm) eggs on foliage; the latter are then consumed by feeding caterpillars (O'Hara, 1985). Host defenses against tachinid flies include avoidance behaviors before and during oviposition and encapsulation by blood cells after the parasitoid enters the host body. However, the ability of most tachinids to attach their posterior spiracles to host tracheae ensures a continuous air supply and allows them to avoid death from encapsulation (Feener and Brown, 1997).

Several tachinid flies are common and effective biological control agents that have often been introduced from one region to another (O'Hara, 1985). The fact that many tachinids are generalists (Feener and Brown, 1997) makes them a risk to non-target species. One species, *Compsilura concinnata*, was introduced repeatedly to North America from 1906 to 1986 to control gypsy moths and has played a role in the decline of silk moth populations in New England (Boettner *et al.*, 2000). It is also common in native luna moths (*Actias luna*) in Central Virginia (Kellogg *et al.* 2003).

Lespesia archippivora is a tachinid fly that parasitizes at least 25 lepidopteran species in 14 families and one species of Hymenoptera (Beneway, 1963). It is widespread throughout North America (Arnaud, 1978) and was purposely introduced into the Hawaiian Islands as a biocontrol agent in about 1898 (Etcheagaray and Nishida, 1975). The female lays macro-type eggs on the host, and soon after oviposition the maggot hatches and bores into the host integument (Stapel *et al.*, 1997). *Lespesia archippivora* maggots go through three larval instars, emerge from their hosts during the late larva or pupa stage and then crawl under leaf litter, where they pupate and eclose within approximately 10–14 d (IG and KO pers. obs.).

Monarch butterflies (*Danaus plexippus*) in the U.S. are frequently parasitized by *Lespesia archippivora* (Prysby, 2004). As part of the Monarch Larva Monitoring Project (MLMP) (Prysby and Oberhauser, 2004), some volunteers collect immature monarchs from the wild to measure parasitism rates. Here, we report on extensive collection efforts of volunteers in the northern (Michigan and Wisconsin) and southern (North Carolina) parts of monarchs' summer range, with summary data from other volunteers and localities. We investigated the biology and prevalence of this monarch parasitoid in wild populations over the eastern half of the U.S. and southern Canada from 1999–2005.

This study has direct relevance for understanding the factors that regulate this charismatic butterfly; most studies of wild populations only take into account mortality that occurs from the egg to the late larval stage, and mortality from *Lespesia archippivora* is not apparent until the prepupa or pupa stage. Additionally, there has been little work on the importance of native parasitoids in regulating non-pest host populations. Our findings address these issues over a broad spatial and temporal scale.

METHODS

The MLMP (Prysbý and Oberhauser, 1999, 2004; Monarch Larva Monitoring Project, 2006) is a Citizen Science project designed to document temporal and spatial variation in monarch egg and larval abundances, compare monarch production across different habitat types and describe variation in egg and larval survival. Since 1997 MLMP volunteers have estimated monarch egg and larva densities on a weekly basis throughout the time that their host plants (milkweed, *Asclepias spp*) are available. Monitoring sites include small backyard gardens, railroad right-of-ways, roadsides, abandoned fields and pastures, natural habitats and restored prairies (*see* Prysbý and Oberhauser, 2004 for detailed methods).

A subset of MLMP volunteers ($n = 77$) in 21 states and one Canadian province collected the data analyzed here. Overall, they collected a total of 2903 monarch larvae. Our analyses include summaries of data collected across North America for most volunteers, with more extensive reports from three focus sites where volunteers collected several hundred monarch larvae over multiple years. Collection sites were arbitrarily divided into regions as follows: Northeast (PA, VT, NY, MA, MD and OH), Southeast (TN, NC, GA, VA and FL), Upper Midwest (MI, WI, MN, IA, IL, IN and Manitoba), Southcentral (TX), Central (MO and NE) and southwest (CA). Because our sample sizes were low in other regions, regional comparisons are only made between the Northeast ($n = 647$), Southeast ($n = 161$) and Upper Midwest ($n = 2054$).

Most volunteers collected fourth or fifth instar monarch larvae, which they reared in their homes. They recorded the date and larval stadium at collection and the outcome (healthy adult, parasitized by fly, parasitized by wasp or died of other cause). The protocol includes characteristics of common fly and wasp parasitoids, and volunteers identify parasitoids to taxonomic order. Volunteers were asked to release healthy butterflies and adult parasitoids back to their sites whenever possible, although it is likely that most flies were not released for reasons expressed by one volunteer, who said, "Sorry, I can't in my own conscience turn them loose to raise more havoc." The USDA Systematic Entomology Laboratory or entomologists from the Universities of Minnesota and Kansas identified several dozen of the parasitoids, and all of these were *Lespesia archippivora* (Tachinidae).

Michigan focus site collections were made by Gebhard from *Asclepias syriaca* (mainly), *A. incarnata* and *A. tuberosa* throughout the summers of 2002–2005. In 2002 and 2003 fourth and fifth instar larvae were collected in a 118 sq m garden bed of prairie forbs and grasses near Kalamazoo MI (2002 $n = 12$, 2003 $n = 10$). All larvae found in the target stadia were collected. In 2004 eggs and all five larval stadia (total $n = 141$) were collected from the above site and surrounding natural area. In 2005 eggs and all larval stadia were collected from the above site and Allegan State Game Area (about 80 km away, and visited during two weeks in June) (total $n = 540$). Several tachinid flies were identified as *Lespesia archippivora*.

Wisconsin focus site collections were made by S. Oberhauser during the summers of 2000–2005 near Leopolis WI. Her monitoring site is along a river; some prairie plantings have been made at the site, but the milkweed (mostly *Asclepias syriaca* with some *A. incarnata*) is growing naturally. Most of the larvae were in the fourth and fifth stadia (373 out of 427). Several tachinid flies from this site were identified as *Lespesia archippivora*.

North Carolina focus site collections were made by Cameron during the late summer and early fall (Aug. 14–Oct. 23) in 2003–2005 from several sites near his home in a Greensboro NC residential neighborhood. Most larvae were collected from gardens and yard areas next to his home containing *Asclepias curassavica*, *A. incarnata*, *A. syriaca*, *A. physocarpa* and *A. tuberosa* ($n = 237$ larvae); a small city park with an unmowed area containing *A. syriaca* ($n = 81$); and an open field near a highway 32 km south of Greensboro containing *A. syriaca* ($n =$

25). Almost all of the larvae collected in NC were fourth and fifth instars (329 out of 343). The majority were found on *A. curassavica* ($n = 99$), *A. incarnata* ($n = 83$) and *A. syriaca* ($n = 127$). Several tachinid flies were identified as *Lespesia archippivora*.

Monarch rearing protocols used by volunteers varied. Monarchs were kept inside homes or on screen porches with ambient light, and their containers were cleaned at least daily. They received fresh milkweed leaves as needed. Surviving monarchs were usually released back into the study sites as adults. Some adult parasitoids were released back into the MI focus site, but many were retained for identification or destroyed after adults eclosed. Flies were not released back to the NC focus sites. Almost all flies were released about 30 m from the WI focus site, although some were retained for identification or research. Cameron and Gebhard reared their monarchs in individual containers, so that the number of flies from each parasitized monarch could be determined. S. Oberhauser reared multiple monarchs in screen cages and did not assess the number of flies from each monarch, although monarchs collected in the same stadium were together to allow tracking of stadium at collection. Whenever monarchs were not reared in individual containers, we only used the following volunteer data: collection date and location, stadium at collection and whether or not the monarch was parasitized.

During the summers of 2003 and 2006 we saved the flies ($n = 61$) that emerged from 19 monarchs collected near the University of Minnesota. We weighed them as pupae within 24 h of emergence from the monarch on a Mettler semi-micro analytical balance, and determined how the number of flies per monarch affects parasite size.

Most statistical analyses are straightforward and described below. In several cases we used chi-square tests to compare numbers of parasitized to unparasitized monarchs between samples. Monarchs that died of causes other than parasitism were included in the unparasitized category. This may underestimate parasitism rates, since some of these monarchs may have been parasitized. Unless otherwise noted, we arbitrarily selected 15 larvae as a minimum sample size for inclusion in comparisons of parasitism rates between sites or regions.

RESULTS

OVERALL TACHINID PARASITISM RATES

Of 703 larvae collected by Gebhard in MI, 84% resulted in healthy adult monarchs. Of the 116 that did not survive, 52 died of causes that included eggs that did not hatch, larval miring in latex (Zalucki *et al.*, 2001), unsuccessful molting or deaths that appeared to be viral or bacterial (fairly fast deaths that often included turning black and limp); 20 died of a syndrome we call "failure to thrive," which is characterized by development that appears normal through one or more stadia, then reduced or no feeding for up to several days, and then death; and one or more tachinid fly larvae emerged from the remaining 44 (6.2%). None of the monarchs from which tachinid flies emerged survived.

Of 427 WI larvae collected by S. Oberhauser, 83% resulted in healthy adult monarchs, 8.4% died of other causes similar to those described above and 8.9% resulted in tachinid flies. Of 341 NC monarchs collected by Cameron, 80% resulted in healthy adult monarchs, 7.3% died of other causes and 13% contained tachinid flies. We did not distinguish the "failure to thrive" syndrome in the WI or NC data sets. Two NC larvae that died of other causes had two or three parasites that were probably mermithid nematodes, two were damaged in handling and one was killed by another larva. Of the 2903 larvae throughout our sampling range from 1999–2005, 80.4% resulted in healthy adult monarchs, 7.4% died of other causes, and 13% contained tachinid flies.

EFFECTS OF LARVAL STADIUM AT COLLECTION

Gebhard collected large numbers of monarchs across all larval stadia, as well as during the egg stage. Three flies emerged from monarchs collected as eggs in her MI Sites; these were identified as *Lespesia archippivora*. Monarchs collected as older larvae by Gebhard were more likely to be parasitized (Fig. 1a). Of the 427 larvae collected by S. Oberhauser from her WI site, 54 were collected as first through third instars. None of those collected as first or second instars ($n = 9$) were parasitized and parasitism rates increased with larval stadium at collection (Fig. 1b). We performed logistic regressions to test the significance of the effect of larval stadium at collection on the likelihood of being parasitized, using stadium in the model as an ordered variable. In both cases, the effect of stadium was significant (odds ratio for MI = 2.17, 95% C.I. = 1.71–2.75; for WI = 1.96, 95% C.I. = 1.05–3.65). The odds ratios indicate that the likelihood of being parasitized roughly doubles for successive stadia.

EFFECTS OF COLLECTION YEAR, DATE AND REGION

Parasitism rates varied with year. When we removed MI and WI focus site data from the Upper Midwestern dataset, there was a significant effect of year (Fig. 2a, $X^2 = 154$, $df = 9$, $P < 0.001$), with higher parasitism rates in 2000 to 2002. There was also a significant effect of year on parasitism rates in the WI focus site (Fig. 2a), with no parasitism in 2000, 2003, 2004 or 2005 and relatively high rates in 2001 and 2002 ($X^2 = 23$, $df = 5$, $P < 0.001$). To a large extent, this mirrored the regional pattern. Monarchs collected from the MI focus site in 2005 were more likely to be parasitized than those in 2004 (Fig. 2a, $X^2 = 5.5$, $df = 1$, $P = 0.019$). We did not include MI parasitism rates in 2002 and 2003 in this analysis due to small sample sizes ($n = 12$ and 10 larvae for 2002 and 2003, respectively). The limited number of years over which Gebhard collected large numbers of monarch larvae make it difficult to compare her site to the region-wide data.

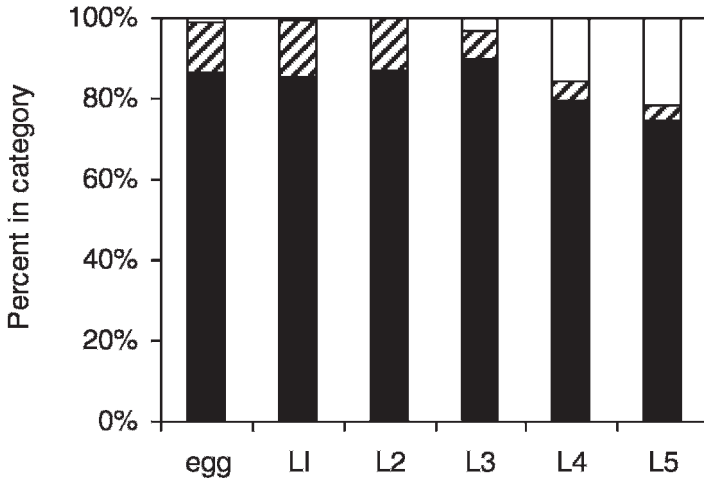
There were no significant differences in parasitism rates across 2003 to 2005 in the Southeastern data with the NC focus site data removed (Fig. 2b, $X^2 = 3.9$, $df = 2$, $P = 0.14$). The trend of an increase across years is opposite that found by Cameron in his NC sites, where parasitism rates decreased from 2003 to 2005 (Fig. 2b, $X^2 = 48$, $df = 2$, $P < 0.001$). However, sample sizes throughout the Southeast (excluding Cameron's data) are low (≤ 65 each year).

We also analyzed parasitism rates seasonally, combining date of collection in the WI and MI focus sites into four periods: May 21–June 24, June 25–July 29, July 30–Aug. 19 and $>$ Aug. 19. In 2001 there was no effect of date at the WI site, and in 2002 there was more parasitism early (Fig. 3a, 2002 $X^2 = 9.2$, $df = 3$, $P = 0.027$). In MI we only used 2005 data from Gebhard's yard; monarchs were only collected from the Allegan site for two weeks in early 2005 and only three monarchs were parasitized in 2004. There was a significant effect of date, with monarchs collected later in the season having a greater likelihood of being parasitized (Fig. 3a, $X^2 = 50$, $df = 3$, $P < 0.001$). We compared summer (\leq Sept. 22) and fall ($>$ Sept. 22) parasitism rates in NC; more monarchs collected in the fall were parasitized each year, but within year comparisons are not statistically significant (Fig. 3b; within year X^2 tests, all $P > 0.15$).

EFFECTS OF PRIOR YEAR SAMPLING

Because many volunteers did not release flies back to their sites, we were concerned that sampling may have caused a decrease in parasitism for sites sampled over multiple years. To test for this effect, we analyzed parasitism rates from sites with multiple years of data from 2000–2005, using only site/year combinations during which ≥ 10 monarchs were collected.

a) MI focus site



b) WI focus site

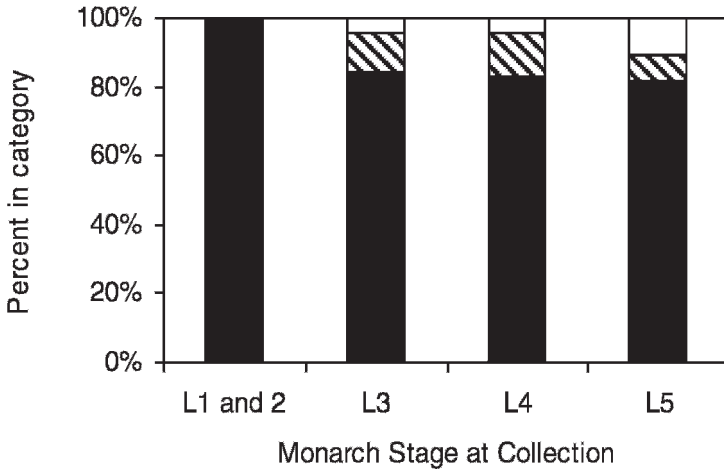
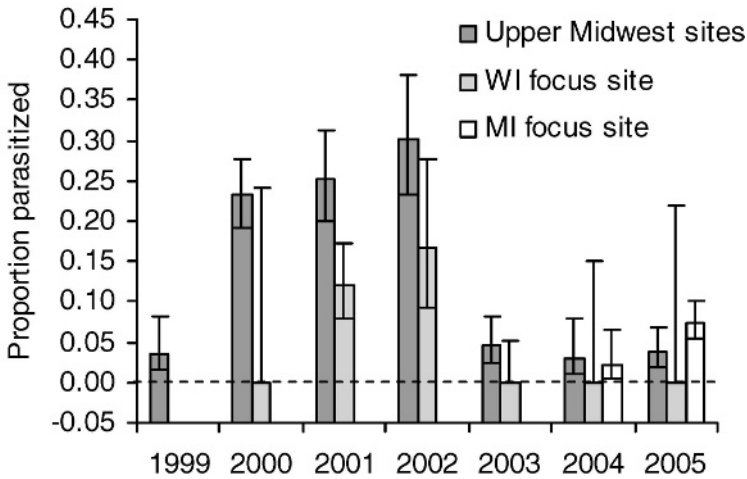


FIG. 1.—Outcomes for monarchs collected during different stages or larval stadia. Dark portions of the bars represent larvae that produced adult monarchs, hatched portions those that died of causes other than fly parasites, and open portions those that died due to tachinid parasitoids in the a) MI and b) WI focus site. The chance of being parasitized increased with age at collection

a) Upper Midwestern U.S.



b) Southeastern U.S.

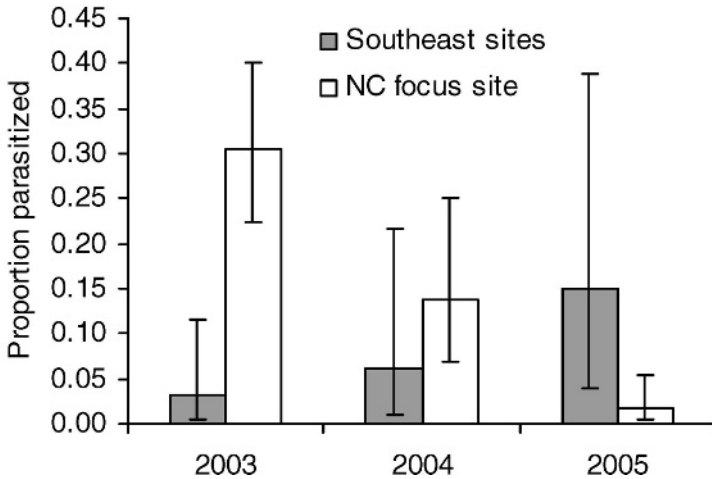
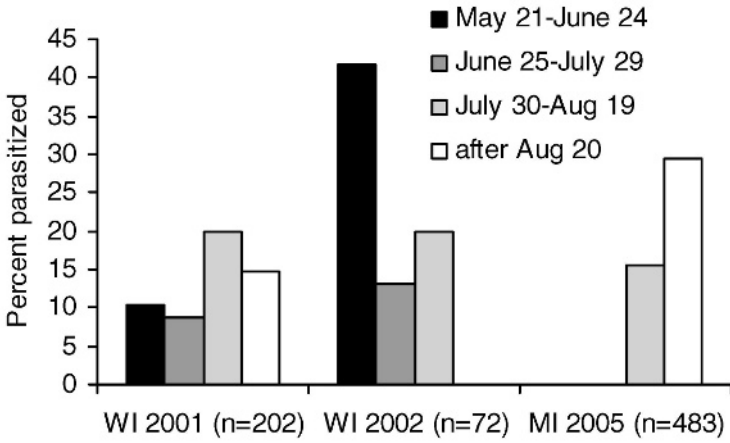


FIG. 2.—Proportions of larvae that were parasitized by tachinid flies as a function of the year of collection. a) Upper Midwestern region and focus sites and b) Southeast region and focus site. Yearly effects in the Upper Midwest are mirrored by the WI focus site, but there are no region-wide patterns in the Southeastern U.S. To differentiate zero values from a lack of data in Figure 2a, the y-axis begins at -0.05 . Error bars represent 95% confidence intervals

a) MI and WI focus sites



b) NC focus site

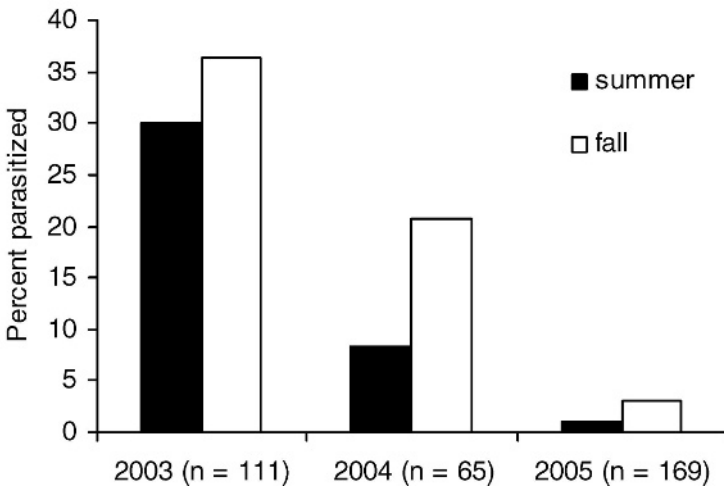


FIG. 3.—Proportions of larvae that were parasitized as a function of date of collection. a) WI focus site in 2001 and 2002, and MI focus site in 2005, b) NC focus site in 2003–2005. The lack of a bar for any time period represents a zero value

TABLE 1.—Parasitism rates (% parasitized, [n]) at MLMP sites, with regional values for comparison

Region	Site#, Location	2000	2001	2002	2003	2004	2005
Northeast	30, Freedom PA			0 (11)	19 (42)	0 (14)	0 (95)
	40, Mayfield Village OH				0* (91)		1.3 (152)
	41, Mayfield Village OH				17 (12)	0 (58)	
	42, Mayfield Village OH				4* (76)		0 (26)
	46, Drumore PA				39 (83)		
Southeast	31, Butner NC			56 (36)	3 (35)		
	83, Greensboro NC				21 (28)	8 (12)	8 (40)
	84, Greensboro NC				13 (15)		0* (10)
	85, Greensboro NC				38 (67)	15 (53)	0* (118)
Upper MW	3, Marquette MI	43 (60)	38 (114)	31 (55)	5 (65)	0 (16)	29 (14)
	4, Appleton WI		8* (13)	8* (12)	0* (38)	0 (23)	18 (11)
	6, Sparta WI	35 (26)	18 (17)	32 (19)	0* (10)	21 (14)	0* (14)
	10, Leopolis WI	0* (16)	12* (202)	17* (72)	0* (91)	0 (28)	0* 12()
	20, New Richmond WI		19 (36)	0* (10)			
	25, Roseville MN		0* (18)	90 (20)	10 (59)	0 (10)	
	29, Dugald Manitoba			0* (14)	0* (12)		0* (30)
	86, Kalamazoo MI			8* (12)	0* (10)	2 (136)	8 (482)
	87, Kalamazoo MI						4 (57)
66, Independence IA						5 (64)	
73, Independence IA						0* (47)	
Region-wide Values							
Northeast	95% CI	0–31		0–27	15–25	0–9	0–3
Southeast	95% CI			38–72	0–12	1–22	4–39
Upper MW	95% CI	18–27	16–23	23–38	2–8	0–8	2–7

All values are rounded to the nearest percent, and those below the regional 95% CI are starred. Regional confidence values are calculated with the entire database, excluding our focus sites (sites 83–85 in NC, 10 in WI and 86–87 in MI) to prevent skewing the data

We analyzed multiple sites close together as separate sites if the volunteer considered them as such; volunteers are told to list sites separately if the distance or differences between them seem “biologically relevant.” We then compared values from individual sites to regional parasitism rates, calculated from all of the data within a given region less focus site data to prevent biasing the region values. Table 1 shows parasitism rates at sites that met these criteria, divided by region. We determined whether each value fell within the 95% confidence interval (Blyth and Still, 1983) for its region, or if it was lower or higher than this range. Lower values are starred in Table 1.

If collecting from a site decreases subsequent year parasitism rates, values should be lower than expected when larvae and thus parasites, were collected from a site during the previous year. This is not the case. Of the 20 values representing the first year of monitoring at a site, eight (40%) were below the regional 95% confidence interval. Of the 36 observations made after a previous year of monitoring, 12 (33%) were below the 95% confidence interval. If anything, there are more low values during the first year of monitoring at a site.

TACHINID FLIES PER MONARCH

Figure 4a illustrates the distribution of the number of flies that emerged from individual monarchs. In the non-focus site database, we were certain of the number of flies that

emerged from 49 parasitized monarchs, and there were a total of 44 and 45 parasitized monarchs collected in our MI and NC focus sites, respectively. We compared the distributions of flies per monarch in the MI and NC focus data to Poisson distributions with mean and variance equal to the average number of parasitoids emerging per host from each site (Table 2), assigning zero values to healthy monarchs and monarchs that died of other causes. Both the MI and NC data were highly divergent from a Poisson distribution ($P < 0.000001$), with more aggregation of flies within monarchs than expected. Because some of the monarchs assigned to the "died of other causes" category may have been parasitized, we also calculated expected values based on a distribution in which these monarchs were omitted from the zero category. Whereas the chi square values were lower (see Table 2), the data were still highly divergent from a Poisson distribution ($P < 0.000001$).

Monarch stadium at collection affected the number of flies per monarch from the MI focus site (Fig. 4b). We collapsed the seven parasitized monarchs younger than fourth instars into a single category and used Analysis of Variance to compare the number of flies in larvae collected as first to third, fourth and fifth instars. Monarchs collected as fifth instars contained more flies per monarch than either other category (ANOVA $F_{2,41} = 6.5$, $P = 0.0034$), but the means for first to third and fourth instars were not significantly different (Tukey's HSD Comparison, $P > 0.05$).

Of the 61 flies collected from monarchs near the University of Minnesota in 2003 and 2006, pupa masses ranged from 0.013 to 0.051 g. There was a significant negative correlation between the mean mass of pupae from each monarch and the number of flies per monarch (Fig. 4c, Pearson correlation coefficient = -0.52 , $P = 0.023$). However, when only fly pupae from monarchs containing one to four flies were considered, this correlation disappeared (Pearson correlation coefficient = 0.19 , $P = 0.46$).

In 2004 Gebhard characterized several monarch larvae as dying after failing to thrive (see above). Hypothesizing that this syndrome might be caused by tachinid parasitoids, she dissected larvae that died of this syndrome in 2005 and 2006. Two (collected as a third and a fourth instar) of the seven that failed to thrive contained a dead tachinid fly larva. One collected as an egg, two as first instars and two as fifth instars failed to thrive but did not contain flies.

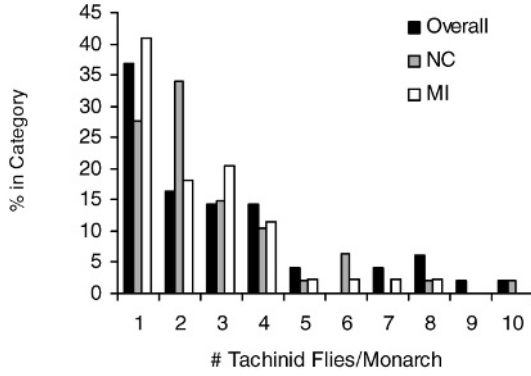
HYPERPARASITISM

Of 38 parasitized monarch larvae collected by Gebhard in 2005, six (16%) were hyperparasitized by wasps. These wasps were identified to the family Perilampidae (verified by Dr. Ann Fraser, Kalamazoo College) and are probably *Perilampus hyalinus* (Prysbey, 2004). Either one ($n = 3$) or two ($n = 3$) wasps emerged from each monarch larva. Two hyperparasitized monarchs produced both tachinid flies and wasps (one produced one fly and one wasp from two fly larvae and one produced two flies and one wasp from three fly larvae).

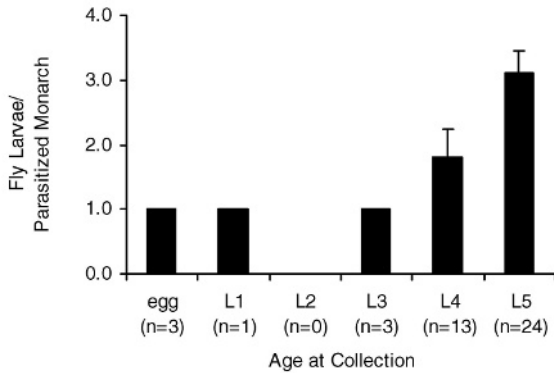
EFFECTS OF HOST PLANT SPECIES

Cameron collected over 80 larvae each on *Asclepias curassavica*, *A. incarnata* and *A. syriaca* from his NC focus sites. The proportions of parasitized larvae collected on each plant (15%, 6% and 17%, respectively) were marginally different ($X^2 = 5.8$, $df = 2$, $P = 0.054$). However, because all three milkweed species were not present at all three of his sites, milkweed species and site may be confounded in this analysis. At the only site that contained all three milkweed species, 31% of 35 monarchs collected on *A. syriaca*, 15% of 98 collected on *A. curassavica* and 5% of 79 collected on *A. incarnata* were parasitized. All of these proportions are significantly different from each other (all $X^2 > 4.2$, $df = 1$, all $P < 0.04$).

a)



b)



c)

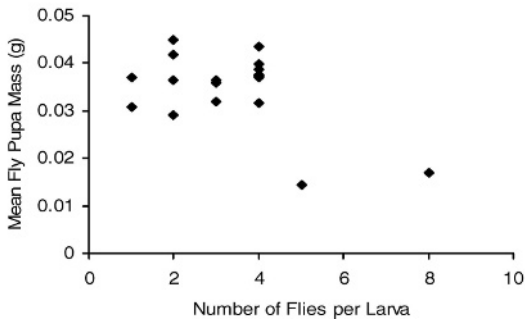


TABLE 2.—Number of monarchs observed with different numbers of tachinid flies compared to the number expected based on a Poisson distribution (mean number of flies per host in NC = 0.40, in MI = 0.17). Both distributions are highly divergent from a Poisson distribution ($P < 0.000001$ in both cases). We also calculated Poisson distributions based on the assumption that monarchs that died of other causes contained undetected tachinid fly larva, and the resultant X^2 values were $1.8E+8$ and $1.1E+8$ for the NC and MI datasets, respectively. These distributions are also highly divergent from a Poisson distribution (both $P < 0.000001$)

Flies/Monarch	NC		MI	
	Obs	Exp	Obs	Exp
0	300	242	653	597
1	13	86	18	92
2	15	15	8	7.0
3	6	1.8	9	0.36
4	5	0.16	5	0.014
5	1	0.011	1	0.00042
6	3	0.00066	1	$1.1E-05$
7	0	$3.32E-05$	1	$2.4E-07$
8	1	$1.47E-06$	1	$4.6E-09$
9	0	$5.77E-08$	0	$7.8E-11$
10	1	$2.04E-09$	0	$1.2E-12$
X^2	$4.9E+8$		$2.2E+8$	

DISCUSSION

Much of our discussion is based on the assumption that all of the parasitoids that emerged from monarchs in this study were *Lespesia archippivora*. It is likely that at least most of them were; all of the flies that we did identify were this species and *L. archippivora* is common throughout the range of the study.

This is the first study to detail the extent and temporal variation of monarch parasitoids over such a broad spatial scale; indeed, there are few, if any, such studies of nonpest hosts and their parasitoids. Because many pest insects are introduced species that tend to occur in densities inflated by host plant monocultures, their interactions with parasitoids may not reflect those that occur between nonpest hosts and nonintroduced parasitoids. The broad distribution of this generalist parasitoid and the fact that it is by far the most common parasitoid of monarchs in the U.S. suggest that it could be one factor that controls monarch populations.

SPATIAL AND TEMPORAL DISTRIBUTION OF PARASITISM

Fly parasitism of monarchs is temporally and spatially patchy. There was a high degree of variation between years; whereas this variation was to a large degree region-wide, at least in the Upper Midwestern U.S., there were cases in which a single milkweed patch produced

←

FIG. 4.—Variation in the number of tachinid flies per monarch larva. a) Distribution of the number of tachinid flies per parasitized monarch larva. b) Effect of the age at monarch collection on the number of flies per larva (mean + SE) from MI focus site. c) Effect of the number of flies per monarch on the mean pupa mass of flies from each larva

parasitism rates much higher or lower than the regional pattern. There are multiple hypotheses for this patchiness. In some cases, one or a few flies may find or emerge in an isolated, small milkweed patch. Stapel *et al.* (1997) and Etchegaray and Nishida (1975) estimated that *Lespesia archippivora* fecundity ranges from about 100–350 and 80–200 eggs, respectively, suggesting that a single female could easily parasitize all of the monarchs in a small milkweed patch. This may have happened in site 25 in Minnesota in 2002, where 90% of 21 larvae collected were parasitized (Table 1). Conversely, a small patch might never be found by a parasitoid.

Region-wide patterns, such as the higher rates of parasitism in 2000 to 2002 in the Upper Midwest, may be due to widespread weather phenomena, such as benign or severe winters. Whereas the way in which *Lespesia archippivora* overwinters is not definitively known, *Lespesia* species generally overwinter as larvae within the pupae of their hosts (Schaffner and Griswold, 1934 as cited in O'Hara, 1999). This could not occur if the host is a monarch, since monarchs do not overwinter as pupae, and are absent from temperate regions during the winter. We assume that *L. archippivora* from monarchs winter as pupae in leaf litter, or they may seek an alternative host at the end of the season. Region-wide patterns may also be driven by population variation in another host, since *L. archippivora* is a generalist parasitoid. Similar variation was not observed in the data from the Southeastern U.S., although our sample sizes, both in numbers of sites and numbers of monarchs collected, are smaller from this region. Sample sizes are also lower in the Northeastern U.S., but there was a fairly consistent trend for higher parasitism rates in 2003 than in 2004 and 2005 (Table 1). The lack of differences between years in the Southeast may reflect less extreme winters, if winter weather influences parasitoid populations.

BIOLOGY OF TACHINID PARASITISM OF MONARCHS

Our finding that larvae collected during later stadia have higher parasitism rates is probably due to longer exposure periods, although it could also result from a fly preference for or higher apparency of larger larvae. Our data do not allow us to discriminate between these nonexclusive hypotheses. In a laboratory study, the flies attacked all monarch stadia, but preferred late second through fourth instars. They rarely parasitized fifth instars successfully because these larvae were able to shake flies off their body (Etchegaray and Nishida, 1975). Our limited data on the failure to thrive syndrome suggest that parasitism of younger hosts may result in premature host death and subsequent parasitoid death; this warrants further study.

The number of flies per host ranged from one to 10, and this number increased with the stadium at collection in the MI focus site. These results are similar to those of Etchegaray and Nishida (1975), who found up to 10 flies per host in field-collected monarchs. The lack of correspondence to a Poisson distribution indicates that females do not lay individual eggs randomly across the host population. The aggregated pattern could be caused by oviposition of multiple eggs at one time by individual female flies. It could also occur if females lay additional eggs in already parasitized hosts (superparasitism) in subpopulations that have higher parasitism rates than the larger metapopulation. The observed increase in the number of flies per monarch with stadium at collection could result from either mechanism; the first if females respond to a larger host by laying more eggs, and the second simply because older monarchs have been exposed to flies for a longer period of time. Our data do not allow us to discriminate between these hypotheses. The first explanation is supported by Stapel *et al.* (1997), who conducted a laboratory study of parasitism of *Spodoptera exigua* (beet armyworms), by *Lespesia archippivora*. They found that female flies laid

more eggs in larger moth larvae; those exposed as second instars produced up to three flies, as third instars up to four flies, and as later instars up to seven flies.

Fly mass was lower when the number of flies within a host was over four, although our data on larger numbers of flies per host are limited. If larger numbers result from superparasitism, our findings suggest that female parasitoids would increase their offsprings' fitness by searching for unparasitized hosts, assuming that fitness correlates with size. However, the fact that superparasitism by dipteran parasitoids is fairly common (Feener and Brown, 1997) suggests that they have limited ability to recognize and discriminate against hosts that are already parasitized.

Hyperparasitism of tachinid flies by Perilampidae wasps, probably the same species, was reported previously in monarchs collected in Minnesota and Wisconsin (Pryby, 2004). Females in this family attach their eggs to foliage or partially embed them in leaves. Planidial first instar larvae emerge and penetrate the skin of the primary host. They search for the larvae of the primary parasitoid (either a tachinid fly or an ichneumonid wasp; Clausen, 1940) within the host and usually do not develop further until the primary parasitoid pupates (Heraty and Darling, 1984). It is not known if females locate and deposit eggs close to primary hosts, or if they simply lay them on appropriate host plants (Clausen, 1940). Whereas the low numbers of hyperparasitoids found in the study suggest that they probably do not have a large impact on tachinid fly numbers over broad spatial and temporal scales, they were relatively common in one site during one year, and may have local effects on this primary parasitoid.

The data from our NC focus sites allowed us to determine if parasitism rates varied among hosts found on different milkweed species. In Cameron's yard, where three species of milkweed grow in close proximity, monarchs collected on *Asclepias syriaca* were most likely to be parasitized, *A. curassavica* had intermediate levels of parasitism and *A. incarnata* had the lowest levels. Cardenolide levels in these three species vary as follows: *A. syriaca*, 50; *A. curassavica*, 1055; and *A. incarnata*, 14 $\mu\text{g}/0.1$ g dry weight of leaf tissue (Malcolm, 1991). Rayor (2004) found that *Polistes* wasps preferred monarch larvae reared on milkweed species with lower cardenolide concentration and suggested that larvae reared on different species represented a spectrum of palatability to which the wasps responded. It is possible that tachinid females oviposited preferentially on larvae found on a species with low cardenolide levels, *A. syriaca*, given the choices of monarch palatability levels that existed in Cameron's yard. However, this association was not perfect, since the parasitism rate on *A. incarnata* was lower than that on the more toxic *A. curassavica*. Ease of finding the host may affect parasitism rates; *A. incarnata* and *A. curassavica* have similar morphology with multiple stems and dense leaves, whereas *A. syriaca* grows in individual ramets with less dense leaves. Additionally, problems for a generalist parasitoid in determining toxicity levels may affect selective oviposition. We did not conduct an experiment specifically designed to measure female choice of hosts, and this pattern deserves further research.

Parasitism was observed in three monarchs that were collected as eggs, but there is no evidence to suggest that the eggs were already parasitized by *Lespesia archippivora* at the time of their collection. Egg parasitism by tachinid flies is unknown, and our results are likely to be due to contamination during the rearing process. Tachinid eggs or first instar larvae could have been inadvertently introduced into the rearing containers when fresh plant material was added.

POTENTIAL IMPORTANCE OF *LESPEZIA ARCHIPPIVORA* IN REGULATING MONARCHS

Whereas there is widespread acceptance of the Hairston, Smith and Slobodkin model (Hairston *et al.*, 1960) that herbivores tend to be regulated by their predators and

parasitoids, empirical and theoretical evidence suggests that multiple factors regulate most herbivores (reviewed in Rosenheim, 1998). Indeed, a commonly held view is that monarchs are protected from most natural enemies by the cardenolide toxins that they sequester from their milkweed host plants. Several authors have suggested that interactions with their milkweed host plants play a large role in regulating monarch populations; larvae often suffer mortality and delayed development that result from the milky latex (Zalucki and Brower, 1992; Zalucki *et al.*, 2001) and possibly the cardenolides (Zalucki *et al.*, 1990) in milkweed. However, monarchs also suffer high levels of mortality from a large suite of invertebrate predators (Prysby, 2004), but only a few studies have quantified the effects of specific predators on monarch populations (Lynch and Martin, 1993; Calvert, 1996, 1999, 2004; Prysby, 2004). The current study suggests that tachinid fly parasitoids could have a relatively important impact on monarchs, killing an average of 13% overall, but up to 90% in some local populations. The interaction with the Perilampinae hyperparasitoids may mitigate the effects of the tachinid flies; as higher order predators, hyperparasitoids could constrain top down control of herbivores (May and Hassell, 1981).

The prevalence of tachinid flies in monarchs suggests that previous studies may underestimate monarch mortality. Because it is difficult to assess population densities of pupae and adults, most studies assess survival from the egg to the late larva stage. However, mortality caused by tachinid flies occurs after this period, and is thus not included in most mortality studies.

SUGGESTIONS FOR FUTURE RESEARCH

Future research should address both the biology of the relationship between monarchs and tachinid flies, and the mechanisms that drive temporal and spatial variation in parasitism rates. Collecting evenly across larval stadia could help to determine the timing of parasitism in wild populations, and laboratory experiments would help to determine the mechanisms for variation in the number of flies per host. The relationship between the failure to thrive syndrome and larval stadium at collection suggests a cost to oviposition on young larvae, and we recommend dissecting larvae that die of other causes to determine if there are fly larvae within them.

Variation in fly parasitism rates from year to year could be caused by both biotic and abiotic factors. It is unlikely that monarch abundance, which itself is variable, drives *Lespesia archippivora* numbers, and it would be valuable to assess parasitism rates in other hosts. Likewise, ecological variables, such as plant density and diversity, will affect the abundance and diversity of other hosts. Additionally, future studies should address *L. archippivora* overwintering strategies and the effects of winter conditions on overwintering survival of the parasitoids.

Acknowledgments.—A study of this scale would have been impossible without the efforts of volunteers throughout the monarch breeding range and we thank all of the MLMP volunteers who have raised hundreds of monarch larvae over the past 6 years, especially S. Payant, S. Duerkop, D. Kemp, D. and R. Hudacsek, D. Marcinski, G. Steffy and S. Cabell. Gebhard thanks E. Chadderdon, B. Csia, J. Leppard and E. Pitcher for help raising and releasing monarchs; and R. Schipper for his help and willingness to share their home with 100's of monarchs. K. Oberhauser thanks M. Prysby, M. Solensky, S. Altizer, R. Batalden, G. Bowers, A. De Anda and other Monarch Lab personnel for the large roles they've played in making the MLMP successful. K. Creasey measured several of the tachinid fly pupae, and A. De Anda, D. Alstad, M. Prysby and two anonymous reviewers made useful comments on the manuscript. This work was supported by the NSF (ESI 0104600), the Monarchs in the Classroom program at the University of Minnesota and the University of Minnesota Extension.

LITERATURE CITED

- ARNAUD, P. H. 1978. A host-parasite catalog of North American Tachinidae (Diptera). *USDA, Misc. Pub.*, **1319**.
- BENEWAY, D. F. 1963. A revision of the flies of the genus *Lespesia* (= *Achaetoneura*) in North America (Diptera: Tachinidae). *Univ. Kansas Sci. Bull.*, **44**:627–677.
- BLYTH, C. R. AND H. A. STILL. 1983. Binomial confidence intervals. *J. Amer. Stat. Ass.*, **78**:108–116.
- BOETTNER, G. H., J. S. ELKINTON AND C. J. BOETTNER. 2000. Effects of a biological control introduction on three nontarget native species of Saturniid moths. *Cons. Bio.*, **14**:1798–1806.
- CALVERT, W. H. 1996. Fire ant predation of monarch larvae (Nymphalidae: Danainae) in a central Texas prairie. *J. Lepid. Soc.*, **50**:149–51.
- . 1999. Patterns in the spatial and temporal use of Texas milkweeds (Asclepiadaceae) by the monarch butterfly (*Danaus plexippus* L.) during fall, 1996. *J. Lepid. Soc.*, **53**:37–44.
- . 2004. The effects of fire ants on monarchs breeding in Texas, p. 47–53. *In*: Oberhauser, K. S. and M. J. Solensky (eds.). *The monarch butterfly: Biology and conservation* Cornell University Press, Ithaca NY.
- CLAUSEN, C. P. 1940. *Entomophagous insects*. McGraw-Hill, New York NY.
- EGGLETON, P. AND R. BELSHAW. 1992. Insect parasitoids: an evolutionary overview. *Phil. Trans: Biol. Sci.*, **337**:1–20.
- ETCHEGARAY, J. B. AND T. NISHIDA. 1975. Biology of *Lespesia archippivora* (Diptera: Tachinidae). *Proc. Hawaiian Ent. Soc.*, **22**:41–49.
- FEENER, D. H., JR. AND B. V. BROWN. 1997. Diptera as parasitoids. *Annu. Rev. Entomol.*, **42**:73–97.
- HAIRSTON, N. G., F. E. SMITH AND L. B. SLOBODKIN. 1960. Community structure, population control, and competition. *Amer. Nat.*, **94**:421–425.
- HERATY, J. M. AND D. C. DARLING. 1984. Comparative morphology of the planidial larvae of Eucharitidae and Perilampidae (Hymenoptera: Perilampidae). *Sys. Entomol.*, **9**:309–328.
- KELLOGG, S. K., L. S. FINK AND L. P. BROWER. 2003. Parasitism of native luna moths, *Actias luna* (L.) (Lepidoptera: Saturniidae) by the introduced *Compsilura concinnata* (Meigen) (Diptera: Tachinidae) in central Virginia, and their hyperparasitism by trigonalid wasps (Hymenoptera: Trigonalidae). *Environ. Entomol.*, **32**:1019–1027.
- LYNCH, S. P. AND R. A. MARTIN. 1993. Milkweed host plant utilization and cardenolide sequestration by monarch butterflies in Louisiana and Texas, p. 107–123. *In*: Malcolm, S. B. and M. P. Zalucki (eds.). *Biology and conservation of the monarch butterfly* Natural History Museum of Los Angeles County, Los Angeles CA.
- MALCOLM, S. B. 1991. Cardenolide-mediated interactions between plants and herbivores, p. 251–296. *In*: Berenbaum, M. R. and G. A. Rosenthal (eds.). *Herbivores: Their interactions with secondary plant metabolites* Academic Press, New York NY.
- MAY, R. M. AND M. P. HASSELL. 1981. The dynamics of multiparasitoid-host interactions. *Amer. Nat.*, **117**:234–261.
- Monarch Larva Monitoring Project. <http://www.mlmp.org>. Accessed 22 May 2006.
- O'HARA, J. E. 1985. Oviposition strategies in the Tachinidae, a family of beneficial parasitic flies. *Agric. For. Bull, Univ. of Alberta.*, **8**:31–34.
- . 1999. Tachinidae (Diptera) parasitoids of bertha armyworm (Lepidoptera: Noctuidae). *Can. Entomol.*, **131**:11–28.
- PRYSBY, M. D. 2004. Natural enemies and survival of monarch eggs and larvae, p. 27–38. *In*: Oberhauser, K. S. and M. J. Solensky (eds.). *The monarch butterfly: Biology and conservation* Cornell University Press, Ithaca NY.
- AND K. OBERHAUSER. 1999. Large scale monitoring of monarch populations, p. 379–384. *In*: Hoth, J., I. Pisanty, K. Oberhauser, L. Merino and S. Price (eds.). *Proceedings of the North American conference on the monarch butterfly* Commission for Environmental Cooperation, Montreal, QC.
- AND ———. 2004. Temporal and geographic variation in monarch densities: citizen scientists document monarch population patterns, p. 9–20. *In*: Oberhauser, K. S. and M. J. Solensky (eds.). *The monarch butterfly: Biology and conservation* Cornell University Press, Ithaca NY.

- RAYOR, L. 2004. Effects of monarch larval host plant chemistry and body size on *Polistes* wasp predation, p. 39–46. In: Oberhauser, K. S. and M. J. Solensky (eds.). *The monarch butterfly: Biology and conservation*. Cornell University Press, Ithaca NY.
- ROSENHEIM, J. A. 1998. Higher-order predators and the regulation of insect herbivore populations. *Annu. Rev. Entomol.*, **43**:421–447.
- SCHAFFNER, J. V., JR. AND C. L. GRISWOLD. 1934. Macrolepidoptera and their parasites reared from field collections in the northeastern part of the United States. *USDA, Misc. Pub.*, **188**.
- STAPEL, J. O., J. R. RUBERSON, H. R. GROSS AND W. J. LEWIS. 1997. Progeny allocation by the parasitoid *Lespesia archippivora* (Diptera: Tachinidae) in larvae of *Spodoptera exigua* (Lepidoptera: Notuidae) *Environ. Entomol.*, **26**:265–271.
- ZALUCKI, M. P. AND L. P. BROWER. 1992. Survival of first instar larvae of *Danaus plexippus* (Lepidoptera: Danainae) in relation to cardiac glycoside and latex content of *Asclepias humistrata* (Asclepiadaceae). *Chemoecology*, **3**:81–93.
- , ——— AND S. B. MALCOLM. 1990. Oviposition by *Danaus plexippus* in relation to cardenolide content of three *Asclepias* species in the southeastern USA. *Ecol. Entomol.*, **15**:231–40.
- , S. B. MALCOLM, T. D. PAINE, C. C. HANLON, L. P. BROWER AND A. R. CLARKE. 2001. It's the first bites that count: Survival of first-instar monarchs on milkweeds. *Austral. Ecol.*, **26**:547–555.

SUBMITTED 6 JULY 2006

ACCEPTED 5 SEPTEMBER 2006