

Prediction of Gypsy Moth (*Lepidoptera*: *Lymantriidae*) Mating Success from Pheromone Trap Counts

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ABSTRACT Mating success of tethered gypsy moth, *Lymantria dispar* (L.), females was measured in 9 newly established, low-density populations in Virginia and West Virginia in 1993–1994. Mating success was correlated with male moth capture rate in milk carton pheromone-baited traps located at the same sites. The instantaneous mating probability for virgin females averaged 0.15 times the mean male capture rate. Average mortality of tethered females caused by predation was 52% per day. Counts of males in pheromone traps combined with expectations of population growth can be used to predict likelihood of persistence (versus extinction) of isolated gypsy moth populations.

KEY WORDS *Lymantria dispar*, mating, extinction

THE GYPSY MOTH, *Lymantria dispar* (L.), is an introduced forest pest in North America and is currently spreading to the west and south at a rate of ≈ 20 km/yr (Liebhold et al. 1992). One of the objectives of gypsy moth management is to slow the spread via suppression of low-density isolated populations beyond the front of the infested area (McFadden and McManus 1991, Leonard and Sharov 1995). Most studies of gypsy moth population dynamics have focused on high-density populations, but little attention has been given to ecological processes specific to low-density populations, which are characteristic of isolated populations near the expanding front (Campbell 1981).

Mating success may be the most important density-dependent factor that affects sparse gypsy moth populations. Campbell and Sloan (1978) suggested that predation by small mammals is also density-dependent in low-density gypsy moth populations. However, experiments by Elkinton et al. (1989) did not support that hypothesis. Mating failure could cause instability in isolated populations because the proportion of nonmated females would increase as population density decreased. The relationship between pheromone trap catch and mating success has never been measured accurately. Knowledge of this relationship would be useful for distinguishing between unstable and established populations.

Granett (1974) measured gypsy moth mating success as the reciprocal of time elapsed to mating of laboratory-reared virgin females placed on tree boles. However, his traps were baited with racemic

disparlure which is much less attractive to gypsy moth males than (+)-disparlure.

Capture rate is affected by trap design (Elkinton and Childs 1983), and thus it is important to relate mating success to male moth capture rate in traps that are widely used in population monitoring. One widely used trap is the USDA milk carton trap, which has large capacity and, therefore, can be used in a wide range of male moth densities (Schwalbe 1981, Elkinton and Childs 1983).

The objective of our study was to quantify the relation between male capture rate in USDA milk carton traps and probability of female mating.

Materials and Methods

Nine study sites were located in Bath, Rockbridge, Amherst (VA), and Pocahontas counties (WV) in the central Appalachian Mountains near the leading edge of the gypsy moth infestation in 1993–1994. Forests were composed of mixed hardwoods, with the proportion of oak (*Quercus* spp.) >30%. Plots were separated by at least 2 km. Some plots were used for several experiments conducted sequentially.

Three kinds of gypsy moth females were used in the study: (1) natural females, collected as pupae near Waynesboro, VA, from a high-density population that caused 30–70% defoliation; (2) irradiated laboratory-strain females; and (3) nonirradiated laboratory-strain females. The laboratory strain of gypsy moth has been kept in the laboratory for >40 generations at the USDA-APHIS Otis Methods Development Center (Otis, MA). Females were irradiated in the pupal stage with 6 krad to prevent egg development. In our laboratory tests, all irradiated females produced only nonviable

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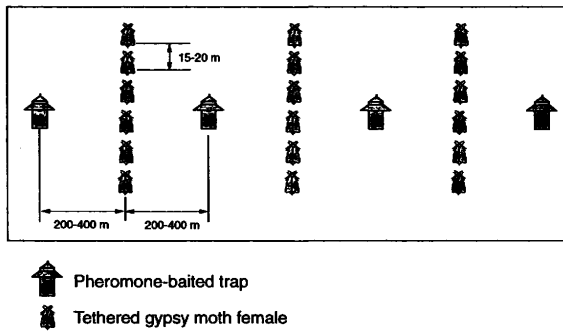


Fig. 1. Experimental plot for the analysis of gypsy moth mating success.

eggs, but mating occurred normally. In 1994, we used only field-collected and nonirradiated laboratory females. Charlton and Cardé (1982) reported similar pheromone release rates in wild and laboratory-reared females. Because no difference in mating ability was found among these different types of females, data were pooled from all females.

One-day-old females were tethered with a 10- to 15-cm length of thread tied at the base of a front wing and attached to tree boles at a 1.5-m height. In the laboratory, none of 30 tested tethered females escaped during 24 h. Tethering was important to prevent female escape in accordance with quarantine regulations. Females typically do not move before mating, but after mating they often search for refuges (Doane 1976). To avoid escapes, Kolodny-Hirsch and Webb (1993) placed females in modified milk carton traps. We selected tethering because females were provided a more natural environment than traps. Males may change their behavior after entering a trap and this could affect mating probability. Laboratory tests indicated that tethering did not interfere with mating behavior. The majority of experiments lasted 24 h; in 1994 some experiments were shorter (from 1 to 5 h).

As a rule, females were placed on oak tree boles; if there was no oak, other tree species were used instead. Three rows of females were located between pheromone-baited traps at each plot (Fig. 1). The distance between females in a row was $\approx 15\text{--}20$ m. The distance from a row of females to the nearest trap was ≥ 200 m. As a rule, each row had 6 females, for a total of 18 females per plot. In several experiments the number of females was increased up to 30. Pheromone traps were standard 1.9-liter USDA milk carton traps baited with synthetic (+)-disparlure. In 1993, we used plastic laminated dispensers (Hercon Environmental, Emigsburg, PA), and in 1994 we used USDA twine dispensers. According to Leonhardt et al. (1992), there was no significant difference in the moth capture rate in traps with these 2 types of dispensers during the 1st 4 wk. Traps were exposed during the same interval that tethered females

were deployed. Trap capture was averaged among the traps, and each experiment was considered as a replicate; 12 experiments were conducted in 1993 and 9 experiments were conducted in 1994.

Fertilization of females was detected by dissection and analysis of spermatheca on the day following the end of the experiment. The last abdominal segment (IX + X) was extracted using forceps to expose the vagina and spermatheca, which are connected by a duct. If the spermatheca was not exposed by this procedure, we dissected the abdomen using scissors and searched for the spermatheca. In fertilized females, spermatheca were filled with sperm, which was white in reflected light and dark in transient light. Sperm was clearly visible under the microscope at $400\times$. In unfertilized females, the spermatheca was transparent and empty. In a few cases sperm was present only in the base of the spermatheca. It took 2–3 min to analyze 1 female.

If a female was killed by predators after oviposition, then the egg mass was collected, and 3 wk later egg embryonation was analyzed under the microscope. A female was considered fertilized if at least some of the eggs were embryonated. This criterion was not applied to egg masses laid by irradiated females because their eggs are nonviable and embryonation is not always detectable. In total, 18 egg masses were used for evaluation of female fertilization.

Mating probability P is the proportion of females mated during time t . Instantaneous mating probability is equal to dP/dt at $t = 0$. We assume that females used in the same experiment had uniform instantaneous mating probabilities, and instantaneous mating probabilities of virgin females in different experiments were proportional to the rate of male moth capture in pheromone-baited traps. Then, the proportion of females $P(t)$ that are mated during time t (days) is equal to:

$$P(t) = 1 - \exp(-s \cdot t \cdot M), \quad (1)$$

where M is male catch per trap per day in the same experiment, and s is a parameter that can be estimated using nonlinear regression of $P(t)$ versus $t \cdot M$. Parameter s can be interpreted as the instantaneous mating probability in the environment where male catch rate is equal to 1 male per trap per day. Another interpretation is that s is the relative success of gypsy moth females in competition with pheromone-baited traps. This success includes male orientation to pheromone source as well as its ability to copulate with a female or to enter a trap. The residual sum of squares was estimated as the sum of differences between actual and theoretical arcsine-transformed mating probabilities. Theoretical mating probability was estimated using equation 1.

The confidence interval for s was found as a region where the residual sum of squares was less than the critical level (CL):

Table 1. Mating success and mortality of tethered gypsy moth females

Exp no.	Date	Plot no.	Male catch, males per trap ($t \cdot M$)	Females tethered	Females analyzed ^a	Mating success, % ($P(t)$)	Mortality, %, caused by predation
1	7 July 1993	1	15.25	18	11	90.9	50.0
2	7 July 1993	2	89.25	18	14	92.9	38.9
3	10 July 1993	3	0.25	18	2	50.0	94.4
4	10 July 1993	4	0.00	18	11	0.0	50.0
5	10 July 1993	5	0.25	18	12	0.0	50.0
6	13 July 1993	6	1.50	18	9	0.0	55.6
7	13 July 1993	7	6.25	18	6	83.3	66.7
8	13 July 1993	8	5.75	18	6	83.3	72.2
9	16 July 1993	6	0.25	18	12	0.0	38.9
10	16 July 1993	7	1.75	24	16	18.8	41.7
11	16 July 1993	9	0.75	23	17	11.8	30.4
12 ^b	27 July 1993	3	4.25	30	10	90.0	76.7
13 ^c	26 June 1994	1	1.00	19	16	12.5	—
14 ^c	26 June 1994	1	3.25	21	20	55.0	—
15 ^c	26 June 1994	6	0.00	20	20	5.0	—
16 ^c	28 June 1994	7	3.25	20	18	33.3	—
17 ^c	28 June 1994	7	1.00	18	18	5.6	—
18	28 June 1994	6	1.00	21	13	7.7	38.1
19	5 July 1994	1	7.25	19	12	58.3	42.1
20 ^c	10 July 1994	2	82.25	17	17	100.0	—
21	12 July 1994	1	11.75	19	16	37.5	36.8

^a Females analyzed means the number of successfully dissected females plus the number of egg masses used for detection of female fertilization.

^b Half of females in this experiment were in 13-mm-mesh cages; however we pooled all the females because cages did not prevent either mating or predation.

^c Experiments were shorter than 24 h, and predation rate is not shown because it is not comparable with that of other experiments that lasted for 24 h.

$$CL = RSS_{min}(1 + F \cdot \nu_1 / \nu_2). \quad (2)$$

where RSS_{min} is the minimum residual sum of squares, F is the critical value for F statistic ($P = 0.05$), $\nu_1 = 1$ and $\nu_2 = N - 1$ are degrees of freedom, and N is the number of experiments (Seber and Wild 1989).

Results

Mean trap capture, $t \cdot M$, ranged from 0 to 89.25 moths per trap (Table 1). Female mating proba-

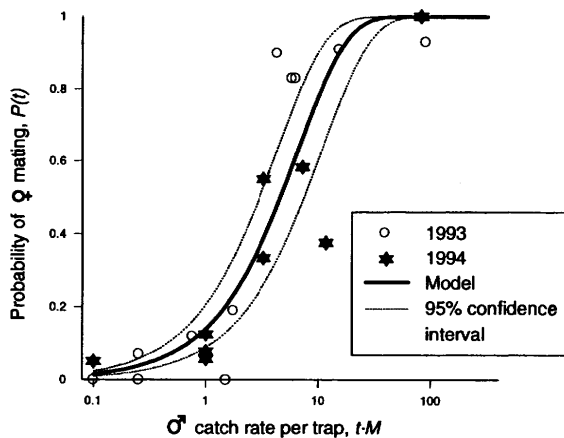


Fig. 2. Mating probability of gypsy moth females in experiments with different male moth catch in pheromone-baited traps exposed for the same time. The model corresponds to equation 1. Zero male capture rates were changed to 0.1 to fit the points to the logarithm scale.

bility, $P(t)$, increased with increasing male catch (Table 1; Fig. 2). In experiment 3, only 2 females survived. Therefore, we pooled the data from experiments 3 and 5 because mean trap catch was the same (0.25 moths per trap).

Maximum likelihood estimation of equation 1 yielded $s = 0.15$, and the 95% CI of s was 0.09–0.23. Theoretical mating probabilities are plotted in Fig. 2.; the confidence interval (dotted lines) was obtained using model 1 with $s = 0.09$ and $s = 0.23$.

Female mortality caused by predation (Table 1) ranged from 30 to 94% per day, with an average of $52 \pm 5\%$. Several times we observed ants eating gypsy moth females or attacking a female that was still alive. We collected the following ant species attacking gypsy moth adults: *Aphaenogaster fulva* Roger, *Componotus* sp., *Prenolepis imparis* Say, and *Formica* sp. (identified by E. R. Day).

Discussion

The instantaneous probability of a virgin gypsy moth female being mated was estimated as 0.15 multiplied by the male moth capture rate in pheromone-baited USDA milk carton traps. There are several factors affecting the relationship between pheromone trap capture and female mating probability. One group of factors is related to pheromone source and trap design, including chemical nature of the pheromone, the type of dispenser, the shape and size of the trap, and the mechanism of killing insects. Our results can only be applied

to USDA milk carton traps and should not be extrapolated to other trap types. However, if the proportional relationship between capture rates of male moths in different trap types is known, then it is possible to relate gypsy moth mating success to the capture rates in other type of traps. For example, Pherocon 1C sticky traps (Zoecon, Palo Alto, CA) were shown to capture about twice as many gypsy moth males as USDA milk carton traps (Elkinton and Childs 1983). Thus, instantaneous mating probability of females should be equal to $0.15/2 = 0.075$ multiplied by capture rate in Pherocon 1C traps.

Another group of factors affecting the mating-trap capture relationship is associated with male moth behavior. Elkinton and Cardé (1983) showed that in a high-density population, males spent more time in tree-oriented vertical flight than in a low-density population. Our results concern only low-density populations, where mating success may be limited. Thus, there is no reason to suspect that population density affects the relationship between mating success and counts of male moths. However, in mating disruption programs male behavior may change because of high concentration of pheromone, and this may affect the relationship between mating success and moth capture in pheromone traps.

The 3rd group of factors is associated with the female calling period. Females are capable of calling for 3 d, but then their ability to call and copulate decreases (Doane 1976, Richerson et al. 1976). However, calling time can be considerably reduced because of predation. In our experiments, average mortality of adult females was 52% per day. In 1 experiment it was as high as 94% per day (Table 1). According to our observations and preliminary predator-exclusion experiments (unpublished data), ants are probably the most important predators of gypsy moth females. Ants are known as important predators on gypsy moth larvae (Weseloh 1989), but their predation on adults was not reported before. Tethering may increase predation rates, and thus, mortality may be overestimated.

The model of the relationship between trap capture and mating success can be used with additional information to predict the minimum number of moths per trap that is associated with stable or increasing populations. These calculations are based on the assumption that at equilibrium densities the average population growth rate should be compensated by lack of mating success. If average population growth rate is equal to $r = E[\ln(N_{i+1}/N_i)]$, where N_i is population density in generation i , then mating probability should be equal to $\exp(-r)$. Now we can solve the equation, which is similar to equation 1:

$$1 - \exp(-s \cdot t \cdot M) = \exp(-r), \quad (3)$$

where s is the parameter, t is female waiting time (days), and M is male moth catch per day in a

pheromone-baited trap. The solution of equation 3 is:

$$M = -\frac{1}{s \cdot t} \log_e [1 - \exp(-r)]. \quad (4)$$

As a rule, the total male moth counts captured during the entire flight season are used for population monitoring. Thus, for practical use, it is better to express male moth capture rate per season rather than per day. The relationship between capture rates per day and per season can be estimated using data on seasonal dynamics of moth abundance reported by Elkinton and Cardé (1984) in a hardwood forest site. Male moth abundance was measured by the number of wild moths captured in pheromone-baited traps, and female moth abundance was measured by female moth counts on tree boles. The average male abundance experienced by a female moth can be estimated as:

$$M = \left(\sum m_i f_i \right) / \left(\sum f_i \right), \quad (5)$$

where m_i and f_i are male and female abundances in day i , respectively. Variable M has the same meaning in equations 4 and 5, except that seasonal dynamics of male flight is ignored in equation 4, and it is assumed that male abundance is uniform through the entire mating period. The ratio of total male counts for the entire season ($\mu = \sum m_i$) to the average male abundance experienced by a female (M) was estimated as 15, using values of m_i and f_i from Elkinton and Cardé (1984, figure 3a). Thus, daily male moth capture M should be multiplied by 15 to get the seasonal capture rate. Seasonal dynamics of gypsy moth flight and, consequently, the value of μ/M , depend on climate, host tree species, and male migration. These may vary from year to year and from site to site. We used the value of $\mu/M = 15$ as an example.

Function 4, multiplied by 15 to convert daily capture rate into seasonal capture rate, is plotted in Fig. 3, assuming $s = 0.15$. Calling time t may be variable among sites with different predation rates. Assuming that female calling time has an exponential distribution, its average is equal to $t = -1/\ln(1-d)$, where d is mortality rate per day. If $d = 0.52$, as in our experiment (Table 1), then $t = 1.36$ d. In the sites with low predation rates, female calling time may increase up to 3 d. Thus, we used 2 extreme values of parameter t , 1.36 and 3 d (Fig. 3).

If trap catches are below the lines in Fig. 3, then gypsy moth populations are likely to go extinct unless there is a stable influx of immigrants (besides males). With trap catches above the lines, populations are likely to establish. The minimum trap capture rate that is associated with stable populations decreases as population growth rate increases. For example, if population density increases 5 times each generation, then $r = 1.61$ and minimum trap captures are 16.4 and 7.4 moths per

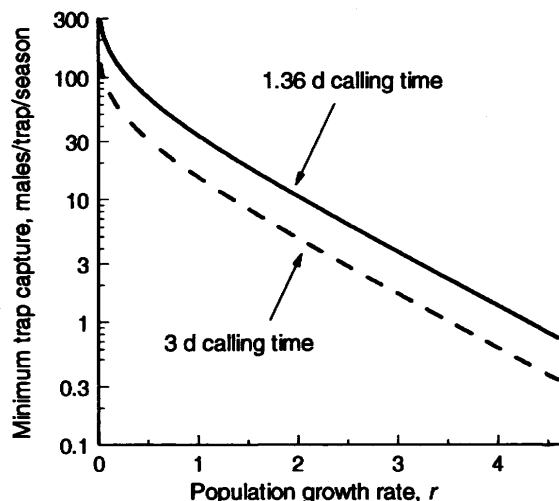


Fig. 3. Minimum gypsy moth male counts in USDA milk carton pheromone-baited traps, which are associated with stable or increasing populations, as a function of average population growth rate.

trap, with and without predation, respectively (Fig. 3). If population density increases 50 times per generation, then $r = 3.91$, and minimum trap captures are 1.5 and 0.7 moths per trap, with and without predation, respectively.

The gypsy moth population growth rate in a particular site or in a set of similar sites can be estimated by comparing egg mass densities in successive years. As an example, we used the data collected by Campbell and Sloan (1978) in Glenville, NY, in 1958–1963. Population growth rate was estimated as the natural logarithm of the ratio of egg density in 1 yr to egg density in the previous year. Only data within the population density interval from 3,000 to 300,000 eggs per hectare were used, because the accuracy of population density estimation was unacceptable below 3,000 eggs per hectare, and populations did not grow exponentially above 300,000 eggs per hectare. For 25 data points, the average population growth rate was $r = 1.65$ (95% CI = 1.15–2.15). This means that on average, populations increased $\exp(1.65) = 5.2$ times each generation. According to Fig. 3, in sites similar to those in Glenville, the minimum (to maintain a stable population) trap capture would be equal to 15 moths per trap with predation or 7 moths per trap without predation.

Gypsy moth population growth rates depend on numerous factors, including the composition of tree species in a forest stand, the density of predators, and climate (see review by Elkinton and Liebhold 1990). Currently there are not enough data to estimate the probability distribution or even the range of average population growth rates in different sites. When these data are available, it will be possible to predict the specific probabilities of gypsy moth population establishment from average counts of male moths in pheromone traps.

Prediction of gypsy moth mating success can be improved by using detailed information on moth capture change in space and time. For example, mating success of females in mating disruption programs changes with season and strata (Kolodny-Hirsch and Schwalbe 1990, Kolodny-Hirsch and Webb 1993). Thus, mating probability should be predicted separately for different dates and strata and then averaged in time and space using the number of females as weights.

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References Cited

- Campbell, R. W. 1981. Historical review, pp. 65–86. In C. C. Doane and M. L. McManus [eds.], *The gypsy moth: research toward integrated pest management*. U.S. Dep. Agric. Tech. Bull. 1584.
- Campbell, R. W., and R. J. Sloan. 1978. Numerical bimodality among North American gypsy moth populations. *Environ. Entomol.* 7: 641–646.
- Charlton, R. E., and R. T. Cardé. 1982. Rate and diel periodicity of pheromone emission from female gypsy moths (*Lymantria dispar*) determined with a glass-adsorption collection system. *J. Insect Physiol.* 28: 423–430.
- Doane, C. C. 1976. Flight and mating behavior of the gypsy moth, pp. 127–136. In J. F. Anderson and H. K. Kaya [eds.], *Perspectives in forest entomology*. Academic, New York.
- Elkinton, J. S., and R. T. Cardé. 1983. Appetitive flight behavior of male gypsy moths (Lepidoptera: Lymantriidae). *Environ. Entomol.* 12: 1702–1707.
1984. Effect of laboratory-reared female gypsy moths, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), on the capture of males in pheromone-baited traps. *Environ. Entomol.* 13: 1377–1385.
- Elkinton, J. S., and R. D. Childs. 1983. Efficiency of two gypsy moth (Lepidoptera: Lymantriidae) pheromone-baited traps. *Environ. Entomol.* 12: 1519–1525.
- Elkinton, J. S., and A. M. Liebhold. 1990. Population dynamics of gypsy moth in North America. *Annu. Rev. Entomol.* 35: 571–596.
- Elkinton, J. S., J. R. Gould, A. M. Liebhold, H. R. Smith, and W. E. Wallner. 1989. Are gypsy moth populations in North America regulated at low density? pp. 233–249. In W. E. Wallner and K. A. McManus [eds.], *The Lymantriidae: a comparison of*

- features of New and Old World tussock moths. U.S. For. Serv. Gen. Tech. Rep. NE-123.
- Granett, J. 1974.** Estimation of male mating potential of gypsy moths with disparlure baited traps. *Environ. Entomol.* 3: 383-385.
- Kolodny-Hirsch, D. M., and C. P. Schwalbe. 1990.** Use of Disparlure in the management of gypsy moth, pp. 363-385. *In* R. L. Ridgway, R. M. Silverstein, and M. N. Inscoe [eds.], Behavior-modifying chemicals for insect management: application of pheromones and other attractants. Marcel Dekker, New York.
- Kolodny-Hirsch, D. M., and R. E. Webb. 1993.** Mating disruption of gypsy moth (Lepidoptera: Lymantriidae) following ground application of high rates of racemic disparlure. *J. Econ. Entomol.* 86: 815-820.
- Leonard, D., and A. A. Sharov. 1995.** Slow-the-spread project update: developing a process for evaluation. *In* S. Fosbroke and K. Gottschalk [eds.], Proceedings of the 1995 Gypsy Moth Research Forum. U.S. For. Serv. Gen. Tech. Rep. (in press).
- Leonhardt, B. A., V. C. Mastro, and E. D. DeVilbiss. 1992.** Evaluation of pheromone dispensers for use in gypsy moth detection (Lepidoptera: Lymantriidae). *J. Entomol. Sci.* 27: 280-284.
- Liebhold, A. M., J. A. Halverson, and G. A. Elmes. 1992.** Gypsy moth invasion in North America: a quantitative analysis. *J. Biogeogr.* 19: 513-520.
- McFadden, M. W., and M. E. McManus. 1991.** An insect out of control? The potential for spread and establishment of the gypsy moth in new forest areas in the United States, pp. 172-186. *In* Y. N. Baranchikov, W. J. Mattson, F. P. Hain, and T. L. Payne [eds.], Forest insect guilds: patterns of interaction with host trees. U.S. For. Serv. Gen. Tech. Rep. NE-153.
- Richerson, J. V., E. A. Cameron, and E. A. Brown. 1976.** Sexual activity of the gypsy moth. *Am. Midl. Nat.* 95: 299-312.
- Schwalbe, C. P. 1981.** Disparlure-baited traps for survey and detection, pp. 542-548. *In* C. C. Doane and M. L. McManus [eds.], The gypsy moth: research toward integrated pest management. U.S. Dep. Agric. Tech. Bull. 1584.
- Seber, G. A., and C. J. Wild. 1989.** Nonlinear regression. Wiley, New York.
- Weseloh, R. M. 1989.** Simulation of predation by ants based on direct observations of attacks on gypsy moth larvae. *Can. Entomol.* 121: 1069-1076.

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