# A Meridic Diet for the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say)[1]

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**ABSTRACT** Foliage of the potato, *Solanum tuberosum* (L.), was analyzed previously to determine its nutritional content. This information was used to help develop a meridic diet for rearing the Colorado potato beetle, *Leptinotarsa decemlineata* Say. Various test diets were prepared, and types and concentrations of proteins, amino acids, carbohydrates, and ascorbic acid were tested for their influence on growth and weight gain of larvae reared from the egg stage on diet. At 2.8% protein or less, neither egg albumin nor casein produced adult beetles. Adults were reared with casein concentrations of 3.0 and 4.2%, but not at 5% casein. An abbreviated list of foliar amino acids was as effective as the complete foliar complement in the rearing of adults. Oat flour or potato flakes were suitable carbohydrate sources for rearing to the adult stage. The composition of a meridic diet is presented which was used for rearing the Colorado potato beetle from the egg to the adult stage, in the absence of host plant material.

**Key Words** Colorado potato beetle, insect rearing, nutrition, protein, amino acids, carbohydrates, minerals, insect pest

The Colorado potato beetle, *Leptinotarsa decemlineata* Say, is a pest of major importance in most temperate zone areas of the world where the cultivated potato, *Solanum tubersoum* (L.), is grown. A reliance on synthetic organic insecticides to control this insect has resulted in the development of resistant populations and, thus, has reduced the number of insecticides that remain effective. The diminishing returns from insecticide use for beetle population control, and the concurrent detrimental ecological consequences of their use, have shifted research efforts toward the development of urgently-needed alternative control methods. For example, natural enemy augmentation and the incorporation of resistance-conferring genes into the host plant genome could be greatly facilitated if all life stages of this insect were commonly available on a year-round basis.

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Currently, most year-round Colorado potato beetle cultures are maintained on greenhouse-grown potato plant foliage, an expensive and labor-intensive effort. The use of foliage in rearing this insect has other disadvantages. The levels of nutrients and feeding stimulants have been shown to vary seasonally in field-grown plants (Hare 1983), and greenhouse conditions may not fully compensate for the flux in natural conditions. Nutrients and feeding stimulants of greenhouse-grown plants also vary significantly among plants, and by leaf age within plants (Domek, unpub. data). The use of host plant foliage may, thus, be an uncontrolled source of experimental error that could confound the effects of test substances coated on leaf tissue and fed to larvae or adults.

The development and use of a meridic diet, free of host plant material and suitable for rearing all life stages of the Colorado potato beetle, has several advantages over current rearing practices. The cost of producing beetles with a synthetic diet should be lower than that of rearing beetles on greenhouse-grown potato foliage. A meridic diet without host plant material may not only satisfy the insect's evolved feeding preferences and nutritional requirements contained in the host plant, but can also provide uniform nutritional content and, thus, avoid the dietary variability inherent in foliage. A meridic diet also allows manipulation of the quantities of all ingredients, which enables a further assessment of host plant-derived substances for possible influences on larval feeding and growth, and adult reproduction. Novel compounds isolated from natural non-host sources can be discovered and evaluated with the aid of a suitable diet. An assessment can be made also of interactions between, for example, nutrients and allelochemicals, which can influence the efficacy of novel substances.

The literature contains few references to the rearing of the Colorado potato beetle on an artificial diet. Hsiao (1966) formulated a diet without plant material that supported the growth for 2 d of fourth-instar larvae, a stage that accepts non-host food more readily than earlier instars. This diet was then used to evaluate plant powders and extracts for their ability to stimulate growth and feeding of fourth instars (Hsiao and Fraenkel 1968a). Many purified compounds, such as sugars and amino acids, were incorporated in an agar-cellulose medium and tested for their ability to stimulate feeding of fourth-instar larvae (Hsiao and Fraenkel, 1968b), and potato plant extracts were similarly evaluated (Hsiao and Fraenkel, 1968c). Wardojo (1969a) used artificial diets to evaluate many nutritive substances for their influence on the growth of larval Colorado potato beetles. He developed an artificial diet that produced beetles which were somewhat smaller in size and slower to develop than were foliage-fed beetles (Wardojo 1969b). However, no successful use of any of these diets for rearing this beetle from egg to adult stages has been reported, despite the potential benefits of such diets.

The purpose of this paper is to describe the development of an artificial diet devoid of host plant foliage or foliar extracts to successfully rear the Colorado potato beetle from the egg to the adult stage.

## Materials and Methods

**Insects.** Egg masses adhering to foliage were collected daily from a lab culture of adult beetles maintained on greenhouse-grown potato plants, then placed in a Petri dish and held at 25°C, with 16:8h light:dark regimen for 2 to 3 days. Before use, egg masses were washed for 30 sec in a 2% v/v solution of bleach containing a few drops of detergent, rinsed in a stream of distilled water and placed on filter paper in a Petri dish. With the aid of a dissecting microscope, fine tweezers were used to separate eggs for solitary placement on a portion of each diet treatment.

**Diets.** Dietary quantities of some nutritionally vital components, such as lipids and vitamins, were primarily based on previously published work in the field of insect nutrition (House 1974, Reinecke 1985) and from diet tests with the Colorado potato beetle (Wardojo 1969a). The ascorbic acid and vitamin A contents of potato foliage were determined, however, for use in development of this diet and tests of some concentrations (0, 36, 108, and 324 mg/100g diet) of ascorbic acid also were done. The foliar content of the major nutrients (protein, carbohydrates), mineral elements, amino acids, and feeding stimulants were previously determined in potato foliage (Domek et al. 1995). This information was used to help formulate a series of meridic diets for the Colorado potato beetle. Suitability of diets was assessed by recording development time, larval or prepupal weight, or adult emergence and adult weight, when applicable.

All diets contained approximately 2.3% agar to retain moisture, distribute ingredients in an ingestible form, and provide a surface firm enough to support larval movement. Agar was cooled to 60°C before combining with other ingredients. Lipids were dissolved in methylene chloride, mixed with cellulose powder, and evaporated under a hood for 6 h. Casein was added to 40 ml water and dissolved by adding 1.1 ml of 3.3 M KOH solution. The remaining ingredients were added, beginning with potassium phosphate, and the pH was adjusted to 5.9-6.2. The mixture temperature was maintained at 60°C in a water bath, followed by addition of 40 ml of 2.3% agar solution and mixing with a homogenizer in a clean-air hood. Diet treatments were poured separately into 15-cm Petri dishes in a clean-air hood and cooled. A sterile spatula was used to cut diet treatments into cubes weighing approximately 60 mg each for the eggs and first instars, and placed singly in sterile 96-well polystyrene tissue culture plates. One surface-sterilized egg was placed on each diet cube. Plates were covered and stored in zip-loc plastic bags in a temperature cabinet at 26°C with 16 h light. Larvae were transferred daily to fresh diet held in clean culture plates and were transferred to 24-hole plates upon reaching the late second or early third instar. At this time, the weight of each diet cube was increased to about 150 mg, and increased again to 300 mg for fourth-instar larvae, which were transferred to 12-well plates to accommodate their larger size and increased amount of diet cube. When fourth instars stopped feeding (usually 10 to 14 d after egg hatch), they were considered to be prepupae and were transferred to sterilized sandy soil for pupation. Soil was held to a depth of about 10 cm in plastic containers with perforated bottoms which allowed soil to hold some moisture but prevented free-standing water from accumulating.

Concentrations of vitamin-free casein (1,4, 2.8, 3.0, 4.0, 4.2, 4.5, 5.0, 6 and 7%), soy protein (2.8, 3.0, 3.2, 4.0, 4.25, 4.75 and 5%), and egg albumin (1.4, 2.8 and 4.2%) were tested alone and in certain combinations to determine if there was an optimal protein type and concentration for promoting growth. The amino acid composition of test diets was modified to determine if specific reductions or increases in concentration produced a change in weight gain or development time. The General Linear Models procedure and Least Square means/t test of difference separation option were used to test for significant mean larval weight differences (SAS Institute 1988). Carbohydrates of several types and dietary concentrations were tested for growth-promoting effects. Rice flour (6.0%), powdered lima beans (6.0%), powdered instant oatmeal (5,6,7 and 8%), powdered instant potato flakes (5,6,7 and 8%), amylodextrin (5,6,7 and 8%), 70% amylose (6%) and amylopectin (6%) were evaluated for suitability. Some subjective testing of polysaccharides on the physical consistency of the diet was done with polygalacturonic acid, pectin, amylopectin, soluble starch, and potato flakes, each in the range from 0.5 to 3.0%. The foliar levels of beta-carotene (analyzed by HPLC in isocratic methanol) and ascorbic acid (Vitamin C) (Vanderslice and Higgs 1988) were evaluated to establish realistic dietary values. The longevity of Vitamin C was tested by preparing two diets with different Vitamin C forms, holding them under refrigeration for 14 d and re-assaying the Vitamin C content. Larvae reared on potato foliage also were analyzed for ascorbic acid content to compare with larvae reared on diet.

## Results and Discussion

**Protein composition tests.** Five levels of casein incorporated into the same basic diet at 1.4, 2.8, 3.0, 4.2 and 5.0 g casein per 100 g diet yielded significantly different weights of fourth-instar larvae (Table 1). From 11 to 15 d after eclosion, the respective weights averaged 32.6 ± 4.2 mg (n = 12), 67.4 ± 4.5 mg (n = 34), 97.3 ± 4.5 mg (n = 30), 126.3 ± 3.7 mg (n = 41), and 75.4 ± 4.1 mg (n = 19). Development to the late fourth instar occurred in 11 d on the 4.2 g casein diet and a few days longer on all other casein levels. A repeat of the 4.2 test diet yielded an average larval weight of 121.9 ± 5.7 mg. Adults were produced from the 3.0 casein diet (n = 15, 8 females, mean = 82.5 ± 9.6 mg, 7 males mean = 59.2 ± 11.2 mg), and both 4.2 casein diets (n = 22, females mean = 92.1 ± 6.4 mg, males mean = 68.6 ± 8.6 mg) and (n = 23, females = 92.1 ± 6.6 mg, males = 70.6 ± 7.5 mg).

Soy protein was tested as an alternative to casein in that, being plant-derived, it might yield better growth than casein. Diets were prepared at soy protein levels of 4.0, 4.25, 4.75, and 5.0 g per 100 g diet. Most larvae reached the second stadium but then grew very slowly and failed to reach the third stadium in all cases.

Egg albumin in powdered form also was tested as a protein source in the diet (Table 1). Quantities of 1.4, 2.8, and 4.2 g per 100 g diet yielded average larval weights of 45.1 ± 8.6 mg (n = 33), 81.2 ± 7.7 mg (n = 25) and approximately 25 ± 9.2 mg, respectively (not shown), although nearly all of the larvae on the 4.2 g diet died during the third stadium.

**Table 1. The influence of protein type and concentration on *Leptinotarsa decemlineata* fourth stadium weight gain.**

| Protein type, g | X mg, 4th stadium, ± SEM, (N) | Days to 4th stadium | Number prepupae | Number adults |
|---|---|---|---|---|
| Casein, 4.2 | 126.3 ± 3.7a*, 41 | 11 | 35 | 23 |
| Casein, 3.0 | 97.3 ± 4.5 b, 30 | 14 | 20 | 15 |
| Casein, 5.0 | 75.4 ± 4.1 c, 19 | 15 | 0 | 0 |
| Casein, 2.8 | 67.4 ± 4.5 c, 34 | 12 | 0 | 0 |
| Albumin, 2.8 | 82.6 ± 4.9 c, 28 | 15 | 0 | 0 |
| Albumin, 1.4 | 45.1 ± 4.1 d, 28 | 15 | 0 | 0 |
| Casein, 1.4 | 32.6 ± 4.2 e, 12 | 12 | 0 | 0 |

*Means with the same letter are not different at $P = .05$, SAS Institute, 1992.

An all-amino acid diet was formulated with approximately the same quantities of amino acids as 4.0 g of casein, plus the usual amount of free amino acids found in potato foliage. After 7 d, none of the 36 larvae had molted, although all the larval abdomens appeared to be engorged and typically colored. Normally, molting to the second stadium occurs after 24 to 36 h of feeding. A diet containing 2.8 g casein and only the essential amino acids and stimulatory amino acids yielded an average of 70.4 ± 4.7 mg (n = 24 - fourth instar) after 12 days of feeding. The addition of 45 mg L-alanine, 100 mg glycine, 100 mg aspartic acid and 24 mg arginine drastically slowed the growth of larvae (mean = 48.4 ± 6.3 mg, n = 45), compared to a diet without these additional amino acids (mean = 118.5 ± 5.7 mg, n = 31). However, a diet without any of the normal foliar complement of amino acids produced small larvae that grew at a reduced rate (mean = 39.4 ± 5.7 mg, n = 35), compared with larvae reared on a normal complement of foliar amino acids (mean = 85.3 ± 4.5 mg, n = 38). Two diets prepared with 4.5% casein and differing in amino acid content yielded similar weight gains in 12-d-old fourth instars (Table 2), despite the fact that one diet contained the normal foliar complement and the second contained only stimulatory amino acids. Two diets were prepared, both with a constant 4.2% casein and 3.2 g oat flour. One diet contained only stimulatory amino acids, while the second contained only essential amino acids. Larvae and adults of similar weight were produced from both diets. (Table 3).

**Protein and carbohydrate tests.** Testing also was done with combinations of protein (casein) and carbohydrate (oat flour) because of likely interactions between levels of these two major nutrients. Casein at 4, 5, and 6 g per 100 g

**Table 2. Effect of changes in some amino acid levels of otherwise complete diets on weight of equal age larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*.**

| Amino acids, | mg | Protein | Larval wt | # insects | age, days | # per instar |
|---|---|---|---|---|---|---|
| L-alanine | 70 | Casein | 70.5 a* | 24 | 12 | 21-4th |
| valine | 14 | 4.5% | | | | |
| gaba | 60 | | | | | |
| serine | 28 | | | | | |
| All found (see table 4) | | Casein | 67.6 a | 28 | 12 | 26-4th |
| in leaf | | 4.5% | | | | 2-3rd |

*Means with the same letter are not different at $P$ = .05, ProcGLM ls means, (SAS Institute, Cary, NC.) 1992.

diet was combined with 4, 5, and 6 g oat flour per 100 g diet to determine the most growth-effective pairing (Table 4). The set of 4 g casein and 6 g oat flour produced larvae with a mean weight of 137 ± 6.0 mg, (n = 35), p = 0.0001, which was greater than all other pairs tested. The set of 6 g casein and 6 g oat flour yielded the lowest mean larval weight (mean = 60.5 ± 6.1 mg, n = 35), with the remaining sets yielding mean weights between these two extremes. The columns in Fig. 1 show the *daily* number of larvae in each stadium throughout the time they were reared on the diet shown in Table 5, which is the 4 g casein and 6 g oat flour diet. These data suggest that while most larvae develop uniformly and pass through the stadia normally, a number of larvae lag behind and fail to grow at a normal rate. This may reflect diet unsuitability or microbial contamination of diet with resulting deleterious effects on some larvae.

A combination of 700 mg sucrose and 2.7 g glucose was substituted for complex carbohydrates (oat flour) to determine if growth was equivalent on both energy sources. At a constant 4.2 g casein, two diets were formulated to contain 3.4 g sugars plus 3.5 g oat flour and no sugars and 6.0 g oat flour. Fourteen-day-old larvae fed the high sugars diet averaged 97.4 ± 7.3 mg (n = 37) in weight, and the same-age larvae fed the oat flour diet averaged 116.6 ± 6.4 mg (n = 39).

In another set of carbohydrate tests with a constant concentration of 4.0 g casein, dried and powdered lima bean, rice flour, potato flakes and oat flour were each compared for their ability to influence larval growth. Thirteen days after eclosion, larvae reared on rice flour grew the least, with a mean weight of 61.2 ± 4.9 mg (n = 19). Oat flour and lima bean powder gave higher mean weights of 121 ± 6.3 mg (n = 22) and 139 ± 6.0 mg (n = 23), respectively. The highest mean weight of 159 ± 7.2 mg (n = 19) was recorded on the potato flake diet. Amylopectin (from corn), purified potato starch, dextrin, whole wheat flour, polygalacturonic acid and pectin also were tested as carbohydrate sources, but none gave results as promising as did potato flakes or oat flour

**Table 3. Weight in mg of 12 day old 4th instar larvae and 2-3 day old adults of the Colorado potato beetle after feeding on four meridic diets using listed combinations of carbohydrates and amino acids per 100 g diet.**

| Diet | CHOs | Amino Acids | | Larval wt, ± SE | Adult wt, ± SE |
|---|---|---|---|---|---|
| 1 | Oat Flour | Alanine | 70 mg | 123.3 ± 4.9 a* | 106.1 ± 5.8 a* |
| | 3.2 g | Arginine | 30 | n = 35 | (18 females) |
| | | GABA | 40 | | 82.1 ± 8.2 b |
| | | Lysine | 23 | | (9.males) |
| | | Cystine | 20 | | |
| | | Threonine | 23 | | |
| 2 | Oat Flour | Above plus: | | | |
| | 3.2 g | Valine | 3 | 116.5 ± 4.6 a | 106.1 ± 8.7 a |
| | | Serine | 25 | n = 35 | (9 females) |
| | | Ornithine | 2 | | 87.4 ± 8.7 ab |
| | | Methionine | 2 | | (9 males) |
| | | Aspartic | 17 | | |
| | | Tyrosine | 3 | | |
| | | Tryptophan | 10 | | |
| | | Asparagine | 57 | | |
| | | Histidine | 4 | | |
| | | Glycine | 4 | | |
| | | Isoleucine | 3 | | |
| | | Glutamine | 47 | | |
| | | Leucine | 2 | | |
| | | Proline | 5 | | |
| | | Glutamic | 52 | | |
| | | Phenylalanine | 5 | | |
| | | As in diet 2 | | | 85.3 ± 4.5 b |
| 3 | Soluble | | | | |
| | Starch 1.5 g | | | | |
| | Pectin 0.5 g | | | | |
| | Polygalacturonic | | | | |
| | Acid 0.8 g | | | | |
| 4 | As in diet 3 | Alanine | 70 | | 39.4 ± 4.6 c |
| | | GABA | 40 | | |

*Means with the same letter are not different at $P = .05$, SAS Institute, 1992.

**Table 4. Diets prepared with combinations of 4,5 and 6 g casein and 4,5 and 6 g powdered instant oatmeal and the resulting weights of fourth stadium larvae of the Colorado potato beetle 12 d after eggs hatched.**

| Casein, g | Oat Flour, g | Mean weight, mg + s.e.m. |
|---|---|---|
| 4 | 4 | 103.7 + 6.0 a* |
| 4 | 5 | 103.7 + 6.3 a |
| 4 | 6 | 137.0 + 6.1 b |
| 5 | 4 | 60.5 + 6.7 c |
| 5 | 5 | 75.5 + 6.2 cd |
| 5 | 6 | 73.1 + 6.3 cd |
| 6 | 4 | 61.3 + 6.0 d |
| 6 | 5 | 93.7 + 5.9 a |
| 6 | 6 | 84.6 + 6.1 acd |

*Means with same letter are not different at $P = .05$. SAS Institute 1992.

(Table 3). Adults did not emerge from the potato flake diet, evidently because of microbial infestation of pupae, as evidenced by blackened, decaying pupae found in the soil-filled pupation containers.

Larvae reared on healthy, greenhouse-grown potato plants contained about 0.15 mg ascorbic acid per gram of body weight. Potato foliage contains approximately 100 mg ascorbic acid per 100 g fresh weight. Diet prepared with pure ascorbic acid produced larvae with a body content of 0.11 mg/g body weight, while larvae reared on a diet with ascorbic polyphosphate (a more stable form of Vitamin C) contained 0.36 mg/g. Fourteen days after preparation and held under refrigeration, ascorbic acid diet contained 42 of the original 100 mg ascorbic added. Ascorbic polyphosphate contained 16 of the original 50 mg of ascorbic acid after 14 d under refrigeration. An attempt was made to rear larvae on a diet devoid of ascorbic acid to determine if diet ingredients or possibly microflora were unaccounted sources of Vitamin C. However, no growth was observed on this diet and larvae died in the first stadium compared with larvae that grew satisfactorily on 36, 108, and 324 mg ascorbic/100 g diet.

Potato leaf was analyzed for beta-carotene (precursor of trans-retinol, vitamin A) content and found to contain about 5 mg per 100 g fresh foliage wt, which is equivalent to about 10 mg Vitamin A/100 g foliage. This was easily supplied in test diets by either powdered forms of Vitamin A or extract from carrot dissolved in vegetable oil.

The possible set of combinations of diet ingredients and amounts is very large. One way to simplify the situation is to use a SAS software titled PROC

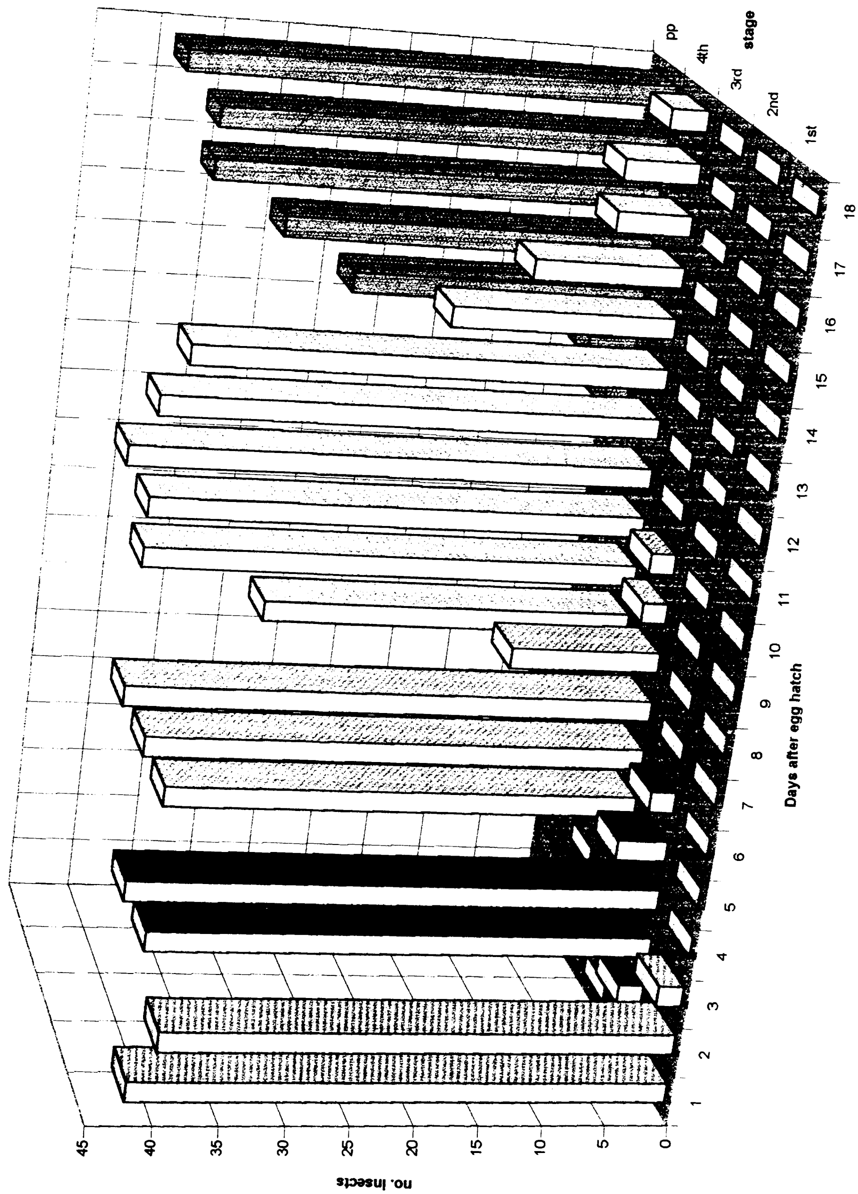


Fig. 1. Daily development of a group of 42 individual Colorado potato beetles from neonate to prepupa on complete meridic diet.

**Table 5. Composition of 100g diet used to rear the Colorado potato beetle *Leptinotarsa decemlineata* from egg to adult stage, using 4 g casein and 6 g oat flour.**

| | | | |
|---|---|---|---|
| Agar | 2.0 g | Phenylaline | 30 mg |
| Cellulose powder | 2.4 g | Asparagine | 9 mg |
| Casein | 4.0 g | Histidine | 36 mg |
| KOH | 0.25 g | GABA | 60 mg |
| Linoleic acid | 30 mg | Glycine | 48 mg |
| Linolenic | 30 mg | Isoleucine | 10 mg |
| Cholesterol | 60 mg | Leucine | 8 mg |
| Sitosterol | 20 mg | Proline | 11 mg |
| Corn oil | 750 mg | Glutamic acid | 130 mg |
| Olive oil | 250 mg | $KH_2PO_4$ | 900 mg |
| 60% Egg lecithin | 200 mg | $MgSO_4$ | 400 mg |
| Vitamin A | 3250 IU | $CaOH_2$ | 300 mg |
| Vitamin E | 130 IU | NaCl | 19 mg |
| Sucrose | 390 mg | $FeSO_4.7H_20$ | 8 mg |
| Fructose | 300 mg | $ZnCl_2$ | 2 mg |
| Glucose | 150 mg | $MnSO_4.H_20$ | 4 mg |
| Oat Flour | 6.0 g | $CuCl_2$ | 1 mg |
| Inositol | 25 mg | Neonycin Sulfate | 5 mg |
| Choline Chloride | 60 mg | Gentamycin Sulfate | 5 mg |
| L-alanine | 60 mg | Sorbic acid | 60 mg |
| Aminobutyric acid | 15 mg | Ascorbic acid | 40 mg |
| Arginine | 10 mg | Thiamine Hcl | 0.83 mg |
| Valine | 14 mg | Riboflavin | 0.53 mg |
| Serine | 28 mg | Calcium Pantothenate | 0.67 mg |
| Ornithine | 5 mg | Pyridoxine HCl | 0.2 mg |
| Lysine | 12 mg | Biotin | 0.02 mg |
| OH-Proline | 12 mg | Folic acid | 0.13 mg |
| Methionine | 2 mg | Cyanocobalamin | 0.02 mg |
| Threonine | 12 mg | Niacinamide | 1.25 |
| Aspartic acid | 40 mg | Water to 100 g | 83 ml |
| Tyrosine | 42 mg | | |

OPTEX, which allows the testing of combinations of diet ingredients in multidimensions (a response surface design). Unfortunately, there are two problems with this approach. First, the diet needs to be well-defined before the design is of any use, or insect responses will be masked by unrealistic levels of test ingredients. Secondly, the insect is more responsive that the statistical software, and it is, therefore, not always a reliable indicator of the direction one should take. Once a diet is well-defined, the software will likely have a meaningful place in more precisely defining the types and quantities of ingredients needed for optimal insect growth.

Table 5 presents the ingredients contained in the most successful diet formulated to this point which utilizes 4 g casein and 6 g potato flakes or oat flour (mean 4th instar weight = 137.1 ± 6.0 mg, $p = .0001$). There is undoubtedly room for improvement in this diet, although when made with proper care against microbial contamination, it will produce many large fourth-instar larvae and numerous adult Colorado potato beetles. The adults produced showed normal mating behavior, and males were clearly attracted to females at close range. However, despite matings, females and males kept in a Petri dish with diet freely available did not produce any eggs. When transferred to potato foliage for 24 h, viable egg masses were produced, suggesting a dietary insufficiency, a behavioral response requiring the host plant, or some combination of both factors.

Casein appeared to be the only suitable protein source among those tested for acceptable growth in the Colorado potato beetle. There is an evidently optimal concentration of casein, between 3.8 and 4.2 g per 100 g diet. Wardojo (1969a) used a combination of albumin and casein for his diet and claimed good growth, although it could not be duplicated here. Other insects have been reared successfully on soy protein diets (Cooper and Schal 1992), although it appears to be unsuitable for Colorado potato beetle rearing. Herbivorous insects are likely to be sensitive to nitrogen deficiencies because plants generally contain less N than animal tissue (Mattson 1980). However, the balance between nutrients contained in a diet is also important and, if not maintained within limits, deleterious effects on growth or reproduction may result (House 1974). This is evident in the poor growth of larvae placed on low or high casein diets, and the generally favorable growth shown by larvae fed a more optimal concentration of casein. Albumin did not produce normal growth at any of the tested concentrations. The amino acid composition may not be as suitable as that of casein, or of leaf protein, or perhaps the optimal concentration was not used. Wardojo (1969a) reported favorable growth of Colorado potato beetle larvae with a combination of 4 g casein and 3 g albumin per 100 g diet which is almost a twofold increase in the optimal protein concentration used here.

It is likely that this insect can accept artificial diets that vary somewhat in amino acid composition, because levels of stimulatory amino acids in potato foliage fluctuate seasonally in nature (Hare 1983). Indeed, the limited data presented here suggest that the full complement of leaf amino acids is not necessary for feeding or growth, if the stimulatory amino acids are present. A number of additional compounds present in foliage are thought to have roles as feeding stimulants (Hsiao and Fraenkel 1968b,c). It may be the completeness of the overall sensory pattern perceived by the insect that helps to determine the degree of feeding, as suggested by Ritter (1967). However, larvae are sensitive to the amino acid content of potato foliage, as evidenced by the variable growth responses on diets with different amino acid compositions. We are uncertain of the optimal set of amino acids to use, but the stimulatory, metabolically-important and protein-component types should be included. It will take a number of trials to determine which are essential and which are complementary to good growth. Manipulation of foliar amino acid levels through genetic recombination techniques may be a fruitful method of decreasing insect feeding damage.

Laboratory-reared Colorado potato beetle larvae grow best on a diet containing a complex carbohydrate source, such as powdered oat flakes or potato flakes, that is quantitatively balanced with the protein content. Approximately 4 g protein and 6 to 8 g carbohydrate/100 g diet appeared to give the best results. This ratio is supported by the relatively low free-sugar content of potato foliage, which is between 3 and 5% of the dry weight of foliage (Domek et al. 1995). Cellulose powder is a beneficial carrier of sterols and lipids in the synthetic diet and makes up a large proportion of the non-starch polysaccharides in potato leaf (Domek et al. 1995). The quantity of sucrose found in potato foliage is about 10 times less than that used in artificial diets published for the Colorado potato beetle (Hsiao and Fraenkel 1968a, Wardojo 1969a). Sucrose was shown to be a feeding stimulant for this insect at .025-0.1 M in an agar medium (Hsiao and Fraenkel 1968c) and at 0.01 M alone or combined with amino acids on agar-cellulose discs (Mitchell 1974). Glucose and fructose had little stimulatory effect (Hsiao and Fraenkel 1968c). Although we have not tried to define an optimal level of sucrose, glucose, or fructose in the diet, it may be more appropriate to use a realistic level of a substance with known stimulatory effect, such as sucrose, and to satisfy a carbohydrate requirement with a polysaccharide. We performed some subjective tests with pectin, polygalacturonic acid, amylopectin, soluble starch, and potato flakes for their effect on the physical consistency of the diet. Larvae placed on these diets were observed for their ability to walk on the surface of a diet cube. Pectin and polygalacturonic acid produced a sticky surface that hindered movement of larvae and would probably interfere with ingestion. The remaining substances appeared acceptable in this regard.

For Vitamin C, a range of concentrations gave similar results. Therefore, from 80 to 120 mg/100 g diet is suitable. It is fairly stable in diet preparations kept under refrigeration. Ascorbic polyphosphate incorporated a very large amount of phosphate to carry a small amount of ascorbic acid, and had a deleterious effect on larval growth.

Table 5 lists the ingredients contained in a meridic diet used to rear the Colorado potato beetle from the egg to the adult stage. The quantities and amounts of lipid components used were based on well-known information, such as the need for insects to have a dietary source of sterols. Plant oils supply linolenic acids, which are essential for many insects (Friend and Dadd 1982). Phosphatidylcholine (lecithin) is an important phospholipid synthesized from choline, which is essential to most animals. Vitamin A is important in animal growth and reproduction, and is a component of the visual pigment rhodopsin.

Vitamin E is an antioxidant for lipids and may have a role in cellular respiration (Dadd 1977). The sucrose, fructose, glucose, and starch content of the diet was based on the levels found in lyopilized leaves (Domek et al. 1995). Powdered oat flour or potato flakes were added as complex carbohydrates in place of the usually gummy non-starch polysaccharides. Inositol is a component of phosphatidylinositol, a membrane lipid which plays a role in calcium mobilization and the activation of various enzymes (Zubay 1988). The amino acids used in the diet are representative of those we found in analysis of lyophilized foliage. Certain sugars and amino acids have been shown to stimulate larval biting and feeding by stimulating specific sensilla on the galea and palpi (Mitchell 1974). The trace metal quantities used are also based on foliar analysis (Domek et al. 1995). Potassium salts have been shown to have phagostimulative value, especially when mixed with sugars (Hsiao and Fraenkel 1968b). The vitamin mixture used was derived from Hsiao (1968a) and Dadd (1977).

Mammalian salt mixtures are frequently high in sodium, calcium, and chloride. Although many insects tolerate these mixtures, they do not reflect the composition of plant tissues, which is often high in potassium, phosphate, and magnesium (Reinecke 1985). There is evidence that both phytophagous and non-phytophagous insects show better growth when reared on diets containing mineral balances similar to their hosts (House and Barlow 1965). Reinecke (1985) noted that the salt mixtures used in insect diets rarely exceed 1% of the total diet weight but that there was no apparent reason for this limit other than presumed empirical evidence. The optimal mineral salt mixture for the Colorado potato beetle remains to be defined, although the mixture presented here may be more appropriate than commercially available salt mixtures.

The plant lipids used in the diet are not typical of the quantity or quality of lipids found in potato foliage. The major diet lipids used are seed oils which contain primarily triacylglycerols. Mono- and di-galactosyldiacylglycerols (galactosyl glycerides) constitute nearly half of the total leaf lipid content of potato foliage, with triacylglycerols present at less than 1% of the total leaf lipids (Moreau et al. 1990). Galactosyl glycerides are sources of linoleic and linolenic acids for phytophagous insects (Harwood 1980). These essential trienoic fatty acids can be cleaved from triacylglycerols, but studies with lepidopterous insects have suggested that insects that feed on leaf tissue are better adapted to use polar lipids (Turunen and Chippendale 1989).

Although the meridic diet presented in this paper can probably be improved with adjustments in the quantities and qualities of at least some of the above-mentioned components, it is noteworthy that many large, fourth-instar larvae and several groups of adult Colorado potato beetles were reared from the egg stage with the present diet. Further work remains to be completed that will more precisely define the types and quantities of ingredients most efficacious to Colorado potato beetle growth. An important immediate goal would be to reduce pupal mortality, which would mean greater adult production. Although antimicrobials were incorporated in all test diets, a persistent contamination problem was present and very difficult to control. Another goal is to have adult beetles feed and lay a normal complement of viable eggs on the diet or on an acceptable substrate. However, at present, the diet presented herein is effective for testing a wide range of substances of potential value in hindering the growth, development, and reproduction of the Colorado potato beetle. The overall goal is to develop a meridic diet that will maintain a year-round culture of Colorado potato beetles with artificial diet, to provide a reliable supply of healthy eggs, larvae or adults to researchers and insect-rearing specialists working in allellochemical development, genetic engineering, or biological control areas.

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