Phenology of *Lymantria monacha* (Lepidoptera: Lymantriidae) Laboratory Reared on Spruce Foliage or a Newly Developed Artificial Diet

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ABSTRACT *Lymantria monacha* (L.) (Lepidoptera: Lymantriidae) is a Eurasian pest of conifers that has potential for accidental introduction into North America. The phenology over the entire life cycle for *L. monacha* individuals from the Czech Republic was compared on *Picea glauca* (Moench) Voss (white spruce) and a newly developed artificial diet. Individuals reared on the artificial diet had lower larval mortality, slightly higher pupal mortality, developed faster, the majority of females went through one less instar, and adults weighed slightly more than those reared on the spruce foliage. Individuals reared on the spruce foliage survived and developed at rates similar to those documented in previous studies on preferred hosts. The host-free rearing methods and artificial diet presented here will allow *L. monacha* to be mass reared year round to aid in the advance of bioassay-based research aimed at preventing introductions and eradicating this pest if it should become established in parts of the world outside its native range. The phenology documented here suggests that managers can expect the timing of peak second instars for *L. monacha* to occur later than that for gypsy moths, *Lymantria dispar* (L.), and the timing of male flight to occur earlier than that of gypsy moth, if both species hatch at the same time and develop under the same environmental conditions.

KEY WORDS *Lymantria monacha*, phenology, rearing, artificial diet

Lymantria monacha (L.) (Lepidoptera: Lymantriidae) is a Eurasian pest of conifers that poses an ever-present threat of being introduced into North America. *L. monacha* has a high potential to be transported via commerce because, although eggs are normally laid in bark crevices, they also could be deposited in crevices on containers, pallets, and ships (M.A.K., unpublished data). The threat of this type of accidental transport is high because both sexes are readily attracted to artificial lights, and females have been observed ovipositing in Russian Far East ports (Munson et al. 1995). *L. monacha* feeds primarily on needles and male cones of conifers (*Picea*, *Pinus*, *Abies*, and *Larix* spp.) but also can develop on leaves of deciduous trees and shrubs (*Fagus*, *Carpinus*, *Betula*, and *Quercus* spp.; Sliwa 1987). In Europe, *L. monacha* prefers and most often damages *Picea abies* (L.) (Norway spruce) and *Pinus sylvestris* L. (Scots pine; Lipa and Glowacka 1995). Evaluation of the survival and development of *L. monacha* on potential North American hosts (Keena 2003) and *Pinus radiata* D. Don (Withers and Keena 2001) has found that they can do well on a broad range of both conifer and broadleaf species not native to Eurasia. Its establishment in North America would have significant ecological and economic effects because of its polyphagous feeding habits, ability to colonize new habitats, and capacity to be spread rapidly by mated flying adult females. Considerable information on the pheromones (Gries et al. 1996), microbial control (Glowacka 1989), and general biology and biological control (Grijpma 1989, Jensen 1996, Sliwa 1987, Švestka 1971) of *L. monacha* in Europe is available to aid in dealing with this pest should it be introduced. The best defenses currently used against this potential pest are to monitor its abundance in Eurasian ports (Munson et al. 1995), develop a good pheromone trap to use in monitoring (Morewood et al. 1999), and educate people on how to identify it (Keena et al. 1998). In addition, a better understanding of the phenology, timing of each instar and stage, on a North American host would improve the predictions of when various eradication strategies should be deployed if this pest is introduced.

There is no adequate artificial diet for rearing *L. monacha* and not all the research that needs to be done on this pest can be done in the field in Eurasia. A method of rearing *L. monacha* on artificial diet would greatly simplify the assessment of biological and chemical control agents; investigations of biology, behavior, and genetics; and increase the reproducibility of results by standardizing rearing methods and diets. Several authors have reared or have attempted to rear *L. monacha* on semisynthetic diets (Grijpma et al.

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1987; Lühl 1973; Salama et al. 1976; Skatulla 1985, 1986; Zakrevskaya 1983; Zethner 1976). The best published diet to date is a modification of the Odell and Rollinson (1966) gypsy moth diet, but there was high first-instar larval mortality and many adults were deformed (Grijpma et al. 1987). We have been working on developing a better diet based on the high wheat germ diet used by Bell et al. (1981). Many modifications were formulated using our knowledge of past *L. monacha* diet testing, published diets of other conifer feeding insects, information about the differences between conifer and deciduous leaves (because the base diet was for a deciduous feeder), and general information on insect nutritional requirements. At each step along the way the best diet available to date was compared against the new modification. The diet presented here has been successfully used for the last 10 generations. To assess how well larval development on this newly developed artificial diet compared with that on a preferred host the phenology on both was compared.

This study compared the phenology of *L. monacha* reared on *Picea glauca* (Moench) Voss (hereafter referred to as spruce) and a newly developed artificial diet. Time in each instar and stage, survival, and adult characteristics were documented in the laboratory. A complete synthetic system for successfully rearing *L. monacha* without host foliage is documented. The potential timing of eradication events in North American forest ecosystems, should *L. monacha* become established, are discussed and compared with gypsy moth, *Lymantria dispar* (L.).

Materials and Methods

Insect Source. Eggs produced by 89 female *L. monacha* (17th laboratory generation) originally from Predin, Czech Republic, were pooled and chilled at 5 ± 1°C and ≈100% RH with a photoperiod of 12:12 (L:D) h for 142 d. From the pool of mixed eggs, eight packets of ≈500 eggs each were made. The eggs were surface sterilized to kill potential pathogens by submerging the egg packets in 10% formalin solution for 15 min, rinsing in lukewarm tap water for 15 min, and letting them dry in a laminar flow hood for no longer than 60 min. The egg packets were put in petri dishes, which were placed on a screen over water in a clear plastic box (water box) to maintain high humidity. The eggs were incubated in a chamber held at 25 ± 1°C, 60% RH, and a photoperiod of 16:8 (L:D) h for 6–7 d. Date of first hatch of each egg packet was recorded.

Once the majority of the eggs had hatched and larvae had begun to move away from the packet, 100 larvae each for the foliage and artificial diet groups were moved with a fine camel's-hair brush into small, tight fitting preweighed petri dishes. Twenty larvae were placed in each petri dish, the larvae and petri dish were weighed together, and then the weight of the dish was subtracted to determine the weight of the larvae. This was necessary because an individual larva weighs less than could accurately be weighed on the available balance. Average initial weight of the larvae in each group was calculated.

The *L. monacha* egg masses used to start the colony were transported under permit to the USDA Forest Service quarantine facility in Ansonia, CT. Voucher specimens of adults were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

Rearing on Spruce Foliage. Branch tips (up to 1 m long) with foliage were clipped from eight different mature spruce trees beginning 10 May when new foliage buds were just opening. For initial setup, all branch tips came from the same tree and included both the current and previous year's foliage. For subsequent foliage changes, foliage was collected from one of the other eight individual spruce trees being used in a sequential rotation. The clipped ends were kept in de-ionized water until used. Insects and debris found on the foliage were removed or washed off with deionized water. If the needles were washed, they were allowed to dry before being used.

Twenty first-instar larvae were placed on a 12-cm-long spruce branch tip, the end of which had been inserted into a 10-ml water pick then wrapped with parafilm to prevent water leakage. The branch tips were laid in a 150-mm-diameter by 25-mm-high petri dish with a 2.5-cm square hole in the top covered with a fine mesh screen. The five petri dishes were taped shut to prevent escape. A 90-mm-diameter filter paper was placed in the bottom of the dish to collect excess moisture and the dish was placed in a water box to maintain the humidity. Dishes were checked daily for molts and mortality. The foliage was changed weekly or more often if needed. All larvae that molted to the second instar were individually weighed and moved to a new container (maximum of 15 per container) that was labeled with instar, number of larvae, and date of molt. The petri dishes and the containers used for larger larvae were both held at 25 ± 2°C, 60 ± 5% RH, and a photoperiod of 16:8 (L:D) h.

For second and all subsequent instars, larvae were held in 3.8-liter rolled paper rearing containers. A piece of clear plastic wrap was used to replace the lid, allowing light to enter the container, and the outer ring of the lid held the wrap tight over the opening. Each container had a 256-ml plastic cup that served as a deionized water reservoir for the branch ends that were inserted through a hole in the plastic lid. The end of each branch was recut underwater before the lid was snapped onto the cup. A perfume and dye free facial tissue was draped over the plastic cup during the second and third instars to ensure that larvae that fell to the bottom could easily climb back onto the branch. In these containers, foliage was changed twice a week while inspected daily for new molts, which were weighed, moved to new containers with fresh foliage, and held in groups of no >15 by date of molt. For all foliage changes everything was removed from the container and old leaves, and debris were searched for live and dead larvae and their numbers were recorded. If diseased larvae were found, all surfaces of the rearing container were cleaned with a 2% benzakonium chloride solution before a new, clean cup containing fresh

Table 1. Ingredients (amount per liter) for artificial diet used in rearing *L. monacha*

	Ingredient	Amt per liter
Initial ingredients	Distilled water (ml)	900
	Agar (*Gracileria* spp.) (g)	15
Premix added at boil	Sucrose (g)	35
	Raw wheat germ (g)	120
	Casein (60 mesh) (g)	35
	Wesson's salt mixture without $FePO_4$ (g)[a]	2.0
	94% Amorphous $FePO_4$ (g)[b]	0.1
	Choline chloride (g)	1
	Methyl paraben (g)[c]	1
	Sorbic acid (g)	2
Added after heat is turned off	Wheat germ oil (g)[d]	2.4
	Raw linseed oil (ml)[d]	2.5
	Cholesterol (g)[d]	1
Added after 5 min of cooling	Vitamin mixture (g)[e]	10
	Ascorbic acid (g)	2

[a] The Wesson's salt mixture was obtained from Purina Test Diets without $FePO_4$.

[b] Product made by Riedel-deHaen (Germany) as iron(III) phosphate extrapure powder (04241).

[c] Full name, *p*-hydroxybenzoic acid methyl ester.

[d] Mixing the two oils and the cholesterol together before adding them helps the cholesterol incorporate better in the diet.

[e] Product of Hoffman-LaRoche (Fresno, CA) as premix 26862.

foliage was placed in the container and all live larvae placed onto the foliage.

Rearing on Artificial Diet. The artificial diet was made in a 12-liter steam-jacketed kettle (in 10-liter batches). Cool deionized water and agar were measured into the kettle and brought to a rolling boil. The diet was continuously mixed from the point that the heat was turned on until the diet was ready to be poured. The premix ingredients (Table 1) were added while the agar–water mixture was boiling, and the mixture was allowed to come back to a rolling boil for 30 s before turning off the heat, after which the diet continued to boil for several minutes. This was necessary to kill bacteria and denature any enzymes in the wheat germ that could degrade the diet. The diet was mixed without heat for 5 min allowing it to cool during which the two oils and cholesterol mixture were added. At the end of the 5-min cooling period the ascorbic acid and vitamins were added. When the last ingredient had been thoroughly mixed in, the mixer was turned off, the diet was dispensed into 2-liter pitchers, and the contents were poured into 150- by 25-mm petri dishes. Diet was allowed to cool to room temperature before storage in plastic bags at 10–15°C until used which was typically within 1 mo. The diet was warmed to room temperature and excess condensation was removed before it was used.

Twenty larvae were placed on a 3-cm-square chunk of diet stuck to the bottom of a 100- by 25-mm petri dish. Metal utensils were flamed and paint brushes and surfaces were disinfected with 2% benzalkonium chloride to prevent diet contamination. The dishes were turned upside down because the larvae naturally crawl to the top of the dish. This ensures they will encounter the diet and allowed their frass to drop to the bottom so it did not contaminate the diet. Dishes were sealed with tape to prevent escape and placed in a water box held at 25 ± 2°C, 60 ± 5% RH, and a photoperiod of 16:8 (L:D) h. All petri dishes were checked daily for new molts that were weighed, moved to new petri dishes with fresh diet, and held in groups of no >15 by the date they molted. Dead larvae were removed daily and the date and instar recorded. The diet was changed once a week or if it shrank to one half its initial size. Second and third instars also were reared in the petri dishes, but they were not placed in water boxes.

New fourth-instar larvae and older larvae were placed on a full petri dish of diet taped to the bottom of a squat 473-ml paper container with a clear plastic lid that had been exposed to UV light for 15 min to surface sterilize the diet. Use of paper containers is critical because the petri dishes are too small and humid an environment for larger larvae. In addition, the larger larvae are not able to cling to the plastic or diet surfaces well and can get tangled in their own silk webbing. Paper containers provided a good surface for them to attach their silk for pupation. Diet was replaced after 3 wk or as needed.

Pupal and Adult Methods. Pupae and prepupae (larvae that shorten and become C-shaped) were removed daily from both the foliage and diet containers and placed in a labeled 473-ml paper container with clear plastic lids. All pupae were weighed individually the day they were found then grouped by ultimate instar, date, and sex. The weight, sex, and pupation date for each individual were recorded. Because the pupae were grouped, a correlation between pupal and adult weights could not be obtained.

Pupae were checked daily, adults were removed, and eclosion date was recorded. Adult containers were labeled with larval diet, setup date, eclosion date, sex, and mating date (single pairs only). Males that eclosed on the same day were placed in groups (maximum of five) in 473-ml paper containers. Adult females were individually weighed and the wing color of both sexes was recorded. The 15 females to be individually mated were placed in 473-ml paper containers that had a 2.5-cm-wide by 30-cm-long piece of smashed single-sided corrugated cardboard that had been folded twice (10 cm long) and stapled to the wall. Females lay their eggs in the deep cracks formed by the corrugated cardboard. One to two males from the same larval diet as the female were placed in with single females. The male was chosen haphazardly from those that had eclosed from that larval diet; males that eclosed >2 d previously were not used unless they were the only males available and they were still capable of flight. In addition, females to be mated in groups (four total groups) were placed in either a white paper bag (two males and two females) or in a 3.8-liter rolled paper container (three to six of each sex, generally more males than females) with three long pieces of smashed single-sided corrugated cardboard (unbleached) that had been folded twice (10 cm long) and stapled to a piece of white butcher paper that lined the walls of the container. Mating pairs were held in the same chamber where the larvae developed.

Approximately 2 wk after mating, all eggs laid by each female or group of females were harvested and embryonation evaluated. This length of time allowed embryonation to proceed to the point that a color change could be observed; embryonated eggs turn dark brown, whereas unembryonated eggs are blue-green (artificial diet) or tan (spruce foliage) and flattened over time due to dehydration. Percentage of successful matings for each larval diet was calculated as the number of matings that produced >10 embryonated eggs divided by the total number of attempted matings.

All eggs from an individual female were placed in a separate glassine envelope and labeled with larval diet, setup date, and egg mass number. Total fecundity was estimated using the relationship between female pupal weight and fecundity developed by Keena (2003). Egg mass embryonation was categorized as all unembryonted, >50% unembryonated, ≤50% unembryonted, or all embryonated. Egg masses with >10 embryonated eggs after a minimum of 22 d were placed at 5 ± 1°C, ≈100% RH, and a photoperiod of 16:8 (L:D) h for 130 d. After incubation at 25 ± 2°C, 60 ± 5% RH, and a photoperiod of 16:8 (L:D) h, the total number of larvae that hatched was recorded for each egg mass.

Statistical Tests. The following dependent variables were analyzed in PROC MIXED (SAS Institute 2009) using restricted maximum likelihood estimation methods, which is a statistical approach to provide unbiased estimates of variance in unbalanced designs: developmental time (days) and instar or stage weights (milligrams). Females and males were analyzed separately for pupal and adult characters. The model for larval characters used larval food source, instar, and the interaction between the two as fixed effects, whereas container was treated as a random effect. The model for pupal characters used larval food source, ultimate instar, sex, and the interaction between the three as fixed effects, whereas container was treated as a random effect. The model for the adult weights used the same model as for pupae, but sex was dropped because only females were weighed. Differences among means were determined using the least squared means test with $\alpha = 0.05$ and a Bonferroni correction (SAS Institute 2009). Percentage mortality and proportions of each sex were compared using pair wise Pearson's chi-square analysis (Analytical Software 2003) between the larval food sources.

Results

Developmental Time and Mortality. The phenology for both larval food sources is given in Fig. 1. Developmental time for the first through fifth instars differed significantly for the interaction between larval diet and instar ($F = 29.14$; df = 4, 667; $P < 0.0001$) (Table 1; Fig. 1). First- and second-instar larvae fed artificial diet developed significantly faster than those that fed on spruce foliage, whereas fifth-instar larvae reared on artificial diet developed slower than those reared on foliage (Table 1). Larvae that went through only four instars took significantly longer to complete

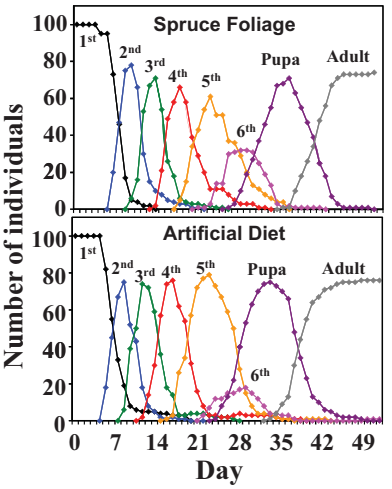

Fig. 1. Phenology of *L. monacha* on white spruce and an artificial diet. (Online figure in color.)

the ultimate instar than those that took five or six instars ($F = 19.51$; df = 2, 120; $P < 0.0001$) (Table 1). The number of days spent as a pupa differed significantly by sex ($F = 17.67$; df = 1, 129; $P < 0.0001$) but not by larval diet or ultimate instar (Table 1). Significantly more females reared on spruce went through six rather than five instars compared with those reared on diet ($\chi^2 = 8.92$, df = 1, $P = 0.0028$). Total time to complete the immature stages differed by larval diet ($F = 5.31$; df = 1, 129; $P = 0.0227$) and by number of instars ($F = 4.68$; df = 2, 129; $P = 0.0110$). Individuals reared on spruce took longer than those reared on artificial diet and individuals that went through six instars took longer than those that went through only five instars (Table 2).

Table 2. Developmental time (d) of immature stages of *L. monacha* [mean ± SE (*n*)]

Instar/stage	Larval diet	
	Picea glauca foliage	Artificial Diet
1	9.6 ± 0.5a (87)	8.5 ± 0.5b (96)
2	4.2 ± 0.5cd (79)	3.7 ± 0.5e (96)
3	4.5 ± 0.5cd (77)	4.7 ± 0.5cd (91)
4	5.7 ± 0.4cd (71)	5.1 ± 0.4cd (89)
5	4.4 ± 0.6d (32)	5.8 ± 0.6ce (25)
Ultimate 4	12.8 ± 0.9a (2)	12.0a (1)
Ultimate 5	8.3 ± 0.2b (40)	8.2 ± 0.2b (65)
Ultimate 6	7.7 ± 0.3b (32)	7.5 ± 0.4b (15)
5 instar ♀ pupa	8.7 ± 0.2bc (11)	9.2 ± 0.2bc (25)
6 instar ♀ pupa	8.7 ± 0.1c (27)	8.8 ± 0.2bc (13)
4 instar ♂ pupa	9.5 ± 0.5abc (2)	10.0abc (1)
5 instar ♂ pupa	9.5 ± 0.1ab (29)	9.9 ± 0.1a (40)
6 instar ♂ pupa	9.5 ± 0.3abc (5)	10.0 ± 0.6abc (2)
5 instar ♀ larva + pupa	39.5 ± 0.7ab (11)	37.3 ± 0.6bc (25)
6 instar ♀ larva + pupa	41.1 ± 0.5a (27)	38.7 ± 0.7abc (13)
4 instar ♂ larva + pupa	39.7 ± 1.6abc (2)	38.1abc (1)
5 instar ♂ larva + pupa	38.0 ± 0.5bc (29)	36.5 ± 0.5c (40)
6 instar ♂ larva + pupa	40.0 ± 1.1abc (5)	38.9 ± 2.2abc (2)

Means within a stage followed by the same letter are not significantly different based on Bonferroni test with $\alpha = 0.05$ (SAS Institute 2009).

Table 3. Weights (milligrams) of stages of *L. monacha* [mean ± SE (*n*)]

Instar/stage	Larval diet	
	P. glauca foliage	Artificial diet
1	0.551 ± 0.002a (100)	0.548 ± 0.002a (100)
2	6.10 ± 0.19b (87)	5.69 ± 0.18b (96)
3	27.5 ± 2.3c (79)	26.0 ± 0.7c (96)
4	92.7 ± 7.6d (77)	101.5 ± 3.9d (91)
5	276.8 ± 14.3e (73)	278.3 ± 9.4e (90)
6	618.1 ± 35.8f (32)	676.1 ± 65.6g (26)
5 instar ♀ pupa	829.5 ± 35.6bc (11)	959.4 ± 25.9ab (25)
6 instar ♀ pupa	928.1 ± 25.5abc (27)	1050.3 ± 34.3a (13)
4 instar ♂ pupa	480.2 ± 82.6d (2)	538cd (1)
5 instar ♂ pupa	448.5 ± 23.1d (31)	511.8 ± 19.4d (40)
6 instar ♂ pupa	481.1 ± 55.2d (5)	471.5 ± 82.2 d (1)
5 instar ♀ adult	474.5 ± 38.4c (11)	596.5 ± 32.9ab (20)
6 instar ♀ adult	532.0 ± 31.3bc (27)	658.2 ± 36.3a (13)

Means within a stage followed by the same letter are not significantly different based on Bonferroni test with $\alpha = 0.05$ (SAS Institute 2009).

Larval mortality was significantly higher on the spruce than the artificial diet ($\chi^2 = 7.62$, df = 1, $P = 0.0058$). In total, 25 larvae died on the spruce; 13, 8, 2, and 2 in the first, second, third, and fourth instars, respectively (and a fifth-instar larva was lost in a foliage change). Only 10 larvae died on the artificial diet; four, five, one, and one in the first, third, fourth, and sixth instars, respectively. There was no prepupal or pupal death for individuals reared on spruce, but eight prepupae (three partially pupated) and five pupae died when reared on the artificial diet. Diet reared prepupae and pupae that died were ones that developed very slowly and were the last ones in their respective containers. There was no significant difference in the percentage of female pupae reared from the two larval diets ($\chi^2 = 0.15$, df = 1, $P = 0.6956$). The only adult that was deformed when it emerged was reared on spruce.

Stage Weights. Larval weights differed significantly for the interaction between larval diet and instar ($F = 6.06$; df = 5, 861; $P < 0.0001$) (Table 3). Each instar weighed significantly more than the previous and the weights on the two larval food sources within an instar only differed significantly in the sixth instar. Pupal weights differed significantly by sex ($F = 255.43$; df = 1, 116; $P < 0.0001$), but not by larval diet, ultimate instar, or the interaction between the three; female pupae weighed more than male pupae (Table 3). Adult female weights differed significantly by larval diet ($F = 26.14$; df = 1, 64; $P < 0.001$) and ultimate instar ($F = 6.29$; df = 1, 64; $P = 0.0147$) but not the interaction between the two. Artificial diet-reared adult females weighed significantly more than spruce-reared females, and females that went through six instars weighed significantly more than those that went through only five instars (Table 2).

Adult Color and Reproduction. For both larval diets, <20% of the females were all black, and the rest ran the full gamut between black with white markings to mostly white with black markings. Males tended to be darker with ≈50% all black, ≈30% black with white markings, and the rest being white with black markings. There was no apparent difference in adult color between ultimate instar groups within a sex.

For both larval food sources, 69% of matings where more than one male was present (either with a single female or with a group of females) produced embryonated eggs, whereas only 29% of matings with only one male resulted in the production of embryonated eggs. Lumping both single or group matings together, 53% of the matings involving spruce reared adults were successful (produced embryonated eggs), whereas only 42% of those involving diet reared adults were successful, but there were more spruce matings that involved multiple males. Average estimated fecundity for all females was 367 ± 63 for the diet-reared females and 317 ± 51 for the spruce-reared females. In total, 2,774 larvae hatched from eggs produced by diet-reared adults (22 females), and 1,454 larvae hatched from spruce-reared adults (16 females). There was no good way to tell how many females in a group mating laid embryonated eggs, but the average hatch per female reared on spruce was 91 larvae and for females reared on diet it was 126 larvae.

Discussion

Individuals reared on the artificial diet had lower larval mortality, higher pupal mortality, faster development, the majority of females went through one less instar, and weighed slightly more as adults than those reared on spruce. Development, survival, and adult weights attained on the spruce were comparable with those on suitable hosts in a previous study (Keena 2003). Development on the artificial diet was faster than on most hosts but slower than on gray birch (*Betula populifolia* Marshall; 34 d for males and 36 d for females, M.A.K., unpublished from 2003 data set). *L. monacha* is reported to go through five to seven instars in the field (Bejer 1988), with five instars and six instars being the norm for males and females, respectively (Lipa and Glowacka 1995). We found that some males only went through four instars but that five instars was the norm, and we did not have any of either sex that went through seven instars. The number of instars that females went through differed with larval food source, indicating that this trait may have a fairly strong environmental component to the variation because the genetic component was randomized across the larval food sources.

Individuals reared on our artificial diet had lower larval mortality (especially in the first instar, 0 vs. 18%), fewer adult deformities, faster development, fewer instars, and higher weights as adults than those reared on the best artificial diet previously evaluated (Odell and Rollinson (1966) evaluated by Grijpma et al. 1987). The faster development is at least partially due to rearing temperature, 25°C in this study and 23°C in a previous study (Grijpma et al. 1987). However, many studies have shown that providing optimal temperature regimes, without changing food quality, will permit insects to grow faster and reduce mortality (Mattson and Haack 1987). In the Grijpma et al.

(1987) study, both males and females went through an average of six instars, one more than in this study, and weighed less compared with those in this study that could have been due to dietary differences or differences between the strains of *L. monacha* used (Czech Republic in this study and Netherlands in the Grijpma et al. (1987) study). The lower larval mortality and decreased adult deformities are probably directly due to dietary differences between the diet in this study and the one used in the previous study. Failure of an insect to develop normally on a certain diet can result from the following nutritional problems (Gordon 1968): 1) food intake is abnormally low because phagostimulants are deficient or phagodeterrents are present, 2) ingested food is poorly digested because necessary enzymes are lacking or inhibited, 3) absorption of one or more nutrients is blocked, or 4) absorbed food cannot be efficiently converted into body substance because one or more essential nutrients are deficient or antimetabolites (perhaps an excess of a nutrient) are present and interfering with conversion. Our diet has increased amounts of sugars, wheat germ, and casein; reduced antimicrobial compounds; and added lipids in the form of oils compared with the Odell and Rollinson (1966) diet used in the previous study (Grijpma et al. 1987). Antimicrobial compounds have been shown to have adverse effects on survival and development if the levels are too high in the diet (Dunkel and Read 1991). Essential fatty acid deficiencies in Lepidoptera have been shown to result in both failure of adults to emerge and malformed wings or scales (Fraenkel and Blewett 1946), and the Grijpma et al. (1987) version of the Odell and Rollinson (1966) diet did not even contain the linolenic acid as called for in the recipe.

The artificial diet and rearing methods presented here was adequate for rearing the Czech Republic *L. monacha* strain (currently in the 22nd laboratory generation) but was not adequate for rearing a strain of *L. monacha* from Morioka, Honshu, Japan (almost no larvae are able to complete development; M.A.K., unpublished data). This is probably due to significant biological and behavioral differences between *L. monacha* from these two geographic regions. For example, the Japanese strain has a different diel periodicity for its pheromone communication than a strain from Bohemia (Gries et al. 2001), and the Japanese strain almost exclusively feeds on *Larix kaempferi* (Lambert) Carriére on which the Czech republic strain could not complete its development (Keena 2003).

Mating success rate (as measured by oviposition of fertile eggs) of single-pair matings of *L. monacha* in the laboratory is generally not very good, 50–60% of the pairings in normal colony rearing and only 29% in this study. Success of matings increases when either a single or group of females is exposed to more than one male at the same time. More research on the reproductive behaviors of this species may be necessary to further improve mating success. Oviposition is normal on the smashed corrugated cardboard substrate provided in this study, as long as it provided deep crevices available to ovipositing females.

The two most critical phenological events that must be accurately predicted for *L. monacha* detection and eradication efforts should this pest enter the United States are peak second-instar populations and male moth flight. The second instar is the preferred stage for applying microbial insecticide treatments and the pheromone traps used for detection need to be deployed before males start flying. Because *L. monacha* is similar to the gypsy moth, and there is no model similar to the gypsy moth life stage model (Gray 2004) for use in this prediction, a simple comparison of the timing of these events in the two species is provided here. The gypsy moth takes an average of 5.6 d to reach the second instar on white oak (*Quercus alba* L.) at 25°C (Sheehan 1992), whereas *L. monacha* takes an average of 9.6 d at the same temperature on white spruce. This time difference may be partly due to the fact that *L. monacha* spends 3–4 d sitting near the egg mass (most of which would have occurred before they were put on the larval food sources in this experiment) and an additional 1–3 d actively crawling before it settles and begins feeding (Grijpma et al. 1987). Gypsy moth males take an average of 47 d from first instar to adult emergence on white oak at 25°C (Sheehan 1992), whereas *L. monacha* males take an average of 38.5 d at the same temperature on spruce (this study). Therefore, managers can expect the timing of peak second instars for *L. monacha* to occur later than for gypsy moth and the timing for male flight to occur earlier than for gypsy moth, if the two species were to hatch at the same time and develop under the same environmental conditions. Should *L. monacha* become established, regions of highest risk in North America, based on host plant availability and climate, include forests west of the Cascade Range, spruce–fir–pines in the Upper Midwest, and northeastern North America (Wallner 1996). In addition, many of the suitable species are conifers and affects of *L. monacha* feeding on conifers are more severe because they destroy more needles than they actually consume, feed on buds until new foliage is available, and conifers are less able than deciduous trees to replace lost foliage and therefore more susceptible to mortality. Continued vigilance to prevent movement of this insect in international trade and to detect any incursion of *L. monacha* in North America is warranted.

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