



This is to certify that the thesis entitled

presented by

Rajakumari Prakasa Rao

has been accepted towards fulfillment of the requirements for

M.S. degree in Entomology

Major professor

Date Feb 28 1992

LIBRARY Michigan State University

PLACE IN RETURN BOX to remove this checkout from your record. TO AVOID FINES return on or before date due.

DATE DUE	DATE DUE	DATE DUE
क्षाप्र8ं		
0714 Î 5		

MSU is An Affirmative Action/Equal Opportunity Institution characteristics.pm3-p.1

HOST SELECTION AND ESTABLISHMENT OF <u>LYMANTRIA DISPAR</u> L. (LEPIDOPTERA: LYMANTRIIDAE) ON SELECTED SPECIES OF PREFERRED AND NON-PREFERRED TREES

By

Rajakumari Prakasa Ra0

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1992

ABSTRACT

HOST SELECTION AND ESTABLISHMENT OF LYMANTRIA DISPAR L. (LEPIDOPTERA: LYMANTRIDAE) ON SELECTED SPECIES OF PREFERRED AND NON-PREFERRED TREES

By

Rajakumari Prakasa Rao

Host preference of laboratory reared third instars of gypsy moth, Lymantria dispar (L.), was studied among red maple, green ash, and paper birch seedlings, trunks and trunk-foliage combinations. Birch foliage was preferred over maple and maple over ash. Among trunk sections, ash and maple were preferred over birch. When trunk-foliage combinations were used, the foliage type and trunk type of each combination significantly influenced host selection by larvae.

In a field test, third instars from an Otis colony (NJF33) and wild type larvae collected in Michigan readily established on preferred trees, but not on non-preferred trees. Few differences were found between third and fourth instars, or between NJF33 larvae and wild type larvae. Therefore, larvae reared from egg masses of NJF33 and other Otis colonies are suitable for most studies.

Dedicated to Kamalamma and Israel, my mother and father, sister Suseela and brother David Raju whose faithful Christian testimony, prayers and support provided a model for my life's journey

ACKNOWLEDGMENT

First, I would like to thank my Lord Jesus Christ, for His grace which enabled me to complete this study.

I am thankful to Dave Smitley my advisor, for his guidance in conducting this study. I thank E. Grafius, J. Miller, Late G. Simmons, members of my advisory committee for their expert advice, and T. Davis and Maria Davis for technical help; M.Berney and J.Davenport for supplying the tree trunk sections; and K. Kearns and M. Eodchick for assistance (Michigan State University). I thank B. Bishop for patiently reviewing of an earlier draft of this thesis. I thank R. Shugart (Zelenka Evergreen Nursery) for supplying seedlings, and Gary Bernon of Otis Air Force Base for supplying laboratory reared egg masses. This research was supported partly by grants from the Michigan Agricultural Experimental Station.

I am thankful to my fellow Christians at the Little Flock Christian Fellowship for their prayers and to my husband, GP, daughter, Lisa, and sons, Esli and Ashish, for their love and support.

TABLE OF CONTENTS

LIST OF TABLES vi
LIST OF FIGURESvii
GENERAL INTRODUCTION
References Cited
CHAPTER I: Relative importance of trunk and foliage stimuli in host selection by <u>Lymantria</u> <u>dispar</u> L. (Lepidoptera: Lymantriidae)
ABSTRACT 1
INTRODUCTION
MATERIALS AND METHODS
Foliage Experiment
DATA ANALYSIS 1
RESULTS AND DISCUSSION 1
REFERENCES CITED 2
CHAPTER II: Comparison of <u>Lymantria</u> <u>dispar</u> (Lepidoptera: Lymantriidae) larvae from a rearing colony with wild type larvae for establishment on preferred and non-
preferred hosts 2
ABSTRACT 2
INTRODUCTION 2
MATERIALS AND METHODS 2
Study Site
DATA ANALYSIS 3

RESULTS	• • • • • • • • • • • • • • • • • • • •	33
DISCUSSION	•••••	34
REFERENCES	CITED	36
APPENDICES		

LIST OF TABLES

Table 1.	Preference of third instar gypsy moth larvae for birch, maple or ash foliage and trunks
Table 2.	Preference of third instar gypsy moth larvae for trunk-foliage combinations 23
Table 3.	Relative importance of trunk or foliage stimuli for attracting gypsy moth larvae determined by analysis of variance 24
Table 4.	Color codes used for marking larvae 38
Table 5.	Gypsy moth larvae observed 24 h, 48 h, and 72 h after release on preferred and non-preferred trees
Table 6.	Distances (cm) larvae traveled from <u>Fraxinus</u> <u>pennsylvanica</u> , <u>Acer rubrum</u> , and <u>Malus</u> 'Radiant' tree species. Data are means distance <u>+</u> SE
Table 7.	Establishment success of gypsy moth third instars from an Otis culture and comparison with wild type larvae from Midland, Michigan.41
Table 8.	Distance traveled (cm) by third instars of Midland and Otis at 24 h, 48, and 72 h from release trees. Data are mean distance ± SE. 42
Table 9.	Establishment of third and fourth instar gypsy moth larvae on Malus 'Radiant' and Acer rubrum. Larvae are all from a wild strain collected in Midland, Michigan. Data are the means ± SE

LIST OF FIGURES

Figure	1.	Gypsy	Moth	Survey	1989	8

LIST OF APPENDICES

Appendix	
----------	--

1.	Record of Deposition of Voucher Specimens	44
1.1	Voucher Specimen Data	45

GENERAL INTRODUCTION

Life History and Biology of Gypsy Moth (Lymantria dispar L.)

Gypsy moth was introduced into North America in 1869 from Europe by Leopold Trouvelot, who intended to interbreed it with silk worms (Forbush and Fernald 1896). It escaped out and soon became established as a defoliator of hardwoods in Massachusetts (Talerico 1978). Massive outbreaks of gypsy moth were first observed in 1889 around Medford (Doane and McManus 1981). From 1889 until 1991 gypsy moth slowly spread across the Eastern United States. The gypsy moth is now the most important defoliator of hardwoods, especially oak, from New England, south to Pennsylvania and west to Ohio (McManus and Zerillo 1978) (Figure 1).

Gypsy moth eggs are laid in clusters under a dense coating of hairs from the female's abdomen. The number of eggs per female varies from about 100 to 1200. Because females are flightless, egg masses are normally found near the empty female pupal cases. The egg embryonation begins soon after oviposition and larvae are fully formed inside the egg in about a month. Eggs are laid in early summer, enter diapause and remain in diapause through winter, and

hatch in spring. Hatch and activity of newly emerged larvae are strongly influenced by temperature. The newly hatched larva is about 3.6 mm and is pale brownish yellow. Later the caterpillar changes color, the tubercles become black, the body reddish-brown. When larvae leave the hatch area they are positively phototactic and negatively geotactic (Doane and McManus, 1981). Larvae generally remain on or near the leaves for the first three instars.

The time between hatching and first molt is about nine days depending on daily temperatures. Growth and development of larvae is also influenced by moisture and quantity and quality of food. The prepupal stage during which larvae void the gut, lasts only for about two days. Pupation occurs at or near daytime resting locations which are usually under tree limbs, on trunks, or on ground litter. The pupa remains cradled in its sparse silken cocoon for about two weeks (16 or 17 days for females). Mating and egg laying occur soon after the emergence of adults.

Effects of Weather and Other Climatic Conditions

Gypsy moth is strongly affected by temperature, moisture, light, and wind. Eggs cannot survive at temperatures

below about -28.9°C (Campbell 1973b). Freezing temperatures after hatch also may kill small larvae and thus can reduce the population. Moisture also affects the gypsy moth population. Heavy rainfall at the time of hatch can wash away larvae that are not yet established at feeding sites. Research has shown that periods of low populations measured on a geographic scale by acres defoliated, are correlated with copious rainfall during early larval development (Leonard, 1971). Gypsy moth larvae are photosensitive. Small larvae feed mostly during the day, whereas older larvae shift their rhythm to feed at night.

Wind is critical for dispersal of larvae. Newly emerged larvae drop on silk from branches and are transported by wind. Although estimates of the distances larvae travel due to wind vary, the importance of dispersal of first instar larvae is well documented (Leonard, 1971; Capinera and Barbosa, 1976; Lance and Barbosa, 1982; Doane and McManus, 1981).

Foliage Preference

The gypsy moth feeds on over 300 species of plants. Some are favored by all instars (Miller and Hanson 1989a).

Among the favored food plants are oak, birch (except yellow and black), and apple. Less preferred albeit acceptable trees are black and yellow birch, tamarack, cherry, elm, linden, hickory, and red and sugar maples (Anderson 1980). Some of the less favored hosts are ash, tulip, and grape (Hoy 1982; Barbosa et al. 1979; Barbosa et al. 1983; Campbell and Sloan 1977; Anderson 1980).

Dispersal

The gypsy moth has one generation per year. Since adult females are flightless, the task of dispersal is left to the larvae (Taylor and Reling 1986). Dispersal has been defined as the movement away from a populated place that results in the scattering of at least some of the original population (Elton 1947). Dispersal is an innate tendency that larvae must satisfy before they initiate feeding.

Larval dispersal provides for a genetic mixing of individuals within subpopulations and assures a thorough redistribution of individuals over a sizable area (Mason and McManus 1981).

Host selection appears to be primarily determined by first instars (Barbosa et al. 1979). However, during diurnal periods of larval movement, late instars often leave the

tree on which they have been feeding and ascend into a new tree; this behavior has been implicated as a mechanism by which late instars are able to utilize a broader range of host plants than do the early instars (Barbosa 1978). However, larval age, gypsy moth population density, and tree phenology may affect dispersal and food consumption. The first, second, and third instars are diurnal and later instars are nocturnal; it is not known if developmental stage or feeding rhythm affects dispersal behavior.

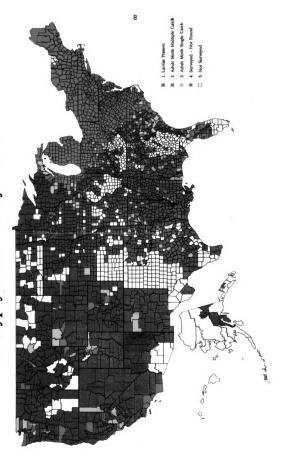
Dispersal is an important process in the population dynamics of the gypsy moth and is still a subject of much controversy. The tendency to disperse seems to be innate in all species of arthropods and may be accentuated by crowding, hunger, actions of predators, or adverse meteriological conditions (Andrewartha and Birch 1954). However, current observations suggest that dispersal is a very complex event; many factors that stimulate gypsy moth's dispersal have been identified and it is recognized that many questions remain to be answered (Mason and McManus 1981).

REFERENCES CITED

- Anderson, J.F. 1980. The gypsy moth. Frontiers of plant science. The Connecticut Agric. Exp. Station, New Haven pg.3.
- Andrewartha, H.G. and L.C. Birch. 1954. The distribution and abundance of animals. University of Chicago Press.
- Barbosa, P. 1978. Host plant exploitation by the gypsy moth, Lymantria dispar (L.). Ent. Ext. Appl. 24: 28-37.
- Barbosa, P. and A.J. Greenblatt. 1979. Suitability, digestibility, and assimilation of various host plants by the gypsy moth, Lymantria dispar (L.). Oecologia (Berl.) 43: 111-119.
- Barbosa, P., M. Waldvogel, P. Martinet and L.W. Douglas. 1983. Development and reproductive performance of the gypsy moth, Lymantria dispar (L.) (Lepidoptera: Lymantriidae) on selected hosts common to Mid-Atlantic and southern forests. Environ. Entomol.12:1857-1862.
- Campbell R.W. 1973b. Forecasting gypsy moth egg-mass density, U.S. Dept Agric. For. Sev., Res. Pap. NE-268.
- Campbell, R.W. and R.J. Sloan. 1977. Forest stand responses to defoliation by the gypsy moth. Forest Sce. Monog. 19:34 pp.
- Capinera, J.L. and P. Barbosa 1976. Dispersal of first-instar gypsy moth larvae in relation to population quality. Oecologia 26:53-60.
- Doane, C.H. and M.L. McManus. 1981. The gypsy moth; research toward integrated pest management. USDA For. Serv. Tech. Bull. 1584, Washington, D.C.
- Elton, C.S. 1947. Animal Ecology, 3rd ed. The McMillan Company, New York.
- Forbush, E.H. and C.H. Fernald. 1896. The gypsy moth. Wright and Potter, Boston.

- Hoy, M.A. 1982. The gypsy moth-here again. California Agriculture, July 1982.
- Leonard, D.E. 1971. Population quality, pp.7-20. Toward integrated control. For. Serv. res. Pap. U.S. Dep. agric. NE-194.
- Mason, C.J. and M.L. McManus. Larval dispersal of the gypsy moth. pp. 161-20 in Doane, C.C. and M.L. McManus. 1981 (Eds.). The gypsy moth: research toward integrated pest management. Forest Service Tech. Bull. 1584, USDA, Washington, D.C.
- McManus, M.L. and Zerillo, R.T. 1978. The gypsy moth. An illustrated biography. U.S. Dept. of Agriculture. Home and Garden Bull. No. 225. Washington, 15 pp.
- Miller, J.D. and P.E. Hanson. 1989a. Laboratory feeding tests on the development of gypsy moth larvae with reference to plant taxa and allelochemicals. Oregon Agri. Exp. station. Bull. 674 Carvallis.
- Taylor R.A. J and D. Reling 1986. Density/height profile and long-range dispersal of first-instar gypsy moth (Lepidoptera; Lymantriidae). Environ. Entomol. 15:431-435.
- Talerico, R.L. 1978. Major hardwood defoliators of the eastern United States. U.S. Dept. of Agriculture, Home and Garden Bull. No. 224, Washington, 22 pp.

Fig. 1 Gypsy Moth Survey - 1989



CHAPTER I

Relative importance of trunk and foliage stimuli in host selection by <u>Lymantria dispar L.</u> (Lepidoptera: Lymantriidae)

ABSTRACT

When third instar gypsy moth, Lymantria dispar L., were reared on artificial diet and placed in cages with red maple, green ash and paper birch trunk sections, the larvae consistently preferred ash and maple trunks over birch. In tests where potted trees of these species were placed beneath cages with slotted floor boards so that only the foliage protruded into the cage, larvae preferred birch foliage over maple and ash, and maple foliage over In a test designed to evaluate the relative importance of trunk and foliage as stimuli to larvae, trunk sections were drilled and split for placement around live tree seedlings. All possible combinations of maple or birch trunks with maple, birch or ash foliage were tested. The percent larvae attracted to combinations of trunks and foliage in descending order were: maplebirch(41), birch-birch(19), maple-maple(15), birchmaple(12), maple-ash(2), and birch-ash (1). Host preference of third instars depends mostly on foliage type, but trunk characteristics also play a significant role.

INTRODUCTION

The gypsy moth (Lymantria dispar L.) became established in the northeastern United States over 100 years ago. It has now spread throughout most of the northeast and midwest United States (Doane and McManus, 1981). Cyclic populations have caused periodic defoliation of large tracts of susceptible forests (Bess et al. 1947). Larvae are highly polyphagous; oaks (Quercus spp), paper birch (Betula papyrifera), and trembling aspen (Populus tremuloides) are among the most preferred tree species (Houston and Valentine 1977, Barbosa and Greenblatt 1979, Mauffette et al. 1983).

Larval behavior is an important component of host selection (Barbosa 1978a). Considerable evidence exists that foliage is an important factor in larval host selection by gypsy moth (Rafes and Ginenko 1973). But Campbell and Sloan (1977) observed that bark texture and availability of resting sites may also affect host selection. However, no studies have attempted to evaluate the relative importance of trunk and foliage in host selection by gypsy moth larvae. The purpose of this study was to compare the importance of trunk and foliage stimuli in gypsy moth host selection behavior.

Three tree species were used for this study: Paper birch (Betula papyrifera) from the most preferred hosts, red maple (Acer rubrum) from the intermediate hosts, and green ash (Fraxinus americana) from the least preferred hosts. One of the most abundant forest trees in the northern United States with an intermediate food value is Acer rubrum, and Fraxinus americana is a widespread forest species that is resistant to gypsy moth (Mosher 1915, Barbosa and Capinera 1977, Campbell and Sloan 1977). Betula papyrifera is consistently classified as a preferred host (Peterson and Smitley 1991).

MATERIALS AND METHODS

Gypsy moth egg masses were secured from the USDA Otis Air Force Base culturing facility in Massachusetts. The egg masses were stored at 4°C in sealed polyethylene bags for two months. Eggs were disinfected by immersing masses in 10% formaldehyde solution for about 60 minutes after which they were washed in running water for another 60 minutes and dried. Dried eggs were collected in sterile polystyrene containers (100 x 15 mm) and kept in growth chambers at 24°C and 60 to 80% RH (LD 16:8). After 75% egg hatch (5-6 days), the neonates were removed with a fine paint brush and placed in 57 ml transparent Solo

plastic souffles cups containing artificial diet. The cups were inverted to force the larvae to feed at the top of the containers and to minimize food contamination by frass. After each molt, larvae were transferred to new diet cups. Diet was prepared using Agar and diet powder from Bioserv. Inc., New Jersey. In preliminary tests gypsy moth larvae were reared to the third instar in ten days after eggs were hatched.

Three wooden cages were constructed for these experiments. Each cage was of 1.5 x 1.5 x 1.0 meters in size. The sides of the cages were covered with a fine mesh screen (10x10 per sq.cm) and the top with plexi glass. The floor of each cage consisted of five sliding wooden planks with 1.3 cm diameter holes centered between boards so that the boards could be placed around trunks. The experiments were conducted in a laboratory lighted by 40 W fluorescent tubes hung from the ceiling, 1.5 m above the cages. lights and the cages were arranged such that the primary light source was directly above the cages. Windows were covered to avoid light from one direction that could attract larvae towards one side of the cage. A few weeks prior to the experiment, seedlings of paper birch, red maple, and green ash were planted in pots and grown in the green house until their leaves were fully expanded. Each

tree required a different time for leaf flush. These times were determined in preliminary tests so that all test trees' growth could be synchronized with larval development.

Foliage Experiment

The pots of tree seedlings were placed below the test cage so that the trunks extended through holes in the cage floor. The seedlings were positioned such that only branches with leaves protruded above the cage floor; this minimized any trunk stimuli. Two seedlings each of birch, maple, and ash were randomly arranged in a circular configuration. Sixty third instars were released in the center of the cage. The numbers of larvae on leaves and branches, or on the floor, or on the sides of the cage, were counted every 12 hours for 36 hours. The experiment was repeated twice for a total of six replications per foliage type.

Trunk Experiment

Two trunk sections (7.5 cm diameter and 60 cm length) each of paper birch, red maple, and green ash were randomly placed in the cage in a circular configuration 8 cm apart

and 20 cm from the center of the cage. A 20 ml plastic container fitted with the previously described diet was placed on the top of each trunk section. Diet cups were replaced daily with cups containing fresh diet. Sixty third-instars were released in the center of the cage. The number of larvae on the trunks, in the cups and on the cage floor or sides were counted every 12 hours for 36 hours. This experiment was repeated twice for a total of six replications of each trunk type.

Trunk-Foliage Combination Experiment

Trunk and foliage types were combined in all the six possible permutations of maple and birch trunks with maple, birch, and ash foliage. Ash trunks were not used because in the previous experiment gypsy moth larvae were equally attracted to maple and ash trunks. To make these combinations possible, 1.0 cm diam holes were drilled longitudinally through 15 cm long trunk sections. Each trunk section was then split into two halves length-wise. The split trunk sections were bound around stems of birch, maple, or ash seedlings to make the desired trunk-foliage combinations. One replication of each possible trunk-foliage combination, for a total of six trunk-foliage units, were arranged in a circle, 8 cm apart from each

other and 20 cm from the center of the cage. Sixty third instars were released in the cage as before. Larvae on leaves and trunks, or on the floor, or on the sides of the cage were counted every 12 hours for 36 hours. The larvae found on the floor and the sides of the cage were also counted. This experiment was repeated six times for a total of six replications of each trunk foliage combination.

DATA ANALYSIS

All percent data were transformed to the $\arcsin \sqrt{z}$ before statistical analyses. If treatment means were significantly different by analysis of variance, they were separated by Tukey's test (Systat 1987). The number of larvae found on the sides of cages were excluded from analysis before differences in treatment means were determined.

RESULTS AND DISCUSSION

When third instar gypsy moth larvae were released in a test cage where tree seedling foliage was projecting through holes in the bottom of the test cage, more larvae

(71%) were found on birch foliage than on maple (14%) and ash (1%) foliage. Larvae preferred birch foliage over maple and ash, and they preferred maple foliage over ash (Tukey's test p=0.05; Anova: F=46.5; df=15; p<0.0001) (Table 1).

This observation is in agreement with the classification given by Mosher (1915) and Casagrande et al. (1987) that birch is a favored host and maple an intermediate host of the gypsy moth. Campbell and Sloan (1977) recognized ash as a least preferred host of gypsy moth.

When the seedlings in the test cage were replaced by trunk section of birch, maple, and ash, more larvae were found on maple (44%) and ash (43%) than on birch (6%). It was evident that the third instar larvae preferred dark and deep structured maple and ash trunks over the smooth and light structured birch trunks (Tukey's test p=0.05; Anova: F=15.5; df=15; p<0.0001) (Table 1). There was no difference between the attractiveness of ash or maple trunks.

These observations agree with the suggestions of Campbell and Sloan (1976) that larvae preferred tree barks which were deeply textured and furrowed. Rossiter (1981), and

Lance and Barbosa (1982) also observed that physical structure of trees was important for larvae to select host trees.

When larvae were released on mixed trunks and foliage combinations of red maple or paper birch trunks with red maple, green ash or paper birch foliage were preferred in the following order: maple-birch (41%), birch-birch(19%), maple-maple(15%), birch-maple(12%), maple-ash(2%), and birch-ash(1%) (Table 2).

Results of analysis of variance indicated influence of both trunk and foliage on gypsy moth host selection was significant (p<.05). The interaction between trunk and foliage was also significant (p<.05) (Table 3).

These observations suggest that foliage type had a stronger effect on gypsy moth host selection. Larvae were more attracted to trees with birch foliage on both trunk types (60%) compared to maple foliage (27%) and ash (3%) foliage on both trunk types. However, trunk type also had some effect on larval behavior because larvae preferred birch foliage with a maple trunk (41%) to birch foliage with a birch trunk (19%).

My results indicate that host preference of third instars depends most heavily on foliage type, but trunk characteristics also play a significant role. Lance and Barbosa (1982) also observed that in endemic sites, larvae remained longer on trees that provided both preferred foliage and suitable daytime resting sites compared with trees that did not provide both.

REFERENCES CITED

- Barbosa, P. 1978a. Host plant exploitation by gypsy moth, Lymantria dispar (L.), Ent. exp. Appl. 24:24-37.
- Barbosa, P. and J.A. Greenblatt. 1979. Suitability, digestibility, and assimilation of various host plants by the gypsy moth, Lymantria dispar (L). Oecologia 43: 111-119.
- Bess, H.A., S.H. Spurr and E.W. Littlefield. 1947. Forest site conditions and the gypsy moth. Harvard Forest Bulletin, 22: 1-56.
- Campbell, R.W. and R.J. Sloan. 1976. Influence of behavioral evolution on gypsy moth pupal survival in sparse operation. Environ. Ent. 5:1211-1217.
- Campbell, R.W. and R.J. Sloan. 1977. Forest stand responses to defoliation by the gypsy moth. supplement to For. Sci. 23, Monograph 19: 1-33.
- Capinera, J.L. and P. Barbosa 1977. Influence of natural diets and larval density on gypsy moth, Lymantria dispar (Lepidoptera: Orgyiidae) egg mass characteristics. Can. Ent. 109: 1313-1318.
- Casagrande, R.A. et al. 1987. Phenological model for gypsy moth, Lymantria dispar (Lepidoptera: Lymantriidae), Larvae and pupae. Environ. Entomol. 16:556-562.
- Doane, C.C. and M.L. McManus. 1981 (Eds.). The gypsy moth: research toward integrated pest management. Forest Service Tech. Bull. 1584, USDA, Washington, D.C.
- Houston, D.R. and H.T. Valentine. 1977. Comparing and predicting forest stand susceptibility to gypsy moth. Can. J. For. Res. 7:447-461.
- Lance D. and P. Barbosa. 1982. Host tree influence on the dispersal of late instar gypsy moths, Lymantria dispar. Oikos 38:1-7.
- Mauffette, Y., M.J. Lechowicz and L. Jobin. 1983. Host preferences of the gypsy moth, Lymantria dispar (L.) in southern Quebec. Can. J. For. Res. 13:53-60.

- Mosher, F.H. 1915. Food plants of the gypsy moth in America. USDA Bulletin, 250.
- Peterson, N.C., and D.R. Smitley, 1991. Susceptibility of selected shade and flowering trees to gypsy moth (Lepidoptera: Lymantriidae). J. Econ. Entomol. 84(3): 587-592.
- Rafes, P.M. & Y.I. Ginenko. 1973. The survival of leaf eating caterpillars (Lepidoptera) as related to their behavior. Ent Rev. 52: 204-211.
- Rossiter, M.C. 1981. Factors contributing to host range extension in the gypsy moth, Lymantria dispar. Ph.D. Dissertation, State Univ. of New York, Stony Brook.
- Systat. 1987. Systat: the system for statistics. Systat, Evanston, 111.

Table 1. Preference of third instar gypsy moth larvae for birch, maple or ash foliage and trunks.

Type	n	Larvae on Foliage alone	Larvae on Trunk alone	
		Mean <u>+</u> SE (%)	Mean <u>+</u> SE (%)	
Maple	6	14 ± 1.33 a(1)	44 ± 5.93 a	
Birch	6	$71 \pm 1.00 b$	6 ± 1.86 b	
Ash	6	1 <u>+</u> 0.67 c	43 <u>+</u> 8.45 a	
Off pl	ant/trunk	14 ± 2.08	7 ± 1.20	

⁽¹⁾ Means within a column followed by the same letter are not significantly different, by Tukey's test (p=0.05).

Table 2. Preference of third instar gypsy moth larvae for trunk-foliage combinations.

Trunk - Foliage Combination	n	Percent Larvae + SE
Maple - Birch	6	41 ± 5.35 a(1)
Birch - Birch	6	19 ± 1.59 b
Maple - Maple	6	15 ± 1.45 b
Birch - Maple	6	12 ± 3.31 b
Maple - Ash	6	2 ± 1.22 c
Birch - Ash	6	1 ± 0.45 c
Off trunk-foliage	combinations	10 ± 1.45

⁽¹⁾ Means within a column followed by the same letter are not significantly different, by Tukey's test (p=0.05).

Table 3. Relative importance of trunk or foliage stimuli for attracting gypsy moth larvae determined by analysis of variance.

Effect	df	MS	F Ratio	p
Trunk	1	0.112	9.043	0.005
Foliage	2	0.700	56.497	0.000
Trunk X Foliage	2	0.041	3.320	0.050
Error	30	0.012	-	-

CHAPTER II

Comparison of <u>Lymantria dispar</u> (Lepidoptera: Lymantriidae) larvae from a rearing colony with wild type larvae for establishment on preferred and non-preferred hosts.

ABSTRACT

Establishment of Lymantria dispar larvae was studied by releasing marked third and fourth instars from Midland, MI-Wild type, and from an Otis colony, NJF33, on 3m-tall maple(Acer rubrum 'Northwood'), ash (Fraxinus pennsylvanica 'Marshall Seedless') and crabapple (Malus 'Radiant') trees. Third instars established more readily on A. rubrum or Malus 'Radiant' than on F. pennsylvanica. Larvae released on F. pennsylvanica were also found in greater frequency on surrounding trees and traveled further than larvae released on Malus 'Radiant'or A. rubrum. In the 3 day time frame of this experiment few differences were found among wild type and NJF33 larvae. At 72 h after release, more third instars from the wild strain were found on Malus trees than third instars from NJF33 (0.05<p<0.10). There were no differences in the number of NJF33 and wild type larvae that established on A. rubrum or F. pennsylvanica. The average distance that larvae moved away from trees they were released on was similar for wild type and NJF33 larvae. At 72 h after release more third instar larvae were found than fourth instar larvae on Malus 'Radiant' trees (6.0 and 2.5 larvae per tree, respectively, p<0.05).

INTRODUCTION

THE GYPSY MOTH, Lymantria dispar L., is established as a periodic forest defoliator in Michigan and from Ohio east to Maine and south to North Carolina. Oak, birch, and poplar forests in newly infested regions are the most likely to suffer heavy defoliation (Mosher 1915). The gypsy moth feeds on over 300 species of plants. Studies have shown that, although gypsy moth is polyphagous, it has a primary preference for certain tree species. Among the favored food plants are oak, apple, crabapple, willows, American beech, trembling aspen, and grey and paper birch (Mauffette et al. 1983; Peterson and Smitley, 1991). Host selection appears to be primarily determined by first instar larvae (Barbosa et al. 1979). However, during diurnal periods of larval movement, late instars often leave the tree on which they have been feeding and ascend into a new tree. This behavior has been implicated as a mechanism by which late instars are able to utilize a broader range of host plants than do the early instars (Barbosa 1978a). Larval age, gypsy moth population density, and tree phenology may affect dispersal and food consumption (Barbosa et al. 1979). Little is known about establishment and movement of third and fourth instars.

Several gypsy moth strains have been established in the laboratory. The oldest strain was established in 1967 at the USDA Animal and Plant Health Inspection Service's (APHIS) Otis Gypsy Moth Methods Development Laboratory, Otis Air Force Base, Massachusetts, and has been reared continuously for 24 generations (ODell, 1984). Many research projects obtain larvae from Otis for their studies, but it is not known if larvae from these cultures behave and disperse the same way as larvae from wild strains do. ODell(1984) suggests that results of studies that use laboratory-reared insects without comparing their performance with their wild counterparts must be interpreted narrowly. Little is known about population dynamics and behavioral responses of most laboratoryreared species; indeed, there are relatively few published tests for deriving such information (ODell, 1984). The purpose of this study was to observe establishment of third and fourth instars on preferred and non-preferred trees, and to compare establishment and movement of third instars from NJF33 colony with third instars of wild type from Midland, MI.

MATERIALS AND METHODS

Study site

The experiment was conducted from 30 May to 2 June 1989 in the city of Midland nursery. The experimental planting site is surrounded on the east, west and north by forest stands and on south by an open field. Nursery growntrees, 3m-tall, were planted on April 23, 1987. Five replications of nineteen landscape tree species were planted in a complete randomized design (5 rows x 19 trees). For this study, four crabapple trees (Malus 'Radiant'), four maple trees (Acer rubrum), and four ash trees (Fraxinus pennsylvanica) were selected as release points.

Laboratory reared egg masses were secured from Otis Air Force Base, Massachusetts. These egg masses were stored at 4°C in two layers of sealed polyethylene bags. Wild strain egg masses were secured from the forest stand surrounding the Midland city nursery. Eggs were dissected from the hairy masses and immersed in 10% formaldehyde solution for about 60 minutes after which they were washed in running water and dried for 60 minutes. Hatching time was tested three months prior to the experiment. It was

earlier than Midland eggs, and that Otis larvae took four to five days less than Midland larvae to reach third instar. On the basis of this information, hatching of Otis and Midland eggs was planned in such a way that the larvae of these two strains reached third instar at the same time and were also synchronized with the development of larvae in the forest adjacent to the nursery site.

Gypsy moth eggs were placed in sterile polystyrene containers (100 x 15 mm) and kept in growth chambers at 24° C, 60 to 80% RH and LD 16:8. Egg masses were held in growth chambers for 5 or 6 days, until egg hatch was 75% complete. When neonates emerged, they were randomly chosen and transferred with a camel's hair brush into 57 ml transparent Solo Plastic Souffles cups containing artificial diet. The cups were inverted to force the larvae to feed at the top of the containers to minimize food contamination by frass. After each molt the larvae were transferred to new diet cups. Rapidly developing larvae were kept at 4°C for one or two days, to delay development long enough to synchronize the batch. Two batches of egg masses from Midland and one batch from Otis (NJF33 Colony) were hatched, reared and grown to the required stadium for release on May 30, 1989. A total of

900 larvae were reared for release; 300 third instars from Midland, 300 fourth instars from Midland, and 300 third instars from Otis sources.

Marking of Larvae

The larvae were made inactive by keeping them at 4°C for 30 min. Using different color codes of water based paints, the larvae were marked on the dorsum of the abdomen. Marks specified the egg source and tree species that larvae were released on. Third instars were marked with one dot on the anterior end, fourth instars were marked with two dots, one on the anterior and another on the posterior end (Table 4).

Release and Observation of Larvae

Nine hundred marked larvae were released on 12 trees from plastic cups fastened around the upper half of the trunk. All the trees in the nursery including the release trees were searched at 24, 48, and 72 hours after release. The nursery trees were small enough (2-3 m tall) that the markings could usually be observed without disturbing the caterpillars. Occasionally, branches were bent down to view the caterpillars. Larvae were identified by their

color codes and grouped by source, instar, and tree type they were released on. In addition to counting larvae, the average distance traveled by larvae was determined by measuring the distance from the trunk of the tree larvae were observed on, to the trunk of the tree they were released on. Thus the data collected relates to two variables: (1) successful colonization of release trees or dispersal away from them and, (2) the average distance traveled by dispersing larvae later observed on other trees.

Data Analysis

Data were subjected to an analysis of variance and means were separated by Tukey's procedure, or LSD test (Systat 1987). A t-test procedure was used to compare means of recaptured third instar wild strain larvae with fourth instars and of third instar larvae from Otis with third instar larvae from a wild strain.

RESULTS

Fewer larvae were recaptured from <u>Fraxinus pennsylvanica</u>
than from <u>Malus</u> 'Radiant' or <u>Acer rubrum</u> at 24, 48, and 72
hours (Table 5). It was found that larvae moved longer
distances from <u>Fraxinus</u> than from <u>Acer</u> and <u>Malus</u> species 24
h, 48 h, and 72 h after release (Table 6).

Third instars from Midland and Otis sources were recaptured on Malus 'Radiant' at the same rate at 24 and 48 h after release. However, at 72 h after release more 'Midland' third instars were found compared with 'Otis' larvae (Midland=6.0; Otis=3.0; t=2.22; 0.05<p<0.10) (Table 7). No such differences were observed among Midland and Otis larvae released on Acer or Fraxinus tree species. There was no difference in mean distance traveled by Midland and Otis third instars either at 24, 48, or 72 h (Table 8).

No differences were found among third and fourth instars of Midland source, with respect to recapture on Malus and Acer species, 24 and 48 h after release (Table 9). There were however, more third than fourth instars found on Malus species at 72 h after release (Midland third=6.0; Midland fourth = 2.5; t =3.36; p<0.05).

DISCUSSION

In this study, both wild and laboratory reared strains dispersed more readily from Fraxinus pennsylvanica, a less preferred tree, than from Acer rubrum, and Malus 'Radiant' which are relatively more preferred trees. This finding is consistent with that of Capinera and Barbosa (1976) that larvae on less acceptable tree species are more likely to disperse than larvae on favored trees. We also found that among larvae leaving release trees, those released on Fraxinus pennsylvanica traveled longer distances than those released on Acer rubrum or Malus 'Radiant'.

Mason and McManus (1981) have observed that though larval activity may be related to repeated dispersal episodes by individuals, it has little effect on the total distance covered by any one larva, implying that there could be other factors that might explain variation in distance covered by larvae. Our study suggests that the distance traveled by any one larva may be related to the type of tree from which it initially moved away. This observation raises a question for further investigation: Do larvae coming from less preferred tree species tend to move longer distances than those coming from more preferred trees?

From our results it appears that fourth instars disperse more readily from their initial locations than third instars. This agrees with the observation of Doane and Leonard (1975) that older larvae can more readily switch host plants than younger larvae. This study showed few differences in establishment and none in the distance traveled by Midland third instars compared with Otis third instars.

REFERENCES CITED

- Barbosa, P. 1978a. Host plant exploitation by the gypsy moth, Lymantria dispar (L). Ent. Exp. Appl. 24: 28:37.
- Barbosa, P., J. Greenblatt, W. Withers, W. Cranshaw and E.A. Harrington. 1979. Host plant preferences and their induction in larvae of the gypsy moth, Lymantria dispar. Ent. Exp and Appl. 26:180-188.
- Capinera, J.L. and P. Barbosa 1976. Dispersal of first instar gypsy moth larvae in relation to population quality. Oceologia (Berlin) 26: 53-64.
- Doane, C.C. and D.E. Leonard. 1975. Orientation and dispersal of late-stage larvae of Porthetria dispar (Lepidoptera: Lymantriidae). Can. Ent. 107: 1333 _ 1338.
- Lance, D.R. Host-seeking behavior of the gypsy moth: The influence of polyphagy and highly apparent host plants. In Ahmed, S. (ed.). 1983. Herbivorous insects. Academic Press Inc., N.Y. pp 201-224.
- ODell, T.M. Production of, use in quality testing in insect rearing. King, E.G. and Leppala, N.C. 1984. Advances and challenges in insect rearing: USDA agricultural research service. Ag. Research Service, New Orleans.
- Mason, C.J. and M.L. McManus. Larval dispersal of the gypsy moth. pp. 161-203 in Doane, C.C. and M.L. McManus (Eds.). 1981. The gypsy moth: research toward integrated pest management. USDA For. Serv. Tech. Bull. 1584, Washington, D.C.
- Mauffette, Y., M.J. Lechowicz and L.Jobin. 1983. Host preferences of the gypsy moth, Lymantria dispar (L.), in southern Quebec. Can. J. For. Res.13:53-60.

- Mosher, F.H. 1915. Food plants of the gypsy moth in America. USDA. Ag. Bull. No. 250.
- Systat. 1987. Systat: the system for statistics. Systat, Evanston, III.

Table 4. Color codes used for marking larvae.

Color combination	r	Releasing tree	Source of larvae	Stadium
One orange dot	25	Malus 'Radiant'	Midland	Third
Two orange dots	25	Malus 'Radiant'	Midland	Fourth
One white dot	25	Malus 'Radiant'	Otis	Third
One blue dot	25	Acer rubrum	Midland	Third
Two blue dots	25	Acer rubrum	Midland	Fourth
One yellow dot	25	Acer rubrum	Otis	Third
One red dot	25	Fraxinus pennsylvanica	Midland	Third
Two red dots	25	Fraxinus penn.	Midland	Fourth
One cream dot	25	Fraxinus penn.	Otis	Third

Gypsy Moth larvae observed 24 h, 48 h, and 72 h after release on preferred and non-preferred trees. Table 5.

		Larvae I		
			24 hours 48 hours 72 hours	72 hours
Malus 'Radiant'	4	41.8±8.4a	11.0±2.0a	11.5 <u>+</u> 1.2a
Acer rubrum	4	26.8±4.4ab	13.8±2.7a	9.5±1.2ab
Fraxinus pennsylvanica	4	18.3 <u>+</u> 4.2b	3.3 <u>+</u> 1.4b	2.8 <u>+</u> 1.3b

Means within a column followed by the same letter are not significantly different by Tukey's test (p=0.05).

Distances(cm) larvae traveled from <u>Fraxinus pennsylvanica, Acer rubrum,</u> and <u>Malus</u> 'Radiant' tree species. Data are mean distance<u>+</u>SE. Table 6.

		Distance	per larva	
Tree	u	24 h.	48 h.	72 h.
Fraxinus sp.	12	89.0 <u>+</u> 22.7a	$186.4\pm15.4a$	$127.1\pm36.8a$
Acer rubrum	12	50.8±21.7b	38.1 <u>+</u> 19.9b	50.8 <u>+</u> 28.7b
Malus 'Radiant'	12	12.7 <u>+</u> 12.7b	12.7 <u>±</u> 12.7b	25.4±17.1b

Means within a column followed by the same letter are not significantly different by Tukey's test (p=0.05).

Establishment success of gypsy moth third instars from an Otis culture and comparison with wild type larvae from Midland, Michigan. Table 7.

or larva n				Larvae pe	Larvae per source ± SE	SE	
		Malus 'Radiant'	Malus 'Radiant'	Acer rubrum	Acer rubrum	mn.	
		24 h.	24 h. 48 h.	72 h.	24 h.	48 h.	72 h.
Midland 4	_	10.0+1.8	8 4.5±1.0	6.0±1.0	8.5±3.0	3.5±0.6	3.8+0.5
otis 4		10.8±2.1	1 3.5±1.2	3.0±1.0	8.0 ± 1.8	4.5±1.7	4.0±0.8
t-value		0.28	99.0	2.22	0.15	0.56	0.26
t-sig.		0.79	0.54	0.07	0.89	09.0	08.0

Distance traveled (cm) by third instars of Midland and Otis at 24 h, 48 h, and 72 h from release trees. Data are mean distance \pm SE. Table 8.

Source			Distance per larva	
or larva	ı c	24 h.	48 h.	72 h.
Midland	12	76.3 <u>+</u> 23.0	82.6±31.8	76.3±39.8
otis	12	38.1 <u>+</u> 20.0	57.2 <u>+</u> 25.1	50.8±21.7
t-value		1.25	0.63	0.56
t-sig.		0.22	0.54	0.58

Establishment of third and fourth instar gypsy moth larvae on Malus Table 9.

rable 9.	Establish 'Radiant' collected	ment of the and Acer in Midlan	ird and i rubrum. d, Michig	Establishment of third and lourth instar gypsy moth larvae on <u>maius</u> 'Radiant' and <u>Acer rubrum</u> . Larvae are all from a wild strain collected in Midland, Michigan. Data are the means ±SE.	Larvae are all from a wild strain Data are the means ±SE.	n tarvae om a wild s <u>+</u> SE.	on <u>marus</u> strain
Instar	1	Malus 'Radiant'			Acer rubrum	Ħ	
or tarvae		24 h. 48 h.	i i	72 h.	24 h. 48 h.	48 h.	72 h.
m	4	10.0±3.6	4.5 ± 1.9	6.0±2.0	8.5+5.9	3.5 ± 1.3	3.8 ± 1.0
4	4	14.3±0.5	4.0±2.8	2.5±0.6	10.3±2.6	5.8+4.4	1.8±2.1
t-value		2.37	0.29	3.36	0.54	0.99	1.76
t-sig.		90.0	0.78	0.02	0.61	0.36	0.13

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

oucher No.: 1991-09
itle of thesis or dissertation (or other research projects): HOST SELECTION AND ESTABLISHMENT OF LYMANTRIA DISPAR L.
(LEPIDOPTERA: LYMANTRIIDAE) ON SELECTED SPECIES OF PREFERRED AND NON_PREFERRED TREES
useum(s) where deposited and abbreviations for table on following sheets:
Entomology Museum, Michigan State University (MSU)
Other Museums:
Investigator's Name (s) (typed) RAJAKUMARI P. RAO
DateOctober 29, 91.

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or

dissertation.

Copies: Included as Appendix 1 in copies of thesis or dissertation.

Museum(s) files.

Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 2 of 2 Pages

Number of:	Museum where depos- ited Other Adults of Adults Pupae Nymphs Larvae Eggs	ory 12	orce	œ		Voucher No. 1991-09 Received the above listed specimens for	Entomology Museum.	Date
	Label data for specimens collected or used and deposited	Eggs collected from Otis Airforce base, Massachusets July 8, 1990, R. Rao, Laboratory reared.	Eggs collected from Otis Airforce base, Massachusets July 8, 1990, R. Rao, Laboratory reared	IV Instar	ary)		Entomology Museum.	\$
	Species or other taxon	Lymantria dispar			(Use additional sheets if necessary)	Investigator's Name(s) (typed) RAJAKUMARI P. RAO		Date October 29, 91.

