SINIGRIN AND THE MONARCH BUTTERFLY: A STUDY IN CHEMICAL ECOLOGY

by

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ABSTRACT

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The larval feeding niche of the monarch butterfly, Danaus plexippus L., is restricted to the family Asclepiadaceae. Proximal explanations such as host finding and oviposition behavior, and larval feeding stimulants adequately account for this restriction in ecological time (i.e. over one generation). The maintenance of this intimate relationship in evolutionary time (i.e. over several generations) may be partially explained by the chemical disparity common among early successional herbaceous plants. The present study investigates the possible role of sinigrin (an allelochemic found throughout the Cruciferae) as a chemical barrier to the monarch butterfly.

Monarch larvae were reared on an artificial diet containing 0.01%, 0.08%, and 0.15% sinigrin (wet weight of diet). Growth performance and food utilization of 5th instar larvae were measured by the nutritional index technique. Monarch larvae reared on 0.08% and 0.15%

sinigrin experienced a 42% and 61% reduction in growth respectively. The reduced growth was attributed predominantly to a reduced rate of ingestion although some toxic effects were evident. Larvae reared on 0.08% and 0.15% sinigrin took longer to develop and exhibited higher rates of mortality than larvae reared on the control and 0.01% sinigrin diets. In addition to the increased rates of mortality, the increased likelihood of predation, parasitism, pathogenesis, etc., associated with the longer larval periods would appear to present an effective barrier to monarch utilization of the Cruciferae as food plants.

Possible mechanisms of monarch tolerance to the proven assimilation - reducing nature of sinigrin are discussed. The decreased rates of ingestion in the presence of sinigrin support the principle that host specificity among oligophagous insects is largely controlled by sensitivity to feeding deterrents.

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LIST OF SYMBOLS

SYMBOL	DEFINITION
Ea	Apparent assimilation efficiency
En	Net growth efficiency
Eg	Gross growth efficiency
i	Specific rate of ingestion
g	Specific rate of growth
a	Specific rate of assimilation
r	Specific rate of respiration
f	Specific rate of egestion

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CHAPTER I

INTRODUCTION

There are many solutions to any given problem in nature and the evolutionary process ensures that many possible avenues are tested (Price, 1984).

The amount of literature concerning the coevolution of plants and animals is overwhelming. Many of the most interesting examples of this intimate process are found in butterfly/hostplant associations. Here natural selection has sculptured some of the more fascinating reciprocal interactions found in nature. In such relationships, the most important questions concern host specificity and plant susceptibility (Beck and Reese, 1976).

Evolution has equipped plants with an effective arsenal with which to combat herbivory. Many plants employ camouflage or diverse leaf structure (disrupts search image) to reduce the probability of discovery, while others utilize mechanical deterrents such as trichomes, stipules, or counterfeit egg masses (Price, 1984). The most studied and controversial plant defense system, however, is that of secondary plant substances (allelochemics). These compounds are chemical byproducts from the synthesis of primary metabolic compounds and are not thought to be essential to plant metabolism,

although some storage and regulatory functions have been suggested (Price, 1984). Allelochemics are nonnutritious chemicals produced by one organism which affects the "growth, health, behavior, or population biology of another organism" (Whittaker, 1970). Interactions mediated by allelochemics may be intra- or interspecific and are not limited to insect - plant associations (Whittaker and Feeny, 1971; Freeland and Janzen, 1974).

Host plant susceptibility and specificity among phytophagous insects involve a myriad of mechanical, behavioral, physiological, ecological, and chemical factors. However, as Ehrlich and Raven (1964) note:

> A systematic evaluation of the kinds of plants fed upon by the larvae of certain subgroups of butterflies leads unambiguously to the conclusion that secondary plant substances play the leading role in determining patterns of utilization.

Allelochemics may affect host utilization by influencing oviposition behavior or larval feeding behavior. Although most workers agree oviposition is a key step in host specificity, little detailed work exists addressing its chemical regulation. However, the central role of allelochemics in oviposition is becoming unquestionably clear. Havukkala and Virtanen (1985) working with the turnip root fly, Delia floralis Fall. (Anthomyiidae), have demonstrated an increase in oviposition elicited by allylisothiocyanate, a mustard oil. Myers (1969) has shown oviposition in the Florida queen butterfly, Danaus gilippus bernice Cramer to be dependent upon tarsal food

plant chemoreceptors. Pyrrolizidine alkaloids sequestered by many danaine butterflies serve in chemical defense as well as the biosynthesis of sex pheremones essential to courtship behavior (Edgar, 1982; Boppre, 1983).

In addition to affecting reproductive behavior and physiology, allelochemics may define host specificity by influencing larval feeding behavior (i.e. phagostimulants and deterrents). A seemingly endless number of allelochemics have been identified as chemical feeding inhibitors (Jermy, 1966; Schoonhoven and Derksen-Koppers, 1973; Bernays and Chapman, 1974; Chapman, 1974; Schoonhoven and Derksen-Koppers, 1976). Such allelochemics act by blocking specific phagostimulatory receptors and/or stimulating specific deterrent cells (Chapman, 1974). Such deterrent cells are capable of becoming "desensitized" to allelochemics with which they have had previous experience (Schoonhoven, 1969; Blaney and Simmonds, 1983). It was suggested over twenty years ago (Jermy, 1966) that phagodeterrents play the dominant role in determining host specificity in oligophagous insects. As Fraenkel (1969) notes, " ... in oligophagy the specific attractant can only lead to success in the absence of repellent substances." Thus, host specialization appears to be a function of both phagostimulants and deterrents with the latter being of paramount importance.

The role of allelochemics as specific phagostimulants has also enjoyed much attention (Thorsteinson, 1953; Fraenkel, 1969; Nault and Stryer, 1972; Kogan, 1977; Aspirot and Lauge, 1981). Traditionally, however, this role has been difficult to demonstrate for a number of reasons: (1) when hungry many insects will feed on nonhost plants, (2) under stress insects will feed on an attractant - free artificial diet, (3) attractants may be labile or difficult to isolate, and (4) several substances may be acting together in a synergistic effect (Fraenkel, 1969). Even with such formidable obstacles, the role of secondary plant substances as attractants cannot be ignored by the serious student of plant - insect interactions. Indeed, one must consider the combined effects of ovipositional attractants and repellents as well as feeding stimulants and deterrents to completely understand such relationships.

In addition to influencing reproductive and feeding behavior, many allelochemics act at a physiological level to affect food utilization. According to Feeny (1976) two basic chemical defense strategies have evolved in plants: quantitative and qualitative. Quantitative allelochemics tend to be digestibility - reducing substances which are effective against all herbivores, present in large amounts, and dose dependent. As Price (1984) notes, such allelochemics are found "... in late successional plants which exist in pure stands of

low diversity for long periods." Such plants are apparent to herbivores and are bound to be found. Selection favors the evolution of quantitative defensive chemicals in such plants where long generation times make the high cost of such defense feasibile (i.e. they can spread the cost out). As Futuyma (1983) notes, "... iteroparous, long lived species can 'afford' to allocate a larger fraction of their energy budget to massive amounts of defensive compounds than can short lived (r - selected) species." Digestibility - reducing substances have such a generalized effect on insect nutrition that they are not easily counteradapted by herbivore detoxification mechanisms (Feeny, 1976). The presence of tannins in many climax plant species is a classic example of such digestibility - reducing compounds in apparent plants (Feeny, 1968).

In contrast, qualitative defenses are typical of early successional plants which are short lived, grow fast, mature early, and effectively disperse (Price, 1984). Such plants are hard to find as they rely on escape in space and time to avoid herbivory. Selection favors the evolution of toxic allelochemics in such plants; as Rhoades and Cates (1976) note, "Ephemeral leaf tissues ... are defined primarily by cheap divergent toxic chemical systems ...." By producing toxic, qualitative defensive compounds, a plant is able to allocate a larger portion of its energy budget for growth and reproduction. The mustard

oil glycosides of the Cruciferae and the cardiac glycosides of the Asclepiadaceae are thought to be two such allelochemics.

It has been suggested that the chemical diversity of early successional herbaceous communities acts to reduce the apparency (i.e. susceptibility to herbivores) of all of its members. As Feeny (1976) notes:

> ... the greater is the diversity of chemical compounds in a community of early successional herbs, the more effective is any one compound likely to be both as an ecological and as an evolutionary barrier to enemies.

In such habitats, disparity among allelochemics would be selected for thereby reducing the risk of exploitation by nonspecialized herbivores. This exquisitely accounts for the chemical diversity characteristic of these communnities, as well as the number of specialists supported by such a community (i.e. Danaus/Asclepiadaceae, Pieris/Cruciferae, Papilio/Umbelliferae etc.).

Allelochemics may act on a behavioral or a physiological level to influence plant susceptibility and host specificity. Deither (1970) notes, "... the first barrier to be overcome in the insect/plant relationship is a behavioral one. The insect must sense and discriminate before nutritional and toxic factors become operative." Futuyma (1983) supports this view since most insects seem quite capable of dealing with a variety of toxic allelochemics. This ability appears to reside in the form of a mixed - function oxidase (MFO) system. This

generalized detoxification system is thought to have evolved in response to plant allelochemics accounting for monophagous insects having lower MFO activity than oligo- and polyphagous species (Krieger et al., 1971; Brattsten et al., 1977). The MFO system is unusually nonspecific prompting the speculation that insect adaptation to plant toxins would require minimal genetic change, if any. As Futuyma (1983) states, " ... host specificity in insects is very largely a consequence of behavioral responses to attractant and repellent chemicals, rather than specialized physiological adaptations." In this context, the unique physiological adaptations possessed by specialists are simply fine tunings following behavioral adaptations.

The monarch butterfly, Danaus plexippus L., offers many unique opportunities to investigate butterfly/plant interactions. This fascinating creature has already contributed much to our understanding of pheremone communication (Ackery and Vane-Wright, 1984); mimicry (Brower, 1969); insect migration (Brower, 1977); and ecological chemistry (Brower, 1969). It is widely distributed in North America (south of the subarctic) with larvae feeding almost exclusively on Asclepias species (milkweed) or more rarely on Apocynum species (dogbane) and Acerates species (green milkweed) (Ehrlich and Ehrlich, 1961). The monarch may be classified as an oligophagous herbivore since its feeding is restricted to

a few plant families. Monarch MFO activity is characteristic of oligophagy being significantly reduced from that of polyphagous herbivores (Krieger et al., 1971). Whittaker and Feeny (1971) suggest such oligophagous insects are more efficient at resource utilization than polyphagous insects since less energy is invested in detoxifying enzymes. However, Schroeder (1976) showed the monarch butterfly to exhibit similar to low growth efficiences as compared to other lepidopteran species.

Perhaps the most interesting aspect of monarch biology concerns the ecological chemistry surrounding its intimate food plant relationship with the Asclepiadaceae. These plants possess a variety of cardiac glycosides (cardenolides) which monarch larvae sequester and use in chemical defense against vertebrate predators (Parsons, 1965; Reichstein et al., 1968; Brower, 1969). The larva, pupa, and aposematic adult may incorporate enough cardenolides into their tissues to cause emesis in avian predators (Brower, 1969). A palatability spectrum exists among monarchs ranging from completely palatable to totally unpalatable (Brower et al., 1968; Duffey, 1970; Brower et al., 1975) resulting in automimicry in which intraspecific palatable mimics exist (Brower, 1969; Gibson, 1984). The sequestered glycosides may also benefit the monarch by reducing larval bacterial infections and parasitism (Urquhart, 1960; Frings et al., 1948).

The Asclepiadaceae (milkweed family) consists of over 200 genera and 2500 species of perennial shrubs and herbs many of which are of ornamental value. They are recognized for their poisonous properties and known to cause death among grazing animals (Kingsbury, 1964). Ovipositing monarchs first locate the plants by sight and then examine them more closely by means of tarsal chemorecptors (Urquhart, 1960; Myers, 1969). The larvae require the presence of a phagostimulant (or absence of a deterrent) to exhibit normal feeding behavior (Brower et al., 1967; Ackery and Vane-Wright, 1984).

Glycosides exert their toxic effects by inhibiting the action of ATPase, an enzyme required for the transport of alkali metal cations (Na^+ and K^+). Inhibition of ATPase may produce lethal effects in nerve and muscle tissue which require rapid movement of Na^+ and K^+ across membranes (Ackery and Vane-Wright, 1984). The monarch may reduce or eliminate these toxic effects by maintaining a high haemolymph K^+ level during feeding permitting storage or excretion of the toxin (Vaughan and Jungreis, 1977). Such detoxifying mechanisms have been suggested to impose a physiological cost on the monarch reducing its efficiency of food utilization. Work by Dixon et al. (1978), however, suggests no significant metabolic cost to cardenolide storage. Moreover, the monarch is able to regulate the type and amount of cardiac glycosides sequestered independently of the type and amount present

in the host plant (Dixon et al., 1978; Brower et al., 1982).

Sinigrin is a widespread mustard oil glucoside (thioglucoside, glucosinolate) found in many cruciferous plants. Upon leaf tissue damage, it is hydrolyzed by myrosinase to yield the volatile mustard oil, allylisothiocyanate (Virtanen, 1965). The hydrolysis of certain other glucosinolates may yield nitriles, thiocyanates, or other derivatives (Bell, 1978). Autotoxicity is prevented by physically separating the enzyme and the glucosinolates in different cells of the tissue. When the plant is damaged (i.e. herbivory), the enzyme hydrolyzes the glucosinolate to its respective mustard oil. The glucosinolates appear bitter to human taste while their mustard oils possess a pungent odor and a strong burning taste (Thorsteinson, 1953). At low concentration the mustard oils are rather pleasant to the taste and used in a number of condiments. These volatile oils are also known to possess antimicrobial activities against moulds and bacteria (Virtanen, 1965).

In addition to the Cruciferae, glucosinolates are found in many other species of the order Capparales: Capparaceae, Resedaceae, and Moringaceae as well as in some species of unrelated families such as: Caricaceae, Tropaeolaceae, and Limnanthaceae (Van Etten and Tookey, 1979). Every species of Cruciferae investigated has been found to possess one or more glucosinolates including:

forages, rapeseed (Brasssica napus Koch and B. campestris L.), cole crops (B. oleracea L.), mustards (B. nigra L. and B. hirta Moench), and horseradish (Armoracia spp.) (Van Etten and Tookey, 1979). Glucosinolates are found throughout the plant but are in highest concentrations in the seeds and young leaves (Rhoades and Cates, 1976; Bell, 1978).

The insect and microbial associates of cruciferous plants are well adapted to Cruciferae chemistry. The typical insect fauna include many species of Lepidoptera, Diptera, Coleoptera, and Homoptera. Herbivores attacking the Cruciferae include: the cabbage white butterflies (Pieris rapae L. and P. brassicae L.), the diamondback moth (Plutella maculipennis Curt.), the vegetable weevil (Listroderes costirostris Klug), the mustard beetle (Phaedon cochleariae Gyll.), the flea beetles (Phyllotreta cruciferae Goeze and P. striolata F.), and the cabbage aphid (Brevicoryne brassicae L.) (Whittaker and Feeny, 1971). The mustard oil derivative of sinigrin, allylisothiocyanate, is known to attract Diaeretiella rapae M'Intosh (Hymenoptera:Braconidae), a parasite of cruciferous aphids, as well as stimulate germination of Plasmodiophora brassicae Wor.; a parasitic fungus (Whittaker and Feeny, 1971).

Many such insects have coevolved with the Cruciferae in such a way as to exploit their characteristic chemistry as oviposition and feeding cues.

Verschaffelt (1910) was the first to identify sinigrin as a chemical attractant of the cabbage butterflies, P. brassicae and P. rapae. Work by Hovanitz et al. (1963) showed P. rapae was attracted specifically to sinigrin's breakdown product, allylisothiocyanate. Since then, sinigrin has been found to stimulate feeding in a variety of insects including the diamondback moth, the mustard beetle, and the flea beetle (Renwick, 1983). Aspirot and Lauge (1981) displayed the stimulatory effect of sinigrin on the feeding rate of Schistocerca gregaria Torsk (Orthoptera: Acrididae). Nault and Styer (1972) induced Hyadaphis erysimi Kaltenbach (whose host range is limited to the Cruciferae) to feed on nonhost plants treated with sinigrin. These workers concluded sinigrin acted to stimulate stylet penetration and phloem sieve element location. Feeny et al., (1970) have demonstrated the stimulatory nature of mustard oils and their glucosides on the feeding behavior of various flea beetles.

Oviposition behavior is also affected by sinigrin. Pieris brassicae will oviposit on broad bean plants or filter paper when treated with sinigrin (Schoonhoven, 1972). The cabbage root fly will oviposit on any substrate if it is treated with sinigrin or allylisothiocyanate (Schoonhoven, 1972). The turnip root fly is stimulated to oviposit by allylisothiocyanate, while sinigrin results in slight inhibition of oviposition (Havukkala and Virtanen, 1985).

Sinigrin deters feeding in many insects which do not usually feed on crucifers. Such is the case for the black swallowtial butterfly, Papilio polyxenes Fabr. (Erickson and Feeny, 1974), and the polyphagous aphids, Aphis fabae Scopoli and Acyrthosiphon solani Kaltenbach (Nault and Styer, 1972). Wearing (1968) found sinigrin to suppress feeding in the polyphagous peach aphid, Myzus persicae Sulzer, while Klingauf et al. (1972) and Nault and Styer (1972) found sinigrin to promote feeding in the very same aphid. Myzus viciae Bekt., a noncruciferous feeding insect, exhibits a similar increase in feeding rate in the presence of sinigrin (Klingauf et al., 1972). Although singrin acts to deter feeding in many noncruciferous insects, such evidence suggests other physical or chemical host determinants are involved in feeding behavior (at least in these aphid species).

In addition to affecting feeding behavior, sinigrin has also been shown to influence food utilization. Rhoades and Cates (1976) suggest sinigrin functions both as a toxin (i.e. qualitative defense) and as a digestibility - reducing substance (i.e. quantitative defense). The products of glucosinolate hydrolysis, isothiocyanates, are known to combine with amino groups in proteins (the basis of the Edman degradation reaction) and may reduce assimilation efficiencies. The toxic effects of sinigrin are well documented as well as its MFO inducing capability. Brattsten et al. (1977) induced a

180% increase in MFO activity over control activity in the southern armyworm, Spodoptera eridania Cramer, by rearing it on a 0.10% sinigrin diet. Erickson and Feeny (1974) demonstrated the lethal effects of sinigrin on black swallowtail larvae (noncruciferous feeding insects) at concentrations found in nature.

The toxic effects of glucosinolates and their mustard oils are not limited to insects. Cattle have been killed by ingesting as little as 0.001% of their body weight of allylisothiocyanate (Kingsbury, 1964). This mustard oil is known to increase blood flow to the skin and irritate mucous membranes (Virtanen, 1965). Vertebrate grazing on large quantities of ground seed containing such oils has resulted in chronic enteritis, hemorrhagic diarrhea, colic, abortion, nephritis with hematuria, apathy, heart paralysis, and respiratory paralysis (Muenscher, 1939). Glucosinolates in general and isothiocyanates specifically have been shown to prevent uptake of iodine in mammals resulting in poor growth and enlargement of the thyroid gland (Van Etten and Tookey, 1979).

Along with the Asclepiadaceae, the prime habitat for the monarch includes species of the Compositae, Leguminosae, Graminae, Umbelliferae, and Cruciferae. These plants are typical of early successional herbaceous communities occurring along roadsides, old fields, pastures, and waste areas. The restriction of the monarch

to the asclepiads is explained in proximate terms by its behavioral responses to specific asclepiad chemical cues (i.e. host finding cues, oviposition cues, and/or phagostimulants). Such elaborate behavioral adaptations are typical of oligo- and monophagous insects ensuring that only the normal host plants are attacked (Erickson and Feeny, 1974). Erickson and Feeny (1974) suggest such proximal adaptations adequately account for host specialization in "ecological" time but not for host restriction in "evolutionary" time. Following this argument, monarch response to ovipositional and feeding cues are simply proximal adaptations ensuring that it remains associated with the Asclepiadaceae to which it is adapted in various ultimate ways. Monarch host specialization in "evolutionary" time would seem to involve factors such as cardenolide storage and the defensive chemistry of plants typical of its habitat.

Many species of Lepidoptera are known to make ovipositional mistakes (Deither, 1959; Kitching and Zalucki, 1983) often with lethal consequences for their larvae (Straatman, 1962; Sevastopulo, 1964). D. plexippus has been known to make such "mistakes" ovipositing on nonasclepiad plants lethal to its larvae (Kitching and Zalucki, 1983). Brower et al. (1967), with much difficulty, reared monarch butterflies fed exclusively on cabbage (Brassica spp.). Such factors prompted the present investigation into the relationship between the monarch

butterfly and the Cruciferae to better understand the larval feeding niche of the monarch butterfly. It is hypothesized that sinigrin or its breakdown product, allylisothiocyanate, acts as a chemical barrier to larvae of Danaus plexippus preventing the utilization of cruciferous plants as hosts.

Plant allelochemics may affect herbivore survivorship, feeding behavior, development, food utilization, and fecundity. Effects on food utilization are best studied by the nutritional index technique with the allelochemic serving as the single variable in a standardized diet (Beck and Reese, 1976). In such designs, the effects of the chemical factor can be determined without equivocation. See Schroeder (1984), McEvoy (1985), Schmidt and Reese (1986), and Reese and Schmidt (1986) for a consideration of the limitations and sources of error associated with the nutritional index technique. Data derived from the nutritional index technique, are helpful in determining the physiological effects of secondary plant substances but do not reveal the specific biochemical modes of action or eliminate the possibility of nonnutritional effects (Beck and Reese, 1976). They do, however, demonstrate that herbivore growth can be inhibited by plant allelochemics in a variety of ways and the nutritional physiology of an insect can be influenced by nonnutritional factors.

Three basic indices are commonly calculated: (1) apparent assimilation efficiency (Ea), (2) net growth efficiency (En), and (3) gross growth efficiency (Eg) (see methods section for formulas). The apparent assimilation efficiency is an estimation of the proportion of ingested food which is assimilated. This index has been referred to as the "Assimilation Efficiency" (Odum, 1971); "Coefficient of Digestibility" (House, 1965); and "Approximate Digestibility" (Waldbauer, 1968). It is only an approximation of the nutrient uptake across the gut wall for it does not account for the presence of urine in the feces or fecal metabolic products such as the peritrophic membrane. As Waldbauer (1968) notes, "The difference between the weight of food ingested and the weight of the feces actually represents the food which is stored or metabolized less metabolic waste discharged in the urine or as fecal metabolic products." However, the total contribution of the urine to the weight of the feces is negligible in most insects and does not significantly affect the apparent assimilation efficiency (Waldbauer, 1968).

The net growth efficiency is a measure of the efficiency with which assimilated food is converted into biomass. Waldbauer (1968) refers to this index as the "efficiency with which digested food is converted to biomass" (E.C.D.). Odum (1971) refers to this index as the "Tissue Growth Efficiency". This efficiency will decrease

as the organism metabolizes a greater portion of its digested food for energy (Waldbauer, 1968).

The gross growth efficiency is an overall measure of the efficiency with which an insect converts ingested food into biomass. Waldbauer (1968) refers to this index as the "efficiency of conversion of ingested food to body substance" (E.C.I.). This index is dependent upon the digestibility of a food as well as the proportion of digested food which is used for growth on the one hand and that used for baseline metabolism on the other (Waldbauer, 1968). Thus, the gross growth efficiency is directly dependent upon the net growth and apparent assimilation efficiencies.

By rearing monarch larvae on a sinigrin - containing artificial diet and monitoring development and food utiliation, we hope to gain insight into the following questions:

I. Does sinigrin act as a chemical barrier to the monarch butterfly?

II. Does the monarch/Cruciferae relationship support the theory of chemical diversity reducing the apparency of early successional herbs?

III. If sinigrin presents a chemical barrier to the monarch butterfly, is it predominantly a behavioral or physiological barrier?

IV. Does the monarch butterfly conform to Jermy's principle that feeding deterrents are of paramount importance in host selection of oligophagous insects?

CHAPTER II

MATERIALS AND METHODS

Stock Culture

A laboratory culture of Danaus plexippus was maintained using methods modified from Glass and Pan (1983). The culture originated from adult monarch butterflies collected in July 1986 from Mahoning and Mercer counties in northeastern Ohio and western Pennsylvania respectively. Adults were housed in a wooden frame cage (ca. 31 x 31 x 31 cm) with the top and sides of the cage covered with 2 mm plastic mesh screening. Cage fronts were secured with doubled layered cheesecloth which permitted easy access. Male and female adults were housed separately (ca. 5 per cage). All life stages were maintained in a walk-in environmental growth chamber (Environmental Growth Chambers, Chagrin Falls, Oh., model 25) at: 25°C ± 1°C, 70% R.H. ± 1%, and long day conditions (LD, 16h:8h).

Each cage was supplied with two containers of a 1:10 honey/water solution which along with a Kimwipe wick were replaced daily. To ensure adequate nutrition for breeding purposes, adults were removed from their cages daily and fed the dilute honey solution. This was accomplished by placing a Kimwipe saturated with the honey

solution into a petri dish and uncoiling the butterfly's proboscis with a teasing needle until it came into contact with the solution. After a few days of such conditioning, the butterflies independently extended their proboscises when their tarsi were placed in the solution.

Upon eclosion, adults were maintained as previously described for seven days before hand pairing was attempted. This period ensured sexual maturation (Urquhart, 1960). Pairings were more successful in the early afternoon and controlled so as to avoid sib-matings. Following mating, females were supplied with a potted common milkweed plant (Asclepias syriaca L.) for oviposition. The mated female and host plant were contained in a wooden frame cage (ca. 31 x 31 x 62 cm) with 1 mm mesh screening covering all sides. The tight quarters of the cage increased the frequency of contact between the female and the host plant and thus oviposition. Eggs were collected twice daily, by excising small portions of leaf, and placed in petri dishes (ca. 50 per dish).

Upon hatching, the larvae were transferred to an artificial diet (Table 1) (Glass and Pan, 1983). Ingredients were combined with 930 ml of 70°C distilled water and blended at high speed for two minutes (Glass and Pan, 1983). The vitamin mixture (Riddiford, 1968) was constantly agitated while being dispensed and stored at 0°C in a small brown bottle. The milkweed leaf powder

TABLE 1.

INGREDIENTS FOR MONARCH ARTIFICIAL DIET

Milkweed leaf powder
Gelcarin,[a] 13 g
Wheat germ, 30 g
Sucrose, 30 g
Alphacel, 5 g
Casein, 30 g
Torula yeast, 20 g
Wesson's salt mixture, 5 g
Cholesterol, 1.5 g
B-Sitosterol, 1.5 g
Choline chloride, 1.2 g
Sorbic acid, 1 g
Methyl-p-OH-benzoat, 1 g
Ascorbic acid, 4 g
Chlortetracycline, 1 g
2% Formalin, 12 ml
Raw linseed oil, 10 ml
Vitamin mixture, 15 ml
10% KOH, 10ml

[a] FMC Corp., Marine Colloids Division Philadelphia, Pa.

originated from Asclepias syriaca leaves. Leaves were kept on ice during transport to the laboratory and lyophilized within an hour of collection. Dried leaves were ground into a fine powder and stored at 0°C.

The diet was cut into 4 x 2 x 0.5 cm strips and placed on the bottom of 100 ml clear plastic containers secured with perforated plastic lids. As many as 25 1st instar larvae were kept in each container. Every two days the diet was replaced and the larvae equally divided so as to prevent crowding and cannibalism. All 5th instar larvae were housed separately. When any larva showed signs of disease (i.e. monarch cytoplasmic polyhedrosis virus), it

was destroyed along with its container and any accompanying larvae (Glass and Pan, 1983).

Pupation within the rearing container was facilitated by wedging a wooden applicator stick between the walls of the container in the upper third of the chamber. Larvae, which invariably suspended from the applicator sticks, were thus easily manipulated. After hardening of the pupal cuticle, the applicator stick with the attached pupa was suspended within the adult cage. Under the controlled conditions, eclosion occurred in approximately 10 days. Upon eclosion, the adult's wings were extremely fragile and susceptible to damage; therefore, the adults were not sexed and separated until 3 hours following eclosion (Urquhart, 1960).

Experimental Cultures

Sinigrin (Aldrich Chemical Co., Milwaukee, Wisconsin) was incorporated into the standard diet at concentrations of: 0.00% (control), 0.01% (treatment A), 0.08% (treatment B), and 0.15% (treatment C) wet weight of the diet. These concentrations span the range of sinigrin found in nature (Lichtenstein et al., 1962; Lichtenstein et al., 1964). Appropriate amounts of sinigrin, which is freely soluble in water, were simply added to the respective diets and blended at high speed for 3 minutes.

Experimental and control diets were poured into 1000 ml beakers, allowed to harden, and stored at 4^{o}C

until needed. Cylinders of the experimental diet were removed with a cork borer and sliced into appropriately sized disks for feeding to the larvae. The selection of the disks from any particular concentration was done randomly to reduce the possible effects of a nonhomogeneous sinigrin concentration. The disks were impaled upon glass rods and placed in rearing containers so as to suspend the diet near the top of the container. This reduced the risk of larvae consuming their own feces. Glass rods were used to suspend the diet since larvae tended to consume applicator sticks along with the diet.

One hundred eight newly hatched monarch larvae were sequentially assigned upon emergence to one of the four treatment groups. Each treatment group consisted of 27 experimental organisms. Ten newly eclosed 5th instar larvae per treatment were sacrificed to determine the dry weight/live weight conversion factor. Food utilization determinations were carried out on the remaining 17 larvae per treatment. Each experimental larva was assigned to an individual rearing container with its date and time of hatching recorded. As experimental larvae originated from three different females, care was taken to equally distribute them among the treatment groups. The rearing containers were maintained at: 25^{o}C, 70% R.H., and long day conditions (LD, 16h:8h). Light in the environmental chamber was supplied by five, four - foot flourescent lamps.

Nutritional Indices

Dry matter budgets of the form: ingestion (I) = growth (G) + respiration (R) + egestion (F) and assimilation (A) = ingestion (I) - egestion (F) were determined for each larvae. The specific nutritional indices were calculated based on 5th instar D. plexippus larvae as described by Waldbauer (1968). Mathavan and Bhaskaran (1975) have shown that D. chrysippus Cramer acquires as much as 82% of its total food intake during this instar.

Initial 5th instar dry weights were determined from the dry weight/live weight ratios of newly molted 5th instar larvae. This conversion factor was determined from an aliquot of 10 larvae per treatment which was sacrificed and dried to constant weight at 80°C for 48 hours. Since the gut is empty prior to molting and the larvae were sacrificed before feeding had resumed, the influence of gut contents on the weight could be ignored (Schroeder, per. comm.). Weighed disks of diet were offered to the larvae each day at which time the feces and uneaten diet were collected and dried to constant weight (80°C for 48 hrs.). The size of the disks offered was such that the larvae would consume a large portion of the diet without completely depleting the supply. The dry weight of the food offered was determined from the dry weight/wet weight

ratio of two samples of diet taken at the time of feeding. The conversion factor did not change significantly within a treatment throughout the experimental period and thus a mean conversion factor for each treatment was utilized in all calculations. All surviving larvae were sacrificed and dried to constant weight as they entered the prepupal stage which was easily identified as a quiescent period associated with cessation of feeding and a marked reddening of the feces (feces are typically light brown when reared on the artificial diet). Likewise, larvae which perished before reaching the prepupal stage were dried to constant weight upon death. All calculations were made on a dry weight basis utilizing a Mettler H70 analytical balance (± 0.1 mg).

The various nutritional indices were calculated according to the following formulas:

$$Ea = \frac{\text{wt. of food ingested (mg) - wt. of feces (mg)}}{\text{wt. of food ingested (mg)}} \times 100$$

$$En = \frac{\text{wt. gained (mg)}}{\text{wt. of food ingested (mg) - wt. of feces (mg)}} \times 100$$

$$Eg = \frac{\text{wt. gained (mg)}}{\text{wt. of food ingested (mg)}} \times 100$$

Although the gross growth efficiency and net growth efficiency are valuable measures of the efficiency with

which ingested or absorbed food is converted into biomass, such ratios often conceal significant similarities or differences in the actual rates which are the "underlying reality" (Gordon, 1968). For this reason, the food utilization variables were also expressed as specific rates employing the conversion suggested by Gordon (1968) based upon the mean exponential larval weight:

Variables

Wf = dry weight of larva at the end of experiment

Wi = dry weight of larva at the beginning of experiment

T = duration of experiment (days)

We = mean exponential larval weight

$$= \frac{\text{wt gained (mg)}}{\ln \frac{Wf}{Wi}}$$

Specific rates

i = specific rate of ingestion

$$= \frac{\text{amount ingested (mg)}}{We \times T}$$

g = specific rate of growth

$$= \frac{\text{wt gained (mg)}}{We \times T}$$

a = specific rate of assimilation

$$= \frac{\text{amount ingested (mg)} - \text{wt of feces (mg)}}{We \times T}$$

Efficiencies of Food Conversion

apparent assimilation efficiency (Ea) = 100 a/i

net growth efficiency (En) = 100 g/a

gross growth efficiency (Eg) = 100 g/i

Larval Durations

The effect of sinigrin on monarch development and survivorship was monitored throughout the experimental period. The experimental cultures were inspected three times daily to determine the particular instar of each larva. The date and time of each molt were recorded with a precision of eight hours. Each instar possesses specific morphological characteristics which allow it to be easily distinguished from others (Urquhart, 1960). Since several of the larvae in treatment groups B and C died during the 5th instar before reaching the prepupal stage, two separate larval durations were calculated: (1) only those larvae which survived to the prepupal stage, and (2) all larvae including those which died before reaching the prepupal stage. In the second case, the date and time of death were utilized in duration determinations. This approach gave a better indication of the effects of sinigrin on the development of the monarch butterfly. Survivorship was represented as the percent of experimental organisms reaching the prepupal stage (i.e.

the ratio of surviving larvae to total larvae per treatment group).

CHAPTER III

RESULTS

Survivorship and Larval Durations

Larval durations and survivorship of D. plexippus maintained on control and experimental diets are shown in Table 2 and Figure 1. The survivorship of larvae reared on control and treatment A diets was 94% and 100% respectively which is similar to larvae reared on an allelochemic - free diet (Glass and Pan, 1983). Survivorship of larva reared on treatments B and C (82% and 76% respectively) was markedly reduced from that of larvae reared on the control diet (Table 2). Linear regression analysis of this relationship resulted in a correlation coefficient of -0.8353 suggesting a general trend towards decreasing survivorship with increasing sinigrin concentration ($0.05 < p < 0.08$).

Analysis of variance of the mean duration of instars L1, L2, L3, and L4 was not significant ($p > 0.05$) with F values of: 0.77, 0.34, 1.43, and 1.44 respectively (Table 2). Thus, the developmental durations of the first four instars were independent of sinigrin concentration. Results of Scheffe's multiple comparison test (SMCT) are presented in Table 2. The analysis of variance of the mean duration of larvae surviving to the prepupal stage

TABLE 2. Duration (days) and survivorship of Danaus plexippus larvae reared on an artificial diet. Control, 0.00%; Treatment A, 0.01%; Treatment B, 0.08%; Treatment C, 0.15% sinigrin. N = number of replicates. Mean values ± standard deviations.

Treatment	Survivorship	Instar Duration[a]						
		L1	L2	L3	L4	L5[b]	L5[c]	$\bar{L}$[d]
Control	94%	3.5 ± a 0.6 N = 27	2.7 ± a 0.4 N = 27	2.6 ± a 0.4 N = 26	3.3 ± a 0.4 N = 26	5.2 ± a 0.7 N = 16	5.2 ± a 0.7 N = 17	16.9 ± a 1.0 N = 17
A	100%	3.4 ± a 0.5 N = 27	2.7 ± a 0.5 N = 27	2.7 ± a 0.4 N = 27	3.2 ± a 0.3 N = 27	5.2 ± a 0.5 N = 17	5.2 ± a 0.5 N = 17	16.9 ± a 1.0 N = 17
B	82%	3.6 ± a 0.6 N = 27	2.7 ± a 0.4 N = 27	2.8 ± a 0.3 N = 27	3.4 ± a 0.6 N = 27	8.0 ± ab 4.3 N = 14	9.8 ± b 5.6 N = 17	21.9 ± b 5.8 N = 17
C	76%	3.6 ± a 0.6 N = 27	2.6 ± a 0.4 N = 27	2.8 ± a 0.3 N = 27	3.2 ± a 0.4 N = 27	10.0 ± b 4.0 N = 13	13.0 ± b 6.7 N = 17	25.2 ± b 6.9 N = 17
F ratio		0.77	0.34	1.43	1.44	9.69	12.82	13.34
Significance		0.5183	0.8013	0.2376	0.2337	0.0001	0.0001	0.0001

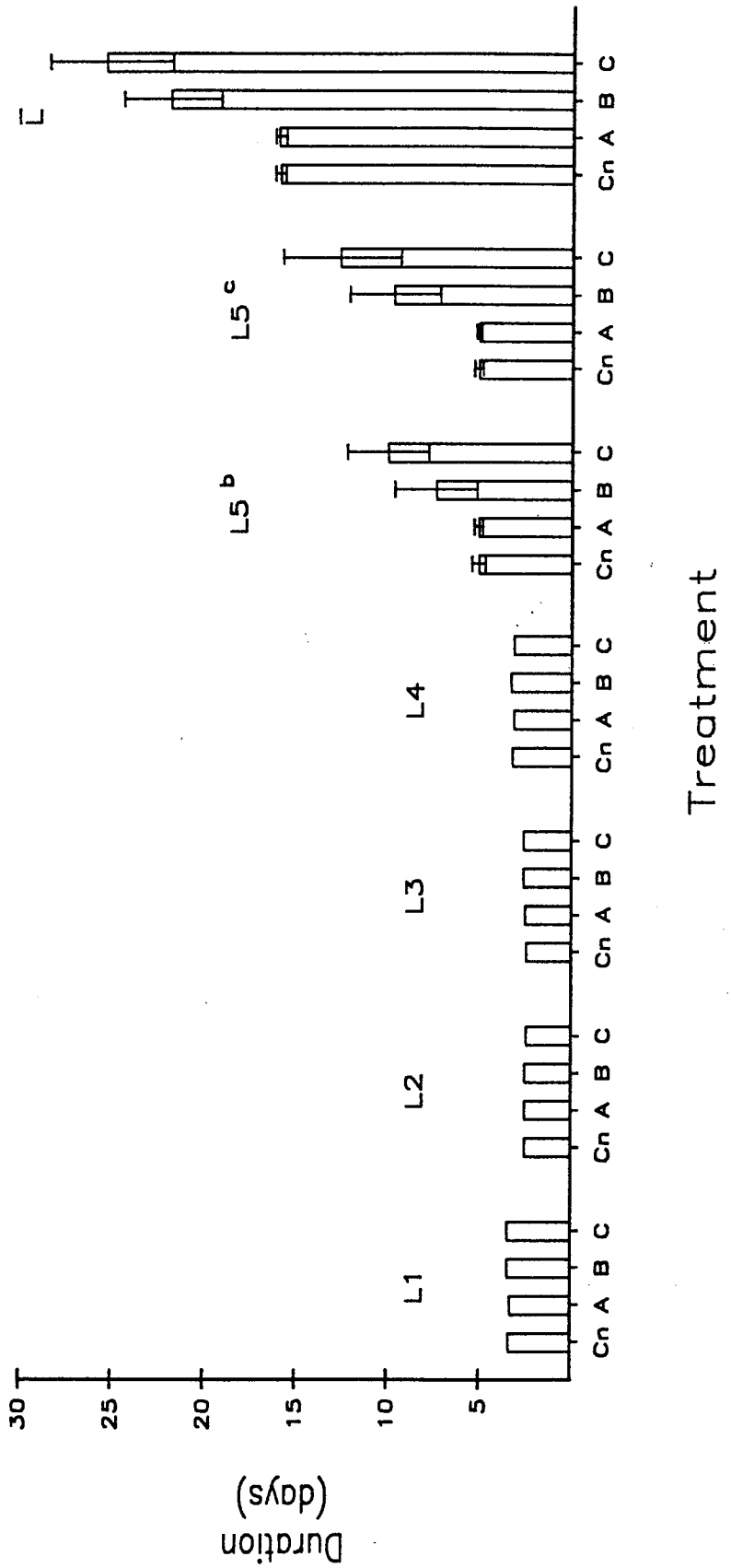
[a] Means followed by the same letter are not significantly different at 5% level (Scheffe's test)

[b] Values based on larvae surviving to the prepupal stage

[c] Values based on all larvae including those which died before the prepupal stage

[d] Mean duration of all larvae

FIGURE 1. Durations of D. plexippus instars: first (L1), second (L2), third (L3), fourth (L4), fifth surviving to the prepupal stage ($L5^b$), all fifth ($L5^c$), and mean duration of all larvae ($\overline{L}$) reared on an artificial diet containing 0.00% (Cn), 0.01% (treatment A), 0.08% (treatment B), and 0.15% (treatment C) sinigrin. Treatments B and C of $L5^b$, $L5^c$, and $\overline{L}$ varied significantly from control and treatment A larvae ($p < 0.05$, Sheffe's test).


Duration
(days)
30
25
20
15
10
5
L1
L2
L3
L4
L5 b
L5 c
L
Cn A B C
Treatment

($L5^b$), all larvae including those which died before reaching the prepupal stage ($L5^c$), and mean larval duration ($\overline{L}$) (Figure 1) proved significant ($p < 0.0001$) with F values of: 9.69, 12.82, and 13.34 respectively (Table 2). SMCT of $\overline{L}$ resulted in two groups: (1) control and treatment A larvae, and (2) treatments B and C larvae. Likewise SMCT of all larvae including those which died before reaching the prepupal stage ($L5^c$) resulted in two groups: (1) control and treatment A larvae, and (2) treatments B and C larvae. However, SMCT considering only those larvae which survived to the prepupal stage ($L5^b$) resulted in two overlapping sets: (1) control, treatment A, and treatment B larvae, and (2) treatments B and C larvae. Thus, treatment B larvae were not exclusively assigned to either group when only larvae surviving to the prepupal stage ($L5^b$) were included. Mean durations of 5th instar larvae including those that died prior to the prepupal stage ($L5^c$) eliminated this ambiguity and provided a more realistic indication of the effects of sinigrin on larval growth rates. The statistical difference in $\overline{L}$ can therefore be attributed solely to the increased 5th instar duration of treatments B and C (Table 2, Figure 1).

Dry Matter Budgets

The mean initial dry weight (grams) ($\pm$ standard deviations) of 5th instar larvae, Wi, reared on control

and treatments A, B, and C diets were 0.0387 ± 0.0061, 0.0392 ± 0.0062, 0.0389 ± 0.0076, and 0.0340 ± 0.0058 respectively. The mean final dry weight (grams) (± standard deviations) of 5th instar larvae, Wf, reared on control and treatments A, B, and C were 0.2099 ± 0.0364, 0.2175 ± 0.0356, 0.1473 ± 0.0553, and 0.1159 ± 0.0425 respectively. The mean percent dry matter (± standard deviations) of control and treatments A, B, and C diets were 14.7 ± 1, 14.5 ± 0.41, 14.4 ± 0.41, and 14.6 ± 0.35 respectively.

Dry matter growth performance variables (g, i, and a) and coefficients (100 a/i, 100 g/a, and 100 g/i) are shown in Table 3 and Figures 2 - 7. The analysis of variance of dry matter growth performance variables (g, i, and a) and coefficients (100 a/i, 100 g/a, and 100 g/i) proved significant ($p < 0.0001$) with F values of: 25.71, 18.82, 23.79, 8.54, 13.46, and 15.98 respectively (Table 3). The growth performance variables of control and treatment A larvae did not vary significantly (SMCT, Table 3). Similarly the growth performance variables of treatments B and C larvae were found to be equitable (SMCT, Table 3). Control and treatment A larvae displayed similar specific rates of growth (g), ingestion (i), and assimilation (a) (Figures 2 - 4). Larvae reared on treatments B and C exhibited significantly reduced specific rates of growth (g), ingestion (i), and assimilation (a) as compared to control and treatment A

larvae (Figures 2 - 4).

Although the analysis of variance of the apparent assimilation efficiency (100 a/i) was significant ($F = 8.54$, $p < 0.0002$), no mutually exclusive sets were identified by SMCT (Table 3, Figure 5). The analysis grouped: (1) control and treatment B, (2) treatments A and C, and (3) treatments A and B. SMCT analysis of both the net growth efficiency (100 g/a) and gross growth efficiency (100 g/i) resulted in two sets: (1) control and treatment A larvae and (2) treatments B and C larvae (Table 3, Figures 6 and 7). Thus, neither the net growth efficiency nor the gross growth efficiency varied significantly among control and treatment A larvae. Likewise, the net growth efficiency and gross growth efficiency among treatments B and C larvae were found to be equitable.

TABLE 3. Dry matter budget of 5th instar Danaus plexippus larvae. N = number of replicates, g = specific rate of growth, i = specific rate of ingestion, a = specific rate of assimilation, 100 a/i = apparent assimilation efficiency, 100 g/a = net growth efficiency, 100 g/i = gross growth efficiency. Mean values ± standard deviations. [a]

Treatment	N	g	i	a	100 a/i	100 g/a	100 g/i
Control	17	0.33 ± a 0.05	1.3 ± a 0.19	0.75 ± a 0.11	58 ± a 2	45 ± a 5	26 ± a 3
A	17	0.33 ± a 0.04	1.2 ± a 0.14	0.66 ± a 0.08	54 ± bc 3	50 ± a 3	27 ± a 2
B	17	0.19 ± b 0.11	0.89 ± b 0.28	0.51 ± b 0.16	57 ± ac 4	34 ± b 16	19 ± b 8
C	17	0.13 ± b 0.10	0.76 ± b 0.30	0.40 ± b 0.15	53 ± b 4	29 ± b 14	15 ± b 8
F ratio		25.71	18.82	23.79	8.54	13.46	15.98
Significance		0.0001	0.0001	0.0001	0.0002	0.0001	0.0001

[a]Means followed by the same letter are not significantly different at the 5% level (Scheffe's test)

FIGURE 2. Specific rate of growth of all 5th instar D. plexippus larvae reared on diets containing 0.00% (Cn), 0.01% (treatment A), 0.08% (treatment B), and 0.15% (treatment C) sinigrin. Treatments B and C larvae varied significantly from control and treatment A larvae ($p < 0.05$, Scheffe's test).

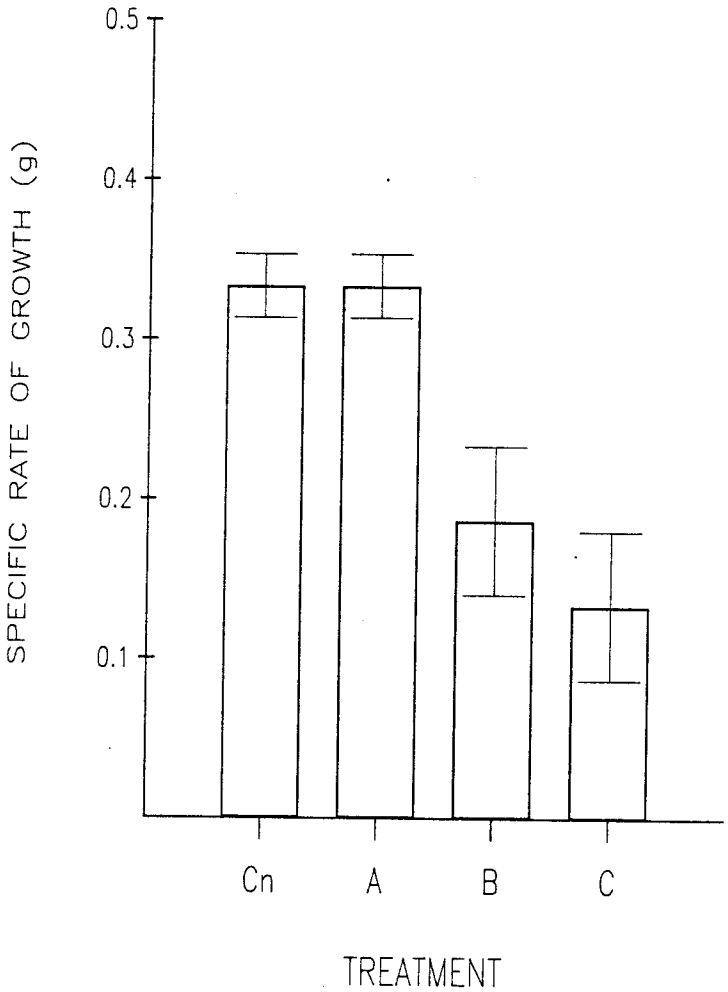

0.5
0.4
0.3
0.2
0.1
SPECIFIC RATE OF GROWTH (g)
Cn
A
B
C
TREATMENT

FIGURE 3. Specific rate of ingestion of all 5th instar D. plexippus larvae reared on diets containing 0.00% (Cn), 0.01% (treatment A), 0.08% (treatment B), and 0.15% (treatment C) sinigrin. Treatments B and C larvae varied significantly from control and treatment A larvae ($p < 0.05$, Scheffe's test).

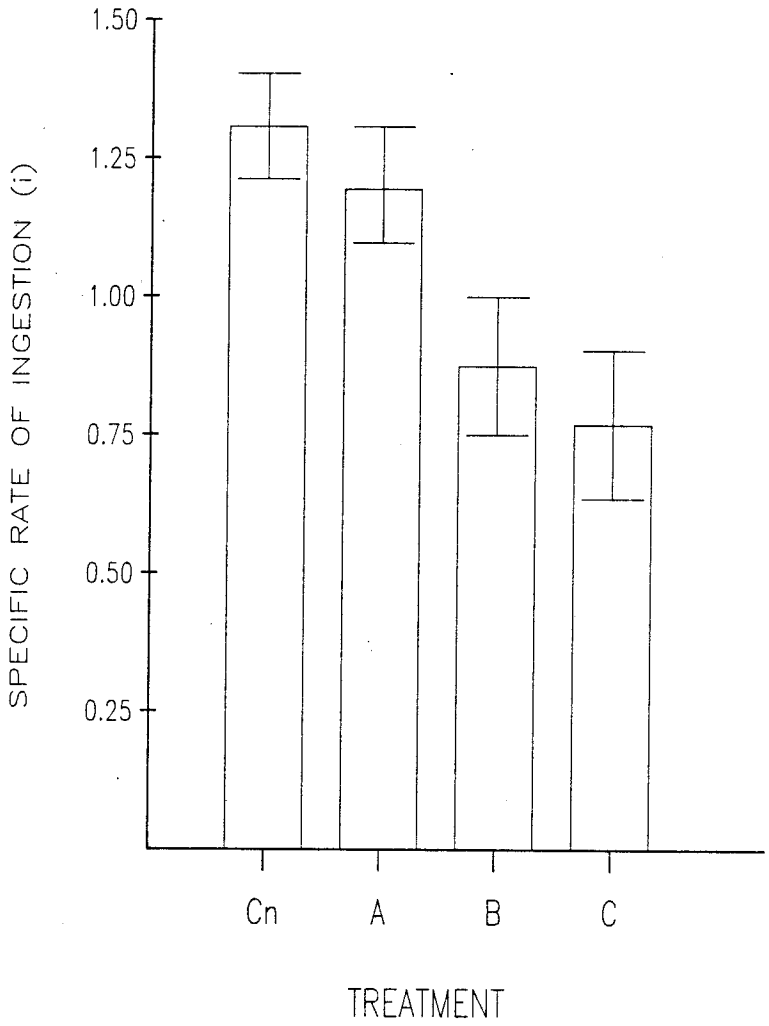

1.50
1.25
1.00
0.75
0.50
0.25
SPECIFIC RATE OF INGESTION (i)
Cn
A
B
C
TREATMENT

FIGURE 4. Specific rate of assimilation of all 5th instar D. plexippus larvae reared on diets containing 0.00% (Cn), 0.01% (treatment A), 0.08% (treatment B), and 0.15% (treatment C) sinigrin. Treatments B and C larvae varied significantly from control and treatment A larvae ($p < 0.05$, Scheffe's test).

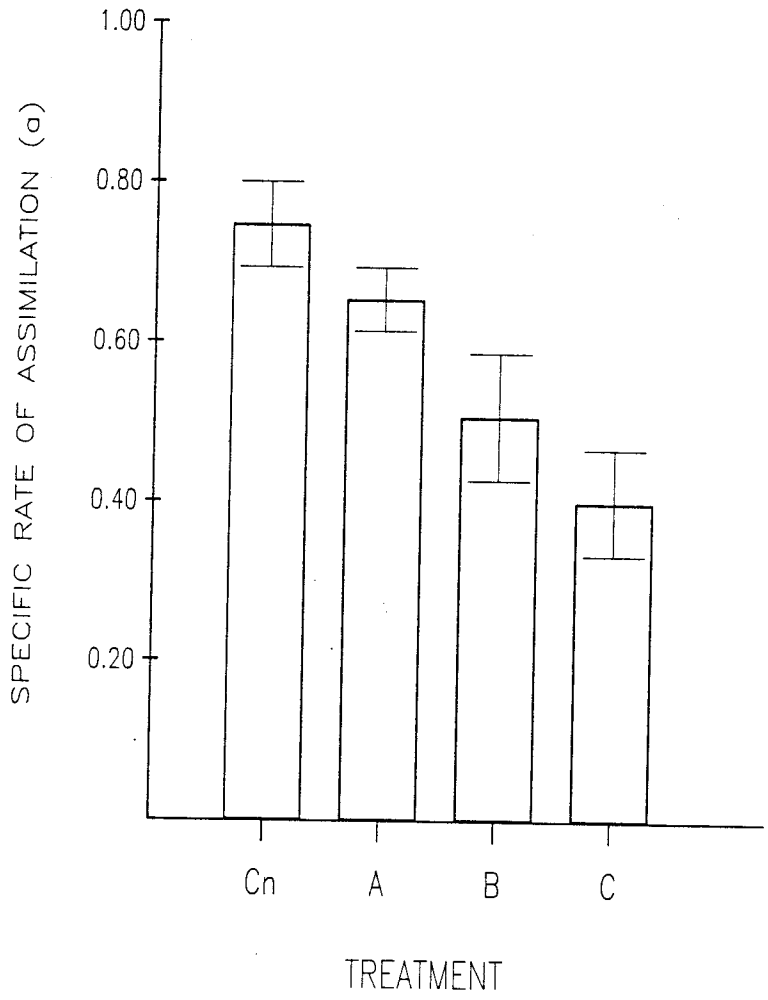

1.00
0.80
0.60
0.40
0.20
SPECIFIC RATE OF ASSIMILATION (a)
Cn
A
B
C
TREATMENT

FIGURE 5. Apparent assimilation efficiency of all 5th instar D. plexippus larvae reared on diets containing 0.00% (Cn), 0.01% (treatment A), 0.08% (treatment B), 0.15% (treatment C) sinigrin. No biologically interpretable groups were identified ($p < 0.05$, Scheffe's test).

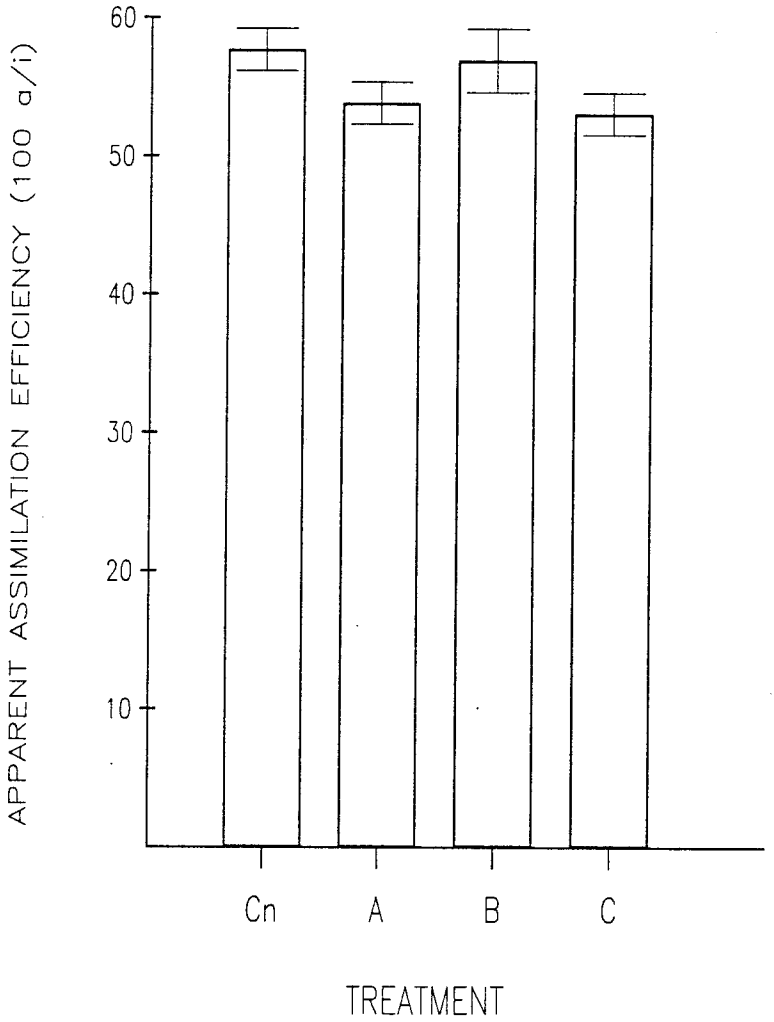

APPARENT ASSIMILATION EFFICIENCY (100 a/i)
60
50
40
30
20
10
Cn
A
B
C
TREATMENT

FIGURE 6. Net growth efficiency of all 5th instar D. plexippus larvae reared on diets containing 0.00% (Cn), 0.01% (treatment A), 0.08% (treatment B), and 0.15% (treatment C) sinigrin. Treatments B and C larvae vareied significantly from control and treatment A larvae ($p < 0.05$, Scheffe's test).

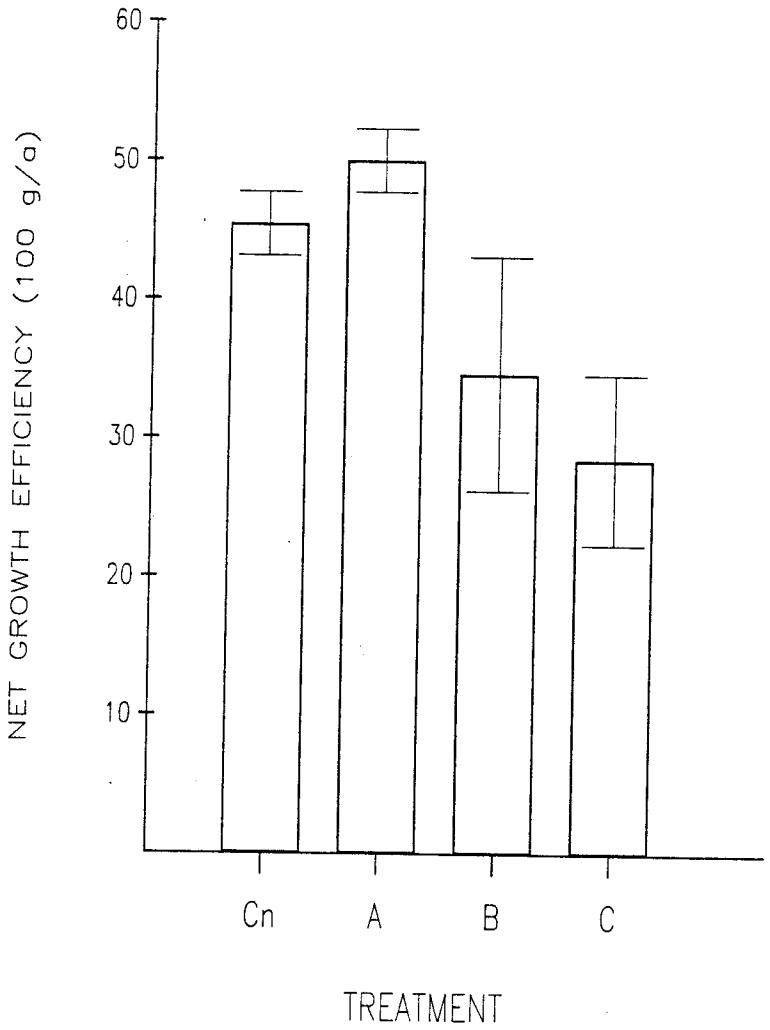
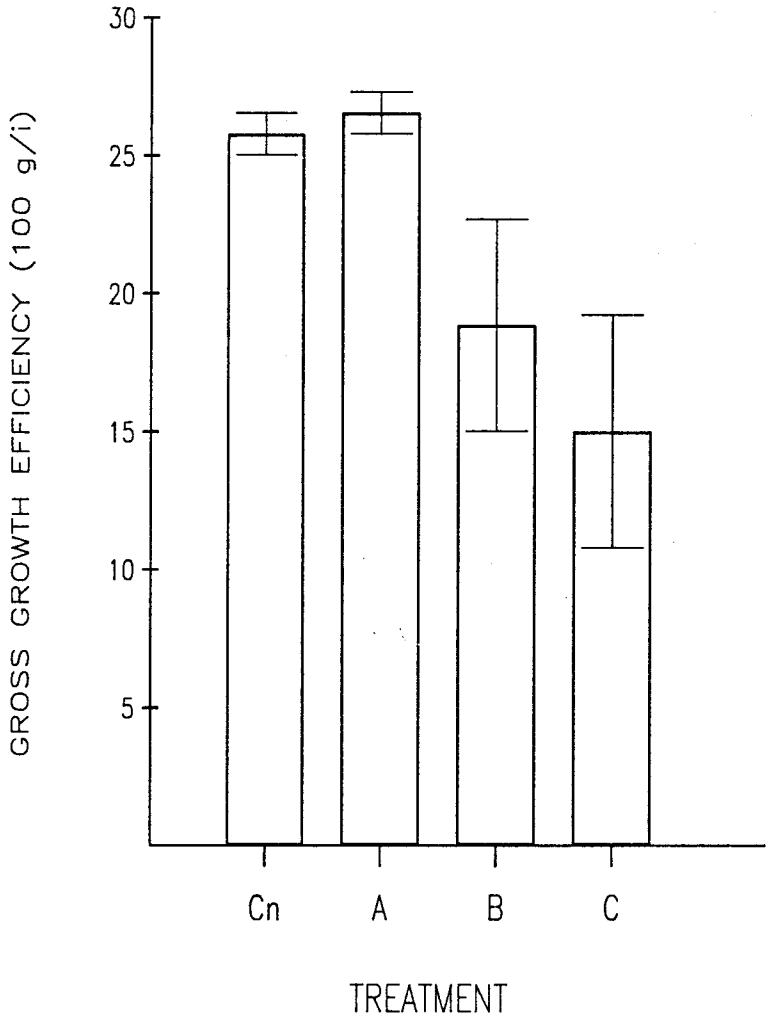

60
50
40
30
20
10
NET GROWTH EFFICIENCY (100 g/a)
Cn
A
B
C
TREATMENT

FIGURE 7. Gross growth efficiency of all 5th instar D. plexippus larvae reared on diets containing 0.00% (Cn), 0.01% (treatment A), 0.08% (treatment B), and 0.15% (treatment C) sinigrin. Treatments B and C larvae varied significantly from control and treatment A larvae ($p < 0.05$, Scheffe's test).


GROSS GROWTH EFFICIENCY (100 g/i)
30
25
20
15
10
5
Cn
A
B
C
TREATMENT

CHAPTER IV

DISCUSSION

Allelochemics may affect insect growth through a variety of avenues. Rates of ingestion may be reduced due to the lack of specific phagostimulants and/or the presence of phagodeterrents. Ingested food may be inefficiently digested if necessary enzymes are lacking or are inhibited by antimetabolites. Reduced growth may also result from impaired absorption or inefficient conversion of assimilated food to larval biomass (Gordon, 1968).

The reduced growth rates experienced by treatments B and C larvae (42% and 61% respectively) were attributed to decreased rates of ingestion (32% and 42% respectively) along with increased rates of respiration (14% and 23% respectively). Thus, larvae reared on the 0.08% and 0.15% sinigrin diets consumed less food and converted what food was ingested less efficiently than control and treatment A larvae. This suggested sinigrin acted both as a feeding inhibitor and a toxin to the monarch butterfly. The reduced rates of ingestion accounted for over two - thirds of the decline in growth while the increased rates of respiration were responsible for nearly one - third of this decline. Therefore, it may be concluded that sinigrin acted predominantly as a feeding deterrent and

secondarily as a toxin to affect the reduction in growth. The relatively large standard deviations associated with the specific rates of ingestion of treatments B and C larvae (0.28 and 0.30 repectively) may reflect the variability in the monarch feeding response to sinigrin as a feeding deterrent. A wide variability in the feeding response of Pieris brassicae larvae to an artificial diet containing glucosinolates was noted by David and Gardiner (1966).

Although the ANOVA of the apparent assimilation efficiency (Ea, 100 a/i) proved significant ($F = 8.54$, $p < 0.0002$), no biologically significant relationship between Ea and increasing sinigrin concentrations was apparent (see SMCT, Table 3). Treatment B larvae displayed an Ea similar to control larvae while treatment A larvae exhibited an Ea similar to treatment C larvae. Thus, any meaningful biological effect of the small but statistically significant difference was not readily apparent. The relatively large number of replicates per treatment and the resulting small error mean square (11.63) may have enabled the ANOVA to detect small differences in Ea (i.e. increased statistical power) which were not biologically interpretable. The slight overall decrease in Ea (58 - 53) supports this contention. Linear regression analysis revealed that only 9% of the total variation in Ea could be accounted for by the variation in sinigrin concentration. Thus, for interpretive

purposes, the apparent assimilation efficiency was considered to be independent of sinigrin concentrations used in the study. Therefore the reduction in the specific rate of assimilation (a) associated with treatments B and C was attributed to the decreased specific rates of ingestion (i.e. a = i - f). Since food was being assimilated with nearly equal efficiency throughout the treatments, a decline in the specific rate of ingestion would be accompanied by a proportional decline in the specific rate of assimilation.

It follows from i = g + r + f and a = i - f that a = g + r. Thus, the net growth efficiency (En, 100 g/a) may be defined as: En = 100 g/g+r. From this equation it is clear that the reduction in the net growth efficiency exhibited by treatments B and C larvae could have resulted from either an increase in the specific rate of respiration (r) and/or a decline in the specific rate of growth (g). The specific rates of respiration (r = a - g) of control and treatments A, B, and C larvae were: 0.42, 0.33, 0.32, and 0.27 respectively. The observed decline in r was expected since r is partly dependent upon the assimilation of nutrients across the gut wall (i.e. all other things being equal, a decrease in the processing of nutrients results in a decreased rate of metabolism). Therefore, the decline in r was expected since the specific rate of assimilation declined from 0.75 (control) to 0.40 (treatment C). Thus, of real interest was whether

or not the observed decline in r was significantly less than the expected decline in r indicating an actual increase in the rate of respiration. The expected values of r were determined from the ratio of r/a of control larvae (r/a = .56). Thus, the expected values of r (if r simply varied with "a" in proportion to control larvae) for the various treatments were determined by solving for "r" in the following equation:

$$\frac{\text{expected "r" of treatment}}{\text{observed "a" of treatment}} = 0.56$$

Thus, the mean expected values of r for treatments A, B, and C larvae were determined to be: 0.37, 0.28, and 0.22 respectively resulting in mean increases of -0.04, 0.04, and 0.05 respectively. Linear regression analysis of this relationship proved highly significant ($p < 0.0001$) with a correlation coefficient of 0.6217. Thus, the reduction in En experienced by treatments B and C larvae resulted from an increase in respiration as well as a decrease in ingestion.

The significantly increased rates of respiration exhibited by treatments B and C larvae suggested sinigrin imposed a metabolic load on these larvae. The specific biochemical origin of this metabolic stress could not be readily identified by the nutritional index technique; however, induction of detoxification enzymes is one possibility. Schroeder (1986), working with Datana ministra (Drury), has suggested larval hemolymph osmotic

imbalances may increase metabolic demands. Schroeder hypothesized accumulation of amino acids and their metabolites in larval hemolymph increased osmotic pressures resulting in water retention. Such a retention of water was noted in monarch larvae reared on the 0.08% and 0.15% sinigrin diets. The mean percent dry matter (dry weight/live weight) ($\pm$ standard deviation) of 5th instar monarch larvae reared on control, and treatments A, B, and C diets were 19.04 $\pm$ 1.5, 18.5 $\pm$ 1.2, 15.8 $\pm$ 1.8, and 15.0 $\pm$ 2.6 respectively. Linear regression analysis of this relationship proved highly significant ($p < 0.0001$) with a correlation coefficient of -0..6563. Therefore, larvae reared on the higher concentrations of sinigrin retained significant amounts of water suggesting sinigrin may have disturbed hemolymph osmotic balances. Such osmotic imbalnces could have resulted from sinigrin (or allylisothiocyanate) interupting the normal excretory process or perhaps directly inhibiting the conversion of assimilated food to biomass.

The gross growth efficiency (Eg, 100 g/a) varies with Ea and En. Since no clear effects of sinigrin on Ea could be demonstrated, the reduction in Eg associated with treatments B and C larvae was attributed to the reduction in En which has been shown to be the consequence of a reduced rate of ingestion and increased rates of respiration.

The mean larval duration of treatments B and C ($\bar{L}$, Table 2) was significantly prolonged as compared to that of control larvae. The prolonged larval development was attributed solely to the increased duration of the 5th instar (Figure 1). Prolonged larval development in insects exposed to dietary stress is well documented (Scriber and Slansky, 1981). Most of the total consumption and growth of phytophagous insects occurs during the penultimate and ultimate instars (Mathavan and Bhaskaran, 1975).

The mortality rate of D. plexippus tended to increase with increasing sinigrin concentrations (Table 2). However, even at the highest sinigrin concentration (0.15%), only 24% of the larvae terminated before reaching the prepupal stage despite the fact that their rate of ingestion decreased by 42%. It is known that partial inhibition of feeding or severe food limitations do not necessarily prevent adult development but simply result in lower adult weights (Trager, 1953).

Sinigrin's role as a feeding deterrent in noncruciferous feeding insects has been well established. Nault and Styer (1972) demonstrated the deterrent nature of sinigrin on two polyphagous aphid species, Aphis fabae and Acyrthosiphon solani, as well as in the Leguminosae restricted species, Acyrthosiphon pisum Harris. Klingauf et al. (1972) showed a 0.1% sinigrin solution to be a strong deterrent to Rhopalosiphum padi L. Feeding in the polyphagous aphid, Myzus persicae, has been shown to be

inhibited by sinigrin even though its natural host plant range includes many Cruciferae (Wearing, 1968; but see Klingauf et al., 1972; and Nault and Styer, 1972).

Sinigrin has also been shown to deter feeding and reduce assimilation in the black swallowtail butterfly, Papilio polyxenes (Erickson and Feeny, 1974). These workers incorporated sinigrin into a normal food plant (Umbelliferae) of the swallowtail at concentrations of: 0.001%, 0.01%, 0.1%, 1.0%, and 2.5%. All larvae on the 1.0% and 2.5% treatments failed to complete the first instar while larvae on the 0.1% treatment terminated during the 5th instar. The rate of ingestion of 5th instar larvae reared on 0.001% sinigrin was reduced nearly 15% from that of control larvae while that of the first four instars reared on this treatment was reduced an average of nearly 33%. The rate of ingestion of 5th instar larvae reared on 0.01% sinigrin was reduced by 15% and that of the first four instars was reduced by an average of nearly 34%. Although the reduction in the rate of ingestion of 5th instar larvae reared on 0.1% sinigrin could not be determined, instars L1 - L4 were found to exhibit an average reduction in ingestion of over 60%. Monarch larvae exhibited reductions in specific rates of ingestion of: 8% (0.01% sinigrin), 32% (0.08% sinigrin), and 42% (0.15% sinigrin).

In addition to reducing the rate of ingestion, Erickson and Feeny (1974) found sinigrin impaired the

efficiency with which ingested food was assimilated from the gut. The apparent assimilation efficiency (Ea) of 5th instar P. polxenes larvae reared on 0.001% sinigrin was reduced 10% from that of control larvae. The first four instars of this treatment exhibited a similar average reduction in Ea. Fifth instar larvae reared on 0.01% sinigrin experienced a 22% reduction in Ea while that of instars L1 - L4 was reduced by an average of nearly 21%. Although the reduction in Ea of 5th instar larvae reared on 0.1% sinigrin could not be determined, instars L1 - L4 exhibited an average reduction of nearly 60%. The apparent assimilation efficiency of the monarch butterfly tended to decrease with increasing sinigrin concentrations; however, the percent reductions were minimal: 7% (0.01% sinigrin), 2% (0.08% sinigrin), and 9% (0.15% sinigrin). Thus, P. polyxenes was much more susceptible to the assimilation - reducing effects of sinigrin than D. plexippus. Indeed this may account for the high levels of mortality experienced by P. polyxenes on the 0.1% sinigrin concentration while a comparable concentration had little effect on the survivorship of D. plexippus.

Brattsten et al. (1977) have shown sinigrin to induce mixed function oxidase activity; such activity (albeit minimal) has been demonstrated in monarch larvae (Krieger et al., 1971). Thus, the relative tolerance of the monarch butterfly to the assimilation - reducing effects of sinigrin may partially reside in this system.

Sinigrin has been shown to induce the MFO system in the southern army worm, Spodoptera eridania (Brattsten et al., 1977) and natural levels of sinigrin do not inhibit growth in this insect (Blau et al., 1978).

Monarch larvae may further circumvent the detrimental effects of sinigrin by maintaining an alkaline midgut. Saxena (1981) reports the midgut pH of 5th instar D. chrysippus larvae to range from 8.0 to 8.3. Drobnica et al. (1977) report isothiocyanate will react only with the SH groups of proteins at a pH $\leq$ 7 but will react with both SH and NH_2 groups at a pH of 10. Thus, if monarch larvae possess a midgut pH similar to D. chrysippus, allylisothiocyanate may react with fewer proteins than it would at a higher gut pH. Therefore, fewer nutrients would be bound and a minimal effect on assimilation would result. Interestingly, Cruciferae specialists also maintain a dilute alkaline midgut. From available data (Berenbaum, 1980), cruciferous specialists are found to possess a mean midgut pH near 8.1. However, many such specialists have been reported to possess some of the lowest midgut pH values found among lepidopteran larvae; e.g. Trichoplusia ni Hubner (7.0 - 7.6), Pieris rapae (7.3 - 7.6), and Plutella maculippennis (7.4 - 7.9) (Berenbaum, 1980). It is of further interest to note that P. rapae larvae were unaffected by the incorporation of a cardenolide (k - strophanthin) into their diet (Usher and Feeny, 1983).

In contrast to D. chrysippus and cruciferous specialists, a 5th instar midgut pH as high as 9.7 - 10 has been reported for larvae of Papilio demoleus L. (Narayanan et al., 1976). If P. polyxenes maintains a similar midgut pH, it may partially account for the reduced assimilation efficiencies experienced by this organism when exposed to increasing concentrations of sinigrin. At such pH levels, allylisothiocyanate may bind to both SH and amino groups of proteins thereby reducing the bio - availability of nutrients and utimately reducing the efficiency of assimilation. Isothiocyanates are also known to be potent inhibitors of enzymes which require thiol groups for their catalytic activity (Drobnica et al., 1977).

The toxic and feeding inhibitory nature of sinigrin appeared to provide an effective barrier to the monarch butterfly. The most dramatic result was the increased rate of mortality. However, the prolonged larval durations may present the real barrier to the monarch. In natural ecosystems, prolonged larval development would result in the increased likelihood of predation, parasitism, and pathogenesis. Such increases would also decrease monarch fitness by decreasing the probability of successful reproduction. Extensions of the larval period would present special problems to the monarch since adults emerging in late summer migrate to overwintering sites. Such migrations are dependent upon

timely development of the imago as well as the acquisition of sufficient energy reserves which partly depends upon the nutritional history of the larvae.

Sinigrin may also exert delayed effects on pupal and adult mortality and fecundity. Erickson and Feeny (1974) found sinigrin increased pupal duration and decreased pupal weight, oviposition, and egg viability in the black swallowtail. Such dependence of adult fecundity on the nutritional history of the larva is quite common (Trager, 1953). Indeed to achieve a complete understanding of the effects of sinigrin on the monarch butterfly many successive generations should be monitored for even subtle effects on pupal and adult stages could provide protection to the Cruciferae (Chew, 1977). Indeed observations made during this study suggested that sinigrin may adversely affect monarch larval - pupal transformations.

Behavioral adaptations modifying the monarch interpretation of sinigrin as a feeding deterrent and physiological adaptations reducing the toxic effects of sinigrin would be required before the monarch could efficiently utilize the Cruciferae as host plants. Such findings support the theory that the chemical diversity of early successional herbaceous communities reduces the "apparency" of each of its members. Thus, as a result of the toxic and feeding inhibitory effects of sinigrin, the Cruciferae are much less likely to be exploited as a food

source by the monarch butterfly.

The fact that the feeding deterrent effects of sinigrin accounted for over two - thirds of the observed reduction in monarch growth supports the suggestion that host plant susceptibility and specificity was largely controlled by behavioral responses to attractant and repellent chemicals. This seems to be true for the monarch/Cruciferae relationship as minimal physiological costs were associated with ingestion of sinigrin at levels common in nature (i.e. less than one - third of the decrease in g was attributed to toxic effects). Thus, modification of the monarch response to sinigrin as a feeding deterrent and appropriate oviposition behavior modifications could be sufficient in themselves to allow utilization of the Cruciferae (if appropriate selectional forces were present).

Finally, the feeding deterrent effect of sinigrin on D. plexippus supports the principle expressed by Jermy (1966) that sensitivity to deterrents is of paramount importance in determination of host specificity among oligophagous insects. Thus, although the diet offered the monarch contained phagostimulants (i.e milkweed powder), the inhibitory nature of sinigrin predominated. As Schoonhoven (1977) notes:

> Each insect species has evolved a sensory machinery that... is optimal in discriminating between acceptable and unacceptable food... When the incoming information differs too much... from the desired pattern, the food is rejected.

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