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The eastern tent caterpillar : overwintering microhabitat and host-plant interactions.

Alejandro E. Segarra-Carmona
University of Massachusetts Amherst

THE EASTERN TENT CATERPILLAR: OVERWINTERING MICROHABITAT AND HOST-PLANT INTERACTIONS

A Thesis Presented

BY

ALEJANDRO E. SEGARRA-CARMONA

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Approved as to style and content by:

Dr. David N. Ferro, Chairperson of Committee

Dr. Chih-Ming Yin, Member

Dr. T. Michael Peters, Member

Dr. John G. Stoffolano, Jr. Acting Dept. Head
Department of Entomology

PREFACE

This thesis is a contribution to the understanding of the biology of the eastern tent caterpillar. It has also been a step towards gaining confidence as a scientist, a great opportunity to understand my limitations. It is improbable, for example, that in a single lifetime any person could gather and correctly interpret all the information needed to comprehend the biology of an organism, especially when biological species do not behave like static protoplasm masses, but are actively evolving entities. This was my saddest realization, it will not be me, but rather all those before and after me, who effort by effort will decode the biology of the eastern tent caterpilllar, to those I wish luck.

The article style in which the text is written was adopted for convenience. Clarity of thought was always my main concern and problem. This concern was aroused by the fact that ideal scientific style demands: a) complete integration of theory and facts, b) precise communication skills and c) elegant style; the three of which are very rare in a single work. Thanks to support and comments from teachers and friends, I confronted this problem with patience and discipline, in the same fashion many others have done successfully before me.

I would like to express gratitude to all who, in one way or another provided their time, effort, and ideas; so essential for the completion of this work, with them I will always be indebted. Those who provided their companionship and their hands in the

research phase: Mr. Jim Brady and Ms. Wren A. Withers. Those fellow students and collegues who constantly exchanged their ideas and comments with me: Drs. Jane Greenblatt and William Calvert. Those who provided their help in the completion of biochemical analyses: Drs. Jean Oberly and W. Nawar. Those who provided moral support, intelectual nourishment, many valuable comments and lots of good times: Drs. David Ferro, T. Michael Peters and Chih-Ming Yin. To Dr. Pedro Barbosa for his support and friendship, with him I will be eternally indebted. Thanks to all who above all were, are and will be my friends. Gracias.

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REVIEW OF LITERATURE

Introduction

The genus Malacosoma Hbn. is a group of Lasiocampid moths restricted to the temperate regions of the Northern Hemisphere. Five species are currently recognized in Europe and Northern Africa, three in Asia and six in North America. Malacosoma americanum (Fab.) is distributed throught the eastern half of the United States and southeastern part of Canada (Stehr and Cook 1968).

Life cycle. The life cycles of all species of Malacosoma Hbn. are very similar. The eggs are laid as a mass encircling or partly encircling small twigs of suitable host plants. As the mass is being deposited, the female covers the eggs with a frothy material called spumaline. This substance is hygroscopic and it's role in protecting the mass from desiccation has been suggested by Hodson and Weinman (1945). Embryological development is completed within 3-4 weeks. At this point the larvae enter a period of aestivation and then hibernation, until the following year. Larval hatching in the spring is related to degree-day summation (Mattson and Ericson 1978), usually not synchronous in any given locality (Britton 1935). The larvae's first meal consist of chorion and spumaline, thereafter they will move to the nearest leaf bud to feed and construct their

silk tents and trails.

The number of larval instars is not constant, but the caterpillars usually go through 5-6 moults before pupating. Towards the end of the last instar the larvae wander, apparently in search for suitable pupation sites. The larvae forms fairly tight cocoons, entering a prepupal stage which lasts 2-3 days. The pupal stage lasts 13-18 days after which the imago emerges. Adult emergence occurs during the late afternoon; swarming and mating occurs at various intervals during the night. Oviposition on host branches occurs at dawn in this species (Laboratory observation). The adults are short lived, the females mate only once and die soon after oviposition (Williams 1939).

Economic importance. This species cannot be considered a pest which causes extensive economic losses, since most of its hosts are of little economic value. Once such hosts, Prunus serotina (Ehrh.) the black cherry, whose wood is valued for furniture, could be considered an important host in some areas. Neglected apple trees are often defoliated but commercial orchard are protected by normal spray schedules. It is the construction of unsightly tents and tree defoliation that results in this species being regarded as a nuisance (Stehr and Cook 1968).

Medical importance. The cocoon of all Malacosoma species is covered with a yellowish or whitish cristalline powder identified as calcium oxalate monohydrate (Ohnishi et al. 1968) and urates (Wigglesworth 1953). This powder causes allergic reactions in

some people, especially in areas moistened by perspiration, in addition there are reports of death to farm animals after ingestion of the cocoons (Stehr and Cook 1968).

Host plants. This species shows strong oviposition preference for pioneer hardwoods of the Rosaceae like black cherry (Prunus serotina (Ehrh.)), choke cherry (P. virginiana L.) and hawthorn (Crataegus spp.). In addition, other species like apples and crabapples (Malus spp.) are also attacked (Britton 1935; Stehr and Cook 1968; Sweetman 1940). Other hardwood species are attacked when its original Rosaceous host has been defoliated, these include: oak (Quercus spp.), maple (Acer spp.), birch (Betula spp.), aspen and poplar (Populus spp.), beech (Carpinus spp.) and willow (Salix spp.). Pin cherry (P. pensylvanica L.) is regarded by some authors as a preferred host plant and an occasional host by others.

Population regulating factors. Malacosoma americanum (Fab.) populations have been shown to exhibit cycles of abundance every 9-11 years (Headlee 1934). The first such outbreaks recorded occurred in 1646. The early colonist referred to such outbreaks as caterpillar years (Britton 1935).

The relative importance of various density regulating factors causing the collapse of populations have always been debated. Polarization occurs between those who solely point at biological factors such as predation and disease (e.g., Britton 1935) and

those who propose that harsh weather conditions are the ultimate regulator (e.g., Blackman 1918; Hodson 1941; Hodson and Weinman 1945; Tomlinson 1938). Whatever the cause, mortality can be very high. Witter et al. (1972) found 97-99% mortality for two generations of the related M. disstria in Minnesota.

Egg mortality. In Malacosoma this stage (including "pharate larvae") often suffers high mortality during unusually hot summer periods (Hodson 1941), others report diapause disruption under extreme heat (Lorimer and Mattson 1979). Other temperature related factors like unusually cold winters or springs can account for mortalities of up to 45% (Stacey et al. 1975; Witter et al. 1972).

Accounts of Malacosoma egg parasitism in the research literature yield similar values ranging from 1-10% mortality (e.g., Witter and Kulman 1972 a; Tomlinson 1938). This probably corroborates the density independent nature of this mortality factor (Iwao 1970). Witter and Kulman (1972 b) reviewed the taxonomy of egg parasitoids and predators of Malacosoma. The major egg parasitoids belong to the order Hymenoptera, in the families: Encyrtidae, Eulophidae, Eupelmidae, Scelionidae, Torymidae and Trichogrammatidae. The egg masses also suffer predation from larger organisms such as birds (e.g., the black capped chicadee) in addition to mice and shrews.

The "pharate larvae" also suffer from transovarially transmitted microsporidial infections, these infected larvae may be

more susceptible to biotic and abiotic stresses than uninfected larvae (Nordin 1976).

Larval and pupal mortality. Late frosts in the spring have been reported to cause the collapse of Malacosoma outbreaks (Blackman 1918; Blais et al. 1955), when the larvae were in early instars. Ives (1973) concluded that suboptimal cumulative total of heat units during the early feeding period was associated with population declines.

Tent caterpillars are also attacked by a variety of predatory organisms. Parasitoids, mainly in the orders Diptera and Hymenoptera, vary seasonally, from site to site and due to habitat type (Witter and Kulman 1972 b). Little is understood about the phenology of parasitization but it is known that most of the attacks occur on late instars, where up to 90% parasitization has been reported (Hodson 1941). The braconid Rogas sp. is among the few reported parasitoids attacking early instars (Witter and Kulman 1972 b), this is probably caused by the unpredictability of young larvae as a resource due to density independent mortality (e.g., late frosts).

Other important insect predators are Calosoma beetles, coccinellids and pentatomid bugs. Birds and mammals also attack larvae. Stomach content analysis of black billed cockoos have yielded up to 200 larvae (Witter and Kulman 1972 b). Nematodes have also been found in M. americanum but seldom causing significant mortality (Op. cit.).

Tent caterpillar populations are also attacked by a variety

of diseases. These diseases mainly affect the larvae in a density-dependent fashion especially in outbreak populations (Iwao 1970). Among the most important pathogens in Malacosoma are a nuclear polyhedrosis virus (NPV) (see Clark 1956) and microsporidians such as Nosema spp. and Thelohania spp. (see Nordin 1976 and Smirnoff 1975). Most of these pathogens are transovarially transmitted to their progeny.

Physiological studies in Malacosoma. In general the quantity of literature dealing with physiological studies in Malacosoma americanum (Fab.) is very small. The first studies delt with the seasonal changes in some chemical constituents such as fats, sulphates, water content, glycogen and weight (Rudolfs 1926, 1927, 1929, 1932).

Since one of the most important mortality factors of the "pharate larval" tent caterpillar is freezing, most physiological studies deal with this topic. Hanec (1966) studied cold hardiness of M. disstria, including glycerol concentrations and supercooling temperatures of the eggs. He correlated rate of decrease of glycerol during the post diapause period with increases in the temperature. Nine years later Mansingh (1974) correlated the increase of glycerol concentrations during diapause with decrease of the supercooling points of the "pharate larvae" of M. americanum, suggesting their casual relation.

Recently Fitzgerald (1976) has discovered the presence of a trail pheromone in the silk trails of M. americanum. Although the

structure and composition of the pheromone is unknown, it is not species specific, demonstrated by the fact that M. disstria responds to it readily (Fitzgerald and Edgerly 1979). The sex attractant is presently under investigation in the Geneva Experiment Station of the Cornell University by Dr. W. Roelofs.

Taxonomic relations of Malacosoma americanum (Fab.)

Kingdom	-	Animalia
Phylum	-	Arthropoda
Class	-	Hexapoda (Insecta)
Order	-	Lepidoptera
Suborder	-	Bombycoida
Family	-	Lasiocampidae
Genus	-	Malacosoma Hbn.
Species	-	Malacosoma americanum (Fab.)

Synonymy (Stehr and Cook 1968)

Bombyx americana Fabricius, 1793.
Phalaena castrensis Linnaeus sensu Smith and Abbot, 1797.
Clasiocampa americana Harris, 1841.
Clasiocampa decipiens Walker, 1855.
Clasiocampa americana (Fab.), Walker, 1855.
Bombyx frutitorum Boisduval, 1868-1869.
Malacosoma americana (Fab.), Dyar, 1898.
Malacosoma americana (Harris), Dyar, 1928.
Malacosoma pensylvanica McDonnough, 1938.
Malacosoma americanum (Fab.), Langston, 1957.

C H A P T E R I I

OVERWINTERING MICROHABITAT AND SURVIVAL ADAPTATIONS OF MALACOSOMA AMERICANUM (FAB) (LEPIDOPTERA: LASIOCAMPIDAE), THE EASTERN TENT CATERPILLAR

Summary

1. This paper describes aspects of the overwintering microhabitat of Malacosoma americanum (Fab.) on black cherry trees and their role in winter survival.

2. Oviposition site preferences are described for tree height, branch diameter and cardinal direction.

3. The insulative and hygroscopic properties of the spumaline are surveyed.

4. Possible adaptive significance of the studied traits is discussed.

Introduction

In the temperate region winter's low temperatures and humidities are major selective forces which shape the evolution of many adaptative strategies. In insects, physiological adaptations such as the presence of cryoprotectants, low supercooling points and reduced metabolic activity (dormancy) are among those factors commonly studied. Microenvironmental adaptations, on the other hand, have been largely overlooked, and thus less information is available on this important aspect of overwintering strategy.

Malacosoma americanum (Fab.) overwinters as a "pharate" larva inside its egg shell. Egg masses are laid on branches of suitable

hosts by early summer and complete embryonation occurs within 3-4 weeks. Mature embryos remain in diapause from late July to April of the following year (Mansingh 1974). The egg masses of all North American species of Malacosoma Hbn., except M. tigris (Dyar), are covered with a froth called spumaline which is secreted by the accessory glands. This material has been shown to be hygroscopic and its role in preventing desiccation by absorbing atmospheric moisture was suggested by Hodson and Weinman (1945).

This investigation describes aspects of the overwintering microhabitat of Malacosoma americanum (Fab.) and its role in winter survival.

Materials and Methods

Tent caterpillar egg masses surveyed were located in Amherst, Mass. (42°23'N-72°32'W) on black cherry trees (Prunus serotina (Ehrh.)), during February and March 1979.

Egg mass position on the tree. Of the cherry trees sampled only those with two or more egg masses were checked for egg mass position. Tree and egg mass height were measured to the nearest 0.05 and 0.01m respectively, using a 5m graduated stick. A relative height index (= Height of the egg mass/height of the tree) was employed to express oviposition frequency as a function of egg mass height on trees. A Vernier caliper was used to measure the thickness of the oviposited branch to the nearest o.1mm. The cardinal direction of the egg mass was determined using a standard compass.

Spumaline measurements. The thickness of the spumaline layer was determined with a Vernier caliper, substracting the radius of the intact spumaline to that after scraping the spumaline off of the center of the egg mass. Temperature measurements of the egg mass were taken with a thermocouple probe to a Cryothermometer model Bat-5 (Bailey Instrument Co.). The probe was inserted inside the spumaline and this temperature compared to that of the surrounding air (0.5°C precision).

In tests evaluating the hygroscopic properties of the spumaline, all parts other than the spumaline were covered with wax to prevent water absorption by these tissues. Water absorption by the spumaline is expressed in terms of weight gain of the whole egg mass. Humidity chambers consisted of closed glass containers (105 x 76 x 76 mm); egg masses where placed in a chamber and suspended on zinc wire above a given aqueous sulphuric acid solution. The vapor pressure inside the chambers was calculated from "International Critical Tables" (1928, Vol. 3:303). These tables provide vapor pressures for corresponding sulphuric acid solutions at temperatures ranging from 0°C to 235°C. To obtain vapor pressures for solutions below 0°C extrapolation was utilized, from a regression of $\log_e$ of vapor pressure vs. temperature. In all regressions, the correlation coefficient, r, was larger than 0.999 ($p < 0.001$). Saturation deficits (SD) were calculated from the formula:

$$SD_t = e_s - e. \qquad \text{(Rosenberg 1974)}$$

where e_s is the saturation vapor pressure at temperature t°C and e is the actual vapor pressure, both in mm Hg. Three temperatures were tested, 0°C, -5°C and -10°C. The egg masses were maintained in the chamber for 24 hrs and their percent of weight gain was calculated.

The reflectance spectrum of the spumaline and chorion was measured with a Shimadzu Spectronic 210 UV Spectrophotometer equipped with an integrating sphere 200 UV attachment for measurement of solid materials.

Results and Discussion

Egg mass position on the tree. Of the 150 black cherry trees surveyed 74 contained 2 egg masses or more. The average tree height was 3.6 ± 0.8m. No correlation was found between tree height and egg mass height. Egg mass vertical distribution departed significantly from random (X^2= 68.73, $p < 0.001$) thus appearing to be aggregated primarily in the upper half of the tree (Fig.1), with an average relative height index of 0.66 ± 0.11 or approximatly 2/3 of the tree height. This result could be related to the vertical branch organization of the canopy. If the proportion of branch area is greatest at the trees maximum horizontal diameter (middle of the canopy) it would be reasonable to expect oviposition to be a function of canopy branch area, as it appears to be.

Cardinal direction of the egg masses is influenced by the shape and habitat of the trees. Black cherry trees are found in the open as well as in forest margins. The latter are characteri-

zed by having most of their branch area on the insolated side, such margin trees were eliminated from consideration; the rest of the trees were assumed to have random branch distribution with respect to the total number of branches. Oviposition departed significantly from random (X^2= 71.53, p <0.001), occurring most frequently on the southern and northern sides of trees; the south being the preferred tree aspect (Fig.2). Oviposition was reduced in the eastern and western sides of trees. The temperature of overwintering habitats above the snow (e.g., twigs) is influenced by the cardinal direction (Harvey 1923; Jensen et al. 1970; Danks 1978). South facing sides tend to have higher daily temperature maxima. This agrees with the southern oviposition preference of the egg masses in this survey but does not account for the high frequency of northern ovipositions recorded.

Diameter of oviposition branches. No direct relationship was found between the size of the egg mass and the diameter of the branches upon which females oviposited. The preferred branch diameter ranged from 2-4mm with a mean of 3.05 ± 0.95mm (Fig.3). No egg masses were found on the trunk of any of the surveyed trees.

The spumaline and its properties.

Thickness. By the time the measurements were taken, some of the egg masses showed severe erosion of the spumaline, this accounted for smaller diameters. Spumaline thickness was independent of egg mass size and its average thickness was 1.24 ± 0.46mm (Fig.4).

Hygroscopic properties. Initial attemps to establish the hygroscopicity of the spumaline at low temperatures and high saturation deficits resulted in water loss to the surrounding environment. Subsequent experiments using desiccated egg masses resulted in the expected water gain.

Water absorption decreased logarithmically with saturation deficit (Fig.5), the less water in the environment the less water the spumaline absorbs at all temperatures tested. If we examine closely this water uptake system, two interfaces are present: the air-spumaline interface and the chorion-spumaline interface, the chorion being also hygroscopic (see Hodson and Weinman 1945). A differential response of each interface absorption rates could account for the logarithmic nature of the increased humidity interaction. The regression analyses of water absorption at $0^{o}C$ ($r= -0.99$, $p < 0.01$), $-5^{o}C$ ($r= -0.98$, $p < 0.01$) and $-10^{o}C$ ($r= -0.99$, $p < 0.001$), show that the water absorption is a direct function of temperature and water concentration. Examination of the slopes of the regression equations reveal an inverse relationship between temperature and the slope's absolute value. This agrees with a fundamental characteristic of hygroscopic materials where a decreasing temperature marks a corresponding increase in the material's ability to absorb water (Hodson 1937).

In addition, immediately after rainfall at temperatures higher than $10^{o}C$ field collected spumaline contains so much water that it can be literally squeezed out of it. This illustrates the extent of spumaline's hygroscopicity at high absolute humidity characte-

ristic of higher temperatures.

Insulative properties of the spumaline. There was a marked temperature difference within the spumaline and the air surrounding the egg masses (Table 1). These differences varied inversely with temperature, in several instances, differences of up to 12°C were recorded. This agrees with a report (Wellington 1950) of differences of up to 5°C on an egg mass of M. disstria during a cold and clear February day. However, during cloudy days and in shady situations, the described phenomena was not observed indicating that solar radiation was a key factor.

The reflectance spectrum of the spumaline and the chorion is presented in Figure 6. The percent reflected light decreased with decreasing wavelength with a reflectance minimum at 342nm, the near ultraviolet region. The fact that the spumaline consistently had high reflectance at the infrared (IR) region of the spectrum suggest that the chorion allows short wavelength radiation to penetrate while reflecting long wavelengths, this could account for a "greenhouse effect" in which reemitted IR is trapped in the spumaline, thus retaining heat and maintaining egg mass temperature above that of the air.

Air temperature range / °C.	Mean temperature difference between the spumaline and air / °C.	Number of egg masses.
9 - 5	+1.6	29
4 - 0	+2.7	47
-1 - -5	+3.0	122
-6 - -10	+3.5	73
-11 - -15	+6.0	67

Table 1. Temperature differences of field egg masses of M. americanum (Fab.) and air temperaures on clear days.

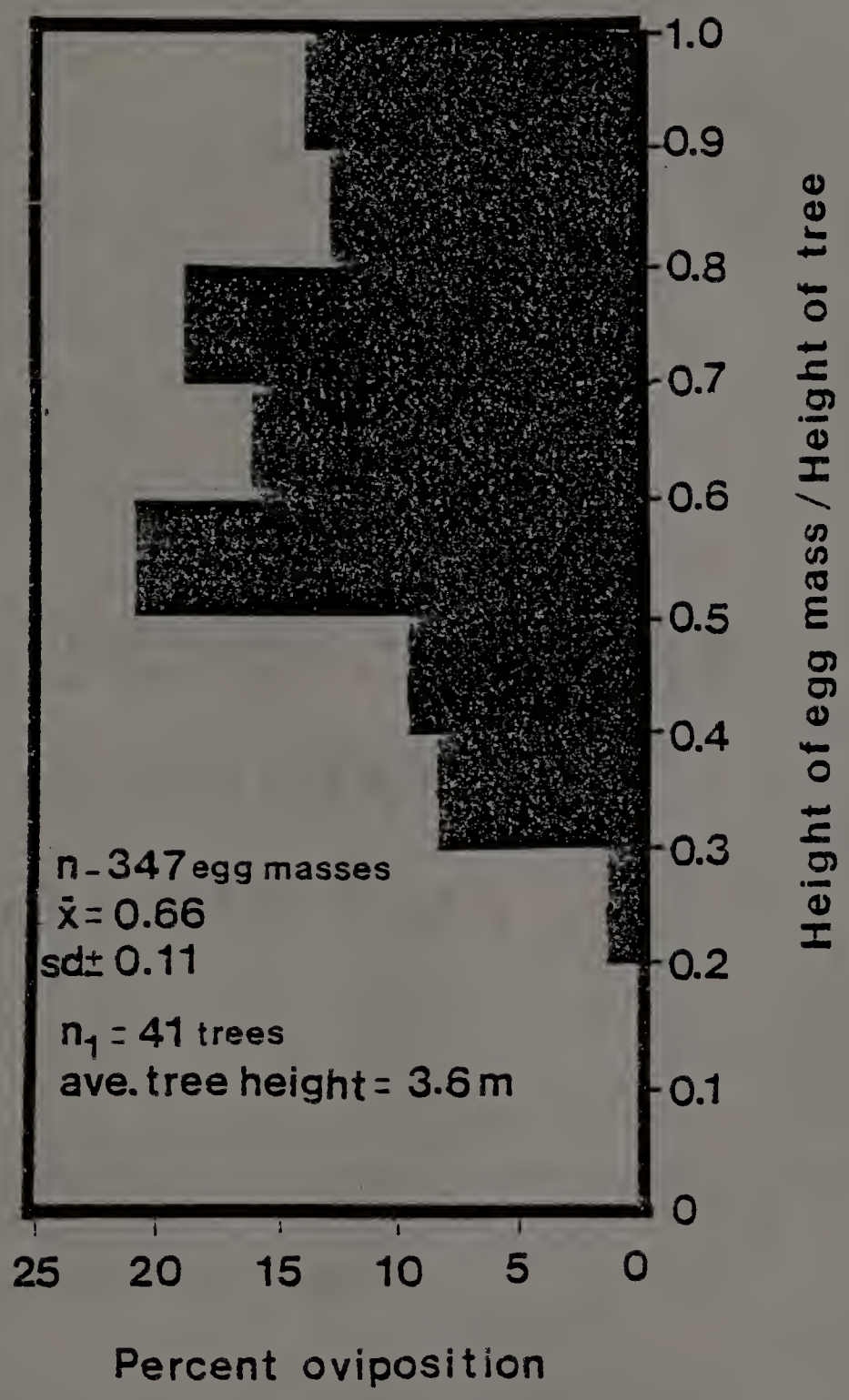

Fig. 1. Height distribution of egg masses of M. americanum on the branches of Prunus serotina (Ehrh.).

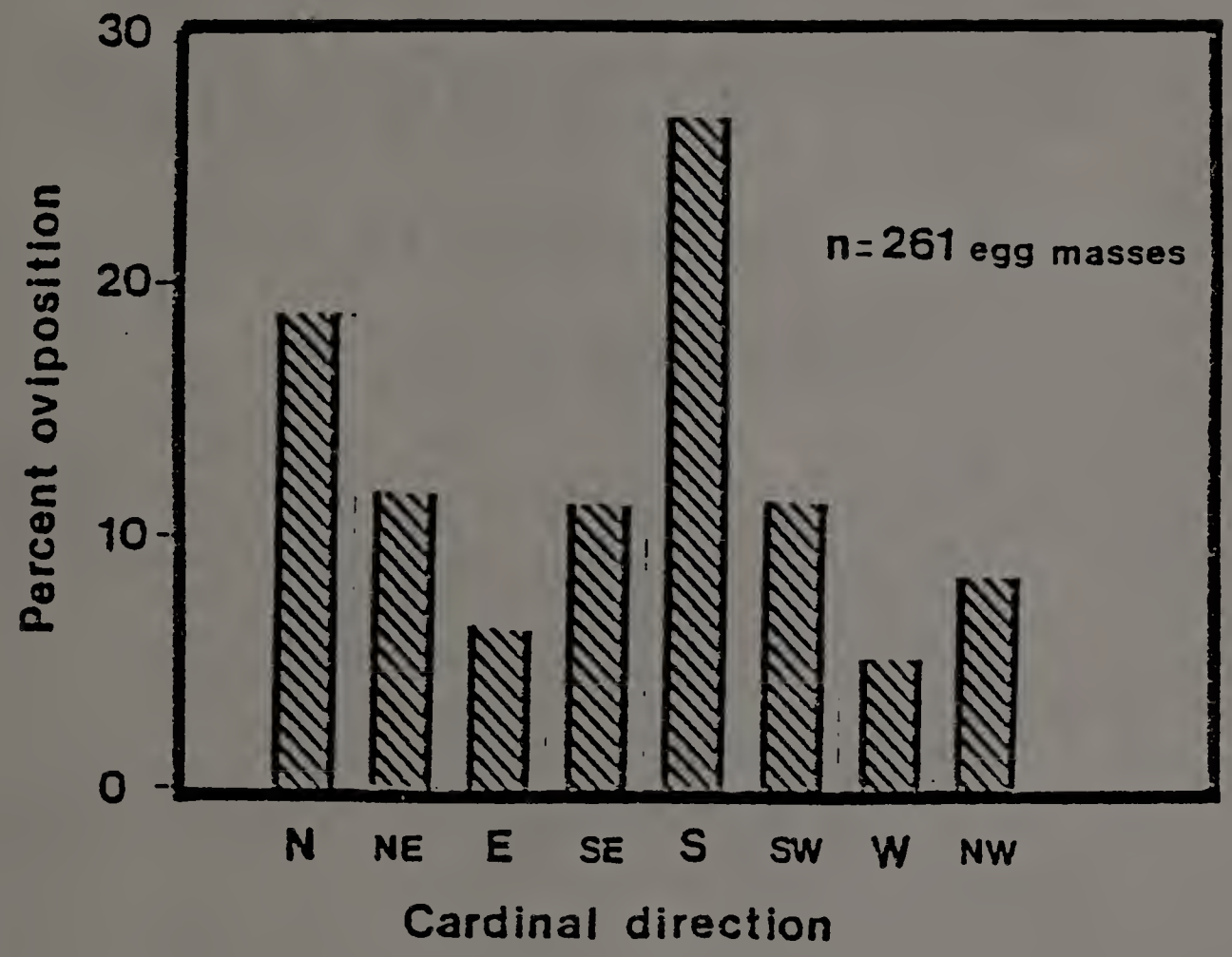

Fig. 2. Positional distribution of M. americanum (Fab.) egg masses on the branches of Prunus serotina found in open habitats.

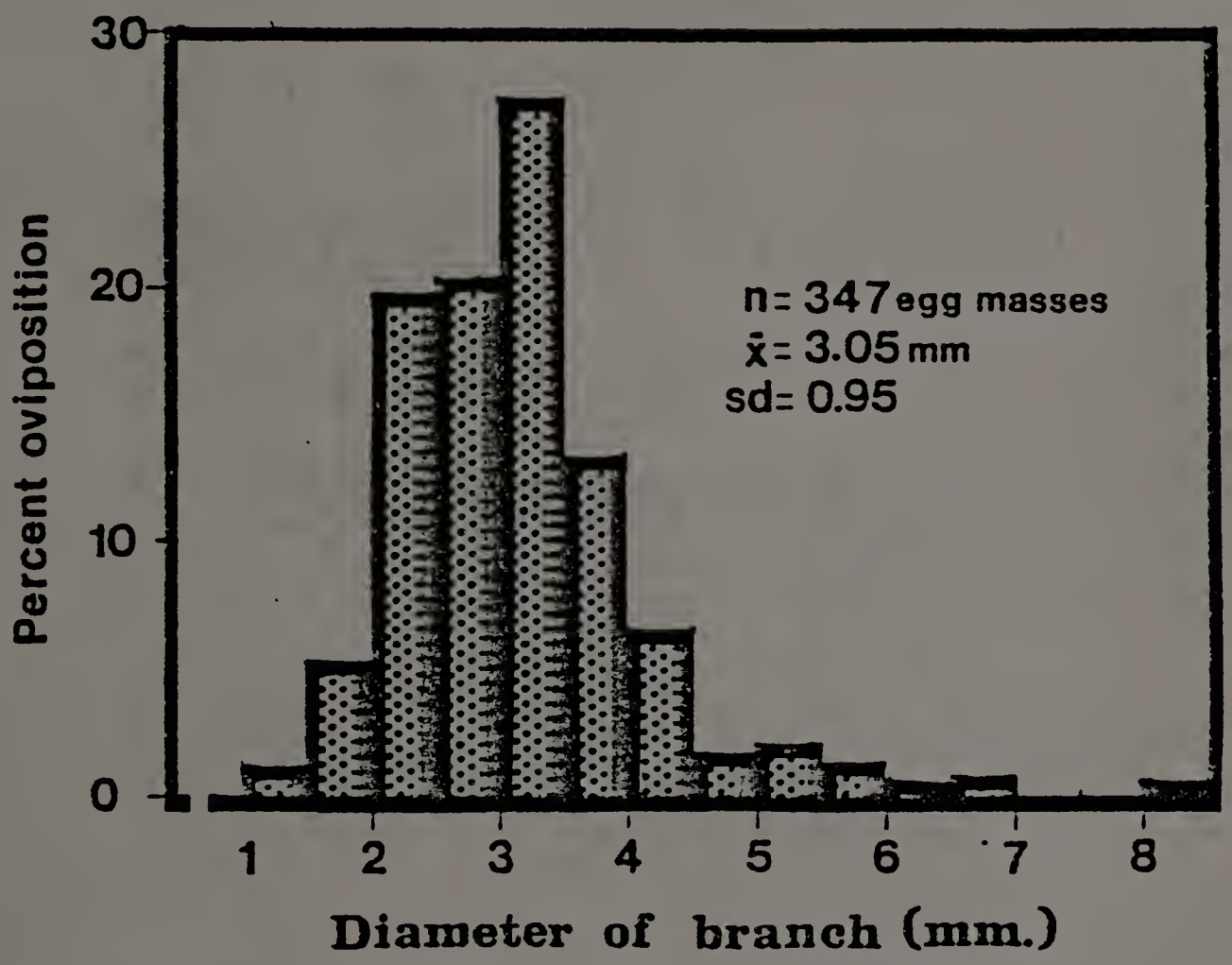

Fig. 3. Diameter of branches of Prunus serotina oviposited by M. americanum (Fab.) and their proportions.

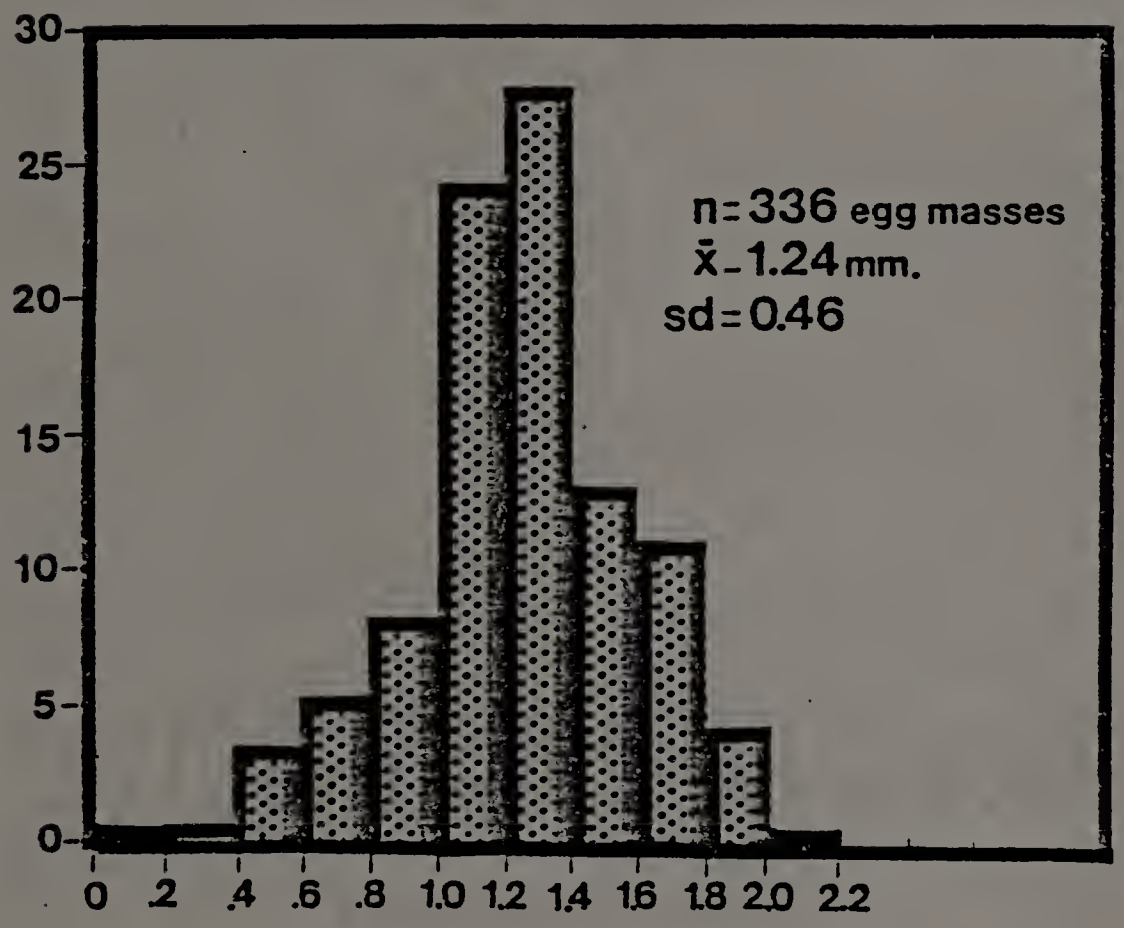

Fig. 4. Frecuency of various spumaline thickness among the egg masses of M. americanum (Fab.) deposited on the branches of Prunus serotina.

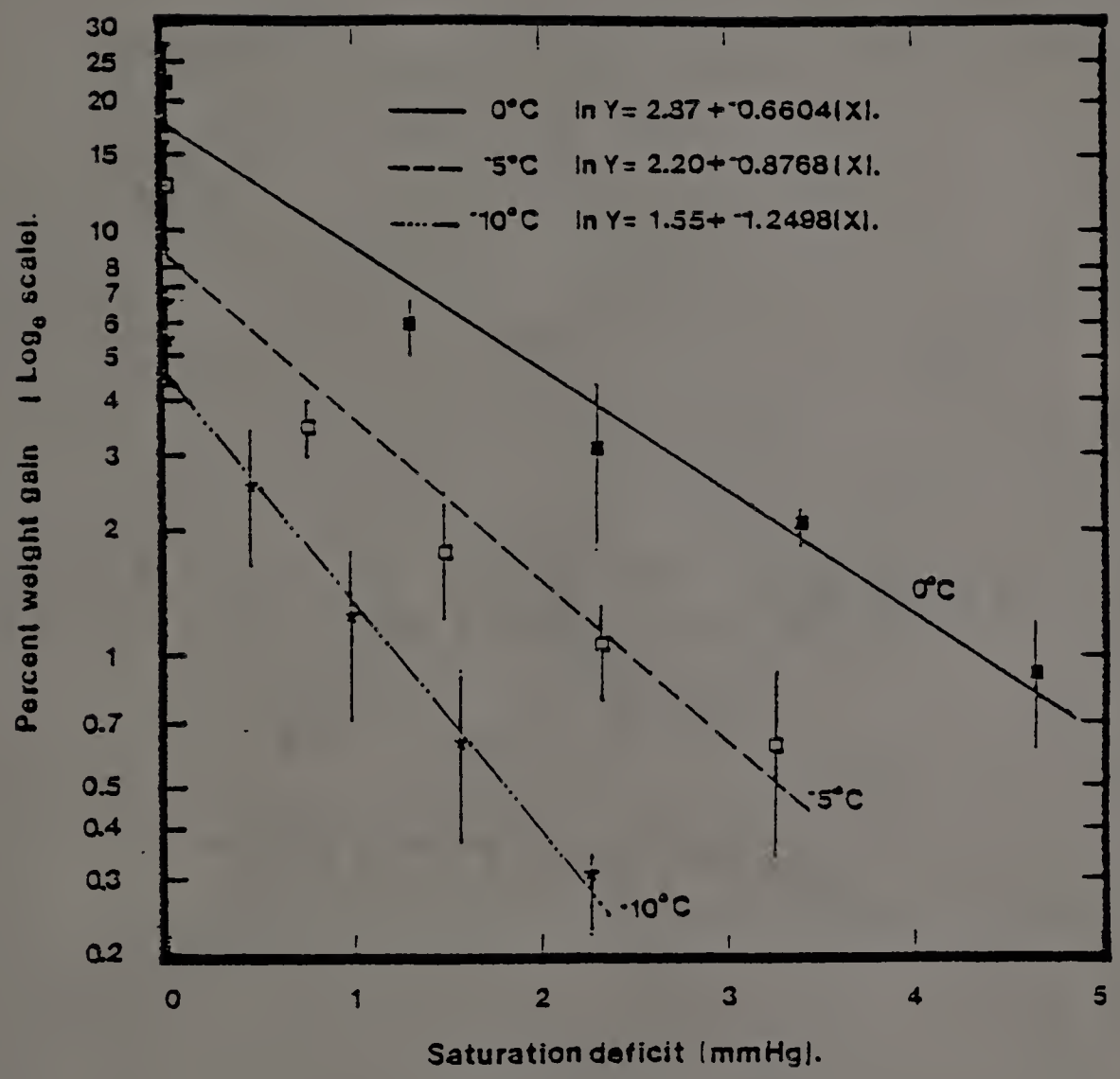

Fig. 5. Log$_e$ of percent of weight gain of M. americanum (Fab.) exposed to different absolute humidities -10°C, -5°C and 0°C for 24 hrs. (mean with standard deviation).

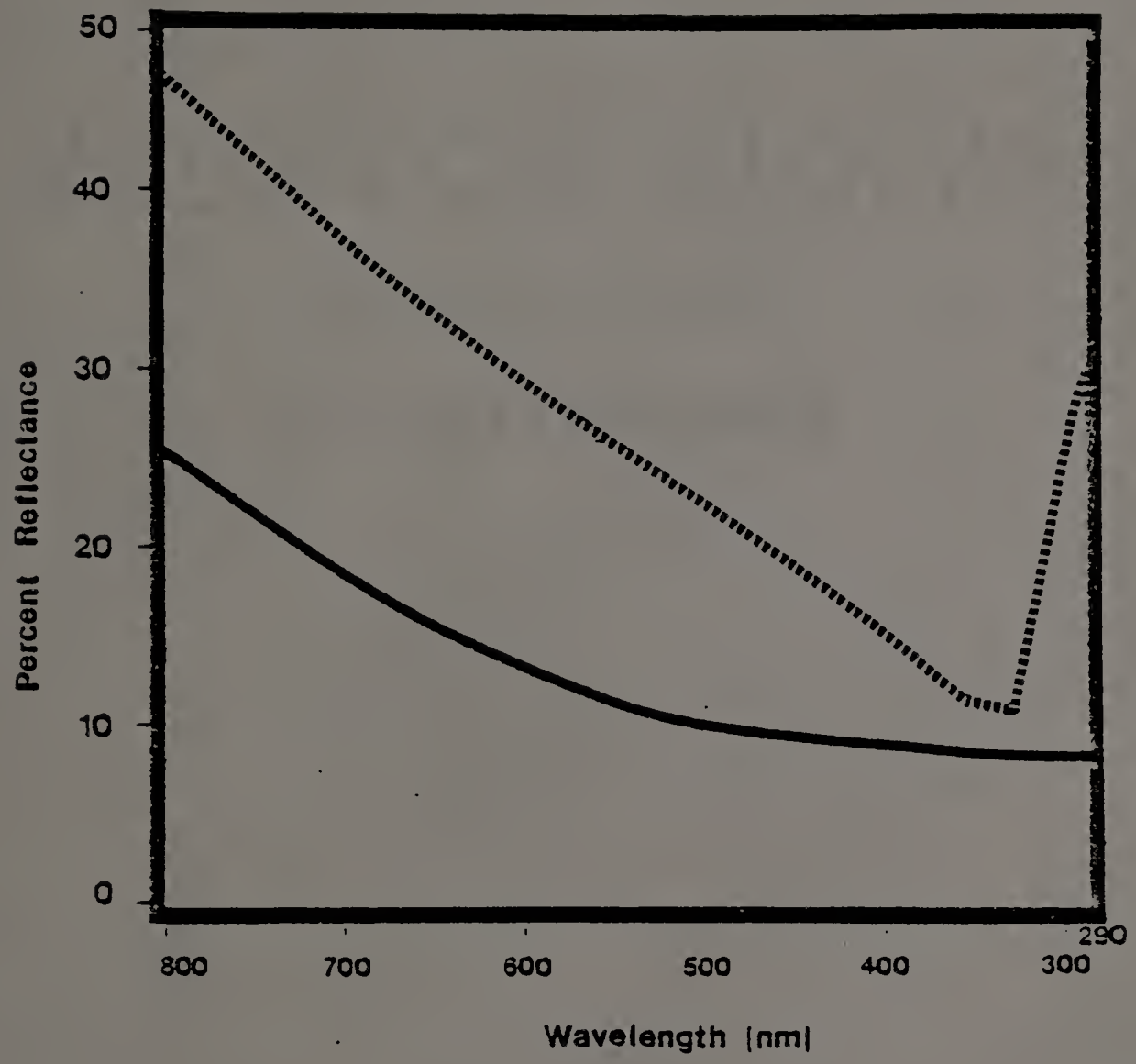

Fig. 6. Percent reflectances of the chorion (▬▬) and the spumaline (▪▪▪▪▪) of egg masses held at 9.8 g/m^3 absolute humidity and 11°C.

Conclusions

The overwintering habitat of many insects is benefited by snow cover, bark, leaf litter or soil; habitats with relatively small temperature fluctuations and excellent insulative qualities (Holmquist 1931). The exposed location of the overwintering stage (the arboreal habitat) of Malacosoma americanum, on the other hand, is one of wide environmental fluctuations and low temperatures. under these conditions, those species like Malacosoma, tend to have very cold-hardy overwintering stages (MacPhee 1964). Several physiological mechanisms of cold-hardiness have been studied in Malacosoma, namely the presence of high cryoprotectant levels and low supercooling points (Hanec 1966; Mansingh 1974). The survival adaptations of overwintering insects are the sum of environmental and physiological adaptations which jointly contribute to overcome or compensate environmental extremes. The relative value can only be determined by a total view of the circumstances, the alternatives present and the interaction of such diverse mechanisms to permit survival.

The egg masses of many overwintering arthropods are often protected from cold environments by structures provided by the maternal parent. The egg of the gypsy moth can withstand temperatures of -50°C if the masses are covered by hair placed on them by the female, but die at -19°C if the hairs are removed (Kulagin 1897 cited in Danks op. cit.). Similarly, the egg sack of Floridia bucculenta protects the egg from desiccation and

flooding (Shaefer 1976). In Malacosoma the presence of the spumaline appears to serve two purposes: to ameliorate environmental temperatures and to prevent desiccation. The spumaline insures the capture of water when the humidity is high and serves as an interphase for slow evaporation when air moisture is low. Extremely dry winters seldom occur in the natural range of M. americanum where precipitation is usually high. The suggestion of Hodson and Weinman (1945) that the spumaline of M. disstria protects from desiccation during short dry periods may apply to this species as well. The occurance of higher temperatures than ambient in the egg masses is apparently a mechanism to compensate for temperature drops during the night since this effect is dependent of solar radiation.

C H A P T E R I I I

THE NUTRITION OF MALACOSOMA AMERICANUM (FAB.) (LEPIDOPTERA: LASIOCAMPIDAE): NUTRIENT COMPOSITION OF FOUR HOST PLANTS AND THEIR EFFECT ON DEVELOPMENT AND FECUNDITY

Summary

1. This paper examines the influence of different host plants on the development and fecundity of the eastern tent caterpillar under laboratory conditions.

2. Larval growth between host cohorts was fastest in the crab-apple fed larvae, pin cherry fed larvae showed sluggish growth.

3. Survival was highest in the black cherry cohort, while pin cherry fed larvae showed highest mortality.

4. Fecundity and pupal weight were unaffected by diet.

5. Nutrient levels of host plant leaves are given for total carbohydrates, fatty acids, nitrogen, energy content and water content.

6. The significance of dietary effects is discussed in the light of evidence presented.

Introduction

Malacosoma americanum (Fab.), the eastern tent caterpillar (ETC), (Lepidoptera:Lasiocampidae) is an extensive defoliator of members of Rosaceae occurring in pioneer hardwood associations in eastern North America and southeastern Canada. It exhibits strong oviposition preference for members of the plant genera: Prunus, Malus, and Crataegus. Other plant species outside the Rosaceae are often attacked especially after defoliation of the tree on

which eggs were originally oviposited (Stehr and Cook 1968). ETC is univoltine. Their eggs hatch by about April and adult development, matings and oviposition are completed during June. Embryonation occurs in 3-4 weeks, and remain in diapause until the following spring.

Different species of host have been shown to affect fecundity, development and survival in many Lepidopterous species (Morris and Fulton 1970, Allen 1973, Hough and Pimentel 1978, and Wagner and Leonard 1979). Some authors have implied the importance of single nutritional components such as leaf nitrogen content, as explanation for such effects (Soo Hoo and Fraenkel 1966; Slansky and Feeny 1977; Scriber 1977).

The relative nutritional suitability of the hosts of ETC in relationship to its performance on these hosts has not been previously evaluated and is assessed in this study.

Materials and Methods

Collection and rearing. Tent caterpillar egg masses were collected in the locality of Amherst, Ma. from black cherry trees (Prunus serotina (Ehrh.) March 1979. The eggs were surface sterilized in 5% sodium hypochlorite. After hatching the larvae of 50 egg masses were allowed to mix for 24 hrs. The larvae were separated into four host cohorts: Crabapple (Malus sp.), apple (Malus pumila (L.)), black cherry (Prunus serotina (Ehrh.)) and pin cherry (Prunus pensylvanica).

The larvae were maintained at 22 ± 3°C, 14 hrs photophase and 70% RH. For the first 10 days of development, they were reared in 16 oz waxed cups at 50 larvae/cup. Thereafter, until pupation they were kept in 8 oz unwaxed cups at 10 larvae/cup. Fresh surface sterilized leaves were supplied daily.

Mating and oviposition occured in plastic screened (1.2 x 0.9 x 0.9 m) wood cages supplied with crabapple branches, these branches were replaced daily.

During the course of the crabapple rearing experiments the trees from which foliage was collected were sprayed with insecticide. Although only a portion of the developmental data had been generated, the evaluation of developmental parameters was continued using field collected larvae off unsprayed crabapple trees. The resulting data is included because colonies remain on a single host unless it is defoliated (and our sampled trees were not defoliated).

Measurements. Weight of larvae pupae and despumalinated egg masses were obtained on an electrobalance. Estimates of the number of eggs per egg mass were obtained by multiplying the average number of eggs per mm^2 (using a dissecting microscope) by the total egg mass surface (obtained by using a portable area meter). These estimates were accurate within ± 5%.

Foliar material analysis. Six trees of each species were used both for feeding and chemical analysis. The leaves were collected

between 9-11 AM from all parts of the canopy. All nutrient analyses were conducted on leaves present during the growth period of field larvae. Water content was obtained by drying the leaves in a forced air oven at 65°C for 48 hrs. The dried leaves were ground in a Wiley mill (40 mesh) and stored at -20°C until analysed.

Total soluble leaf carbohydrates were extracted from 3 replicates of 100 mg for 3 hrs in a micro-soxhlet in 70% Ethanol. The carbohydrates were quantified by the micro-Kjeldahl procedure of McKenzie and Wallace (1954). Energy content of foliar material was determined from 5 replicates of 3-5 mg using a micro bomb calorimeter (Phillipson 1964).

Fatty acid extraction was conducted in a micro-soxhlet in chloroform for 6 hrs. Fatty acid methyl esters were prepared according to Metcalfe et al. (1966) and quantified with a Perkin Elmer 881 gas chromatograph using a flame ionization detector. The carrier gas was nitrogen and the column was stainless steel, 183 cm long, 4 mm (OD), packed with 10% (w/w) EGSS-X, 100/120 mesh on Chromosorb-P.

Results

Larval development and survival. Figure 7 reveals that the rate of weight gain for the crabapple cohort (Ca) was faster than any other host cohort (an estimated average of 7.89 mg/day). The pin cherry cohort (Pp), on the other hand, grew slowest (an estimated average of 4.69 mg/day), while black cherry (Ps) and apple (Mp) exhibited intermediate development rates. Mean time to pupation (= larval developmental period) was significantly shorter in the black cherry cohort compared with the pin cherry or apple cohorts (Table 2). Survival was also greatest in the black cherry cohort, apple was intermediate in survival and the pin cherry group had lowest survival (Table 2).

Pupal weight and fecundity. Due to the poisoning incident, field reared crabapple larvae were recruited to the experiment. Pupal weights and fevundity of these crabapple reared larvae were significantly higher than those of any other cohort (Table 2). The pin cherry, apple and black cherry cohorts did not differ in their pupal weights nor in female fecundity. The pin cherry cohort's mean egg weight was significantly higher than that of the black cherry and apple cohorts (Table 2).

Host plant nutrient analysis. Water content in leaves of the four species was essentially similar, slowly decreasing during the course of the season. The values ranged from 62 to 73% water content (Fig. 8).

Energy content was higher in pin cherry leaves although not significantly different from those of the other host plants (Fig. 9.). Foliar nitrogen generally decreased during the course of the season (Fig. 10.). There were no significant differences among any of the host plants. Black cherry leaves contained the highest nitrogen content throughout the test period. Pin cherry (Fig. 10.) had the lowest nitrogen content during most of the caterpillar growing period.

Soluble leaf carbohydrates levels rose early in the sampling period and rapidly stabilized later in the season. This pattern was most pronounced in pin and black cherry cohorts. Pin cherry leaves had significantly more soluble sugar than any other host ($p<0.01$), followed by black cherry which in turn had significantly higher levels than apple or crabapple ($p<0.05$). Sugar levels of apple and crabapple foliage did not differ significantly (Fig. 11.).

Foliar fatty acids accumulated by the 8th of May in host species that are listed in Table 3. Crabapple leaves had the highest total fatty acid levels, not significantly higher than those of black or pin cherry. Apple leaves contained significantly lower fatty acid concentration ($p<0.01$). The most common fatty acid in all species was myristic acid followed by palmitic and linolenic acids. Percentages vary from species to species.

Table 2. Percent survival, time to pupation, pupal weights, number of egg per female and mean egg weight of M. americanum (Fab.) reared on four different host plants. (1).

Diet	Percent survival (2)	Mean time to pupation (days)	Male pupal weight(mg) mean ± se (3)	Female pupal weight(mg) mean ± se (3)	No.eggs/ female mean ± se (4)	Mean egg weight(mg) mean ± se (4)
Crabapple*	-	-	347.8±3.5a	597.4±7.4a	234.5±4.4a	.327±.005ab
Apple	70.6	45.98b	233.8±2.6b	389.4±5.1b	170.4±4.6b	.317±.005b
Black cherry	88.7	41.06a	227.9±4.5b	405.0±5.0b	169.4±7.2b	.318±.006b
Pin cherry	61.6	46.29b	233.9±2.9b	390.9±4.4b	164.2±5.6b	.343±.005a

* This cohort was field collected in their last larval instar.

1. Any two means followed by the same letter are not significantly different based on Duncan's multiple range tests (p 0.05).

2. N= 1140 larvae per host-cohort.

3. N= 80 pupae per host-cohort.

4. N= 45 egg masses per host-cohort.

Table 3. Mean concentrations of individual fatty acids (mg/g dry wt.) in the foliage of four hosts of the eastern tent caterpillar as of May 8, 1979. (Percentage of total fatty acid composition in parenthesis).

	Black cherry	Crabapple	Apple	Pin cherry
Myristic (C14:0)	23.50 (58.40)	30.60 (65.70)	21.20 (64.40)	20.19 (45.90)
Palmitic (C16:0)	9.22 (22.80)	4.70 (10.10)	4.52 (13.70)	7.85 (17.90)
Stearic (C18:0)	1.24 (3.08)	1.26 (2.70)	1.63 (4.95)	2.50 (5.69)
Oleic (C18:1)	0.95 (2.36)	1.10 (2.36)	0.44 (1.34)	1.92 (4.41)
Linoleic (C18:2)	1.58 (3.93)	1.52 (3.26)	1.47 (4.46)	3.47 (7.89)
Linolenic (C18:3)	3.46 (8.60)	7.03 (15.10)	2.75 (8.34)	7.30 (16.60)
Arachidic (C20:0)	0.24 (0.60)	0.33 (0.71)	0.83 (2.52)	0.68 (1.55)
Unidentified	0.03 (0.10)	0.05 (0.11)	0.10 (0.30)	0.06 (0.14)
Total (mg/g)	40.23	46.59	32.94	43.97

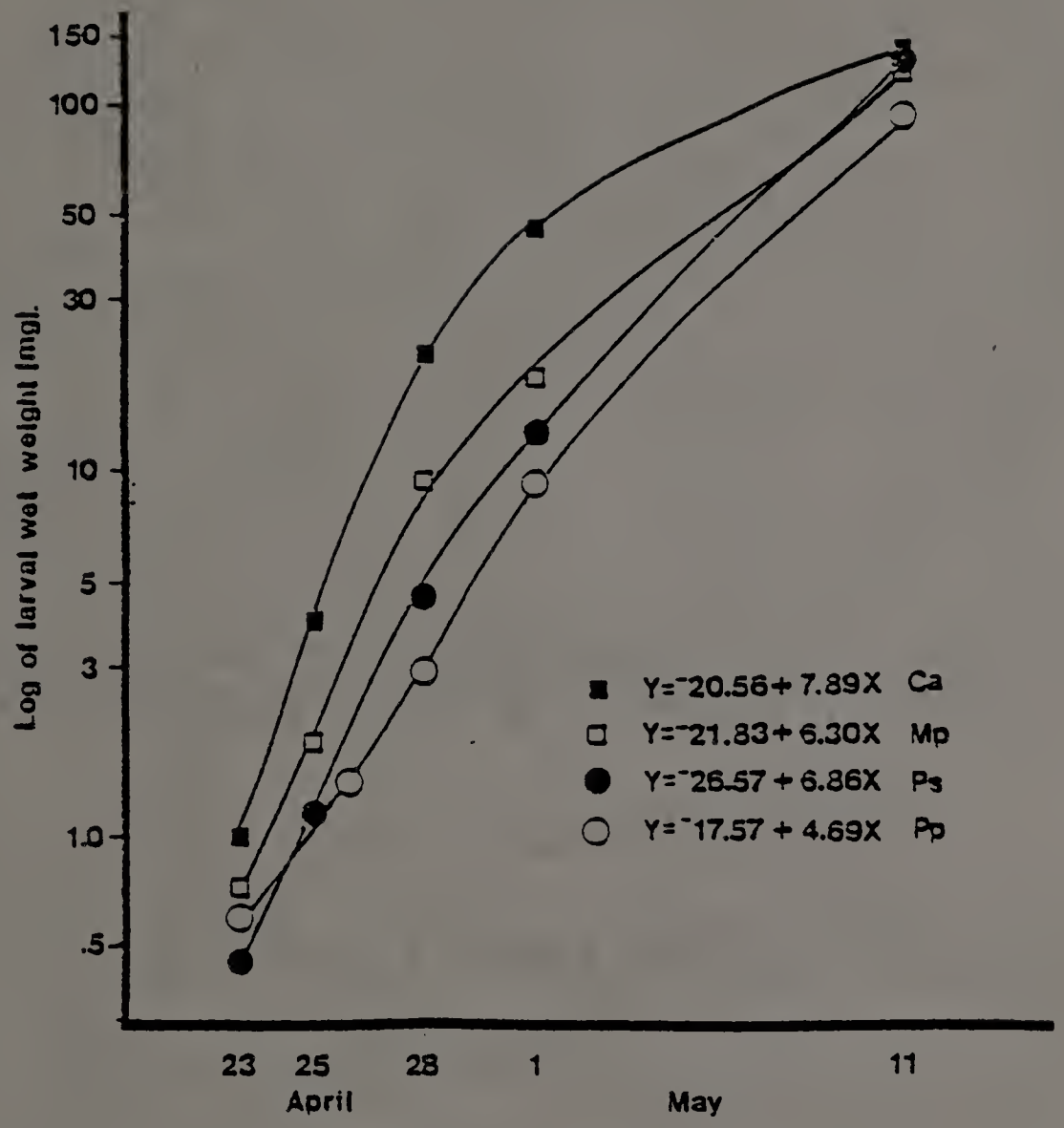

Fig. 7. Growth curves of the larvae of Malacosoma americanum (Fab.) reared on foliage of: Crabapple (Ca), apple (Mp), black cherry (Ps) or pin cherry (Pp), during the first 20 days after eclosion.

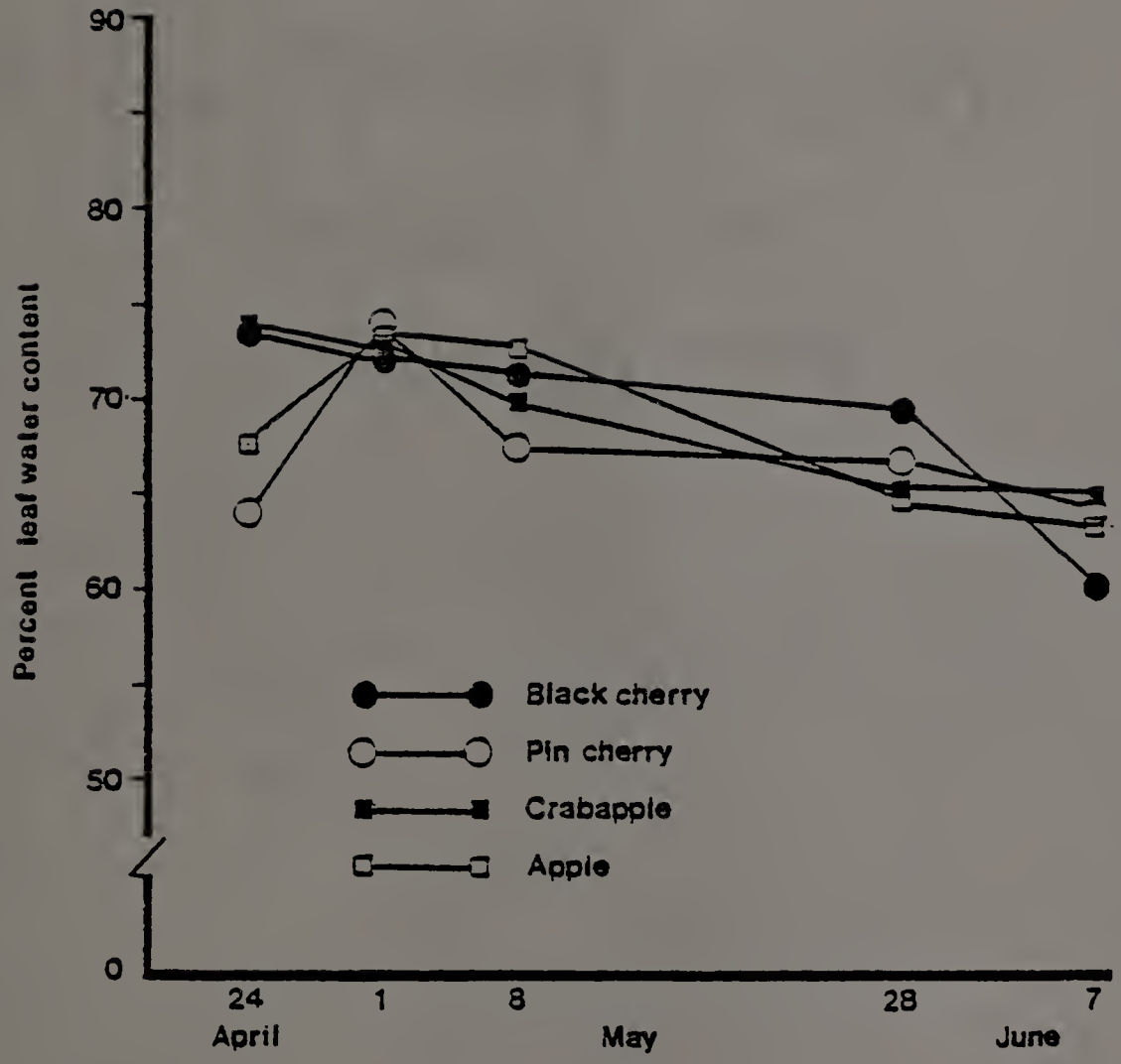

Fig. 8. Water content in the foliage of four host species during the growing period of Malacosoma americanum (Fab.).

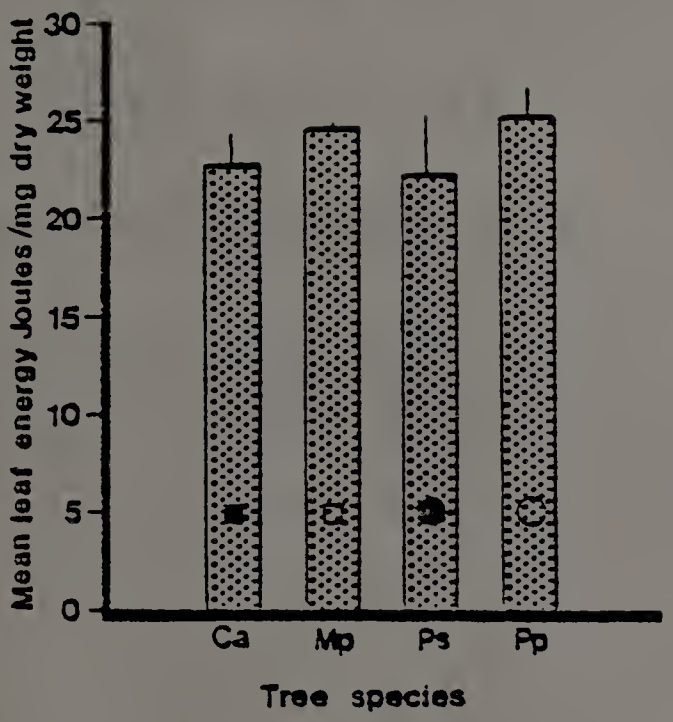

Fig. 9. Energy content in joules/mg dry wt. in the foliage of crabapple (Ca), apple (Mp), black cherry (Ps) and pin cherry (Pp) as to May 8, 1979. (1 joules= 4.183 cal).

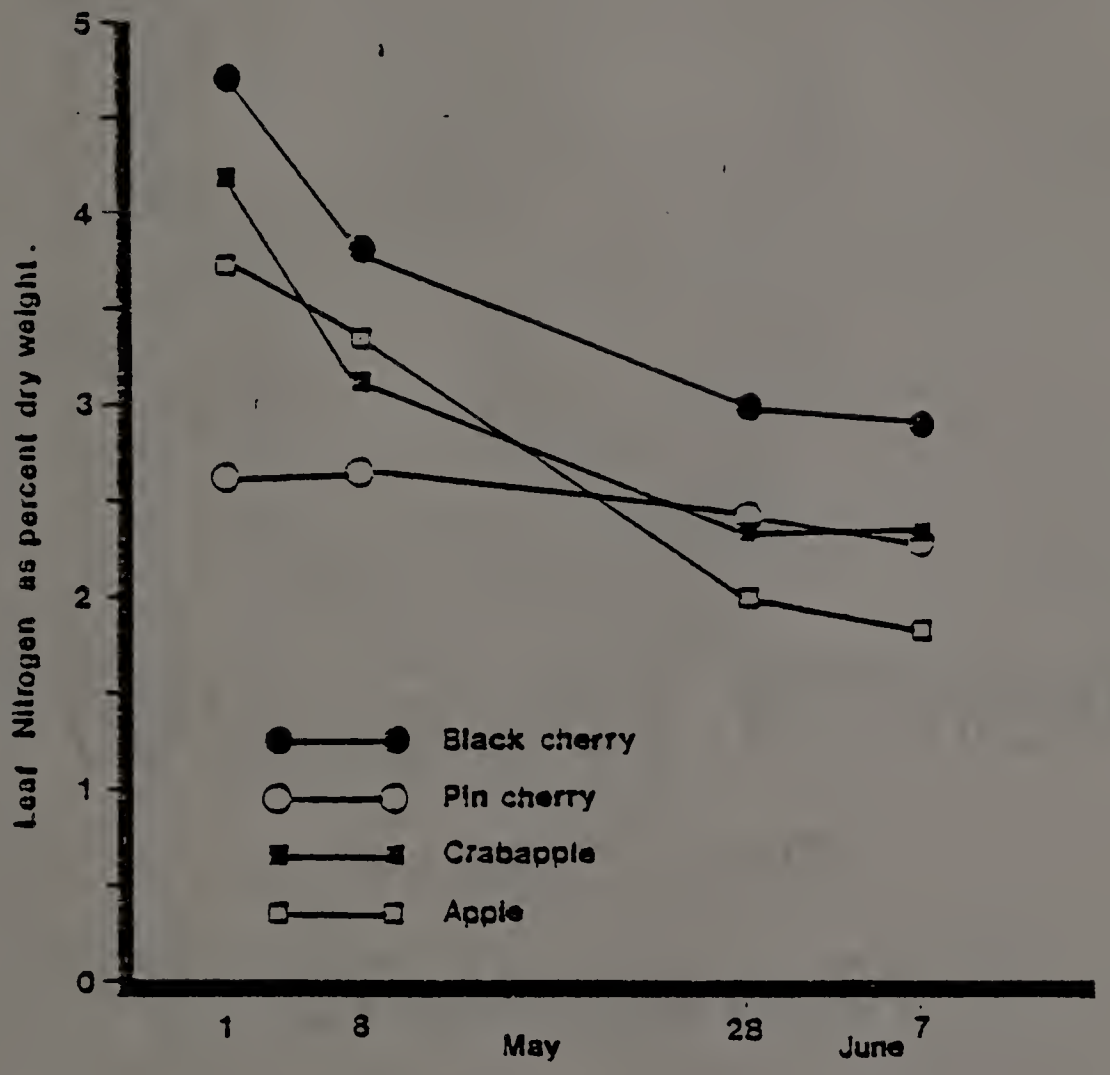

Fig. 10. Total nitrogen as percent dry wt. in the foliage of four host plant species during the growing season of Malacosoma americanum (Fab.).

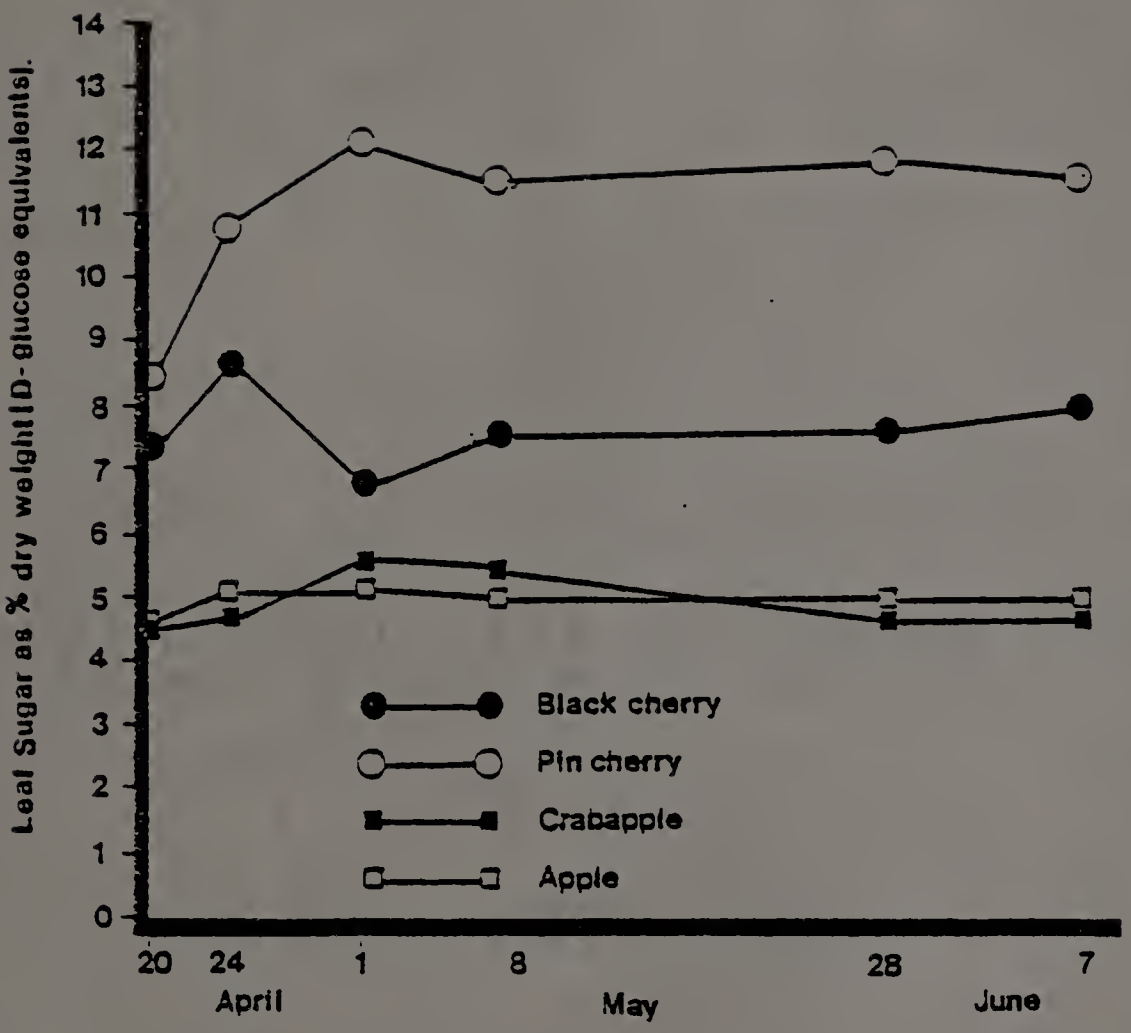

Fig. 11. Soluble leaf carbohydrate in the foliage of four plants during the growing period of Malacosoma americanum (Fab.).

Discussion

It was often very difficult to establish the proper relationship between developmental parameters in the ETC and the major nutritional components of their diets. Water concentration in the foliage of all the host plants tested was similar, hence not direct casual factors in developmental differences between cohorts. Energy content in the leaves of the different hosts do not vary significantly and were in reverse rank order of results on larval development. This agrees with the findings of Slansky and Feeny (1977), who observed the same on the developmental interactions in the cabbage butterfly, Pieris rapae. The total soluble sugar content, too is unlikely to be a limiting factor for larval growth and survival. For example, pin cherry maintained the highest carbohydrate levels, but its corresponding cohort grew slowest of all groups. Total nitrogen content may not by itself explain why the crabapple and the black cherry cohorts developed faster, had high survival and short larval growth periods, but may explain why the pin cherry cohort developed slowly and showed the highest mortality, since leaves fed to this cohort contained the lowest nitrogen concentrations.

It is important to point out the fact that "total nitrogen" comprises a large array of biochemical entities, which include such important cellular components as amino acids, amines and nitrogenated bases in addition to a number of less abundant substances.

The usefulness of this widely used parameter must be weighed with the above in mind. The total fatty acid content (or myristic acid conc.) could explain why the pupation time of the apple cohort was longer and might explain why the crabapple cohort developed faster, since its diet contained the highest fatty acid levels.

It becomes evident that not one single factor is by itself limiting to growth and development but that most likely such a phenomenon is responsive to a combination of factors. An alternative hypothesis is that growth and development are not solely linked to biochemical composition of host plants but reflect an adjustment to host, host habitat limitations or the influence of other biotic and abiotic forces. Thus in addition, finding out what biochemicals effect growth one may need to ask, What is the importance of faster development in Malacosoma americanum? It could be speculated that such a development would be of advantage if the build up of body materials help the larvae escape the effects of late spring frosts. This trend of differential mortality was in fact observed by Mansingh (1974) in the ETC, were the susceptibility of larval mortality due to chilling was inversely related with age (i.e. the instar). It could also be speculated that since the nutritional quality of the foliage decreases as the season progressed, it is advantageous to feed on a host were efficient assimilation could be achieved before the nutrient level falls from a level of minimum suitability for ETC.

In the field black cherry appears to be the preferred host plant species (Britton 1935; Sweetman 1940). Crabapple and apple

appear to be attacked to the same extent as black cherry, but this is yet to be corroborated with numerical data. The fact that the ETC does not seem to be more abundant may be due to the patchy distribution of its hosts which tend to be ornamental or orchard trees. Pin cherry on the other hand is seldom attacked under field conditions (personal observation; Waage, unpublished). It is interesting to point out that both Britton and Sweetman includes pin cherry in the preferred host list. These authors wrote their publications during a period when population outbreaks were evident. This author has observed that as the population levels of ETC soared into the outbreak of 1979 the number of egg masses of those found on the pin cherry also seemed to increase.

The reason why ETC do not exploit this host as often as it exploit others remains uncertain, particularly in light of our data on caloric, carbohydrate and fatty acid levels. In addition fecundity of females feeding on these species are comparable to those feeding on black cherry and apple. The limiting aspects of pin cherry utilization, however could include the fact that survival on pin cherry is low. This is perhaps related to the long development time of ETC on this host which may expose them to additional mortality factors. In our attemps to artificially infect pin cherry trees, the resulting tents were loosely constructed when compared with those of black cherry colonies. This could reflect the nutritional condition and/or delayed development of the larvae and could be detrimental to the extent that the tent is utilized as refugia from environmental stresses and predators.

C H A P T E R I V

THE EFFECTS OF PARENTAL DIET ON THE CRYOPROTECTANTS OF MALACOSOMA AMERICANUM (FAB.) PROGENY

Summary

1. This paper examines the influence of parental diet on the levels of carbohydrate yolk, sugars and cryoprotectant in the overwintering stage of the eastern tent caterpillar.

2. No significant differences were found in cryoprotectant levels among the different host cohorts tested.

3. In the light of these findings, factors subject to natural selection are suggested.

Introduction

The accumulation of glycerol and other cryoprotectants in the bodies of temperate diapausing insects is a phenomenon reported by many investigators (e.g. Salt 1957, 1959; Wyatt and Meyer 1959; Chino 1957, 1958; Baust and Miller 1972; Sømme 1963). Initial evidence of the role of glycerol as an antifreeze in cryobiological systems came from "in vitro" experiments with human erythrocites (Lovelock 1953). These experiments concluded that the cryoprotective effect of glycerol is due to its osmotic properties, which reduce the fraction of frozen water in the system by colligative means (Zachariansen 1979).

Evidence for the advantages of having greater glycerol concentrations for cold hardiness in Malacosoma americanum (Fab.) was presented by Mansingh (1964), where an increase in the ability to

supercool was directly related to an increase in the glycerol concentrations. This relation has been shown in other insects (Sømme 1969).

The qualitative effects of parental diet on progeny characteristics can be influential factors on the quality of the population as a whole. For example, gypsy moths reared on red maple foliage showed reduced fecundity and egg size as compared with red oak fed individuals (Capinera and Barbosa 1977). In addition, large eggs were found to contain significantly lower protein yolk concentration when compared with small eggs (Capinera et al.1977).

In the light of such results it was sought to ascertain the effect of maternal diet on the levels of carbohydrate yolk, sugars and glycerol in the "pharate" larvae (overwintering stage) of M. americanum (Fab.).

Materials and Methods

Tent caterpillar egg masses were collected from the vicinity of Amherst, Mass., in March 1979, from black cherry branches. These masses were kept at 5°C until field hatching was observed. They were surface sterilized with 5% sodium hypochlorite. The caterpillars were reared in unwaxed 8 oz cups, 10 larvae/cup and fed with fresh leaves daily. The adults were mated in screened cages (3 x 3 x 4ft), the egg masses retrieved and stored at 25°C for 50 days, at 10°C for 25 days, and at 0°C for 65 days. Three host-cohorts, namely: black cherry (Prunus serotina Ehrh.), pin

cherry (Prunus pensylvanica L.) and apple (Malus pumila L.) were reared as described. A fourth host-cohort, crabapple (Malus sp.) was not laboratory reared, instead it was collected from the field in their last larval instar, mating and egg mass harvesting were accomplished as described above.

Two replicates of 4 egg masses per host-cohort were homogenized in 70% ethanol, in a glass-teflon homogenizer under ice water. The homogenate was centrifuged at 500 g for 5 min, the supernatant collected and evaporated under nitrogen gas at 70°C. Aliquots of this extract were applied on silica gel H (20 x 20 cm) glass plates 250 um thick, and developed in a 9:6:3:1 (n-butanol-glacial acetic acid - ethyl ether - water) solvent system. All compounds were visualized spraying half of the plate with 0.5% (w/v) $KMnO_4$ in 1N NaOH (Briggs et al. 1956). The unsprayed "spots" were collected with aspirators, suspended in distilled water, and the silica removed by centrifugation. Sugar concentrations were determined using the phenol sulphuric acid reagent (Dubois et al. 1956); glycerol was determined using Bok and Demain (1977) polyol determination method using metaperiodate reaction and Nash reagent for formaldehyde quantification. Samples were taken at 32, 58, 85, 106 and 140 days after oviposition. Glycogen was also quantified at day 20. Due to the asynchronous nature of oviposition, the day when 50% of the egg masses were obtained is arbitrarily chosen as day 0. For detailed procedures see Appendix 1.

Results

This experiment was performed in 1978 and repeated in 1979. The sugar concentrations obtained in 1978 were significantly lower than those of 1979. In 1979, using more sensitive techniques, sorbitol was identified in the carbohydrate compliment of the egg masses. This polyol was not previously described by Mansingh (1974). The difficulty of separating sorbitol from glucose due to very close R_f values (R_f values - sorbitol = 0.30, glucose = 0.32) and interference of glucose with the metaperiodate oxidation of sorbitol, did not enable us to determine sorbitol concentration.

No significant differences ($p < 0.05$) were found in the glycerol concentrations of the different host cohorts (Fig.12). The pin cherry cohort is noteworthy because of its low rate of glycerol production during the first 85 days of treatment. All cohorts responded similarly to the decrease in temperature, i.e., significant increases in the glycerol levels ($p < 0.01$).

Glycogen concentrations on day 20 were significantly higher in the pin cherry cohort ($p < 0.05$) compared with the remaining cohorts (Fig.13). This difference, however, was not maintained as the experiment progressed. All cohorts showed steady decreases in glycogen concentration (Fig.13).

Trehalose and glucose concentrations followed the decreasing pattern exhibited by glycogen (Figs.14 & 15), no significant differences in their concentrations were observed between the cohorts.

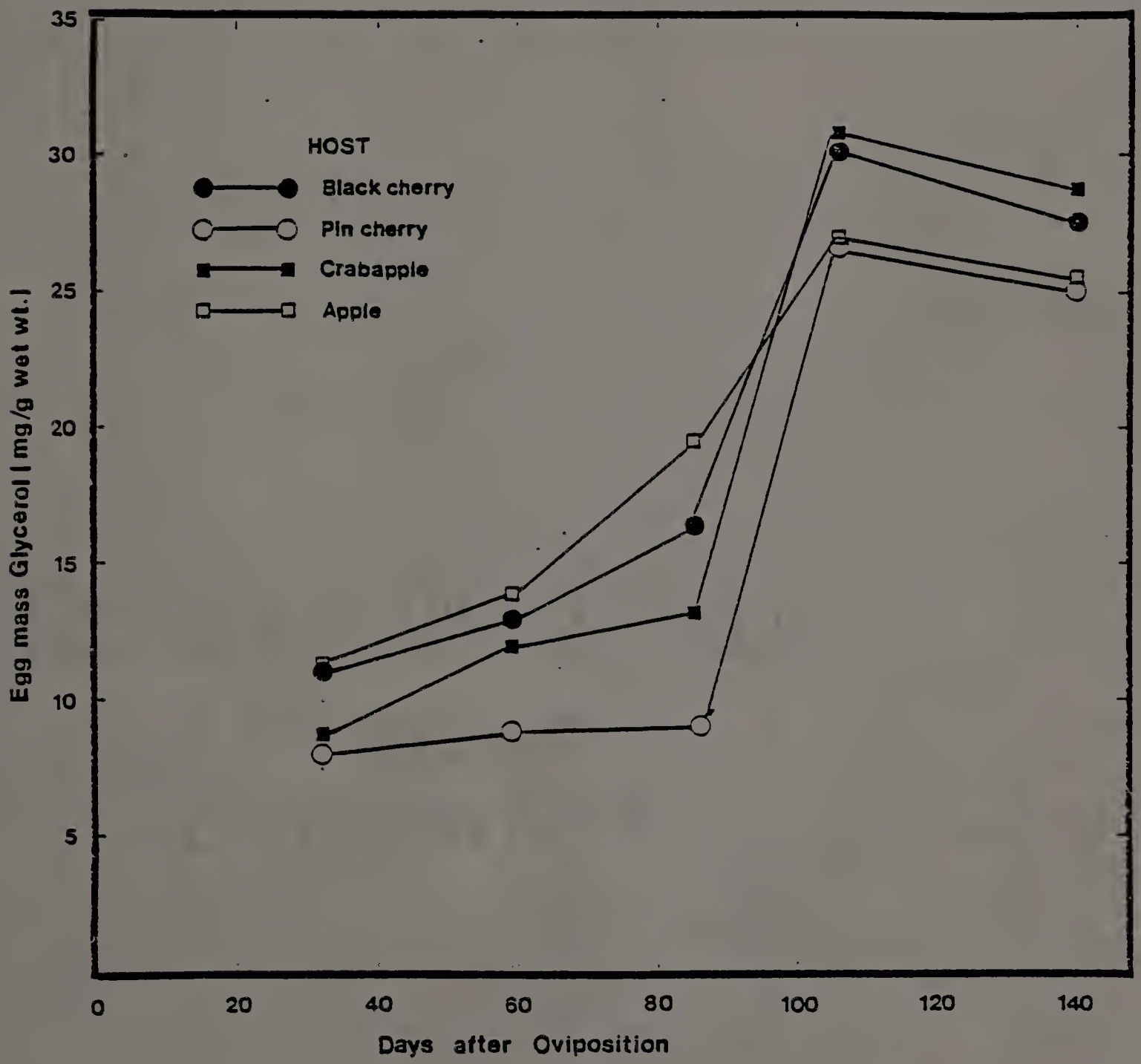

Fig. 12. Glycerol concentrations in eggs produced by Malacosoma americanum (Fab.) females reared on various host plants.

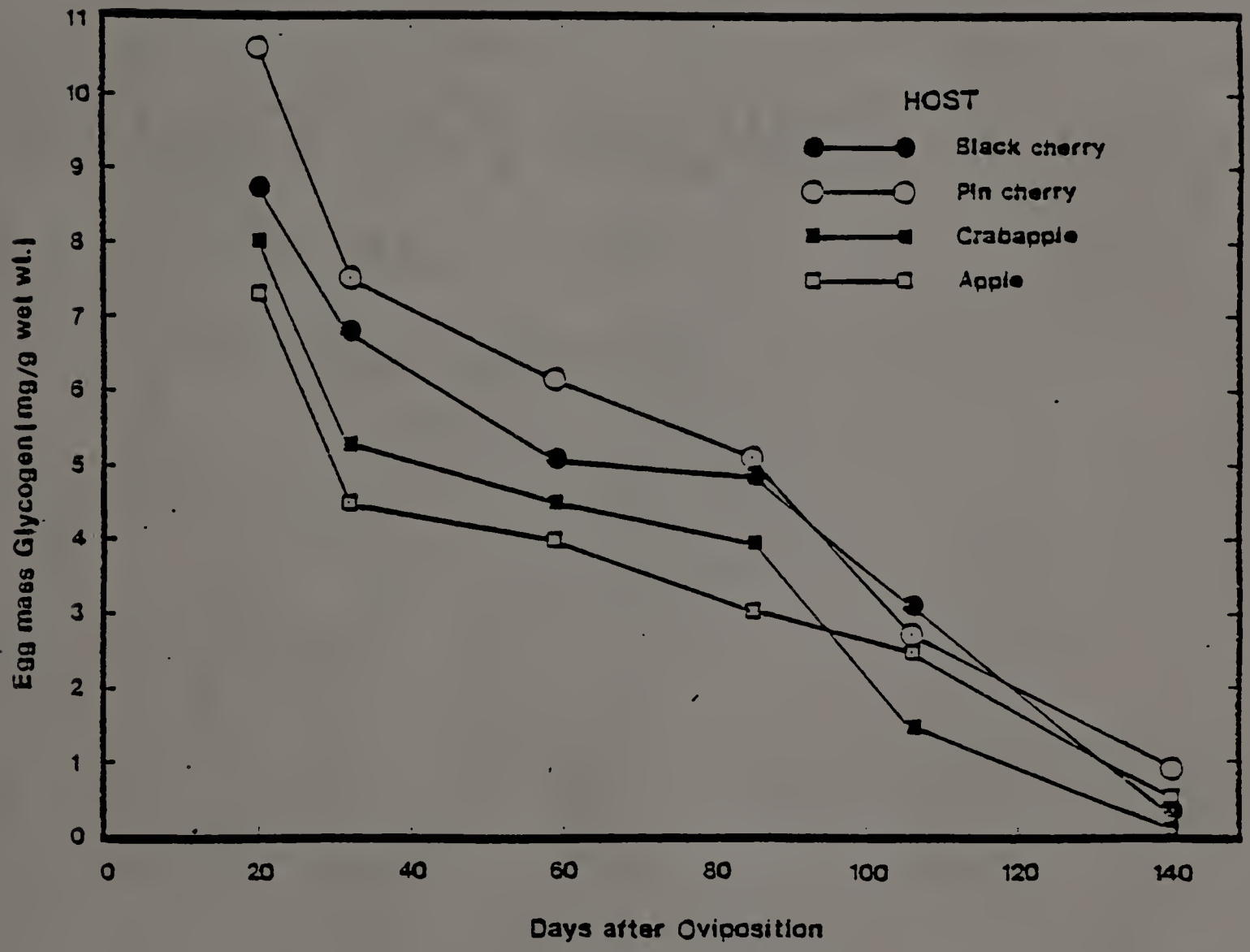

Fig. 13. Glycogen concentrations in eggs produced by Malacosoma americanum (Fab.) females reared on various host plants.

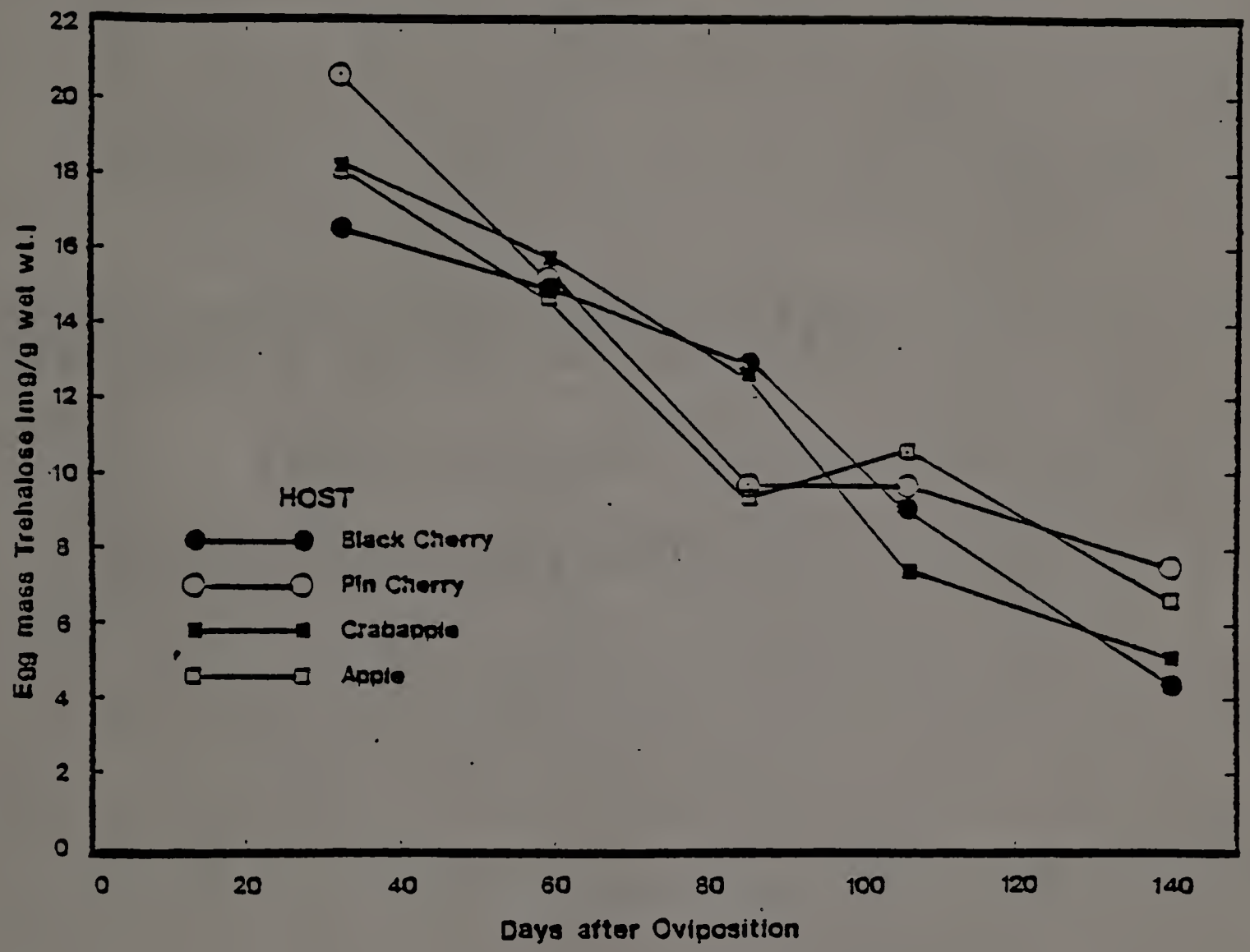

Fig. 14. Trehalose concentrations in eggs produced by Malacosoma americanum (Fab.) females reared on various host plants.

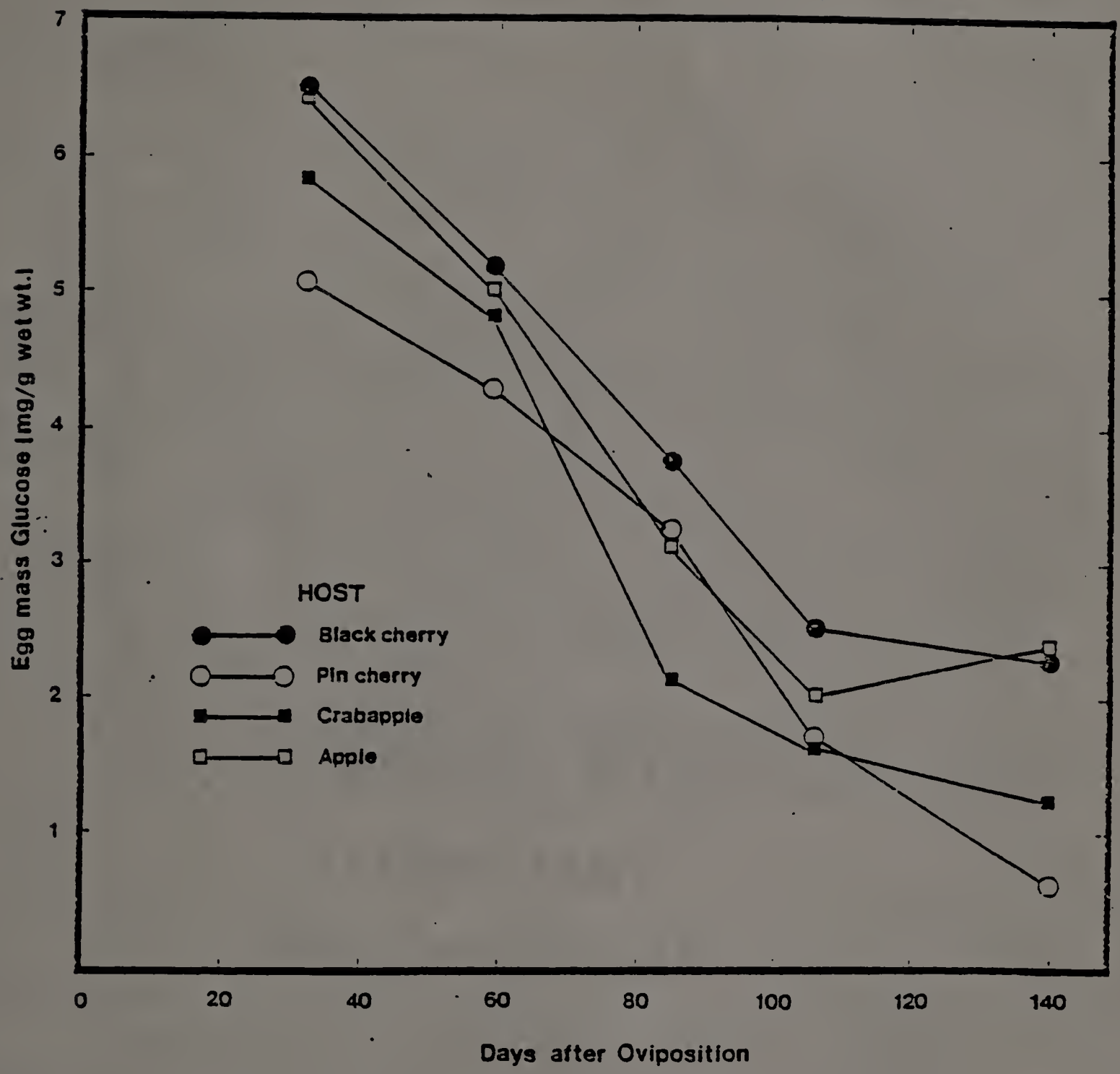

Fig. 15. Glucose concentrations in eggs produced by Malacosoma americanum (Fab.) females reared on various host plants.

Discussion

Unusually cold weather, is the most important mortality factor affecting Malacosoma "pharate" larvae (Witter and Kulman 1971; Blais et al. 1955; Tomlinson 1938). Stacey et al. (1975) found 11.6% mortality of "pharate" larvae among Arkansas M. americanum, while Witter and Kulman (1971) found up to 38% mortality, with significant variation from year to year. However, most of the investigations dealing with this mortality factor, suggest that mortality occurs after diapause is terminated and glycerol concentrations are below protective thresholds. Our experiments have demonstrated that levels of cryoprotectants are not host plant dependent. The fact that glycerol concentration in diapausing larvae is not affected by parental diet can be construed to suggest that selection for this trait, in a given geographical region, is probably a function of parental genotypes and selective mortality. Hanec (1966) states that supercooling points during diapause in M. disstria are well below the natural temperature range in a given area. Thus, other aspects subject to selection might be: the time of diapause termination and the rate of glycerol convertion back to glycogen in the post diapause larvae. In New England, larvae of M. americanum eclose while host plant buds are closed. Thus, the relationship between the above mentioned aspects of diapause and the relative nutritional value of leaves or buds becomes the next issues of importance in the life history of ETC. Further research is needed on the evolutionary

balancing act between the temperature related mortality associated with early diapause termination compared to synchronization of egg hatch with bud break.

List of References

Allen,D.C. 1973. Fecundity of the saddled prominent, Heterocampa guttivitta. Ann. Ent. Soc. Am. 66:1181-1183.

Baust,J.G. and L.K. Miller. 1972. Influence of low temperature acclimatation on cold hardiness in the beetle, Pterostichus brevicornis. J. Insect Physiol. 18:1935-1947.

Blais,J.R., R.M. Prentice, W.L. Sippel and D.R. Wallace. 1955. Effects of weather on the forest tent caterpillar Malacosoma disstria Hbn., in central Canada in the spring of 1953. Can. Ent. 87:1-8.

Blackman,M.W. 1918. Apple tent caterpillar. J. Econ. Ent. 11:432-433.

Bok,S.H. and A.L. Demain. 1977. An improved colorimetric assay for polyols. Anal. Biochem. 81:18-20.

Briggs,D.R., E.F. Garner and F. Smith. 1956. Separation of carbohydrates by electrophoresis on glass filter paper. Nature 178:154-155.

Britton,W.E. 1935. The eastern tent caterpillar. Conn. Agric. Exp. Sta. Bull. 378:65-82.

Calvert,W. 1979. Personal communication. Dept. of Biology, Amherst College, Amherst, Mass.

Capinera,J.L. and P. Barbosa. 1977. Influence of natural diets and larval density on gypsy moth, Lymantria dispar (Lepidoptera:Orgyiidae), egg mass characteristic. Can. Ent. 109:1313-1318.

----------, P. Barbosa and H.H. Hagedorn. 1977. Yolk and yolk depletion of gypsy moth eggs: Implications for population quality. Ann. Ent. Soc. Am. 70:40-42.

Chino,H. 1957. Conversion of glycogen to sorbitol and glycerol in the diapause egg of the Bombyx silkworm. Nature 180:606-607.

-------. 1958. Carbohydrate metabolism in the diapause egg of the silkworm, Bombyx mori-II. Conversion of glycogen into sorbitol and glycerol during diapause. J. Insect Physiol. 2:1-12.

Clark,E.C. 1956. Survival and transmission of a virus causing polyhedrosis in Malacosoma fragile. Ecol. 37:728-732.

Danks,H.V. 1978. Modes of seasonal adaptation in insects. I. Winter survival. Can. Ent. 110:1167-1205.

Dubois,M., K.A. Gillis, J.K. Hamilton, P.A. Rebers and R. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-352.

Ebell,L.F. 1969. Variation in total soluble sugars of conifer tissue with method of analysis. Phytochemistry 8:227-233.

Fitzgerald,T.D. 1976. Trail marking larvae of the eastern tent caterpillar. Science. 194:961-963.

----------, and J.S. Edgerly. 1979. Specificity of trail markers of the forest and eastern tent caterpillar. J. Chem. Ecol. 5:565-574.

Hanec,W. 1966. Cold-hardiness in the forest tent caterpillar, Malacosoma disstria Hbn. (Lepidoptera:Lasiocampidae) J. Insect Physiol. 12:1443-1449.

Harvey,R.B. 1923. Cambial temperatures of trees in winter and their relation to sun scald. Ecology 4:261-265.

Headlee,T.J. 1934. Cycles of abundance of the eastern tent caterpillar. (Malacosoma americana F.) N.J. Agric. Exp. Sta. Bull. 579:1-2.

Hodson,A.C. 1941. An ecological study of the forest tent caterpillar, Malacosoma disstria Hubner in northern Minnesota. Minn. Agr. Exp.Sta. Tech. Bull. 148. 60p.

----------. 1937. Some aspects of the role of water in insect hibernation. Ecological Monogr. 7:271-315.

----------, and C.J. Weinman. 1945. Factors affecting recovery from diapause and hatching of eggs of the forest tent caterpillar, Malacosoma disstria Hbn. Univ. Minn. Agric. Exp. Sta. Tech. Bull. 170: 31pp; illus.

Holmquist,A.M. 1931. Studies of arthropod hibernation. III. Temperatures in the forest hibernacula. Ecology 12:387-400.

Hough,J.A. and D. Pimentel. 1978. Influence of host foliage on development, survival and fecundity of the gypsy moth. Env. Ent. 7:97-102.

Ives,W.G.H. 1973. Heat units and outbreaks of the forest tent caterpillar, Malacosoma disstria (Lepidoptera:Lasiocampidae). Can. Ent. 105:529-543.

Iwao,S. 1970. Analysis of contagiouness in the action of mortality factors on the western tent caterpillar by using $\hat{m}$-m relationship. Res. Pop. Ecol. 12:100-110.

Jensen,R.E., E.F. Savage, and R.A. Hayden. 1970. The effect of certain environmental factors on the cambium temperatures of peach trees. J. Am. Hort. Sci. 95:286-292.

Kulagin,N. 1897. Zur Biologie Ocneria dispar in Russland. Illust. Wschr. Ent. 2:418-420. (Original text not seen).

Lorimer,N. and W.J. Mattson. 1979. Prediapause hatching of the forest tent caterpillar (Lepidoptera:Lasiocampidae) in the laboratory. Great lakes Ent. 12:119-122.

Lovelock,J.E. 1953. The mechanism of the cryoprotective effect of glycerol against hemolysis by freezing and thawing. Biochim. biophys. Acta 11:28-36.

MacPhee,A.W. 1964. Cold-hardiness, habitat and winter survival of some orchard arthropods in Nova Scotia. Can. Ent. 96:617-625.

Mansingh,A. 1974. Studies on insect dormancy. II. Relationship of cold-hardiness to diapause and quiscence in the eastern tent caterpillar, Malacosoma americanum (Fab.) (Lasiocampidae: Lepidoptera). Can. J. Zool. 52:629-637.

Mattson,J.M. and G.W. Ericson. 1978. Degree-day summation and hatching of the forest tent caterpillar, Malacosoma disstria (Lepidoptera:Lasiocampidae). Great Lakes Ent. 11:59-61.

Metcalfe,L.D., A.A. Schmitz and J.R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for Chromatographic Analysis. Anal. Chem. 38:514-515.

Morris,R.F. and W.C. Fulton. 1970. Model for the development and survival of Hyphantria cunea in relation to temperature and humidity. Mem. Ent. Soc. Can. no.70. 60p.

McKenzie,H.A. and H.S. Wallace. 1954. The Kjeldahl determination of nitrogen: a critical study of digestion-temperature, catalyst and oxidizing agent. Aust. J. Chem. 7:55-70.

Nordin,G.L. 1976. Influence of natural Nosema sp. infections on field populations of Malacosoma americanum (Lepidoptera: Lasiocampidae). J. Kansas Ent. Soc. 49:32-40.

Ohnishi,E., S. Takahashi and H. Sonobe. 1968. Crystals from cocoons of Malacosoma neustria testacea. Science 160:783-784.

Phillipson,J. 1964. A miniature bomb calorimeter for small biological samples. Oikos 15:130-139.

Rosenberg,N.J. 1974. Microclimate: the biological environment. John Wiley & Sons, New York, N.Y. 315p.

Rudolfs,W. 1926. Studies on the chemical changes during the life cycles of the tent caterpillar (Malacosoma americana Fab.) I. J. N.Y. Ent. Soc. 34:249-257.

---------. 1927. Studies on the chemical changes during the life cycle of the tent caterpillar (Malacosoma americana Fab.) III. Soluble ash and sulphates. J. N.Y. Ent. Soc. 35:219-229.

---------. 1929. Studies on the chemical changes during the life cycle of the tent caterpillar (Malacosoma americana Fab.) V. Weight and Data. J. N.Y. Ent. Soc. 40:481-489.

Salt,R.W. 1957. Natural occurence of glycerol in insects and its relation to their ability to survive freezing. Can. Ent. 89: 491-494.

--------. 1959. Role of glycerol in the cold-hardening of Bracon cephi (Gahan). Can. J. Zool. 37:59-69.

Schaefer,M. 1976. An analysis of diapause and resistance in the egg stage of Floridia bucculenta (Araneida:Linyphiidae). Oecologia (Berl.) 25:155-174.

Scriber,J.M. 1977. Limiting effects of low leaf water content on the nitrogen utilization, energy budget and larval growth of Hyalophora cecropia (Lepidoptera:Saturniidae). Oecologia 28: 269-287.

Slansky,F. Jr. and P. Feeny. 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated plants. Ecol. Monogr. 47:209-228.

Smirnoff,W.A. 1975. Histological studies on the development of the microsporidian Thelohania pristiphorae in the larvae of Malacosoma disstria and Malacosoma americanum. J. Invert. Pathol. 26:401-403.

Sømme,L. 1963. Effects of glycerol on cold-hardiness in insects. Can. J. Zool. 42:87-101.

-------. 1969. Mannitol and glycerol in overwintering aphid eggs. Norsk. ent. Tidsskr. 21:131.134.

Soo Hoo,C.F. and G. Fraenkel. 1966. The consumption, digestion,

and utilization of food plants by a polyphagous insect, Prodenia eridania (Cramer). J. Insect Physiol. 12:711-730.

Stacey,L., R. Roe, and K. Williams. 1975. Mortality of eggs and pharate larvae of the eastern tent caterpillar, Malacosoma americana (F.) (Lepidoptera:Lasiocampidae). J. Kansas Ent. Soc. 48:521-523.

Stehr,W.F. and E.F. Cook. 1968. A revision of the genus Malacosoma (Hubner) in North America (Lepidoptera:Lasiocampidae): Systematic , biology, immatures and parasites. U.S. National Museum Bull. 276. 321p.

Sweetman,H.L. 1940. The value of hand control for the eastern tent caterpillars, Malacosoma americana (Fab.) and Malacosoma disstria (Hbn.) (Lepidoptera:Lasiocampidae). Can. Ent. 72: 245-250.

Tomlinson,W.E. 1938. Fluctuations in the tent caterpillar abundance and some of the factors influencing it. Masters Thesis (unpublished) Library of the Univ. of Mass., Amherst, Mass. 43p.

Wagner,T.L. and D.E. Leonard. 1979. The effects of parental and progeny diet on development, weight gain, and survival of prediapause larvae of the satin moth, Leucoma salicis (Lepidoptera:Lymantridae). Can. Ent. 111:721-729.

Wellington,W.G. 1950. Effects of radiation on the temperatures of insects and habitats. Sci. Agri. 30:209-234.

Wigglesworth,V.B. 1953. The principles of insect physiology. Methuen and Co., Ltd., London. 546p.

Williams, J.L. 1939. The mating and egg laying of Malacosoma americana. Ent. News. 50:45-50.

Witter.J.A. and H.M. Kulman. 1972 a. Mortality factors affecting eggs of the forest tent caterpillar, Malacosoma disstria (Lepidoptera:Lasiocampidae). Can. Ent. 104:705-710.

----------. 1972 b. A review of the parasites and predators of tent caterpillars.(Malacosoma spp.) in North America. Minn. Agri. Exp. Sta. Tech. Bull. 289, 48pp.

Witter,J.A., H.M. Kulman and A.C. Hodson. 1972. Life tables for the forest tent caterpillar. Ann. Ent. Soc. Am. 65:25-31.

Wyatt,G.R. and W.L. Meyer. 1959. The chemistry of insect hemolymph. III. Glycerol. J. Gen. Physiol. 42:1005-1011.

Zachariassen, K.E. 1979. The mechanism of the cryoprotective effect of glycerol in beetles tolerant to freezing. J. Insect Physiol. 25:29-32.

APPENDIX

CHEMICAL ANALYSIS PROTOCOL

Protocol for carbohydrate isolation

(Haruo Chino.1958. J.Insect Physiol. 2:1-12. Modified.)

1. Homogenize 100mg of eggs in 4ml of 80% Ethanol.
2. Collect homogenate and wash homogenation tube with 4ml of 80% Ethanol. Add this portion to rest of homogenate.
3. Centrifuge homogenate at 2000g for 5 min.
4. Collect supernatant and resuspend the precipitate in 2ml of 80% Ethanol.
5. Centrifuge the suspension at 2000g for 5 min. Reunite both supernatants.
6. Dry in rotary evaporator at 55°C until residue is less than 0.1ml..
7. Resuspend in 3ml 0.1% Benzoic acid solution.
8. Store at 0°C.

Protocol for carbohydrate quantification

Thin-layer chromatography:

1. Place 10ul of sample on a previously developed and activated plate.
2. Run plate in a n-butanol: acetic acid: ethyl-ether: water (9:6:3:1) solvent system, in a saturated chamber.
3. Develop spots with 0.5% $KMnO_4$ in N NaOH.
4. Elute unsprayed spots in 3ml distilled water.
5. Centrifuge at 2000g for 5 min. to remove silica.

Trehalose and glucose determination with the Phenol-Sulphuric acid Reagent. (Dubois et al. 1956):

1. Add 1ml of 5% Phenol to 1ml of sample. Mix well.
2. Add 5ml of H_2SO_4 and carefully mix.
3. Allow test tubes to cool for 20 min.
4. Read absorbance at 490mu.

Determination of glycerol:
(Song Hae Bok and A.L. Demain.1977. Anal. Biochem. 81:18-20)

1. Mix 1ml of sample and 1ml of 0.015M $NaIO_4$ in 0.12M HCL. Allow 10 min. at room temperature.
2. Add 2ml of 0.1% Rhamnose (to remove excess periodate).
3. Add 4ml of Nash reagent and develop color for 15 min. at 53°C.
4. Measure absorbance at 412mu.

Nash Reagent:
a) 75g Ammonium Acetate.
b) 2ml Glacial Acetic Acid.
c) 2ml pentane-2,4-dione.
d) Bring total volume to 1l with dist. water.

Protocol for glycogen isolation

1. Homogenize 100mg of egg masses in 1ml of saline (0.7%).
2. Add 2ml of 5% Trichloroacetic acid (TCA). Mix well and let stand for 10 min.
3. Transfer and centrifuge at 7000g for 10 min. (This tube should be weighed).
4. Collect supernatant and resuspend pellet in 2ml TCA 5%.
5. Centrifuge at 7000g for 10 min. Reunite supernatants.
6. While stirring add 8ml of 95% Ethanol, allow to stand for 15 min.
7. Sediment the precipitate by centrifuging at 1000g for 5 min., discard supernatant.
8. Dissolve the precipitate in 5ml of dist. water and reprecipitate with 95% Ethanol.
9. Centrifuge and discard supernatant.
10. Dry in oven at 60°C. Weigh dried tube.

Protocol for glycogen quantification

1. Dissolve sample in 2ml of dist. water.
2. Mix 1ml of sample and 4ml Anthrone reagent. (1g of anthrone in 500ml of conc. H_2SO_4).
3. Develop color at 100°C for 10 min.
4. Allow to cool for 20 min.
5. Measure absorbance at 620mu.

Protocol for lipid isolation

1. Three previously weighed egg masses are homogenized in a 2:1 (v/v) mixture of chloroform-methanol using a Potter-Elvehjem type homogenizer.
2. The homogenate is filtered through a fat-free filter paper and the homogenization tube washed with one ml of the chloroform-methanol mixture.
3. The extract is then thoroughly mixed with 1ml of a normal saline solution (0.7% NaCl) to form a biphasic system. The upper phase is discarded by siphoning. This procedure is repeated as a washing step.
4. The extracted lower phase is then evaporated to be saponified. ($60^{o}C$)

Saponification and Esterification of the non-polar fraction

1. The residue of evaporation is mixed with 3ml of 0.5N KOH in methanol and boiled at $100^{o}C$ for 5 min.
2. Once saponification is completed, 4ml of BF_3-methanol are added. This solution should be boiled for 2 min. Esterification should be completed at this point.
3. Transfer the contents of this test tube to a separatory funnel. Add 2ml of saturated NaCl and 3ml of Hexane in order to form a biphasic system.
4. Remove the hexane phase and evaporate the same at $60^{o}C$ in evapomix.
5. After evaporation is complete, add 2ml of hexane to the residue, mix thoroughly, and add a small BHT crystal.
6. Storage of methyl esters in teflon capped vials at $-20^{o}C$ is recommended.