LABORATORY REARING OF THE NANTUCKET PINE TIP MOTH, *RHYACIONIA FRUSTRANA* (COMSTOCK), ON ARTIFICIAL DIETS

By

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PREFACE

This study is concerned with rearing the Nantucket pine tip moth larvae to the pupal stage on artificial diets in the laboratory. A successful technique in mating the male and female moths in captivity is necessary before the Nantucket pine tip moth can be mass reared in the laboratory.

Sincere appreciation is extended to my committee members, Dr. R. D. Eikenbary, Dr. R. R. Walton, and T. H. Silker, for their advice and criticism of this manuscript.

I would like to express my appreciation to my wife, Jan, for her encouragement and understanding during the preparation of this thesis.

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INTRODUCTION

The Nantucket pine tip moth, *Rhyacionia frustrana* (Comst.), infests most native pines of Eastern and Southeastern United States. Of the four major southern pines, only shortleaf and loblolly are seriously damaged by the tip moth (Yates 1966).

The larvae bore into, and feed on, the growing shoots of young pine trees. In boring, the larva severs the conductive tissue and causes death of the shoot. The death of the tip may cause damage in four ways: deformation of the tree, loss or retardation of height growth, reduction of cone crops, and sometimes mortality of infested trees (Yates 1960). Coyne (1968) found that if shortleaf pine seedlings were infested more than one year in a row the size of the roots was reduced by 50% or more.

There are disagreements by researchers as to the degree of damage the Nantucket pine tip moth inflicts. Some researchers contend that damage is transitory, while others say that damage is permanent. Korstian and Maughan (1935) found that the rotation age of trees infested by the Nantucket pine tip moth was increased by five years.

Further research of the Nantucket pine tip moth would be enhanced if a constant supply of the moths were available throughout the year.

The purpose of this study was to develop an artificial diet able to sustain the moths from the egg to the pupal stage within the laboratory. Although artificial diets are the first step in rearing insects, a successful technique in mating the captive insects is necessary before a complete generation can be reared in the laboratory.

REVIEW OF LITERATURE

There has been very little published work on artificial diets or nutritional requirements for *Rhyacionia*, the genus of the Nantucket pine tip moth. Most of the work published on artificial diets of Lepidopterous larvae has been in genera other than *Rhyacionia*.

Developing an Artificial Diet

Bailey (1964) has made an excellent review on the history of the development of artificial diets. The literature reviewed here will pertain more to information used particularly in this study.

Adkisson et al. (1960) developed a wheat germ diet for the pink bollworm, *Pectinophora gossypiella* (Saund.). This diet produced normal growth of the pink bollworm larvae and had an average of 81.5% adult emergence. Vanderzant et al. (1962) modified Adkisson's wheat germ diet to rear the larvae of the bollworm, *Helothis zea* (Boddie). Sodium alginate was omitted and ascorbic acid was added to the diet for normal growth of the bollworm larvae. Berger (1963) used a slight modification of Vanderzant's diet in rearing the bollworm along with rearing the tobacco budworm, *Heliothis virescens* (Fabricius).

The spruce budworm, *Choristoneura fumiferana* (Clem.), was successfully reared in the laboratory using Berger's wheat germ diet (McMorran 1965). Previously the spruce budworm had been reared in the laboratory on balsam fir buds with an adult emergence of 54.9%. The adult emergence on the artificial wheat germ diet was 75%.

Brinton et al. (1969) reared the codling moth, *Carpocapsa pomonella* (L.), on an artificial diet which was a modification of a wheat germ diet used by Ignoffo (1963) for rearing the cabbage looper, *Trichoplusia ni* (Hubner). Wood sawdust, wheat flour, and wood pulp replaced the agar as a binding medium because of the relatively high cost of agar. Brinton obtained a 52% adult emergence and concluded that the yield could have been improved if more of the neonatal larvae could have been induced to enter the medium. Spraying the surface of the medium with volatile extracts from apples did not induce feeding. Baking the surface of the medium after scarification did stimulate larval entry into the medium.

The forest tent caterpillar, *Malacosoma disstria* (Hubner), was reared in the laboratory on an artificial wheat germ diet (Addy 1969). The diet was a modification of a diet used by Chippendale et al. (1965) to rear the cabbage looper. Addy obtained 80% adult emergence and attributed the mortality to an inadequate removal of excess fecal material from the rearing containers.

Schroeder et al. (1969) successfully reared the hickory shuckworm, *Laspeyresia caryana* (Fitch), through seven generations on a pinto bean diet. The bean diet was a modification of a diet used by Shorey and Hale (1965) in rearing nine species of the noctuid family. The adult emergence of the hickory shuckworm ranged from 71 to 85% for the seven generations. Schroeder noted that in the first five generations it took less time for male larvae to develop than female larvae.

The bluegrass webworm, *Crambus teterrellus* (Zincken), was reared in the laboratory by Ward (1969). The diet was a modification of Adkisson's wheat germ diet. Ward obtained 40.8% pupation and 35.8%

adult emergence from the best of her diets. A 52% pupation and 50.1% adult emergence was obtained by using a bluegrass diet.

The European pine shoot moth, Rhyacionia buoliana (Schiffermuller), is the only species in the genus Rhyacionia that has been successfully reared on an artificial diet (Chawla and Harwood 1968). Modified wheat germ diets of Berger (1963) and modified bean diets of Shorey and Hale (1965) were used in rearing the European pine shoot moth. The bean diets proved not as satisfactory as the wheat germ diets. Chawla and Harwood obtained an average of 65.4% pupation of larvae that were dissected from pine tips in the second or third instar and placed on the wheat germ diets.

Nutritional Requirements for Lepidopterous Insects

The nutritional requirements of insects differ among species. Hinton (1959) concluded that specific nutritional requirements of genetically different strains of Drosophila were determined by the individual's genetic constitution.

Wigglesworth (1965) has given an excellent general review on nutritional requirements of insects. The following information covers some of the important facts that Wigglesworth discussed. All insects require water, but the amount needed depends upon the rate at which it is lost by the body. Salts are believed to be required for normal growth of an insect. The usual salts are NaCl and $CaCl_2$, but some insect species have been reared on a diet containing only K_2HPO_4 and $MgSO_4$. Carbohydrates can be a source of energy to the insect. It has been shown that larvae of the honey-bee can survive 4 to 7 times longer when given carbohydrates than when given only water. Amino acids are among the most important raw materials for growth. Drosophila larvae are able to synthesize their essential amino acids by using ammonium salts as their sole source of nitrogen. Whereas, Aedes aegypti need ten essential amino acids in their diet for normal growth. Nucleic acids do not appear essential in the insect's diet but additions of ribonucleic or desoxyribonucleic acid may accelerate the rate of growth. A source of sterol (mainly cholesterol) in the diet is usually required, but there is a qualitative and quantitative difference in the need for the sterols depending on the species. In general, the phytophagous species of insects can convert C_{28-29} sterols of their food to cholesterol; whereas, the obligatory carnivorous species must have a supply of cholesterol in their diet. Some insects have been shown to need the presence of fatty acids in their diet. The absence of the unsaturated fatty acid linoleic acid in the diet of the Mediterranean flour moth, Ephestia kukniella (Zeller), will cause the scales to fall from their wings before the adult moths have emerged. Requirement of ascorbic acid (vitamin C) in the diet of insects is dependent upon the species. Drosophila and cockroaches have been reared for years on a diet free from ascorbic acid. The locusts in the genus Schistocerca have been shown to need ascorbic acid in their diet. In an ascorbic acid free diet, no locust nymph survived to the adult stage (Dadd 1960). Ascorbic acid is also necessary for growth and normal egg production in the bollworm. The vitamins of the B group comprised of thiamine (B_1), riboflavin (B_2), nicotinic acid, pyridoxine, pantothenic acid, choline, inosital, p-aminobenzoic acid, and biotin are required by most insects. Thiamine, riboflavin, nicotinic acid, pyridoxine, and pantothenic acid will greatly reduce the growth rate of an insect if any of the vitamins are deficient in the diet. Choline and biotin have less effect if deficient in the diet than those previously mentioned. Inositol and p-amino-benzoic acid are of minor importance in the diet. The fat soluble vitamins (vitamins A and D) are not ordinarily required in an insect's diet.

Chawla and Harwood (1968) showed that additions of fatty acids in the diet (more than is normally found in wheat germ) did not appear to be essential for normal growth of Rhyacionia buoliana larvae. The additions of chlorogenic acid, B_{12}, folic acid, or gibberellic acid did not improve growth or percentage pupation. The exclusion of salt mixture W in the diet appeared to be detrimental in rearing first instar larvae.

In rearing the pink bollworm, Pectinophora gossypiella, Ouye (1964) showed that a lack of calcium pantothenate, folic acid, nicotinamide, pyridoxine, riboflavin, and thiamine in a casein medium diet resulted in a 100% mortality. A complete larval mortality occurred in a wheat germ diet if nicotinamide or calcium pantothenate were not added to the diet. The pink bollworm larvae developed with or without the addition of vitamin B_{12} to the diets.

Environmental Requirements for Lepidopterous Larvae

The environmental factors of temperature, humidity, and light (photoperiod) are of importance in rearing insects on artificial diets. Photoperiod may be of little importance in rearing the Nantucket pine tip moth because it feeds within the stem of pine trees where light penetration is insignificant.

Tzanakakis and Phillips (1969) successfully reared three generations of the oriental fruit moth, Grapholitha molesta (Busch), in the laboratory on an artificial diet. Diets were maintained in an incubator at 25-27 C (77-81 F), with a 90-95% relative humidity, and a 16-hour daily photoperiod. The hickory shuckworm, *Laspeyresia caryana* (Fitch), was successfully reared on an artificial diet at a temperature of 25-29 C (77-84 F) and a 40-100% relative humidity (Schroeder et al. 1969). The optimum temperature for rearing the corn earworm, *Heliothis zea* (Boddie), has been reported to be 73-80 F (Callahan 1962). The optimum temperature for rearing the cabbage looper, *Trichoplusia ni* (Hubner), has been found to be 73-77 F (McEwen and Hervey 1960). Ditman et al. (1940) showed that there was a 1.6% loss of corn earworm pupae at 100% relative humidity; whereas, at 0% relative humidity there was an 18.6% loss of corn earworm pupae. The European pine shoot moth, *Rhyacionia buoliana* (Schiffermuller), was reared successfully on artificial diets at a temperature of 25 C (Chawla and Harwood 1968).

Miscellaneous Problems on Rearing Insects on Artificial Diets

There are many problems that may be encountered when rearing insects on artificial diets. Such problems as mold contamination, hydrogen ion concentration, and water condensation within the rearing containers may cause reduction in growth or even death of an insect.

In rearing the cabbage looper, McEwen and Hervey (1960) had to use strict sanitation methods to prevent outbreaks of the polyhedral virus. Vanderzant et al. (1962) used p-hydroxybenzoate as a mold inhibitor in rearing the bollworm under non-aseptic conditions. Methyl p-hydroxybenzoate was used successfully in inhibiting mold growth, and aureomycin was successful in inhibiting yeast growth on an artificial diet used in rearing the cabbage looper larvae (Ignoffo 1963). Sorbic acid, methyl p-hydroxybenzoate and butyl p-hydroxybenzoate at a 0.1%

concentration and formaldehyde at a 0.05% concentration were used to control microbial growth for 15 days when incorporated into a nutrient agar medium (Ouye 1962); however, the first three antimicrobial agents were less effective in a wheat germ medium. Increased concentrations of the antimicrobial agents were found to lengthen larval and pupal development of the pink bollworm significantly. Contaminants could not be controlled in nutrient agar medium by using potassium sorbate and sodium benzoate.

Auclair (1965) showed that a variation in pH above 8.0 or below 7.0 in the artificial diet for the pea aphid, *Acyrthosiphon pisum* (Harris), usually resulted in reduced growth, reproduction, and survival. The optimum pH for the best growth and survival of the pea aphid was from 7.4 to 7.7; whereas, reproduction was highest at pH 7.5.

Allen et al. (1968) reported that growth chambers caused moisture condensation on the feeding containers used in rearing the jack-pine budworm, *Choristoneura pinus*. The larvae had difficulty in feeding and many were drowned in the moisture condensation. Ruth and Hedlin (1969) reported that the small larvae of the Douglas-fir cone moth, *Barbara colfaxiana* (Kearfott), were unable to free themselves from the moist surface of the diet, resulting in high larval mortality.

MATERIALS AND METHODS

Source of Larvae Used in Diet Studies

Gravid female Nantucket pine tip moths, *Rhyacionia frustrana* (Comstock), were collected during June and July of 1969. They were found on loblolly pines near Lake Carl Blackwell in Payne County, Oklahoma. The moths were placed in individual 1-dram glass vials with polyethylene stoppers (Figure 1) and were allowed to oviposit on the inside of the vials. The moths were removed from the vials after the eggs had been oviposited and the vials set aside for the development of the fertilized eggs.

The larvae were removed within 2 hours after emergence. A number "0" red sable artist's brush was used in removing the larvae from the glass vial to the diet. Only one larva per cup was used in recording the development of each larva. The total numbers of larvae placed on each diet were recorded (Table 1). The larvae did not appear to be cannibalistic; therefore, in mass rearing, more than one larva could be placed in each cup.

Diet Composition and Mixing

The artificial diets used (Table 2) were a modification of diets developed by Vanderzant et al. (1962), Berger (1963), and Bailey (1964). Mayo (1969) had successfully used diet D_1 in rearing the corn earworm. Diet D_6 was a further modification of diet D_1. Chawla and Harwood (1968) had successfully used diets D_2 and D_3 in rearing the

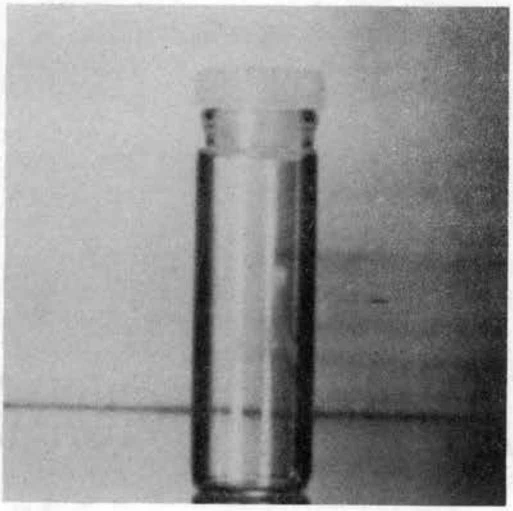

Figure 1. One-dram glass vial, with polyethylene stopper, used as a container for female moths to oviposit within.

Table 1. Total number of larvae placed on each diet

Diets	Larvae
D_1	297
D_2	299
D_3	295
D_4	295
D_5	398
D_6	298
D_7	300

Table 2. Composition of the diets used in rearing the Nantucket pine tip moth

Constituents*	Amount						
	D_1	D_2	D_3	D_4	D_5	D_6	D_7
Wheat germ	18	12	24	15	18	18	12
Sucrose	16	10.5	10.5	10.5	16	16	10.5
Casein	21	14	28	28	21	21	14
Salt mixture W	6	1	2	4	6	6	1
Alphacel	3	6	12	12	3	3	6
Soybean flour	-	40	-	-	-	-	55
Dextrin	-	-	10.5	10.5	-	-	-
Brewer's yeast	-	13.3	-	-	-	-	-
Pine tissue	-	-	-	35	-	35	-
KOH (22.5%)	3	4	4	4	1.5	3	4
Formaldehyde (10%)	2.5	1.4	1.1	1.1	1.2	2.5	1.4
Propionic acid	0.9	-	-	-	0.5	0.9	-
Methyl-p-hydroxybenzoate+	6	8	8	6	3	6	8
Sorbic acid	-	0.8	0.8	0.8	0.4	-	0.8
Tetracycline	125	-	-	-	62.5	125	-
Vitamin diet fortification mixture	6	4	8	8	6	6	8
Ascorbic acid	2.5	2.8	2.8	2.8	5	2.5	2.8
Agar	13.5	13.5	16	16	13.5	13.5	13.5
Water (distilled)	500	570	585	585	500	500	570

*Liquid constituents in milliliters, dry constituents in grams, excluding tetracycline which is in milligrams.

+A mixture of 60 grams of methyl-p-hydroxybenzoate in 400 milliliters of 95% ethyl alcohol.

European pine shoot moth, Rhyacionia buoliana. Diets D_4, D_5, and D_7 were a modification of diets D_2 and D_3.

The major sources of the constituents of the diets were Nutritional Biochemical Corporation (Nutritional Biochemicals Corp., 26201 Miles Ave., Cleveland, Ohio, 44128) and W. H. Curtin and Company (W. H. Curtin & Co., Tulsa, Oklahoma). The pine tissue used in diets D_4 and D_6 was obtained by macerating the succulent tips of loblolly pines in a Waring blender. The pine tips were washed thoroughly and soaked in a 5.25% sodium hypochlorite solution for 15 minutes before macerating.

The dry constituents were weighed on a Mettler balance, Type K5 (Mettler Instrument Corp., Highston, New Jersey) (Figure 2). The liquid constituents were measured in a 500 ml or a 10 ml graduate cylinder.

The diets were prepared as follows:

1. The following dry constituents were mixed together and placed into a Waring blender:

Wheat germ
Sucrose
Casein
Salt mixture W
Alphacel
Soybean flour (if used)
Dextrin (if used)
Brewer's yeast (if used)
Pine tissue (if used)

2. The following constituents were mixed together in 50 milliliters of distilled water and poured into the blender with the dry constituents:

KOH
Formaldehyde
Propionic acid (if used)
Methyl-p-hydroxybenzoate

Figure 2. Mettler balance, Type K5, used in weighing dry constituents of diets.

3. The above constituents were blended for approximately 2 minutes.

4. The agar was placed in a 1000 ml beaker along with the required amount of water minus 50 ml. The agar was poured into the blender after the beaker with the agar had been placed in boiling water for 30 minutes.

5. The agar with the other constituents were blended for approximately 3 minutes.

6. The diet was cooled, below 150 F, before the following constituents were added:

Vitamin diet fortification mix
Ascorbic acid
Sorbic acid (if used)
Tetracycline (if used)

7. The diet was blended for approximately 2 minutes and immediately poured into a 12 x 8 x 2 inch pyrex plate.

Upon cooling, the diet was cut into squares and placed into 1-oz plastic medicine cups with plastic-coated paper lids (Premium Plastics Inc., Chicago, Illinois) (Figure 3).

The hydrogen ion concentration (pH) of each diet (Table 3) was determined by means of a Beckman, Model G, pH meter (Beckman Instruments, Inc., Fullerton, California) fitted with a 1-drop capacity glass electrode assembly (Figure 4). The diets were first filtered through a buchner funnel using Whatman No. 42 ashless filter paper.

Environmental Conditions During Rearing

After the larvae had been placed on the diet, the cups were stacked on shelves in a 12 x 10 ft room. The room was maintained at a temperature of 74 to 78 F.

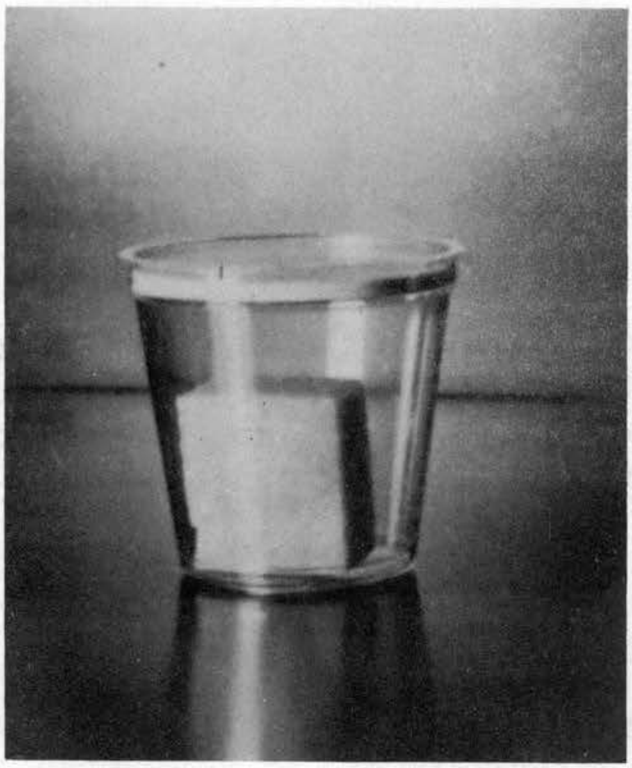

Figure 3. Plastic cup, with diet, used in rearing the Nantucket pine tip moth.

Table 3. The hydrogen ion concentration (pH) of diets used in rearing the Nantucket pine tip moth

Diets	pH
D_1	4.8
D_2	6.2
D_3	4.6
D_4	4.9
D_5	4.1
D_6	4.6
D_7	6.7
Loblolly pines*	3.8

*The filtrate of macerated loblolly pine tips and needles.

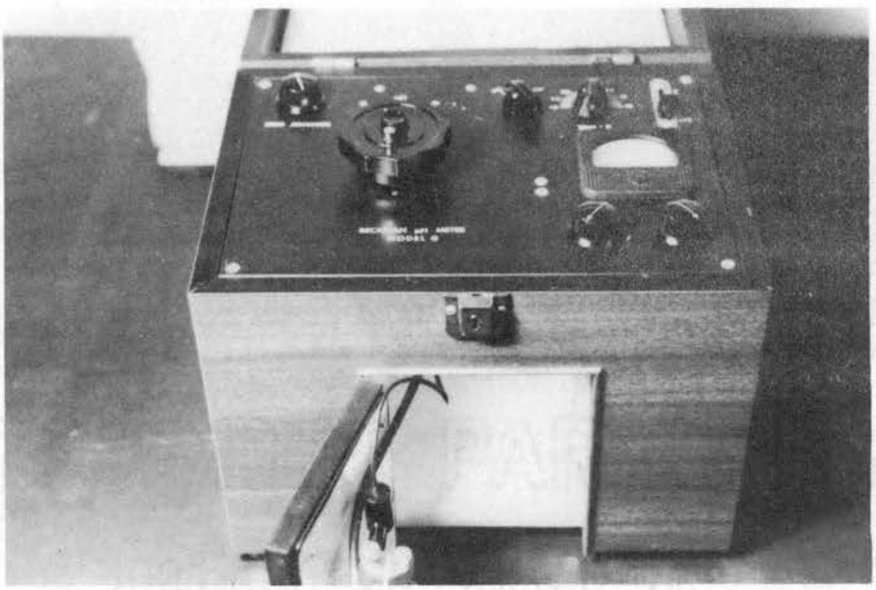

Figure 4. Beckman, Model G, pH meter used in determining hydrogen ion concentration (pH) of diets.

The diet was kept at a temperature of 34 to 38 F if it was not immediately used. The diet was warmed to room temperature before the larvae were placed on it.

Determination of Larval Stage at Death on Diet

The head capsules of all larvae that died, while feeding on the diets, were measured. This was to determine the instar in which each larva had died.

The measurements were made by the use of a calibrated ocular micrometer fitted into the right hand side of a binocular microscope. The micrometer was calibrated when the binocular microscope was at 30 power magnification. The calibration showed that one millimeter was equal to 4 units on the ocular micrometer; therefore, each unit on the micrometer was equal to .25 mm.

Anderson (1962) determined that *Rhyacionia frustrana* had five larval instars. The mean of each instar was calculated by Anderson, using Dyar's principle as explained by Comstock (1964). The means were as follows: first instar = 0.184 mm, second instar = 0.258 mm, third instar = 0.364 mm, fourth instar = 0.512 mm, and fifth instar = 0.720 mm. The range within each instar was determined by subtracting two successive instars, dividing the remainder by two, and adding the quotient to the smaller instar mean. The ranges were as follows: first instar = 0 to 0.22 mm, second instar = 0.23 to 0.31 mm, third instar = 0.32 to 0.44 mm, fourth instar = 0.45 to 0.62 mm, and fifth instar = 0.63 + mm.

Determination of Growth After Pupation

All pupae were weighed that had pupated on the diet. This was to determine if there were any growth differences between diet reared pupae as compared with pupae that had developed in their natural environment. The latter were collected from infested loblolly pine tips near Lake Carl Blackwell in Payne County, Oklahoma.

A total of 100 male and 100 female pupae were used to determine the field-collected mean weights. The pupae were weighed on a Type H16 Mettler analytical balance with a sensitivity of 1/10 mg (Figure 5).

Determination of Sex of Adults and Pupae

The sex was determined on all moths that were successfully reared on the artificial diets. The purpose of the study was to determine if one sex was better adapted to survival on artificial diet.

Adult sex determinations were made on the basis of the external appearance of genitalia as described by Anderson (1962) (Figure 6). The sex of the pupae were determined by a method described by Bronson (1941). The two genital openings of the female pupae come together to form one opening on the 8th abdominal segment; the male pupae have a single genital opening situated on the 9th abdominal segment.

Figure 5. Mettler analytical balance, Type H16, used in weighing pupae.

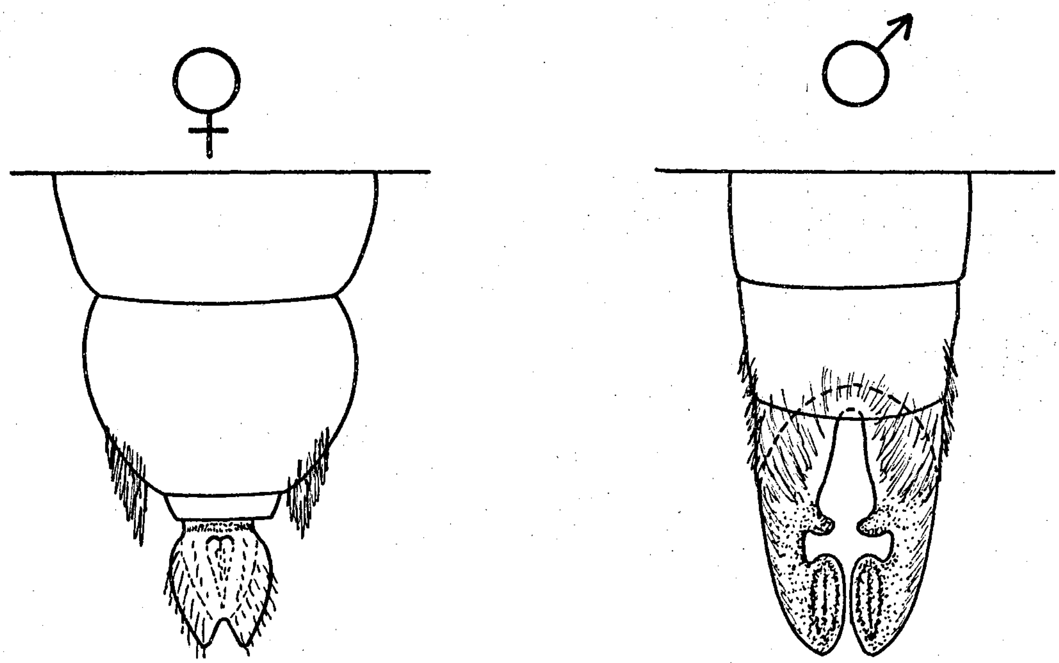

Figure 6. External appearance of the male and female genitalia of the adult Nantucket pine tip moth.

RESULTS AND DISCUSSION

General Feeding and Pupating Habits

The most critical period of the newly emerged Nantucket pine tip moth larvae is the first couple of days. The larvae will not survive on artificial diet if they have not begun to utilize the diet within one to two days. Newly hatched larvae would wander over the diets and rearing containers when first placed on them. In many instances the larvae would become trapped within droplets of water, that had condensed on the inside of the rearing containers, and being unable to free themselves would die. A high moisture content within the diet was also detrimental to newly hatched larvae.

The first instar larvae usually began to feed on the upper edges of the diet and worked their way towards the bottom of the diet as feeding progressed. Most of the third and fourth instar larvae were found at the bottom of the rearing containers and under the media (Figure 7). During the last instar, the majority of larvae would crawl to the top of the rearing containers and construct a hibernaculum before pupating (Figure 8).

Larvae Reared on Diet D_1

The percentage pupation of larvae feeding on diet D_1 was not as high as obtained with diets D_3 or D_5 but the percentage survival to the adult stage was only surpassed by diet D_3 (Table 4). The mean number of days for development of larvae on diet D_1 was 56 days. This was

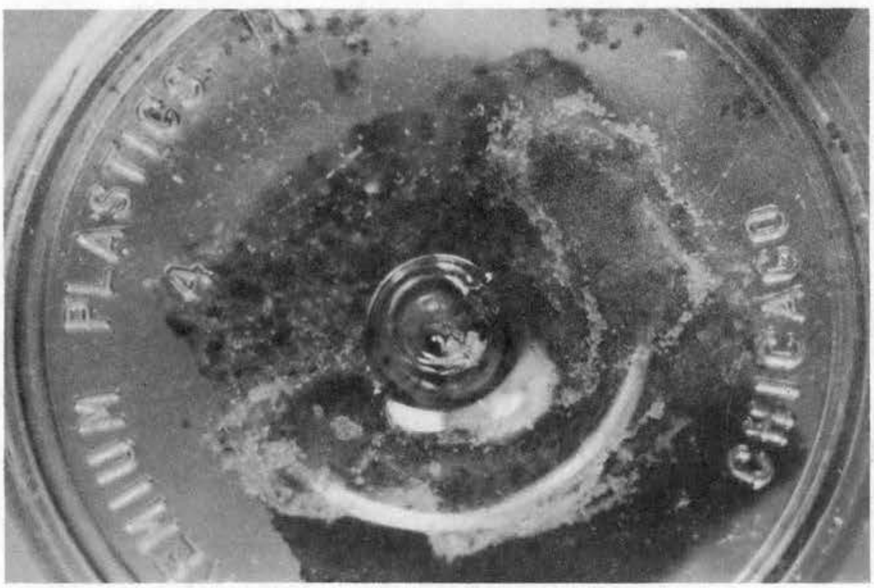

Figure 7. A gallery made by the Nantucket pine tip moth larva while feeding on the underside of an artificial diet.

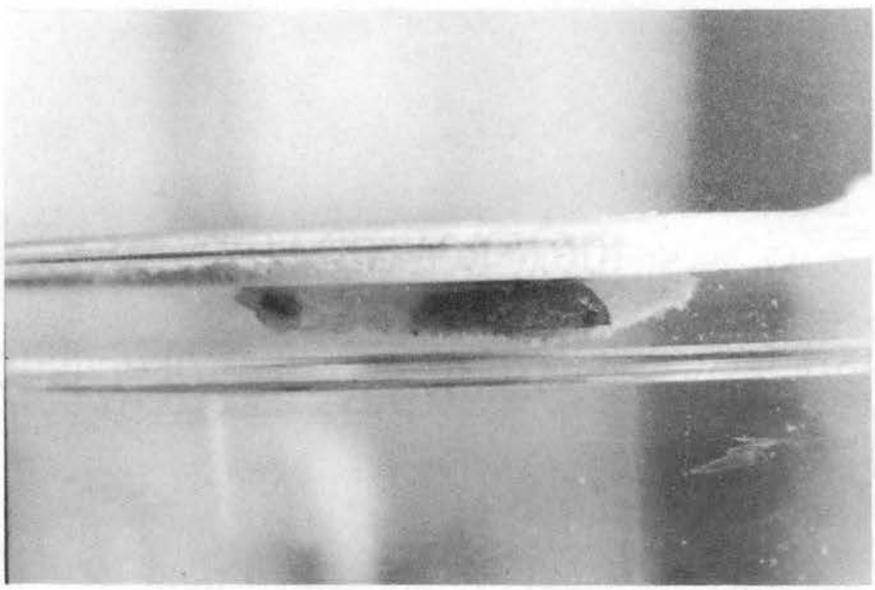

Figure 8. Nantucket pine tip moth pupa with its hibernaculum attached to the side and lid of rearing container.

Table 4. Percentage survival to the pupal and adult stages

Diets	Survival to pupal stage	Survival to adult stage
D_1	6.2	5.6
D_2	0.3	0.0
D_3	28.4	9.2
D_4	5.0	0.3
D_5	17.6	3.0
D_6	5.7	0.7
D_7	1.0	0.0

four days longer than the mean of the seven diets (Table 5). The mean pupal weights were 4.1 mg for males and 6.0 mg for females (Figure 9). The mean weights were 91% and 83%, respectively, of the mean weights of field-collected male and female pupae. The percentage mortality during each larval instar was 66% for the first, 53% for the second, 34% for the third, 55% for the fourth, and 21% for the fifth instar (Figure 10). Therefore, larvae feeding on diet D_1 had the highest mortality in the first instar and the lowest mortality in the fifth instar.

The pupae and adult moths obtained from larvae reared on diet D_1 appeared normal in comparison with field-collected pupae and adult moths.

Larvae Reared on Diet D_2

The percentage pupation of larvae feeding on diet D_2 was the lowest of the seven diets and no adults emerged from this diet (Table 4). There was insufficient data (only two larvae pupated) to determine the mean number of days for development of larvae to the pupal stage. The mean weight for the two female pupae obtained (no male pupae were obtained on this diet) was 3.6 mg (Figure 9). The mean weight of the two female pupae was only 50% of the mean weight of field-collected female pupae. The percentage mortality during each larval instar was 86% for the first, 63% for the second, 20% for the third, 33% for the fourth, and 63% for the fifth instar (Figure 10). Therefore, larvae feeding on diet D_2 had the highest mortality in the first instar and the lowest mortality in the third instar.

The two pupae that were obtained from diet D_2 were not normal. Both pupae retained their larval head capsules after pupation

Table 5. Mean number of days it took larvae to develop to pupal stage

Diets	Days
D_1	56
D_2	Insufficient data[1]
D_3	51
D_4	60
D_5	51
D_6	54
D_7	Insufficient data[1]
Total	52

[1]Only 3 larvae survived to the pupal stage in each of the diets.

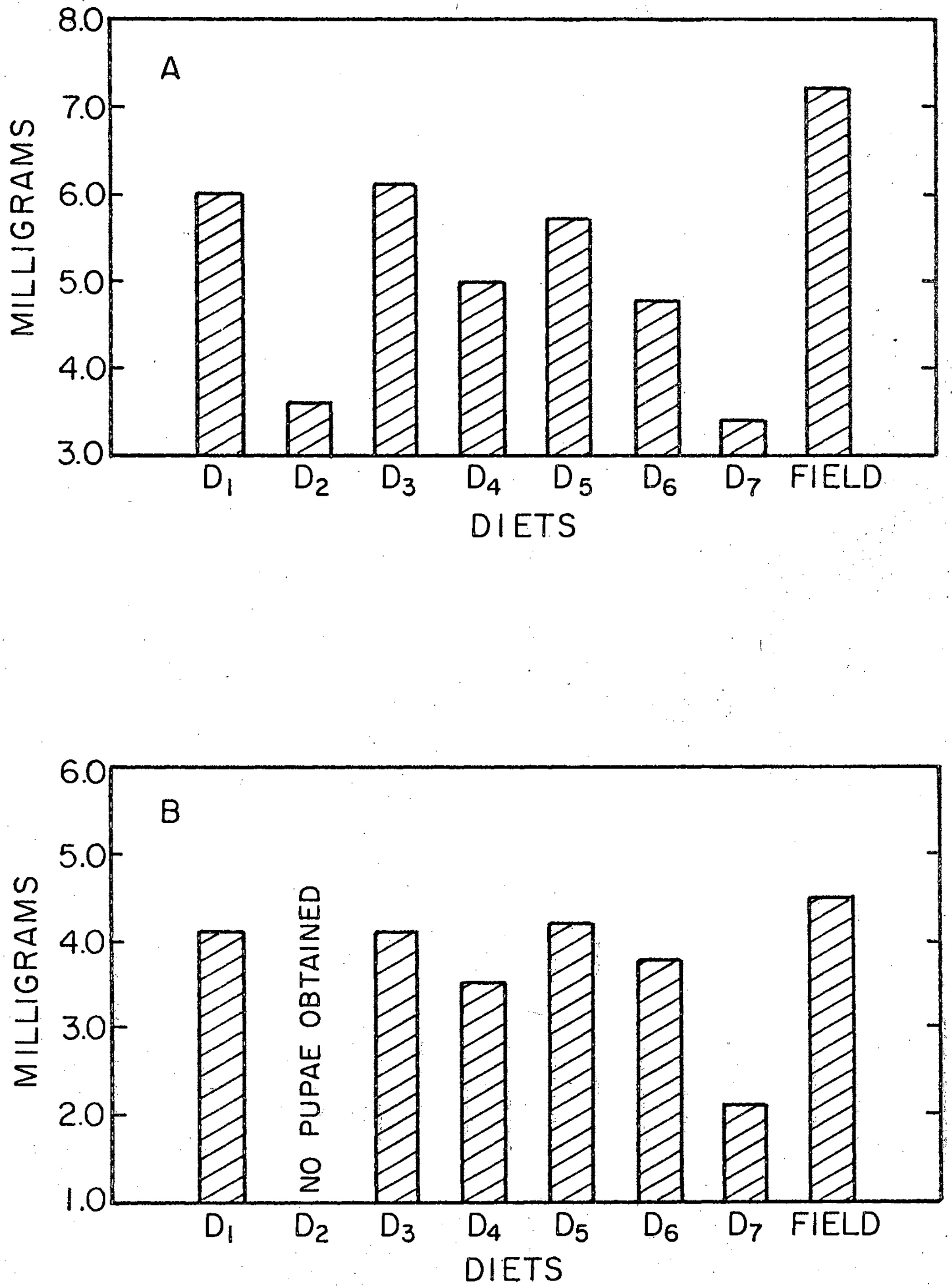

Figure 9. Mean weights of female (A) and male (B) pupae reared on artificial diets compared with pupae obtained from pine tips in the field.

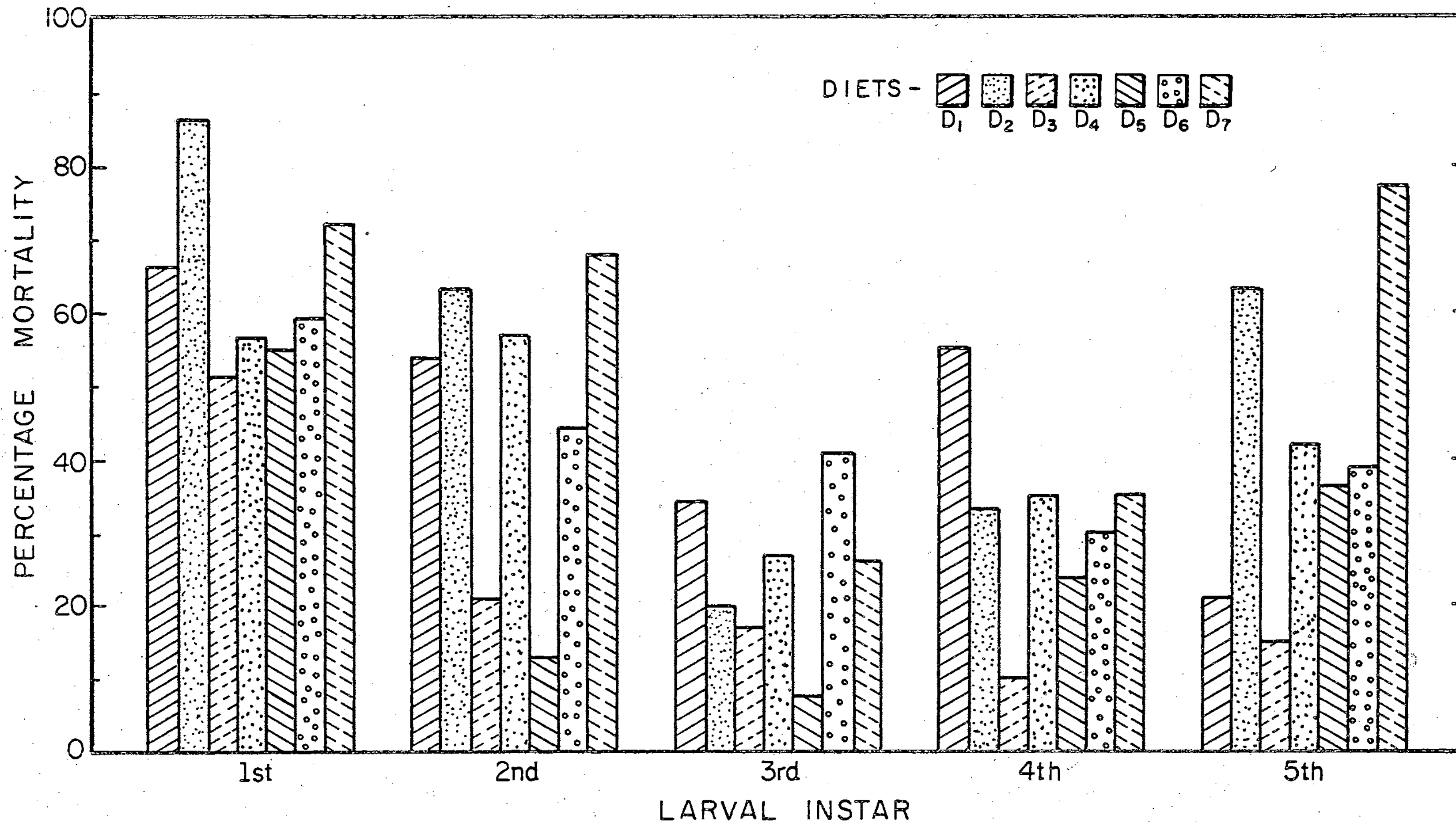

Figure 10. Percentage mortality during each larval instar of Nantucket pine tip moth larvae when reared on artificial diets in the laboratory.

(Figure 11). Wigglesworth (1959) calls this abnormal condition "metathetely" and defines it as "the retention of larval characters in the adult stage." Wigglesworth believes the abnormality is due to disturbances in the secretion of juvenile hormone by the corpus allatum of the insect. In the case of metathetely, there is too high a concentration of juvenile hormone during the pupating process and some larval characteristics are retained.

Larvae Reared on Diet D_3

The percentage pupation of larvae feeding on diet D_3 was the highest of the seven diets and the percentage survival was also the highest of the seven diets (Table 4). The mean number of days for development of larvae on diet D_3 was 51 days. This was one day less than the mean of the seven diets (Table 5). The mean pupal weights were 4.1 mg for males and 6.1 mg for females (Figure 9). The mean weights were 91% and 85%, respectively, of the mean weights of field-collected male and female pupae. The percentage mortality during each larval instar was 51% for the first, 21% for the second, 17% for the third, 10% for the fourth, and 15% for the fifth instar (Figure 10). Therefore, larvae feeding on diet D_3 had the highest mortality in the first instar and the lowest mortality in the fourth instar.

The pupae and adult moths obtained from larvae reared on diet D_3 appeared normal in comparison with field-collected pupae and adult moths.

Larvae Reared on Diet D_4

The percentage pupation of larvae feeding on diet D_4 was not as high as obtained with diets D_1, D_3, D_5, and D_6 (Table 4). The

Figure 11. A deformed Nantucket pine tip moth pupa showing the retention of the larval head capsule after pupation.

percentage survival to the adult stage was surpassed by diets D_1, D_3, D_5, and D_6 (Table 4). The mean number of days for development of larvae on diet D_4 was 60 days. This was 8 days longer than the mean of the seven diets (Table 5). The mean pupal weights were 3.5 mg for males and 5.0 mg for females (Figure 9). The mean weights were 78% and 69%, respectively, of the mean weights of field-collected male and female pupae. The percentage mortality during each larval instar was 57% for the first, 57% for the second, 27% for the third, 35% for the fourth, and 42% for the fifth instar (Figure 10). Therefore, larvae feeding on diet D_4 had the highest mortality in the first and second instars and the lowest mortality in the third instar.

The pupae and adult moths obtained from larvae reared on diet D_4 appeared normal, except for their smaller size, in comparison with field-collected pupae and adult moths.

Larvae Reared on Diet D_5

The percentage pupation of larvae feeding on diet D_5 was surpassed only by diet D_3, but the percentage survival to the adult stage was surpassed by diets D_1 and D_3 (Table 4). The mean number of days for development of larvae on diet D_5 was 51 days. This was one day less than the mean of the seven diets (Table 5). The mean pupal weights were 4.2 mg for males and 5.7 mg for females (Figure 9). The mean weights were 93% and 79%, respectively, of the mean weights of field-collected male and female pupae. The percentage mortality during each larval instar was 55% for the first, 13% for the second, 8% for the third, 24% for the fourth, and 36% for the fifth instar (Figure 10). Therefore, larvae feeding on diet D_5 had the highest mortality in the first instar and the lowest mortality in the third instar.

The pupae and adult moths obtained from larvae reared on diet D_5 appeared normal in comparison with field-collected pupae and adult moths.

Larvae Reared on Diet D_6

The percentage pupation of larvae feeding on diet D_6 was not as high as obtained with diets D_1, D_3, and D_5; also, the percentage survival to the adult stage was surpassed by diets D_1, D_3, and D_5 (Table 4). The mean number of days for development of larvae on diet D_6 was 54 days. This was 2 days longer than the mean of the seven diets (Table 5). The mean pupal weights were 3.8 mg for males and 4.8 mg for females (Figure 9). The mean weights were 84% and 67%, respectively, of the mean weights of field-collected male and female pupae. The percentage mortality during each larval instar was 59% for the first, 44% for the second, 41% for the third, 30% for the fourth, and 39% for the fifth instar (Figure 10). Therefore, larvae feeding on diet D_6 had the highest mortality in the first instar and the lowest mortality in the fourth instar.

The pupae and adult moths obtained from larvae reared on diet D_6 appeared normal in comparison with field-collected pupae and adult moths.

Larvae Reared on Diet D_7

The percentage pupation of larvae feeding on diet D_7 was next to the lowest (exceeded only by diet D_2) of the seven diets and no adults emerged from this diet (Table 4). There were insufficient data (only three larvae pupated) to determine the mean number of days for development of larvae to the pupal stage. The mean pupal weights were 2.1 mg for males and 3.4 mg for females (Figure 9). The mean weight for both male and female pupae was 47% of the mean weights of field-collected male and female pupae. The percentage mortality during each larval instar was 72% for the first, 68% for the second, 26% for the third, 35% for the fourth, and 77% for the fifth instar (Figure 10). Therefore, larvae feeding on diet D_7 had the highest mortality in the fifth instar and the lowest mortality in the third instar.

The three pupae obtained from diet D_7 were not normal. All three pupae retained their larval head capsules after pupation (Figure 11) and were similar to those pupae obtained from diet D_2.

General Discussion of Diets

The percentage pupation was calculated on the number of first instar larvae initially placed on the diets. Many researchers calculate the percentage pupation after a specified time, due to the fact that many larvae are injured during their transfer to a diet and subsequently die. This is an unrealistic approach in calculating percentage pupation because it gives a false economic success.

Diets consisting of a soybean flour base, diets D_2 and D_7, were unsuitable for rearing the Nantucket pine tip moth larvae. The use of pine tissue in the media, diets D_4 and D_6, did not increase the percentage pupation or the weights of male and female pupae.

A diet with a high pH may be detrimental in rearing Nantucket pine tip moth larvae. The pH of diets D_2 and D_7 was about one hundred times more alkaline than the other five diets and both of these diets proved to be unsuitable in rearing the larvae.

Molds were successfully inhibited on all of the diets. Only 0.2% of the cups with the diets developed mold growth.

SUMMARY AND CONCLUSIONS

Mass rearing of the Nantucket pine tip moth larvae in the laboratory on an artificial diet is possible, though some problems do exist.

The tip moth larvae were reared successfully on diets containing a wheat germ base, but diets containing a soybean flour base proved to be detrimental in rearing the larvae. The percentage pupation on the best wheat germ diet was 28.4%. When mortality during the first instar was excluded, a percentage pupation of 54% was obtained from the best wheat germ diet.

In two of the diets, the mean weights of male and female pupae were close to the weights of field-collected pupae.

It was found that larval mortality was greatest during the first and fifth instars and least during the third instar while feeding on artificial diets.

The mean number of days for development of larvae, depending on the diet used in rearing, ranged from 51 to 60 days.

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