NUTRITIONAL STUDIES ON *CEUTHORRHYNCHIDIUS HORRIDUS* (COLEOPTERA: CURCULIONIDAE) AN INTRODUCED WEEVIL FOR THE BIOLOGICAL CONTROL OF *CARDUUS* THISTLES

by

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INTRODUCTION

Ceuthorrhynchidius horridus (Panzer) is a thistle-rosette feeding weevil in the family Curculionidae, subfamily Ceutorhynchinae. Adults of this species have been imported into the United States of America from Europe in an attempt to control thistles in the genus *Carduus*. These plants, specifically *C. nutans* and *C. acanthoides*, encroach upon pastures where domestic animals are grazed. Cattle will not feed on and will tend to avoid areas infested by these plants due to their sharp spines, thus lessening both the productivity and value of the land. Although stringently applied chemical and cultural controls can reduce thistle populations, reinfestation readily occurs from thistle infested roadsides and waste areas. Because of the potential of *C. horridus* to control thistle rosettes, and its inherent capacity for self-perpetuation, the propagation and systematic release of this species has been undertaken to help alleviate the expense of a continual control program for thistles.

Since these accidentally introduced weeds were imported from Europe without their natural enemies, they are susceptible to classical biological control techniques. The primary aim of this research is to increase knowledge of the weevil's dietary requirements and thereby improve techniques for weevil production in the laboratory.

Studies on the nutritional requirements of insects supply information toward several other important objectives. According to McGinnis and Kasting (1972) these include: 1) the increased productivity of economically beneficial insects such as silkworms and honeybees; 2) an improved ability for the economic production of parasites and predators as well as those pest species which can be controlled by autocidal techniques; and 3) the development of new controls based on a detailed understanding of an insect's nutritional requirements. Also, predicated on the development of artificial diets for insects, nutritionally standard test animals are now available in quantity throughout the entire year (Yonce et al. 1973). There is also an academic interest in the nutrition and metabolism of insects as well as comparative aspects with mammals (McGinnis and Kasting 1972). The nutritional studies on C. horridus presented in this thesis are designed to confirm and increase available information on these objectives. In addition, this thesis contains a comprehensive literature survey of artificial diets for the family Curculionidae, and biological studies on C. horridus.

II

LITERATURE REVIEW

The literature survey for this thesis has been presented as follows: A) reviews of literature on artificial diets; B) initial successes in development of artificial diets for phytophagous insects; C) development of synthetic media for Curculionidae. Section D includes a literature survey of biological studies on C. horridus.

A. Reviews on Artificial Diets

Considerable work on insect nutrition has been accomplished during the last few decades. Exhaustive reviews provide excellent sources of information of early work (Wigglesworth 1950; Trager 1953; Lipke and Fraenkel 1956; Friend 1958; Fraenkel 1959; House 1961, 1962, 1965; and Vanderzant 1966). Appearing within the last ten years are detailed accounts relating to nutritional requirements of insects (Waldbauer 1968, House 1969, Hodgson and Rock 1971, Beck 1972, Dadd 1973, and Vanderzant 1974). More recently, compilations of titles and abstracts of papers on artificial diets have become available (House 1967, House et al. 1971, Singh 1972, 1974).

B. Initial Successes in the Development of Artificial Diets for Phytophagous Insects

Many of the initial successes in the development of artificial diets for phytophagous insects occurred in the 1940's and early 1950's. According to Vanderzant (1966), the first synthetic diet for a plant-feeding insect was by Bottger in 1942. This diet,

designed for the European cornborer Ostrinia nubilalis (Hub.), incorporated many of the basic nutritional groups commonly used today; a protein source (casein), carbohydrates, mineral salts, vitamins (A, B_1, C, D, and E), and fats (including corn and wheat germ oils) (Bottger 1942).

In 1949, Beck et al. managed to develop a more chemically defined medium which allowed the laboratory propagation of the European cornborer under nearly aseptic conditions. Beck's techniques involved autoclave sterilization of the medium and glassware, but microorganisms were not excluded from the test larvae. One year later, using a similar technique coupled with egg sterilizing solutions (0.1% mercuric chloride or 2% sodium hydroxide plus 2% formaldehyde), Beck and Stauffer (1950) aseptically reared the European cornborer.

The development of a successful aseptic rearing process was an important step in advancing nutritional studies on insects. The use of aseptic conditions, coupled with antibiotics, permitted research into the dietary requirements of various insects in the absence of nutrition producing microorganisms (endosymbionts, intestinal flora and fauna). The synthetic medium utilized by Beck in the late 1940's and early 1950's has been the basis for many of the artificial diets developed later (Vanderzant 1974).

Friend (1954) successfully reared the onion maggot, Hylemya antigua (Meig.), on a medium of known composition. According to

Vanderzant and Reiser (1956), this was the first aseptic rearing of a phytophagous insect on a chemically defined diet.

In the following year, 1955, Ishii and Hirano determined the amino acid requirements for the larvae of the rice stem borer, Chilo simplex (Butler). Friend et al. (1957) claimed that this was the first use of chemically defined artificial diets under aseptic conditions for studies on the amino acid requirements of an insect. In this same paper, Friend et al. published the second study on the amino acid requirements of a phytophagous insect. By aseptically rearing the onion maggot on chemically defined diets and adding and subtracting various components, the essential amino acids were determined. It was subsequently confirmed that the nutritionally important amino acids were levorotatory.

C. Development of Artificial Diets for Curculionidae

Beginning in the late 1950's considerable emphasis has been placed on development of artificial diets for weevils of the family Curculionidae. This interest is based on: 1) lack of availability of specimens for research throughout the year; 2) difficulty in interpreting research data because of a wide variety of natural characteristics, and 3) shortage of suitable plant material for continuous rearing (Hsiao and Hsiao 1974b, Vanderzant and Davich 1958, and Yonce et al. 1973).

Initial work in this field concentrated on developing diets for the boll weevil, Anthonomus grandis (Boh.) (Brazzel et al. 1959;

Earle et al. 1966, 1967; Moore et al. 1967; Sterling and Adkisson 1966; Sterling et al. 1965; Vanderzant 1959, 1963a, 1963b, 1965, 1967; Vanderzant and Davich 1958, 1961; and Vanderzant et al. 1959.)

The first reported artificial diet for this weevil included mostly soybean protein, sucrose and powdered cellulose (Vanderzant and Davich 1958). Although this medium was successful (77% of eggs become adults), efforts were made to improve it because of the economic importance of the weevil. In 1959, Earle et al. produced a larval diet for the boll weevil based on an acetone powder of cotton. Use of the weevil's normal protein source plus soybean protein aided the development of adults larger than those reared on casein or soybean protein alone. Later that year, Vanderzant et al. reported that pollen, wheatgerm or germinated cottonseed permitted increased growth in larval diets. Six years later, Vanderzant reared the weevil axenically on defined diets. This allowed for the study of amino acid, carbohydrate and mineral requirements through the use of deletion experiments (Vanderzant 1965). In the same year Sterling et al. (1965) reported on a cottonseed-meal diet (modified from a pink bollworm diet used by Adkisson et al. 1960), which produced more vigorous adults and shortened developmental time as compared to a cottonleaf-meal diet. In 1967, Moore et al. used egg albumin as a protein source for both adult and larval diets. However, a cottonleaf powder thought to be a phagostimulant was also used and may have provided more protein to the diet.

Nash and Tombes (1966) evaluated five artificial diets for the alfalfa weevil. Although a colony could be successfully maintained using four of the five diets tested, larval developmental time was increased and percentages of larvae reaching adults were quite low. In 1974, Hsiao and Hsiao compounded an artificial diet capable of greater than 70% yield. This was attributed to a better understanding of the feeding behavior of the alfalfa weevil. The chemical stimulants important to alfalfa weevil feeding were discussed by Hsiao (1969) who worked with adenine and related substances, and by Yamamoto and Cambell (1972) who studied feeding responses to sugar and extracts of alfalfa.

The first synthetic medium for rearing larvae of the plum curculio, Conotrachelus nenuphar (Herbst), was reported by Yonce et al. in 1971. A pinto bean medium developed by Burton (1969) proved to be the most promising of 6 diets tested. Later work by Yonce et al. (1973) indicated that the pinto bean diet produced a higher yield of adults per larvae inoculated (77%) than did the weevil's natural host, apples (52%). However, the weevils preferred to oviposit in apples.

Shanks and Finnigan (1971), attempted to make an artificial diet for the blackvine weevil Otiorhynchus sulcatus (F.). The initial diet, based on powdered milk, was not acceptable as a rearing medium due to detrimental effects on the adults produced, as well as low returns of adults from the inoculated larvae. In 1973, Shanks and Finnigan described a more successful diet for the

blackvine weevil. This medium, with a pinto bean base, resulted in over 30% of the larvae becoming normal adults. No mention was made of adult fecundity or attempts to rear successive generations.

According to Baker and Mabie (1937a, 1973c), nutritional information on stored product weevils of the genus Sitophilus was available before the early 1970's, but few attempts were made to rear these weevils before this time. Chippendale (1972) studied the importance of dietary carbohydrates to the adult rice weevil S. oryzae (L.) using a synthetic diet based on corn starch. The following year a corn starch based diet was produced which permitted larval development of S. granarius (L.) (Baker and Mabie 1973a). Subsequently, Baker and Mabie (1973b) prepared a modified version of this diet which permitted development of S. zeamais Motschulsky and S. oryzae as well as S. granarius. They later published results of tests utilizing modifications of their first successful diet for S. granarius. This was an attempt to produce diets suited to studying growth and fecundity in relation to specific nutrients (Baker and Mabie 1973c).

In 1975, Brown and Chippendale altered Chippendale's earlier diet for S. oryzae and were able to study effects of various nutrients, larval reserves and symbionts on survival of adult S. zeamais. Baker (1975) improved Baker and Mabie's 1973b meridic larval diet for S. oryzae through replacement of casein with amino acids. He claimed that though larval developmental rates were reduced, the use of amino acids was significant toward the production of a holidic diet for S. oryzae larvae.

Once a diet has been developed which permits deletion experiments, specific nutritional requirements can be studied. This type of test has been used to determine the amino acid requirements for several phytophagous insects, including a weevil (Vanderzant 1966). According to Vanderzant (1963b, 1965, 1966), ten amino acids are essential for growth and oviposition of the boll weevil. These include arginine, histidine, isoleucine, leucine, lycine, methionine, phenylalinine, threonine, tryptophan, and valine. Omission of any one results in a failure to pupate. Removal of any eight additional amino acids, alanine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine, or tyrosine, causes a reduction in adult emergence of up to 81% of the larvae utilized (Vanderzant 1966). Further experiments by Vanderzant and Chremos (1971) indicated that arginine could be replaced by citrulline. This suggested that citrulline could be converted to arginine by the boll weevil. Subsequent tests determined minimum amounts of the ten essential amino acids needed to produce a 70% yield of adults (Vanderzant 1973).

The importance of fatty acids in rearing weevils has been studied by many insect nutritionists. Earle et al. (1967) found that boll weevil larvae developed satisfactorily on fat deficient diets but the resulting adults laid fewer eggs. Lambremont and Stein (1964) examined the interconversions of isotopically-labeled fatty acids in the boll weevil. Results indicated that linoleic acid was not metabolically produced from either stearic or oleic acid. Inositol (included with the fatty acids due to its presence

in phospholipids) was found to be indispensable for A. grandis development (Vanderzant 1959, 1966). Although specific requirements for fatty acids have not been shown for the alfalfa weevil, they have been included in artificial diets for this insect (Hsiao and Hsiao 1974b). Other work in this area includes the development of fatty acid profiles and determinations of amounts of lipids in granary weevils (Yadava et al. 1972, Yadava and Musgrave 1972).

In the past fifteen years, the importance of carbohydrates to three species of Curculionidae has been examined. Vanderzant (1965) reported that fructose and sucrose produced the best larval growth for the boll weevil. Ribose, melibose, galactose, maltose, cellobose, starch, lactose, and glucose were less useful. Larvae did not survive in the absence of a carbohydrate source. Although sucrose, fructose, and glucose stimulated feeding by alfalfa weevils, sucrose was superior as a phagostimulant (Yamamoto and Cambell 1972). Chippendale (1972) discovered that metabolically useful dietary polysaccharides were feeding stimulants of the rice weevil. Disaccharides and polysaccharides producing poor growth were classed as feeding deterrents or as producing an unsuitable texture in the synthetic medium. Experiments comparing the useful polysaccharides concluded that the branched chain amylopectin fraction of the starch in the rice weevil's normal diet served as a feeding stimulant and an essential nutrient.

Although most commercially available mineral mixtures were designed for vertebrates, they have been successfully used by insect nutritionists (Vanderzant 1966). Many of the diets developed

for Curculionidae included salt mixtures in an effort to satisfy the insect's biological needs (Earle et al. 1959, Hsiao and Hsiao 1974b, Nash and Tombes 1966, Vanderzant and Davich 1958).

The maize weevil, rice weevil, and boll weevil have been examined with respect to the importance of minerals in their diets. Vanderzant (1965) determined that magnesium was essential for proper growth of A. grandis. In diets lacking magnesium the yield of adults was low (47%), and the average larval weight was considerably reduced. Without the addition of sodium, calcium, iron, copper, zinc, manganese, molybdenum, cobalt or iodine salts, satisfactory growth still occurred. However, these minerals may have been present in other constituents of the diet. Brown and Chippendale (1975) produced similar results utilizing the adult maize weevil. While tests indicated that diets containing mineral concentrations of 0.5% did not affect adult longevity, a 1% concentration was shown to decrease the adult lifespan. Somewhat different results were found by Baker (1975). He showed that larvae developed faster on diets containing 1.0% minerals than on diets with a mineral content of 0.1%. Baker and Mabie (1973c) speculated that a 2% dietary mineral concentration was inhibitory to the granary weevil.

Gueldner et al. (1975) utilized an interesting approach to determine the importance of minerals to the boll weevil. Using atomic absorption spectrophotometry, a comparison of the mineral contents of boll weevils, cotton buds, and two artificial diets was undertaken. Results suggested that the larval diet tested was low

in calcium and manganese as compared to cotton squares. An artificial diet for adults appeared to be low in manganese as well. This method of study may serve to help optimize diets for mass propagation programs.

Whereas mineral requirements for insects may differ, scientists agree that a source of sterols, either dietary or from associated symbionts, is necessary for growth and reproduction (Dadd 1973, Robbins et al. 1971, Vanderzant 1974). This need is apparently due to the lack of a biosynthetic pathway for sterols (Robbins et al. 1971). The ability of phytophagous insects to modify plant or dietary sterols to produce physiologically important compounds is crucial to insect survival in the absence of a sterol manufacturing pathway (Robbins et al. 1971, Vanderzant 1966). Experimental measurements of sterol usage are complicated by the ability of at least one insect, S. zeamais, to live on a cholesterol free adult diet due to a sterol carry-over from the larval stage (Brown and Chippendale 1975). However, Earle (1964) found that adult A. grandis required cholesterol for egg production and normal longevity. The possibility exists that S. zeamais might not be capable of egg production without an adult cholesterol source. Further tests utilizing successive generations and known amounts of cholesterol per generation may resolve this difficulty in measuring sterol usage.

A. grandis, S. oryzae, and S. granarius have been demonstrated to utilize more than one sterol effectively. Vanderzant (1963a)

showed that cholesterol, stigmasterol, and sitosterol were equally useful to the boll weevil. Minett (1973) determined that the plant sterols campesterol and β-stigmasterol could be converted to cholesterol by the rice weevil. The capacity of S. oryzae larvae to use β-sitosterol, ergosterol, cholesterol, cholesterol acetate, and 7-dehydrocholesterol was demonstrated in subsequent experiments (Baker 1974). These tests indicated that the mycetomal microorganisms in S. oryzae might possibly modify cholestanol to the benefit of the weevil. The poor ability of the aposymbiotic granary weevil to use cholestanol was considered supporting evidence. Larvae of S. granarius utilized β-sitosterol and cholesterol equally well, but cholesterol acetate, ergosterol, and 7-dehydrocholesterol delayed growth (Baker 1974).

In similar tests, Baker (1975) demonstrated some of the vitamin requirements for S. oryzae. Deletion tests using an amino acid diet indicated that S. oryzae required thiamine, nicotinic acid, pyridoxine, folic acid, and biotin for normal growth. A closely related species, S. zeamais, survived longer on an adult diet containing 4.0% vitamin mixture than on a diet without additional vitamins. The 4.0% vitamin mixture was used in addition to the vitamins found in wheat germ, a constituent of both diets. Thus, a quantitative requirement for the vitamin mixture could not be established.

Deletion experiments with a vitamin-free casein diet demonstrated that A. grandis larvae required six B-vitamins, pantothenic acid,

thiamine, riboflavin, pyridoxine, niacinamide, and folic acid (Vanderzant 1966). Unlike S. oryzae, biotin was not required by A. grandis (Vanderzant 1973). Vanderzant and Davich (1961) stated that ascorbic acid increased egg production and affected hatch rate. Subsequent tests suggested that there was no synthesis of ascorbic acid during any developmental stage of A. grandis (Vanderzant et al. 1962). Actual requirements for ascorbic acid may be small since boll weevil eggs contain enough to permit adult development. Complete development also occurred in diets free of Vitamin E (Earle et al. 1967). The authors, after rearing the weevil for three consecutive generations, concluded that there was no Vitamin E requirement for the boll weevil.

In order for growth and development to proceed, the insect must first feed. The production of a nutritionally adequate diet might not be sufficient for a feeding response to occur (Davis 1966). However, many of the necessary nutrients for insects were found to act as phagostimulants (Vanderzant 1966). These include sugars, proteins and amino acids, and lipids, as well as several miscellaneous chemicals (Davis 1966).

The literature contains specific examples of phagostimulatory substances. Keller et al. (1963) showed that the volatile materials removed from lyophilized cotton were attractive to the boll weevil. Earlier tests demonstrated that some phagostimulants from cotton were extractable with water (Keller et al. 1962). Other experiments showed that water extracts from different species and varieties of

cotton varied as feeding stimulants (Jenkins et al. 1963). Related studies indicated that the response of boll weevils to phagostimulants was affected by light, pH, age, sex, and diet (Hedin et al. 1966).

Hsiao (1969) discovered that adenine and adenosine acted as feeding stimulants for the alfalfa weevil. Subsequent experiments showed that sucrose as well as fructose and glucose to lesser extents, stimulated feeding by the adult alfalfa weevil (Yamamoto and Cambell 1972). In more recent tests Hsiao and Hsiao (1974a) found that freeze-dried or acetone extracted alfalfa leaf powders improved larval development when compared to diets without this leaf material.

D. Biological Studies of Ceuthorrhynchidius horridus (Panzer)

The initial description of C. horridus was published in 1801 with the generic name Curculio (Panzer 1801). In 1954, a more detailed description of the genus (which was previously modified to Ceuthorrhynchidius) and species was presented by Haufman. The generic name has remained unchanged since that time.

The field biology of C. horridus has been studied in Central and Southern Europe (Frick 1969, Haufman 1954). According to Haufman (1954) C. horridus overwinters in the adult stage in Central Europe. Auber (1960) stated that overwintering adults may become active as early as April. Oviposition lasts at least through June and the resulting adults emerge in September (Sherf 1964). A similar

life cycle occurs in the warmer climates of Southern Europe (Frick 1969). However, adults apparently undergo an aestival diapause during hot summer months which causes the southern population to be seasonally as well as geographically isolated from northern populations.

Since 1972, considerable emphasis has been placed on laboratory studies of C. horridus to determine the weevil's potential for biological control of thistles. Extensive feeding tests were undertaken to determine the host specificity and larval survival rates of first instars (Ward et al. 1974). Results of tests with thirty-five species of economic or aesthetically important plants indicated a narrow range of acceptability. Only the tribes Cynareae and Cichorieae of the family Compositae supported growth to the third instar. Two agriculturally important plants, globe artichoke (Cynara scolymus L.) and lettuce (Lactuca sativa L.), permitted limited larval development. As a host, lettuce is considered inconsequential since: 1) C. horridus has not been recorded as a pest of lettuce, 2) the essentially bland nature of lettuce permits feeding by many stenophagous insects, and 3) lettuce produced very low yields of last instars (Ward et al. 1974). Subsequent tests revealed that larvae reared on globe artichoke matured slowly and did not complete development to the adult stage (Kok 1975). Also, C. horridus has not been reported as a pest of artichoke, although the weevil commonly occurs in regions where artichoke is grown.

Utilizing a laboratory colony of C. horridus (genetic stock from Italy), basic biological information for this insect was determined by Kok et al. (1975). Adult lifespan averaged over 30 weeks, with males living approximately 6 weeks longer than females. Oviposition averaged slightly over 800 eggs per female with a preoviposition period of approximately 5 weeks. The white opaque eggs were found inserted in the leaf midrib singly or in clusters consisting of 2 to 14 eggs. The egg stage averaged 13.5 days at day-night temperatures of 21°-10° C. Upon eclosion larvae burrowed down the midrib toward the root crown where larval development through the third (last) instar was completed. Duration of larval development averaged 65 to 68 days at 21° C. Pupal stage averaged 18.7 days and pupation occurred in loose cells made of silk and soil near the plant crown.

Ward and Kok (1975) studied the effect of varying photoperiods on oviposition by C. horridus. Results indicated that oviposition occurred with photophases declining from 16 to 8 hours, or if subjected to an initial 8 hour photophase for 6 weeks followed by declining photophases from 12 to 8 hours. Oviposition also occurred with constant photophases lasting 8 or 9 hours. Weevils subjected to constant light or dark produced no eggs, while those subjected to 14 or 16 hour photophases oviposited poorly or not at all.

III

LABORATORY PROPAGATION OF C. HORRIDUS

Materials and Methods

A colony of C. horridus was maintained in the V. P. I. and S. U. quarantine laboratory to provide a reliable source of eggs for experimentation. Adult weevils, imported from Rome, Italy, were incorporated into the colony at least twice a year to insure genetic diversity and supplement laboratory populations. Upon arrival, imported weevils were placed in an air-conditioned quarantine room (13°-16° C), given fresh plant material, and observed for 2 weeks for disease or parasites. For egg production adults were sexed, sorted into groups with female:male ratios of 3:2, and placed in 0.4 liter styrene cages fitted with 25 mesh wire screen windows at the top and opposite sides. Feeding and oviposition occurred on bouquets of plant material, the ends of which were immersed in a jar of water via a 1.3 cm plastic tube through the cage bottom. Cheesecloth was wrapped firmly around the stems and prevented the weevils from falling into the water.

Each cage contained a musk thistle leaf (Carduus nutans L.) and a leaf of either globe artichoke (Cynara scolymus L.), plumeless thistle (C. acanthoides), or bull thistle (Cirsium vulgare (Savi) Tenore). All thistle leaves were from plants grown in the greenhouse and washed thoroughly before use. The weevils were most productive when provided with musk thistle and globe artichoke, and maintained

under short photoperiods of light-dark (LD):9-15 that were syncronized with day-night thermoperiods of 21°-10° C. A Controlled Environments Inc.® model E-7-H chamber was used for this purpose. The plant material was changed weekly; the old leaves were dissected to obtain weevil eggs.

Eggs were maintained in 9.0 cm diameter plastic petri dishes on filter paper moistened with distilled water. The filter paper rested on a thin layer of damp cotton which served as a moisture reservoir. Daily inspection of each petri dish permitted the addition of distilled water and the removal of any newly eclosed larvae.

In order to maintain laboratory populations, first instars were inoculated into the growth point of thistle rosettes in which a forcep puncture had been made. Larvae were transferred using a double '0' camel hair brush. A small section of thistle leaf was then wedged in the puncture to prevent the larvae from leaving and subsequently dehydrating. After six weeks, the inoculated rosettes were enclosed in 51 x 20 cm clear polyethylene bags. Newly emerged weevils generally exhibited a positive phototaxis and were easily detected.

IV

LARVAL DEVELOPMENT ON ARTIFICIAL DIETS: NUTRITIONAL EXPERIMENTS

A. Developmental strategy for designing artificial diets

Introduction

The development of diets for C. horridus was predicated on results of nutritional studies on many insects, especially those on the alfalfa and blackvine weevils. The alfalfa weevil, the closest relative for which synthetic diets have been prepared, offered an insight into the nutritional needs of a weevil in a closely related subfamily. Studies on the blackvine weevil, an insect with a life history similar to C. horridus suggested techniques for maintaining suitable larval and pupal environments throughout long developmental periods.

The early diets for C. horridus (numbers 1-12) were designed to produce a diet which would induce larval feeding. For this purpose several types of plant material were incorporated into the diets. Upon formulation of a diet which induced larval feeding, refining of the test diets was based on a two part strategy propounded by Davis (1972). First, a systematic modification of diets which permitted suboptimal feeding was undertaken. This allowed for substitutions, deletions, and additions in each of the major nutritional categories (carbohydrates, proteins, etc.) (Table I).

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus.

Ingredients[a]	Diet #									
	1	2	3	4	5	6	7	8	9	10
Basic Components:										
Agar	15[b]	15[b]	6.4[b]	6.4	6.4	6.4	6.4	15	15	6.4
Ascorbic acid	2.0	2.0	1.63	1.63	1.63	1.63	1.63	2.0	2.0	1.63
Casein hydrolysate					5.0					5.0
Cholesterol				0.5					1.0	2.0[d]
Methyl-p-hydroxybenzoate	0.9	0.9	1.0	1.0	1.0	1.0	1.0	0.9	0.9	1.0
Sorbic acid	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.5
Wheat germ			25	25	25	25	25			25
Wesson Salt Mixture 'W'				0.5	0.5				1.0	0.5
Brewers yeast	20	20						20	20	
Torula yeast			16	16	16					16
Carduus nutans	25	60[c]	10	10	10	10[d]	10[d]	10	10	10
Distilled Water (ml)	600	250	320	480	320	330	330	425	425	330
Potassium hydroxide					1.0					1.0
Other Ingredients:										
Vitamin Mixture				1.0		1.0	1.0[d]			2.0[d]
Pinto beans	60	60						60[c]		
Benlate				0.1		0.1	0.1			
Corn oil (ml)				2.0		2.0	2.0			
Lima Beans									60[e]	
Formaldehyde 40% (ml)			1.0	0.5						1.0
7-dehydro-cholesterol						0.5	0.5[d]			

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus (cont.).

Ingredients	Diet # 11	12	13	14	15	16	17	18	19	20
Basic Components:										
Agar	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
Ascorbic acid	1.63	1.63	1.63	1.63	1.63	1.63	2.63[f]	1.63[d]	1.75[d]	1.75[d]
Casein hydrolysate	5.0	5.0	5.0[d]	5.0	5.0	5.0	5.0	5.0		7.5
Cholesterol	2.0	2.0[d]	2.0[d]	2.0	2.0			1.0[d]	1.0[d]	1.0[d]
Methyl-p-hydroxybenzoate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sorbic acid	0.5	0.5	0.5			0.5	0.5	0.5	0.5	0.5
Wheat germ	25	25	25	25	25	25	25	25	25	25
Wesson Salt Mixture 'W'	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Brewers yeast										
Torula yeast	16	16	16	16	16	16	16	16	16	16
Carduus nutans	10	10	10	10	10	10	10	10	10	10
Distilled Water (ml)	340	340	340	340	340	470	320	340	350	360
Potassium hydroxide	1.0	0.5	1.0	0.5	0.5	1.0	1.0	1.0	1.0	1.0
Other Ingredients:										
Vanderzant-Adkisson Vitamin Mix			2.0[d]				2.0[d]	1.0[d]	1.0[d]	1.0[d]
Corn oil (ml)					2.0					
Inositol							1.0[d]			
Sucrose-D							3.0[d]			
Choline chloride								3.0		
Soy hydrolysate									7.5	5.0
Linolenic acid (ml)										2.0

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus (cont.).

Ingredients	Diet #									
	21	22	23	24	25	26	27	28	29	30
Basic Components:										
Agar	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
Ascorbic acid	1.75[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]
Casein hydrolysate		5.0	5.0	5.0	5.0	5.0[d]	5.0	5.0[d]	5.0[d]	5.0[d]
Cholesterol	1.0[d]	1.0[d]	1.0[d]	1.0[d]	1.0[d]	1.0[d]	1.0[d]	2.0[f]	1.0[d]	1.0[d]
Methyl-p-hydroxybenzoate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sorbic acid	0.5	0.5	0.5	0.5	0.5	0.75	0.5	0.5	0.5	0.5
Wheat germ	25	25	25	25	25	25	25	25	25	25
Wesson Salt Mixture 'W'	0.5	0.5	0.5	0.5	0.5	0.5[d]	0.5	0.5	0.5	0.5
Brewers yeast										
Torula yeast	16	16	16	16	16	16[d]	16	16	16	16
Carduus nutans	10	10	10	10	10	10[d]	10	10	10	8.0
Distilled Water (ml)	360	360	360	360	360	360[d]	360	360	360	360
Potassium hydroxide	1.0	1.0	1.0	1.0	1.0	1.0[d]	1.0	1.0	1.0	1.0

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus (cont.).

Ingredients	Diet #									
	21	22	23	24	25	26	27	28	29	30
Other Ingredients:										
Vanderzant-Adkisson Vitamin Mix	1.0[d]	1.0[d]	1.0[d]	1.0[d]	1.0[d]	1.0[d]	1.5[d]	2.0[f]	1.0[d]	1.0[d]
Soy hydrolysate	1.0[d]									
Inositol		2.0[d]			1.0[d]	1.0[d]				
Arginine-L		0.5[d]							0.5[d]	1.0[d]
Histidine-L	0.5[d]								0.5[d]	
Isoleucine-L	0.5[d]								0.5[d]	
Leucine-L	0.5[d]								0.5[d]	
Lycine monohydrochloride	0.06[d]								0.5[d]	
Methionine-L	0.5[d]								0.5[d]	
Phenylalanine-L	0.5[d]								0.5[d]	
Threonine-L	0.5[d]								0.5[d]	
Tryptophan-L	0.5[d]								0,5[d]	
Starch		10								
Sucrose-D		3.0								
Choline chloride					1.0		0.75			
Linoleic acid (ml)					2.0					
Corn oil (ml)					3.0				2.0[d]	
Biotin							0.25			
Folic acid							0.25			
Nicotinic acid							1.0			
Vitamin B-12 with Intrinsic Factor							0.25			
Valine-L									0.5[d]	
Glutamic acid										1.0[d]

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus (cont.).

Ingredients	Diet #									
	31	32	33	34	35	36	37	38	39	40
Basic Components:										
Agar	6.4[d]	6.4[d]	6.4[d]	6.4[d]	6.4[d]	6.4[d]	6.4[d]	6.4	6.4[d]	6.4[d]
Ascorbic acid	1.0[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]	1.75[d]		1.75[d]	1.75[d]
Casein hydrolysate	5.0[d]	5.0[d]	5.0[d]	5.0[d]	5.0[d]	5.0[d]	5.0[d]			5.0[d]
Cholesterol	0.5[d]	1.0[d]	0.5[d]	1.0[d]	1.0[d]	1.0[d]	1.0[d]		1.0[d]	1.0[d]
Methyl-p-hydroxybenzoate	1.0	1.0	1.0	1.0	1.0	1.0	1.0		1.0	1.0
Sorbic acid	0.5[d]	0.5	0.5	0.5	0.5	0.5	0.5		0.5	0.5
Wheat germ	25	25	25	25	25	25	25			25
Wesson Salt Mixture 'W'	0.5	0.5[d]	0.5	0.5	2.5	0.5	0.5		0.4	0.5
Brewers yeast		16[d]								
Torula yeast	16		16	16	16	16	16		16	16
Carduus nutans	10	10	10	10	10	10	10	5.0	10	7.0
Distilled Water (ml)	360	360	360	360	360	360	360	300	300	360
Potassium hydroxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0			

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus (cont.).

Ingredients	Diet #									
	31	32	33	34	35	36	37	38	39	40
Other Ingredients:										
Vanderzant-Adkisson Vitamin Mix	1.0[d]	1.0[d]	1.0[d]	4.0[d]	1.0[d]	1.0[d]	1.0[d]		1.0[d]	3.0
Alphacel						10				
Soy hydrolysate							5.0[d]			
Arginine-L							1.0[d]			
Histidine-L							1.0[d]			
Isoleucine-L							1.0[d]			
Leucine-L							1.0[d]			
Lycine monohydrochloride							1.0[d]			
Methionine-L							1.0[d]			
Phenylalanine-L							1.0[d]			
Tryptophan-L							1.0[d]			
Threonine-L							1.0[d]			
Valine-L							1.0[d]			
Sucrose-D							2.0[d]	2.0		
Linoleic acid (ml)							1.0[d]			
Adkisson-Vanderzant Diet Mix								30	25	
Bicarbonate of Soda								1.5		1.5

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus (cont.).

Ingredients	Diet # 41	42	43	44	45	46	47	48	49	50
Basic Components:										
Agar		22.5	8.0	6.4	6.4	12	15	15	6.4	6.4
Ascorbic acid	3.0	3.75[d]	3.75	1.75[d]	1.75[d]	2.0[d]			1.75[d]	1.75[d]
Casein hydrolysate	8.0[i]			5.0[d]	5.0[d]	5.0			5.0[d]	5.0[d]
Cholesterol	0.5			1.0	1.0		0.25[d]	0.25[d]	2.0[f]	1.0
Methyl-p-hydroxybenzoate	1.0	4.5[dj]	4.5[dj]	1.0	1.0	0.9[d]	0.85[d]	0.85[d]	1.0	1.0
Sorbic acid	0.5	4.5[dj]	4.5[dj]	0.5	0.5	0.4[d]	0.44[d]	0.44	0.5	0.5
Wheat germ	25			25	25				25	25
Wesson Salt Mixture 'W'	2.0			0.5	1.0	2.0			0.5	0.5
Brewers yeast										
Torula yeast	16			16	16				16	16
Carduus nutans	10	10[d]	10[d]		5.0	5.0		4.0		
Distilled Water (ml)	360	657	360	360	360	200[d]	400	400	360	360
Potassium hydroxide	1.0	4.5[dk]	4.5[dk]	1.0	1.0	0.6[d]	5.2[dk]	5.0[dk]	1.0	1.0

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus (cont.).

Ingredients	Diet #									
	41	42	43	44	45	46	47	48	49	50
Other Ingredients:										
Vanderzant-Adkisson Vitamin Mix	3.0	2.25[d]	2.25[d]	1.0[d]	1.0[d]	2.0[d]	2.75[d]	2.75[d]	2.0[f]	1.0[d]
Arginine-L	1.0				1.0					
Sucrose-D	1.0									
Glutamic acid	1.0				1.0					
Alphacel	5.0						25[d]	25[d]	10	10
Adkisson-Vanderzant Diet Mix		100[d]	100[d]							
Choline chloride 10% (ml)		9.0[d]	9.0[d]							
Formaldehyde 10% (ml)		3.75[d]	3.75[d]							
Streptomycin sulfate		0.125[d]	0.125[d]				0.75[d]	0.75[d]		
Cynara scolymus				10	5.0					
Pinto beans						60				

Table I. Summary of Composition of 50 Diets Used in Feeding Tests of Ceuthorrhynchidius horridus (cont.).

Ingredients	Diet #									
	41	42	43	44	45	46	47	48	49	50
Instant Milk									6.5[d]	6.5[d]
Cysteine									0.5[d]	0.5[d]
Corn oil (ml)									2.5[d]	2.5[d]

[a] g unless specified
[b] Used NBC instead of Difco
[c] Thistle not lyophilized
[d] Added after autoclaving
[e] Soaked overnight in 160 ml distilled water
[f] Includes 1.0 g added after autoclaving
[g] Standard cabbage looper diet
[h] After Shanks and Finnigan (1971)
[i] Titrated in 35 mls of 95% ETOH onto 1.0 g sugar and 5.0 g alphacel
[j] 38% in 95% ETOH - values in ml
[k] ml of 4.0 Molar solution

Second, intuition was used when creating a new diet or altering a previous one, thereby opening substantial possibilities for variation in the diets.

B. Laboratory preparation of artificial diets

Materials and Methods

Diet ingredients were U. S. Certified Pure or reagent grade unless otherwise specified in Appendix A. Those compounds susceptible to heat denaturation were stored in a refrigerator at 4° C. The remaining commercially available ingredients were maintained at 20° C in light-excluding cabinets. Thistle or artichoke, grown without use of pesticides, was lyophilized for at least 48 hours in a Virtis® Universal Sub-Mobil #15 freeze dryer before incorporation into test diets. Upon removal from the lyophilizer, the plant material was powdered in a Waring Blender® and sealed in a 0.4 liter styrene container with a polyethylene lid. The plant material was stored at -8° C in a Futura® freezer. Application of this technique produced a product of consistent moisture and texture throughout the duration of these experiments.

Standard procedures for diet production were as follows. Initially, components were weighed on a Sartorius® balance Model #1106. Constituents to be added after sterilization were placed in sterilized beakers and set aside until needed. The other ingredients were then blended for 2 minutes with 350 ml distilled water in a Waring Blender® . The resulting mixture was drained into a 1,500 ml

Pyrex® beaker capped with Reynolds® heavy duty aluminum foil. The mixture was then autoclaved at 121° C and 21 psi for 17 minutes in an Amsco® Medallion Series sterilizer. After removal, the diet was allowed to cool to 60° C before incorporation of any further ingredients (Table I). The diet was reblended in a Waring Blender (sterilized with 95% ETOH) and transferred to either 30 ml plastic containers (Premium Plastics) or to 15 ml #31 clear plastic boxes (Durphy Packaging Co.). The #31 friction-top containers (sterilized in 95% ETOH and rinsed in 90°-98° C distilled water) were filled to a depth of 1.0 cm (approximately 9 ml diet). The 30 ml plastic containers were similarly sterilized and filled to a depth of 2 cm (15 ml). The diet was allowed to cool for 2 hours with the containers uncapped in an alcohol and blacklight sterilized plexiglas drying cabinet. The cabinet (32.5 x 35 x 60 cm), was equipped with 2 sleeve access ports, a Formica® bottom and a G. E. germicidal blacklight #G875 (Figure 1). After cooling, the diets were capped and stored in a refrigerator at 4° C. Diets not used within 30 days were discarded.

C. Testing of artificial diets - efficacy and x-ray analysis

The efficacy of C. horridus diets was tested using two techniques. First, diets were examined for larval growth and development, and second, the EDAX International® (Energy Dispersive X-ray analysis) unit of the AMR® model 900 scanning electron microscope was utilized to determine the presence or absence of selected minerals.

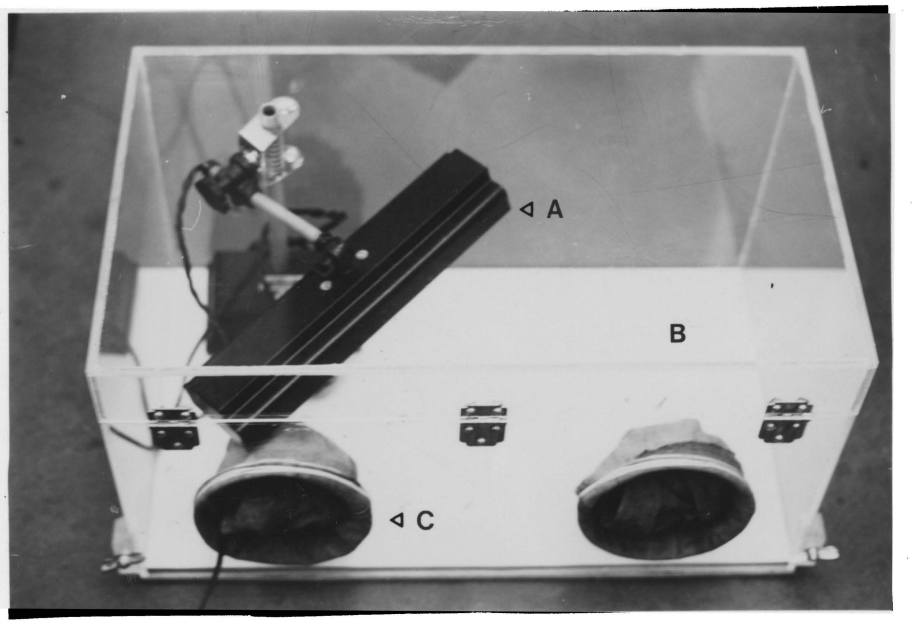

Figure 1. Sterile Plexiglas Sleeve-cage Equipped With Germicidal Blacklight. A) Germicidal Blacklight, B) Formica® Base, C) Sleeve access.

Prior to the larval experiment the surface of each diet was abraded with flame sterilized forceps. Sixty newly eclosed C. horridus were transferred from egg-containing petri dishes to each diet with a double '0' camel hair brush at the rate of 5 larvae per diet container. The rough surface aided larval penetration into the diet. Four diet containers (2 each of the 15 and 30 ml plastic containers) were placed in each of 3 light excluding chambers (61 x 61 x 61 cm) set for photoperiods of light-dark (LD):9-15, 12-12, and 0-24 hours respectively. Each chamber (Figure 2) contained an internal rotary fan which circulated air over a heating coil. Activity of the heating coil was controlled by a variable thermostat. Placing the chambers in an air-conditioned room (13°-16° C) permitted temperature maintenance at 21° C $\pm$ 1° C. Weekly examinations of diet containers were made for signs of contamination, frass production, and larval growth. Diets were terminated only because of contamination or death of the larvae.

The EDAX unit was used to determine the presence or absence of magnesium, sodium, phosphorus, chlorine, potassium, and calcium in thistle leaves and selected artificial diets. These minerals were chosen on the basis of their importance in living systems and the limitations of the EDAX. Comparisons between successful and unsuccessful diets, and between diets with or without thistle material were conducted on selected diets. Because the scanning electron microscope operates in a vacuum, the samples were lyophilized

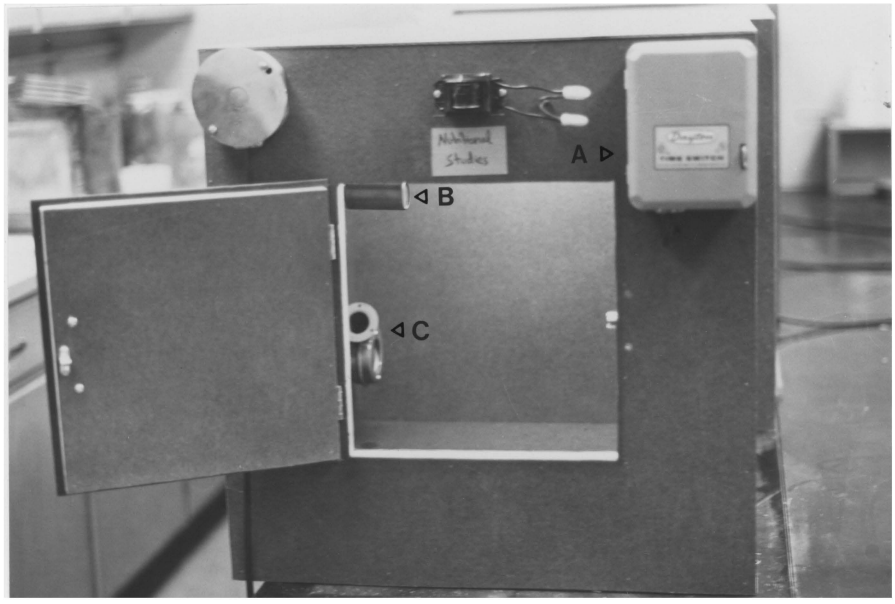

Figure 2. Dual-walled, Temperature Controlled Photoperiod Chamber With Internal Rotary Fan. A) Photoperiod Clock, B) Heating Coil, C) Rotary Fan.

for 48 hours in a Virtis® USM-15 before use. Several locations on each sample were scanned. Scanning size was 3 mm^2 surface area by 150 Å in depth. Polaroid® pictures were then taken of representative readouts from each sample.

Results and Discussion

Feeding responses of first instars on various diets are summarized in Table II. Twenty-eight of the diets tested did not support growth past the second instar, and were therefore unacceptable. Failure of the diets to support feeding may have been due to: physical texture, nutrition, contamination, lack of feeding stimulants, presence of feeding deterrents, or a combination of the above. These diets were not included in subsequent experiments.

Major components of successful diets included: wheat germ, ascorbic acid, cholesterol, brewers or torula yeast, Wesson's salt mixture 'W', soy or casein hydrolysate, lyophilized musk thistle, and agar. The best larval development was achieved on diets in which a vitamin supplement, cholesterol and ascorbic acid were added after sterilization. Methyl-p-hydroxybenzoate and sorbic acid were incorporated to reduce fungal contamination. Diet pH was adjusted to 6.2 - 6.4 with potassium hydroxide. The addition of formaldehyde, amino acids, or plant material other than musk thistle proved unsatisfactory.

As development of the larvae to the last instar occurred more often in photoperiods of LD:0-24 than LD:9-15 or LD:12-12, subsequent

Table II. Development of Ceuthorrhynchidius horridus larvae on 50 Diets Maintained at Different Photoperiods.

Diet #	# Wks to Frass Production	% Mold	Maximum[a] Growth (Instar)	# Third[b] Instars Produced	Photoperiod[c] Producing Last Instars
1	-	100	-	-	-
2	-	25	-	-	-
3	3	0	3	4	2,3
4	5	42	2	-	-
5	3	42	3	2	1,3
6	-	0	-	-	-
7	-	0	-	-	-
8	-	25	-	-	-
9	-	92	-	-	-
10	-	0	-	-	-
11	3	17	3	3	3
12	3	50	3	4	1,2,3
13	3	25	3	7	3
14	3	83	3	2	3
15	-	25	-	-	-
16	-	75	-	-	-
17	-	0	1	-	-
18	-	0	1	-	-
19	4	0	3	2	3
20	4	25	3	3	3
21	3	67	3	5	3
22	3	92	3	3	1
23	4	25	3	6	1,2,3
24	3	17	3	3	1,3
25	3	50	3	3	2,3
26	4	67	2	-	-
27	5	0	3	1	3
28	3	0	3	4	3
29	4	92	1	-	-
30	-	100	2	-	-
31	-	100	1	-	-
32	3	0	3	6	1,2,3
33	3	58	3	5	2,3
34	3	0	3	6	1,2,3

Table II. Development of Ceuthorrhynchidius horridus larvae on 50 Diets Maintained at Different Photoperiods (cont.).

Diet #	# Wks to Frass Production	% Mold	Maximum[a] Growth (Instar)	# Third[b] Instars Produced	Photoperiod[c] Producing Last Instars
35	3	83	3	7	1,3
36	3	42	3	3	2,3
37	-	83	1	-	-
38	-	100	-	-	-
39	-	100	1	-	-
40	-	33	1	-	-
41	-	0	-	-	-
42	-	0	-	-	-
43	-	58	1	-	-
44	3	8	3	3	3
45	-	0	1	-	-
46	-	100	-	-	-
47	4	92	2	-	-
48	3	42	2	-	-
49	3	100	2	-	-
50	3	25	3	3	2,3

[a]- refers to first instars which did not feed, 1 refers to actively feeding first instars.
[b]Based on 60 larvae/diet.
[c]1 = LD:9-15, 2 = LD:12-12, 3 = LD:0-24.

experiments requiring larval development on artificial diets were carried out in constant darkness. Another result of this test was that the 15 ml friction-seal containers were superior to the 30 ml plastic cups in controlling dehydration of the diets.

Frass was produced in all diets which permitted larvae to develop to the last instar. Figure 3 indicates that diets showing frass production in 3 weeks supported more development to the last instar than those with frass in 4 or 5 weeks. Differences in mean number of last instars per diet are significant between those producing frass in 3 versus 4 weeks (t test:$P < .01$). Diets in which frass production did not occur until 5 weeks rarely supported larval development to the last instar; a single third instar was found in only one of the diets. From the preceding evidence, diets which showed frass production at 3 weeks, rather than 4 or 5 weeks, were considered when planning subsequent experiments.

D. Mineral content of selected diets: energy dispersive x-ray analysis

Results of experiments utilizing the EDAX attachment to the scanning electron microscope are shown in Figures 4 through 8. These are mineral traces for artificial diets #23, #28, #34, #50; and for C. nutans leaf respectively. The major nutritionally important minerals present in C. nutans were present in all four diets examined. Specifically, minerals detected included: magnesium, sulfur, phosphorus, chlorine potassium, and calcium. When comparing diets #23, #28, and #34 with diet #50 (which differs from diet #34 through

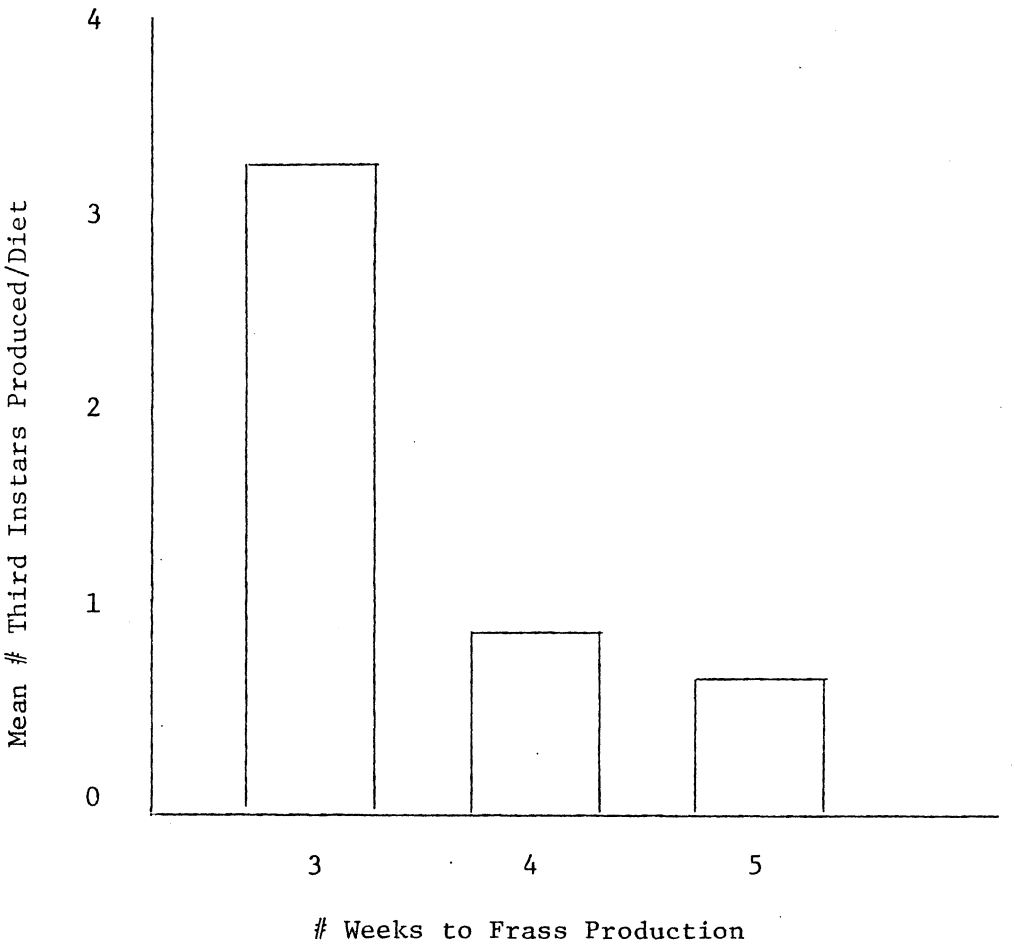

Figure 3. Graph Showing # Weeks to Frass Production Versus Mean Number of Third Instars Produced per Diet Tested.

replacement of thistle with mineral-free alphacel), the variation in relative amounts of minerals appears minimal; especially since all peaks fit on the smallest vertical scale (i.e., 250). Thus, the thistle material incorporated into diets #23, #28, and #34 did not greatly influence the mineral content present in these diets. Although Figures 4 through 8 represent single locations in each sample, several readings were taken for each sample without detecting noticeable variations. The similar mineral traces for each diet tested also indicates that the blending procedures were adequate.

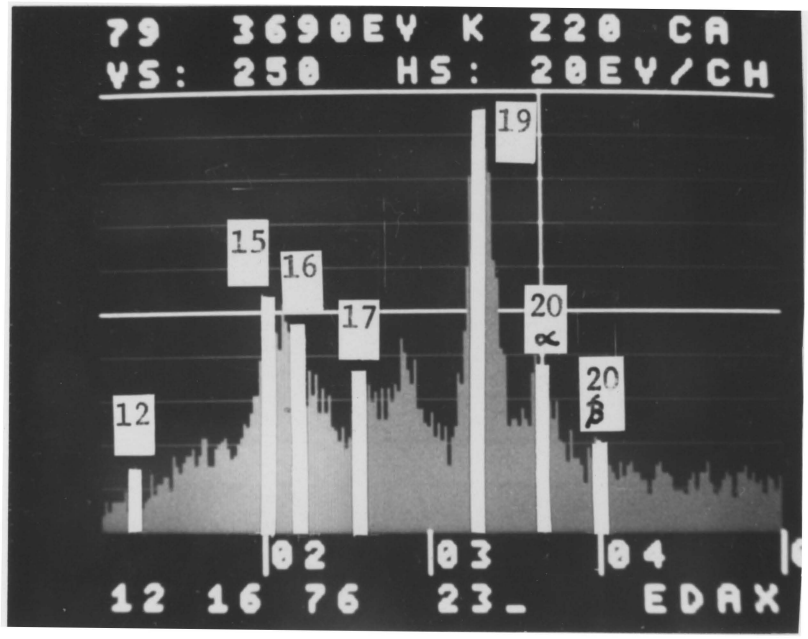

Figure 4. EDAX Mineral Tracing for Diet #23 With Magnesium (12), Phosphorus (15), Sulfur (16), Chlorine (17), Potassium (19), and Calcium (20) α and β Highlighted (Vertical Scale 250).

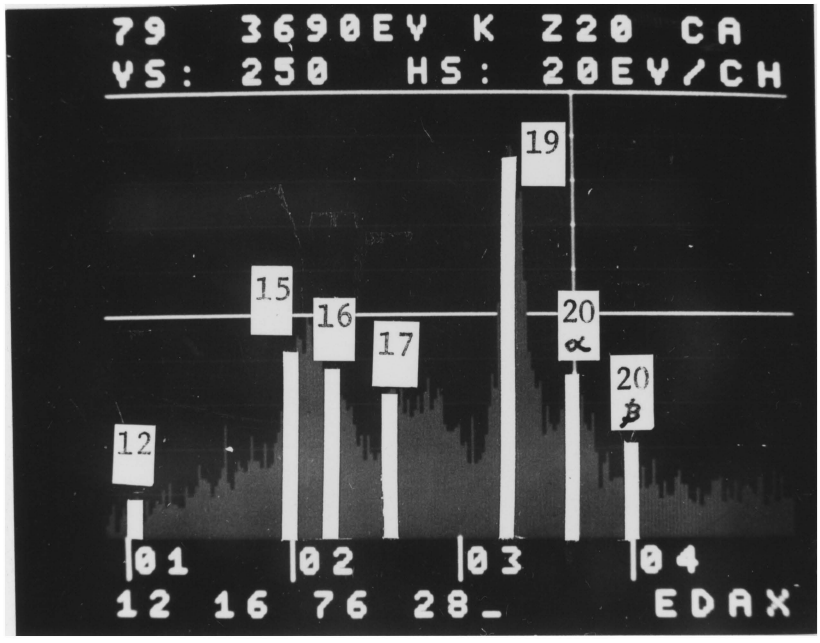

Figure 5. EDAX Mineral Tracing for Diet #28 With Magnesium (12), Phosphorus (15), Sulfur (16), Chlorine (17), Potassium (19), and Calcium (20) α and β Highlighted (Vertical Scale 250).

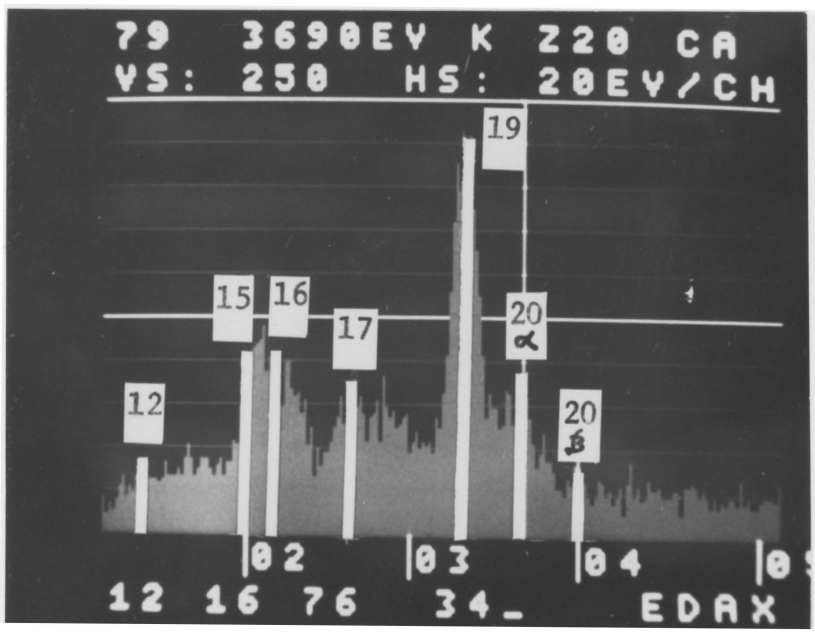

Figure 6. EDAX Mineral Tracing for Diet #34 With Magnesium (12), Phosphorus (15), Sulfur (16), Chlorine (17), Potassium (19), and Calcium (20) α and β Highlighted (Vertical Scale 250).

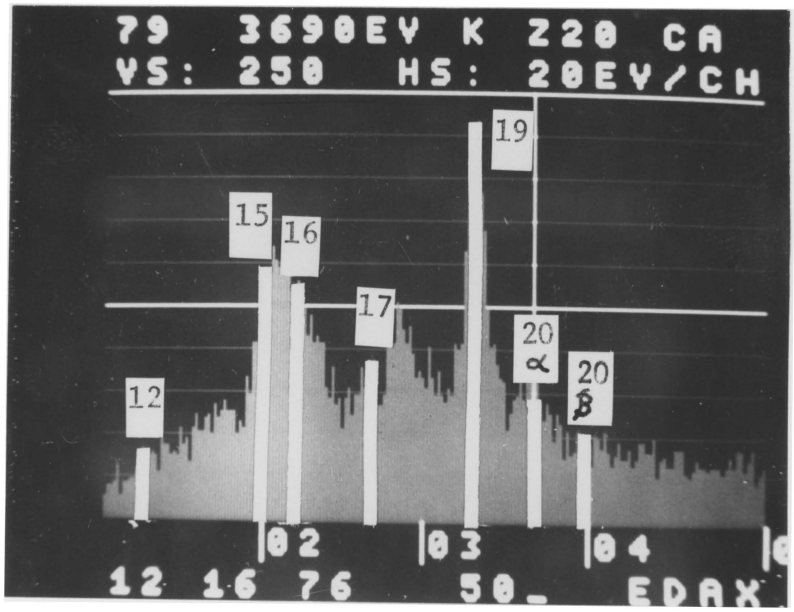

Figure 7. EDAX Mineral Tracing for Diet #50 With Magnesium (12), Phosphorus (15), Sulfur (16), Chlorine (17), Potassium (19), and Calcium (20) α and β Highlighted (Vertical Scale 250).

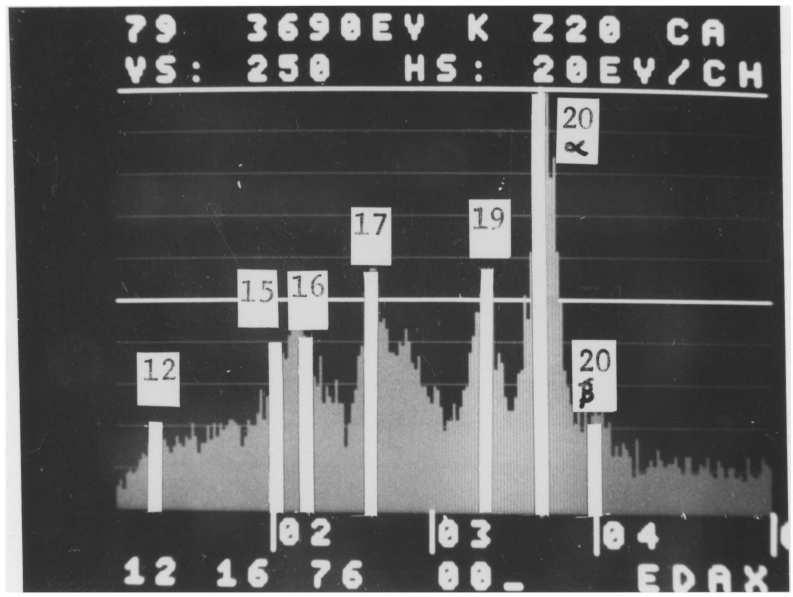

Figure 8. EDAX Mineral Tracing for A Musk Thistle Leaf With Magnesium (12), Phosphorus (15), Sulfur (16), Chlorine (17), Potassium (19), and Calcium (20) α and β Highlighted (Vertical Scale 250).

V

PUPATION EXPERIMENTS

A. Photoperiodic induction of undesirable rhythms

Introduction

Although twenty-two of the artificial diets tested in the nutritional experiments produced last instars, pupation was not observed. Several explanations appear plausible, and are examined individually in this section.

One possibility for lack of pupation was that the repeated admission of light during observation of diets in the LD:0-24 chamber was causing either a photoperiodic induction of diapause or simply stressing the insects through asymmetric alteration of photoperiodically induced rhythms. According to Beck (1962) and McLeod and Beck (1963), photoperiod is an important factor in inducing diapause in many insect species. Also, evidence indicates that many motor activity rhythms, including feeding and locomotion, are influenced by photoperiod (Beck 1968). This hypothesis was tested experimentally.

Materials and Methods

Thirty newly eclosed larvae, 2 replicates of 15, were placed on 21 diets which had produced last instars in the previous experiment. Because diet #50 was formulated after the completion of this test, it could not be included. Five larvae were placed in each diet container. The diet containers were wrapped in heavy duty

Reynolds® aluminum foil to exclude light. After 6 weeks all diet cups were checked for signs of contamination, larval development, and pupation. Monthly checks were made for 10 months thereafter to determine if pupation had taken place. Diets were discarded only if badly contaminated. After 10 months the diets were carefully dissected for signs of pupation. The data from the nutrition experiments LD:0-24 chamber was used to compare the effect of interrupted and uninterrupted darkness on larval development and contamination.

Results and Discussion

Results of this experiment are presented in Table III. Since no signs of pupation were discovered during the test, the limiting factor for pupation does not appear to be induced by photoperiod. However, the possibility that photoperiod played a secondary role in inhibition of pupation was not eliminated. In addition, uninterrupted darkness was not beneficial to larval development. Ten of the 21 diets subjected to uninterrupted darkness produced live third instars at 6 weeks whereas all but one of the diets exposed to interrupted darkness did so. Also, the percentage of last instars alive at 6 weeks for the treatment in interrupted darkness was over twice that for larvae in uninterrupted darkness. This suggests that the admission of light to the LD:0-24 chamber may have had a positive rather than negative effect.

Although contamination appeared to be generally higher in treatments of uninterrupted darkness, this might have been effected by

Table III. Growth of Ceuthorrhynchidius horridus in, and Contamination of, Diets Subjected to Complete and Interrupted Darkness for Six Weeks.

Diet #	Maximum Growth Stage I[a]	Maximum Growth Stage II[b]	% Third Instars per Diet I	% Third Instars per Diet II	% Diets Contaminated I	% Diets Contaminated II	% Live Larvae I	% Live Larvae II
3	2[c]	3	0	25	0	0	0	5
5	1[c]	3	0	20	87.5	25	0	5
11	1	3	0	15	100	0	0	15
12	3[d]	3	16.7	25	25.0	50	11.7	15
13	-[d]	3	0	35	87.5	0	0	35
14	-	3	0	10	100	75	0	10
19	2	3	0	10	25.0	0	0	10
20	1	3	0	10	87.5	50	0	5
21	3	3	1	25	50.0	50	10.0	15
22	2	3	0	15	62.5	100	0	5
23	3	3	6.7	25	37.5	0	3.3	25
24	3	3	18.3	15	75.0	25	16.7	5
25	1	3	0	5	87.5	75	0	0
27	3	3	6.7	5	0	0	6.7	5
28	3	3	3.3	30	25.0	0	3.3	20
32	3	3	25.0	30	50.0	0	15.0	15
33	3	3	18.3	25	75.0	50	15.0	15
34	3	3	11.7	25	0	0	11.7	15
35	3	3	16.7	35	100	75	0	25
36	3	3	6.7	15	75.0	25	5.0	10
44	2	3	0	5	50.0	0	0	15

[a] Uninterrupted darkness - based on 30 larvae/diet.
[b] Interrupted darkness - based on 60 larvae/diet.
[c] Refers to actively feeding enlarged first instar.
[d] Refers to first instar without feeding or growth.

environmental contamination during inoculation of larvae into diets. In spite of weekly sterilization of floors with 10% bleach and daily bench sterilization with 95% ETOH, the maintenance of a wax moth and a false Colorado potato beetle colony in the same room may have affected contaminant levels adversely. Because of this, accurate association between contamination and interrupted versus uninterrupted darkness cannot be predicted.

B. Accumulation of Toxic Materials

Introduction

Another hypothesis, tested concurrently with the previous experiment, speculated that toxic materials, either larval waste or by-products of diet degeneration, were becoming increasingly concentrated during the 6 week larval developmental period. Conceivably these materials could prevent pupation through physical damage to the larvae. Because only one other insect with a developmental period of 2 to 3 months has been reared on artificial diets (Shanks and Finnigan 1973), little information was available to support or contradict this assumption.

Materials and Methods

The procedures used in this test were similar to those employed in the previous experiment. A total of 110 larvae was tested on each of 21 diets which had produced last instars in the nutritional experiment. Diet containers were wrapped in aluminum foil and

placed in LD:0-24 chambers maintained at 21° C ± 1° C. After 4 weeks any live larvae present were transferred to a fresh diet of the same composition. These were wrapped in foil and checked monthly for evidence of pupation. After 10 months the diets were carefully dissected.

Results and Discussion

The results of this experiment were generally negative. No adults emerged, and no sign of pupation was detected. Therefore, the buildup of toxic materials during larval development was not the sole factor inhibiting pupation. Also, since the foil-wrapped diet containers were disturbed only once during 6 weeks, the combination of photoperiod and toxic substance accumulation was probably not the principal cause for the absence of pupation.

C. Detrimental effects of diet shape and depth

Introduction

Since the previous experiment failed to explain the lack of pupation, a third hypothesis was tested. It stated that the diet's shape or depth may have affected larval feeding or pupation. Although these phenomena have not been reported for weevil larvae, Yonce et al. (1972) found that adult female plum curculios preferred to feed on curved surfaces. Also, Vanderzant and Davich (1961) determined that adults of both sexes of the boll weevil preferred to feed on curved rather than flat-surfaced diets. No information was available on how

deep these weevils tunneled in artificial diets. Observations during experiments with C. horridus indicated that a few larvae tunneled to the lower extent of the diet. Thus, restricted diet depth could interfere with larval feeding patterns or normal prepupation movements.

Materials and Methods

Based on this hypothesis, diet #34 was used, but with a curved surface and a greater depth. This diet was formulated as in Table I and poured into sterile 17 ml cylindrical vials. By tilting and gently rotating the vial while pouring, a large curved surface was produced. A meniscus 3 to 3.5 cm from the vial's base was formed with 8 to 9 ml of diet. Diets were allowed to cool for 2 hours in the sterilized plexiglas drying cabinet described previously. Vials were sealed with 95% ETOH sterilized double 'O' neoprene stoppers. One hundred larvae were inoculated at the rate of 5 per vial and held in a photoperiod of LD:0-24. To limit the spread of contaminants, the vials were separated into groups of 5 and placed in 0.4 liter styrene containers with polyethylene lids. Weekly observations were made for larval growth, contamination and signs of pupation. Diets were terminated only when contaminated or when all larvae died.

Results and Discussion

Nine per cent of the larvae inoculated developed to the last instar but none pupated. This suggests that the curved surfaces of the diet did not stimulate feeding. Difficulty in larval penetration due to the smooth unscored surfaces may be responsible for the small

number reaching the last instar. Contamination was minor, affecting only 15% of the diet containers. No larva was observed to tunnel deeper than 1.0 cm. This is less than the media depth available in the nutritional experiments. For this reason, an increase in diet depth beyond 1.0 cm was not considered an important factor for stimulating either feeding or pupation.

D. Pupation substrate test

Introduction

An examination of pertinent literature provided a fourth hypothesis explaining the absence of pupation. Shanks and Finnigan (1973) discovered that few larvae (1%) of the blackvine weevil pupated when left on artificial diets. However, when last instar larvae were transferred to peat moss, the majority pupated. Yonce et al. (1971) found that a soil-vermiculite mixture served as an acceptable pupation medium for the plum curculio.

Materials and Methods

An experiment was designed to test the usefulness of various pupation media for C. horridus. Approximately 200 larvae (range 200-255) were inoculated per diet tested in the previous experiment. Diet preparation and sterilization was carried out as described previously. Plastic friction-seal cups were used as diet containers. Each container was inoculated with 10 newly eclosed first instars and held in a chamber set for LD:0-24 and 21° C $\pm$ 1° C. After six

weeks, larvae were removed from the diets and placed on an assortment of pupation substrates. These included: sterilized Weblite®, a commercially available potting soil; a non-sterile mixture of soil and vermiculite; sterilized sand; peat moss; soil from a thistle infested area; and shredded construction paper. Sterilized materials were autoclaved 1 hour at 21 psi and 121° C. Factory sterilized petri dishes, 8.0 cm diameter with four equal compartments, were filled to 1.25 cm with each of these materials. A single last instar was placed in each compartment. Distilled water was added as necessary to prevent the soil from drying out. Temperature was regulated at 21° C $\pm$ 1° C. Photoperiod, as with the blackvine weevil, was not considered to be a factor since the pupal stages are subterranean (Shanks and Finnigan 1973).

After 2 weeks the containers were observed daily for evidence of pupation or adult emergence. Following a minimum of 60 days, dishes were carefully examined under a dissecting microscope. Prior to this containers were discarded only if all larvae died or became contaminated.

Results and Discussion

Of the 6 pupation substrates tested, 4 proved non-productive almost immediately. The dry environment provided by shredded construction paper caused the larvae to dessicate; none living beyond 24 hours. A similar problem was encountered using peat moss. Because of the low moisture content of commercially available peat

moss, larvae succumbed rapidly to dehydration. Attempts to adjust moisture levels were unsuccessful. After prolonged soaking peat moss would become saturated. Several hours of drying produced an acceptable environment, but moisture levels continued to drop and more water could not be added without prolonged soaking. No C. horridus larvae survived even one such treatment. Attempts to induce pupation using a soil-vermiculite mixture or soil from a thistle-infested area were unsuccessful. Although larvae burrowed readily into these compounds, fungus formation on the larvae and surrounding soil resulted in death within 2 days.

Sterile sand or Weblite® appeared more promising as pupation substrates. However, after repeated additions of distilled water the sand became compacted and unacceptable to the larvae. Sterile Weblite® did not become compacted, developed less mold than unsterilized soils and permitted maintenance of necessary moisture levels. Consequently, the majority of larvae produced (75%) for this test were placed on sterile Weblite®.

As indicated in Table IV, diets #28 and #34 produced larvae capable of becoming adults when placed on sterile potting soil. This proved that at least 2 of the diets were nutritionally adequate for adult development. This also indicated that substrate texture was an important factor for inducing pupation.

Table IV. Use of Sterile Soil as a Pupation Substrate for Third Instars of Ceuthorrhynchynchidius horridus Maintained on 21 Artificial Diets.

	Diet #																				
	3	5	11	12	13	14	19	20	21	22	23	24	25	27	28	32	33	34	35	36	44
# Third Instars Tested on Sterile soil[a]	0	0	0	16	3	0	6	0	4	1	8	6	1	4	6	0	9	11	12	3	2
% Third Instars Becoming Adults	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.7	0	0	9.1	0	0	0

[a]Those diets showing no larvae tested produced no usable third instars from approximately 200 first instars inoculated

VI

FEASIBILITY TESTS USING SIX SELECTED DIETS FOR C. HORRIDUS PROPAGATION

Materials and Methods

In order to determine the feasibility of artificial diets for laboratory propagation of C. horridus, 600 larvae were placed on each of 6 diets selected on the basis of performance in previous tests. Main components common to these diets included: agar, casein or soy hydrolysate, Wesson salt mixture 'W', wheat germ, torula yeast, and lyophilized Carduus nutans. Also, a vitamin supplement, cholesterol, and ascorbic acid were incorporated post-sterilization. Sorbic acid and methyl-p-hydroxybenzoate were included as fungal inhibitors. Diet pH was adjusted to 6.2 - 6.4 with potassium hydroxide.

Sterilized friction-seal containers, filled with diet to 1.0 cm, were inoculated with 10 larvae each. After a 6 week developmental period, last instars were placed on sterilized potting soil (Weblite ®) in 100 x 15 mm sterile quartered petri dishes. Larvae were maintained in a photoperiod of LD:0-24 and temperature of 21° C ± 1° C throughout the test. Distilled water was added periodically to keep the soil from drying out. Daily observations were made for evidence of pupation or adult emergence. After 2 months the soil was examined under a dissecting microscope.

This test was repeated using the same procedures stated earlier, using 50 dram snap-cap vials as substrate containers instead of

100 x 15 mm quartered petri dishes. These were filled to a depth of approximately 5 cm with sterile soil.

Results and Discussion

The results of the first and second tests are presented in Table V and VI respectively. The percentage of last instars per larvae inoculated was generally low; only diet #34 exceeding 10% in both tests. Although diet #23 produced the greatest yield of last instars (9.0% and 21.7%), no living adults were recovered from this diet. Based on this, diet #23 was eliminated as a suitable artificial media for C. horridus propagation.

Because some last instars died before transfer to sterile soil (disease, failure to feed, etc.), the per cent of usable last instars is an important consideration. Excepting diet #12, the percentage of usable last instars in the first trial was greater than 50%, but only diet #13 exceeded 50% in the second. The introduction of wax moths into the C. horridus rearing rooms may have accounted for the higher levels of disease and contamination seen in Table VI. Contamination was low to moderate, with only one diet, #12 exceeding 50%. Diets #13, #23, and #34 proved the most satisfactory with less than one third becoming contaminated in either test.

Table V indicates that diets #28 and #34 produced adults. These were the same diets which produced adults in the previous experiment (see Table IV). However, neither test included the effects of pathogenic fungi of the genus Penicillium on prepupal third instars.

The effects of this fungus, presented in Table VI, were considerable. Losses of last instars ranged from 10% (diet #28) to 38.7% (diet #23). When infected larvae were discounted, the percentage of last instars producing adults more accurately reflected the nutritional adequacy of the artificial diets. Thus, part of the increase in percentage of third instars becoming adults seen in Table VI versus Table V can be attributed to subtracting the effects of Penicillium.

Some of this observed increase may be due to the use of a different substrate receptacle. Many larvae placed in the deeper receptacles in the second test burrowed more than 3.0 cm, with a few reaching approximately 5 cm. This is deeper than the 1.0 cm soil depth provided in the previous test, and clearly affected prepupal movements. In addition, these containers did not require supplementary distilled water. Less variation in moisture levels and fewer mechanical disturbances may have affected larval propensity toward pupation.

Results of averaged values from Table V and VI indicate that laboratory colonies of C. horridus could be maintained on artificial diets if fungal contamination was eliminated. Although complete elimination is unlikely, use of a room solely for C. horridus rearing, coupled with practical sterilization procedures, would reduce fungal contamination to manageable levels. Based on average fecundity determined for laboratory reared females by Kok et al. (1975), each female could produce at least 3 other adults via artificial diets.

This value is based upon: 1) the percentage of usable last instars shown in Table VI, which accounted for larvae lost to Penicillium and 2) diet #28 (3.8 adults/female) and #34 (3.3 adults/female) which have consistently produced adults. Assuming that one half of the emerging adults are female, as indicated by adults generated in the substrate and feasibility tests, the colony would expand. However, this assumes that diet-reared adults are as productive as plant reared, and little information is available on this as yet. Therefore, the use of artificial diets could be feasible as a supplement to natural hosts when: 1) laboratory populations exceed host availability; 2) greenhouse space is limiting for potted thistles; or 3) ambient temperatures in the greenhouses surpass larval tolerance limits.

Table V. Feasibility TestI: Contamination, Third Instar and Adult Production For Six Selected Ceuthorrhynchidius horridus Diets.[a]

	Diet #					
	12	13	19	23	28	34
% Diets Contaminated[b]	6.6	0	33.3	23.3	25.0	5.0
% Third Instars per Larvae Inoculated[c]	7.8	4.7	4.5	9.1	7.8	10.3
% Third Instars Usable	38.9	53.6	77.8	57.1	65.7	63.3
% Usable Third Instars Producing Adults	0	0	0	0	4.3	5.3

[a] Pupation substrate placed in 100 x 15 mm quartered petri dishes.
[b] Based on 60 diet containers per diet - 10 larvae per container.
[c] Does not include larvae from contaminated diets.

Table VI. Feasibility Test II: Contamination, Third Instar and Adult Production For Six Selected Ceuthorrhynchidius horridus Diets[a].

	Diet #					
	12	13	19	23	28	34
% Diets Contaminated[b]	58.0	3.3	18.3	30.0	50.0	31.7
% Third Instars per Larvae Inoculated[c]	4.4	10.5	4.7	21.7	12.0	12.9
% of Third Instars Usable	36.4	50.0	34.8	34.1	27.8	34.0
% Third Instars Contaminated in Soil	25.0	12.9	25.0	38.7	10.0	22.2
% Usable Third Instars Producing adults[d]	0	3.7	16.6	5.3[e]	11.1	7.1

[a] Pupation substrate placed in 50 dram vials.
[b] Based on 60 diet containers per diet - 10 larvae per container.
[c] Does not include larvae from contaminated diets.
[d] Does not include third instars contaminated in soil.
[e] Failed to reach soil surface - died in soil.

VII

COMPARISON OF *CARDUUS NUTANS* AND ARTIFICIAL MEDIA AS HOST SUBSTRATE FOR *CEUTHORRHYNCHIDIUS HORRIDUS*

Introduction

Upon development of nutritionally adequate diets, experiments were designed to compare the growth of diet-reared versus plant-reared weevils. These comparisons included larval weight at 4 weeks and adult lengths. Also, using data on plant-reared weevils available from Kok et al. (1975), and a plant-reared female mated with a diet-reared male (test adults), comparisons were drawn between: 1) width x length of eggs, 2) viability of eggs, and 3) first instar head capsule width.

Materials and Methods

To compare larval weights after 4 weeks, a total of 1,200 first instars were inoculated onto the artificial diets used in the feasibility tests. Also, 70 newly eclosed larvae were inoculated into musk thistle rosettes of about 45 cm diameter at the rate of 10 larvae per plant. Diets and thistle rosettes were maintained at photoperiods of LD:0-24 and LD:9-15 respectively, and 21° C. Based on previous experiments and established rearing routines, these represented optimum growing conditions for the larvae. After four weeks the larvae were dissected out of the plants and diets, and weighed individually on a Mettler® balance.

Measurements were made using an ocular micrometer and a dissecting microscope. Mean egg measurements and per cent viability from the plant reared female and the diet reared male were based on observations of 50 eggs. The mean first instar head capsule width was based on 19 newly eclosed larvae. Adult lengths for diet reared C. horridus were based on 5 weevils of each sex.

Results and Discussion

Variation in egg size and viability, presented in Table VII, was insignificant between plant-reared adults and test adults. Egg widths and lengths were the same; the smaller sample sizes in this study resulted in slightly larger standard deviations for eggs from the test pair. Viability differed only by a single percentage point, and indicates that diet-reared males can be as fertile as plant-reared males.

Table VII shows that first instar head capsule widths were larger for offspring from the test pair than for progeny from plant-reared weevils (t test:$P < .01$). However, larvae measured by Kok et al. (1975) were not recorded as being from a single female, as were larvae of the test pair. Thus, the different head capsule widths could be explained by lack of genetic variation in the test pair's progeny, and may not be significant as suggested by the data.

Based on the results shown in Table VII, larval weight at 28 days is significantly (t test:$P < .01$) greater on plants than on diets. In contrast, larval weights at 30 days for plant grown

Table VII. Comparison of Plant-Reared Versus Diet-Reared Ceuthorrhynchidius horridus.

	Plant[a] $\bar{X} \pm SD$	Diet $\bar{X} \pm SD$
Eggs:		
Width x Length (mm)	.54 ± .01 x .33 ± .01	.54 ± .03 x .33 ± .02[b]
Viability of Eggs (%)	69.0 ± 24.4	70.0 ± 9.5[b]
Larvae:		
First Instar		
Headcapsule Width (mm)	.26 ± .02[d]	.29 ± .01[c]
Weight at 4 Wks (mg)	10.5 ± 0.3[d]	7.3 ± 0.8[e]
Adults:		
Length of Female (mm)	4.3 ± 0.3	3.8 ± 0.1[f]
Length of Male (mm)	3.9 ± 0.2	3.5 ± 0.3[f]

[a] Plant reared means from Kok et al. 1975 - except larval weight at 4 wks.
[b] $\bar{X}$ of 50 eggs - plant-reared female mated with diet-reared male.
[c] Based on 19 first instars.
[d] Based on 15 larvae.
[e] Based on 23 larvae.
[f] Based on 5 individuals.

C. horridus Kok et al. (1975) were not significantly different (t test:$P < .01$) from these diet reared larvae. This suggests: 1) a deviation in host suitability between rosettes used for this test and rosettes utilized by Kok et al.; 2) wide variation in larval growth rates requiring large sample sizes for accurate interpretation; 3) dissimilar environmental conditions, other than temperature which was the same, which could have affected larval development; or 4) a combination of the preceeding. Therefore, host substrate alone may not have caused the larval weights to be significantly different.

The variance between diet-reared and plant-reared C. horridus was confirmed when adult measurements were compared. Plant-fed adults were significantly larger (t test:$P < .01$) than male or female diet-reared weevils. Although environmental conditions for adult development were almost certainly different, the low return of adults per inoculated larvae coupled with smaller adult sizes when using a diet implies nutritional defects in the artificial media. These apparent defects remain the foremost obstacles to a "mass" production program for C. horridus.

VIII

USE OF DIET-SELECTED LARVAE FOR PROPAGATION OF C. HORRIDUS

Introduction

In a test using 100 larvae, Kok (1975) found that 26% of the larvae inoculated into musk thistle rosettes produced adults. Because demand for rosettes occasionally outstripped supply, an experiment was designed to determine the effect on yield of adults from musk rosettes when larvae were initially selected by use of artificial diets.

Materials and Methods

This hypothesis was tested by inoculating 300 larvae on each of 6 artificial diets chosen on the basis of performance in previous experiments. Diets initially containing 100 larvae were removed from photoperiod chambers at 1, 2, and 3 weeks, and the larvae dissected out for transfer to musk thistle rosettes of 42 to 48 cm in diameter. A maximum of 10 larvae were inoculated per rosette. Plants were enclosed in polyethylene bags when total larval developmental time reached 6 weeks. Observations were then made every other day for adult emergence. Larvae were allowed at least 90 days for development before the experiment was terminated.

Results and Discussion

As seen in Table VIII, difficulty was encountered in producing adequate numbers of live larvae to provide sample sizes large enough

to be meaningful. Of the larvae obtained, the majority were killed by fungi. A return of less than 7 per cent was considered insufficient for analytical purposes.

Table VIII does show that larvae from diet #28, if transferred at 3 weeks, would produce adults at a rate of 28.5 per cent of larvae inoculated into plants. Although this represented a greater return from musk thistle than found by Kok (1975) (26%), larvae lost during the initial 3 weeks on the diet caused a net loss in actual adult production. Therefore, this technique was not effective for increasing productivity. However, if contamination were eliminated, this method could prove valuable for maintaining larval life up to 3 weeks while rosettes developed to a useful size.

Table VIII. Percentage of Adults Produced from Larvae Inoculated into Musk Thistle Rosettes After 1, 2 and 3 Weeks on Six Selected Diets.

Duration in Diets (wks)	% Inoculated Larvae Producing Adult Weevils					
	Diet #					
	12	13	19	23	28	34
1	4	5	0	11.1	12.5	0
2	-[a]	0	0	12.5	--	11.1
3	-	-	-	0	28.5	--

[a]Indicates sample sizes too low to be meaningful.

IX

GENERAL SUMMARY AND CONCLUSIONS

Nutritional studies on C. horridus have shown that the weevils can derive adequate nutrition needed for complete development from diets consisting of less than 15% (by dry weight) of host plant material. Main components common to these diets include: agar, casein or soy hydrolysate, Wesson salt mixture 'W', wheat germ, torula yeast, and lyophilized Carduus nutans. Also a vitamin supplement, cholesterol and ascorbic acid were incorporated post-sterilization. Sorbic acid and methyl-p-hydroxybenzoate were included as fungal inhibitors. Diet pH was adjusted to 6.2 - 6.4 with potassium hydroxide. Only diets in which the vitamins and cholesterol were added after sterilization produced adults. Diets including the largest amounts of vitamins were the most productive. This offers a basis for further studies on the nutritional requirements of C. horridus.

The successful larval development of this weevil on artificial media represents only the second occurrence of the use of diets for rearing an insect with immature stages lasting in excess of 60 days; the other being the black vine weevil (Shanks and Finnigan 1972). Two of the diets tested are capable of maintaining and increasing colonies of C. horridus if: 1) fungal contamination is minimized and, 2) diet-reared females are as productive as plant-reared females. Since egg production frequently exceeds host plant supply,

particularly during the winter months, excess larval populations could be maintained on diets until plant material became available.

Comparisons between plant and diet-reared weevils showed that eggs resulting from a diet-reared male mated with a plant-reared female were not significantly different in either size or viability from the eggs of plant-reared weevils. Although plant-reared larvae were heavier (t test:$P < .01$) than diet-reared larvae after 4 weeks, the latter were not significantly lighter than plant-reared larvae reported by Kok et al. (1975). However, adult body lengths of diet-reared weevils were significantly smaller (t test:$P < .01$) than plant-reared adults.

Tests examining the lack of pupation produced mixed results. Evidence showed that pupation was not inhibited by: 1) photoperiodic induction of undesirable rhythms, 2) buildup of toxic materials or gases or, 3) diet shape and depth. Instead, the texture of the diets proved unacceptable as a pupation substrate. Subsequent tests indicated that sterilized potting soil was suitable for pupation.

Several useful techniques were developed during the course of this research. Mineral detection via energy dispersive x-ray analysis offered a rapid and effective technique for comparison of mineral composition of synthetic diets and host material. In addition, this technique could be used to test for trace minerals in diet constituents, a critical factor for accurate determination of nutritional requirements using holidic diets.

Other techniques also proved useful. A plexiglas drying cabinet, sterilized with a germicidal blacklight, aided in reducing contamination while diets cooled. Dehydration of synthetic media was prevented by using 15 ml friction-top diet containers. These containers permitted larvae to develop to the last instar without repeated transfer to undehydrated diets.

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APPENDIX

APPENDIX A

Diet Components and Sources

Diet Ingredients:	Source:
Adkisson-Vanderzant Insect Diet	United States Biochemical Corp. (USBC)
Agar	Difco & Nutritional Biochemicals Corp. (NBC)
Alphacel	NBC
Arginine-L	NBC
Ascorbic acid	NBC
Benlate® fungicide	Dupont
Bicarbonate of soda	Arm and Hammer
Biotin	NBC
Casein hydrolysate	NBC
Cholesterol (USP)	NBC
Choline chloride	NBC
Corn oil	Mazola
Cystine-L	NBC
7-dehydrocholesterol	NBC
Folic acid	NBC
Formaldehyde 40% (USP)	Fisher
Glutamic acid	NBC
Histidine-L	NBC
Isoleucine-L	NBC
Inositol	NBC
Instant nonfat dry milk	Carnation
Linoleic acid 75%	NBC
Linolenic acid 55%	NBC
Leucine-L	NBC
Lima beans	Jack Rabbit Brand
Lycine monohydrochloride	NBC

APPENDIX A (cont.)

Diet Ingredients:	Source:
Methionine-L	NBC
Methyl-p-hydroxybenzoate	NBC
Nicotinic acid	NBC
Pinto beans	Jack Rabbit Brand
Potassium hydroxide (USP)	Fisher
Sorbic acid	NBC
Soy hydrolysate	NBC
Starch (Reagent grade)	NBC
Streptomycin sulfate	NBC
Sucrose-D	NBC
Threonine-L	NBC
Tryptophan-L	NBC
Valine-L	USBC
Vitamin B12 with Intrinsic factor (USP)	NBC
Vitamin mixture - NBC	NBC
Vitamin mixture - Vanderzant-Adkisson	USBC
Wheat germ flakes	USBC
Wesson salt mixture 'W'	NBC
Yeast - Brewers	NBC
- Torula	NBC
- extract	NBC
Other Ingredients	
Cirsium vulgare (Savi) Tenore	V. P. I. greenhouse
Carduus nutans L. (fresh and lyophilized)	V. P. I. greenhouse
Cynara scolymus L. (lyophilized)	V. P. I. greenhouse
Distilled Water	V. P. I. Entomology Department

APPENDIX B

Diets Attempted Prior to the Nutritional Studies Experiment

Diet Ingredients[a]	Diet									
	A	B	C	D	E	F	G	H	I	J
Alphacel	5.0	5.0	5.0	5.0			5.0[b]	5.0[b]	5.0[b]	5.5[b]
Agar	15	15	15	15	15[c]	15[c]	15	15	15	15
Ascorbic acid	2.0	2.0	2.0	2.0	2.0	2.0	2.0[b]	2.0	2.0[b]	2.0[b]
Benlate										0.2[b]
Brewers yeast	20	20	20	20	20	20	20[b]	20[b]		
Carduus nutans	20	20	20	20	30[d]	20[d]		10[b]	30[d]	7.0[b]
Cholesterol	0.5	1.0	1.0	0.5	1.0	1.0	0.5[b]	0.5[b]	1.0[b]	1.0[b]
Corn oil (ml)										1.0[b]
7-dehydro-cholesterol	1.0									
Distilled Water (ml)	640	640	640	640	450	475	635	635	460	460
Formaldehyde 40% (ml)							1.0[b]	1.0[b]	1.0	
Methyl-p-hydroxybenzoate	0.9	0.9	0.9	0.9	0.9	0.9	0.9[b]	0.9[b]	0.9[b]	0.9[b]
Pinto beans	60[e]	60[e]	60[e]	60[e]		60[e]	60[eb]	60[eb]	60[eb]	60[eb]
Stigmasterol	1.0									
Sitosterol		1.0	0.5	0.5						
Sorbic acid	0.4	0.4	0.4	0.4	0.4	0.4	0.4[b]	0.4[b]	0.4[b]	0.4[b]
Soy hydrolysate					30				10[b]	10[b]
Streptomycin sulfate								0.3[b]	0.3[b]	0.5[b]
Sucrose-D								2.0[b]	2.0[b]	2.0[b]
Wesson's salt Mixture 'W'					1.0	1.0	1.0[b]	1.0[b]	2.0[b]	2.0[b]
Wheat germ								5.0[b]	5.0[b]	5.0[b]
Yeast Extract Powder									2.0[b]	2.0[b]

APPENDIX B (cont.)

Diet Ingredients[a]	Diet									
	A	B	C	D	E	F	G	H	I	J
NBC vitamin mixture				1.0	1.0	1.0	1.0[b]	1.0[b]	1.5[b]	2.0[b]
Cirsium vulgare										7.0[b]

[a] In grams unless specified.
[b] Added after autoclaving.
[c] Used NBC not Difco.
[d] Thistle not lyophilized.
[e] Soaked overnight in 160 ml distilled water.

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NUTRITIONAL STUDIES ON *CEUTHORRHYNCHIDIUS HORRIDUS* (COLEOPTERA: CURCULIONIDAE) AN INTRODUCED WEEVIL FOR THE BIOLOGICAL CONTROL OF *CARDUUS* THISTLES

by

John Thomas Trumble

(ABSTRACT)

The larval development of *Ceuthorrhynchidius horridus* (Panzer), an imported thistle-rosette weevil, was tested on 50 synthetic media. Pupation and adult emergence occurred from 4 diets consisting of less than 15% (by dry weight) of host plant material. Major ingredients included: wheat germ, casein or soy hydrolysate, torula yeast, vitamin and salt mixtures, ascorbic acid, cholesterol, agar and lyophilized *Carduus nutans*. Methyl-p-hydroxybenzoate and sorbic acid were incorporated to inhibit fungal contamination. Diet pH was adjusted to 6.2 - 6.4 with potassium hydroxide. Sterilized potting soil proved adequate as a pupation substrate. Emerging adults had a 1:1 sex ratio. A laboratory colony of *C. horridus* could be maintained on artificial diets if: 1) fungal contamination were minimized and, 2) diet-reared females are as productive as plant-reared females.

Eggs resulting from a diet-reared male mated with a plant-reared female were not significantly different in either size or viability from the eggs of plant reared weevils. Although plant reared larvae were heavier (t test:$P < .01$) than diet-reared larvae

after 4 weeks, the latter were not significantly lighter than plant-reared larvae reported by Kok et al. (1975). However, adult body lengths of diet-reared weevils were significantly smaller (t test: $P < .01$) than plant-reared adults. Experiments showed that buildup of toxic materials or diet shape and depth did not inhibit pupation.

Mineral detection via energy dispersive x-ray analysis offered a rapid and effective new technique for comparison of host plant material and synthetic media.