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SOME FACTORS RELATING TO THE  
LARVAL GROWTH OF THE COLORADO  
POTATO BEETLE, *LEPTINOTARSA*  
*DECEMLINEATA* SAY (COLEOPTERA:  
CHRYSOMELIDAE), ON ARTIFICIAL DIETS

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## 1. INTRODUCTION

Almost half of the known insect species feed on living plants or plant parts, and are classified as phytophagous insects. The diversity in their feeding habits has long been recognized, hence their classification into monophagous, oligophagous and polyphagous insects. An insect that feeds on only one plant species, however, is exceptional. In the case of oligophagy, only some plant species belonging to one or a few related families are eaten, while polyphagy denotes the feeding of many plant species belonging to very diverse taxonomic groups. Polyphagous insects may feed and grow for generations on numerous plant species, but they always show some preference for one or a few plant species. Under certain abnormal conditions, on the other hand, oligophagous insects may feed on plant species, which are normally rejected. Thus the difference between the last two categories is merely a matter of degree, although the above mentioned arbitrary division is universally used.

The association between insects and their host plants is thought to have been established by a long period of natural selection during which the insects had to adjust their physiological constitution to the physical and chemical characters of the plants that provided them with food and shelter. Since the host plants are by no means always aggregated in certain areas, the insects must provide some way to find them. Generally, host plant selection is attributed to their visual, tactile and chemical senses. Chemical senses seem by far the most important in this respect, especially those involved in olfaction and gustation.

Host plant selection, in the sense of selection of plant species to be attacked, is generally an adult function. It is the ovipositing female that performs the selection. The larva, after hatching, is bound either to accept the plant or to reject it. The physical and chemical characters of the plant determine the degree of feeding, while its nutritive adequacy determines the pattern of growth, development and reproduction of the insect.

With regard to the role of plant chemicals in insect feeding behaviour, a distinction is usually made between secondary plant substances or 'odd substances' and nutrients. The chemosensory basis of host plant selection has recently been reviewed by SCHOONHOVEN (1968).

Since the work of VERSCHAFFELT (1910), who reported that the feeding of the larvae of the cabbage butterfly, *Pieris rapae* (L.) was stimulated by mustard oil glucosides contained in their food plants, insect-plant relationships are thought to be characterized by the crucial role of secondary plant substances during the food plant selection (FRAENKEL, 1959). On the other hand there are examples suggesting the important role of sapid nutrients. It seems that in most cases, as suggested by KENNEDY (1958, 1965) in his 'dual discrimination theory', both types of plant substances may play a role.

Feeding habits seem, to a certain extent, to be physiologically modifiable. Larvae of the oligophagous tobacco hornworm, *Manduca sexta* (Johann.), reared on tomato leaves till the second instar, will feed on an artificial diet and

pupate (SCHOONHOVEN, 1967). When fifth instar larvae were transferred from the diet to some normally rejected plants, feeding occurred.

Apart from the problems related to the chemosensory relationships between the insect and its host plant, nutritional requirements of this vast horde of phytophagous insects are still far from being understood. Studies of the potential host plants and of the correlation between insect growth and certain substances contained in the host plants do not provide the information necessary for a fundamental knowledge of insect nutrition. Only when the composition of the food is thoroughly known and can be regulated, a better insight can be achieved. A diet composed of known chemicals which induces feeding and support growth of the insect will be suitable for this purpose.

For several decades, efforts have been made to develop artificial diets for phytophagous insects with the aim of studying the nutritional requirements in terms of substances of known chemical composition. The prevailing idea that secondary plant substances are of great importance in initiating and sustaining feeding by phytophagous insects, leads to the inclusion of crude materials from the host plant in the diet. Replacing crude plant material by plant extracts or pure chemicals is the following step in the development of an artificial diet to reach a defined diet for nutritional studies. This empirical method in developing diets for insects is derived from the principles outlined by BECK (1956).

■ In the meantime there is an increasing number of diets containing crude plant materials, which are developed for practical purposes, viz. for a continuous rearing of phytophagous insects.

In many cases it is possible to rear phytophagous insects on diets without any plant extract. As a protein source in the diet usually an animal protein is used, i.e. casein. In some cases proteins derived from soybean are included in the diet. For the study of amino acid requirements it is necessary to replace the protein by pure amino acids. Up till now in only a few cases has such a replacement been successfully achieved. The amino acid diets developed for the pink boll worm, *Pectinophora gossypiella* Saunders (VANDERZANT, 1957, 1958) and the boll weevil, *Anthonomus grandis* Boheman (VANDERZANT, 1965), made it possible to study the importance of different constituents for growth and reproduction according to the procedure generally applied with vertebrates, viz. by omitting the nutrients concerned from the diet.

It has been pointed out by BECK (1956) that the problems related to the development of artificial diets for phytophagous insects comprise two main aspects, i.e. the physical and chemical factors essential for the release of normal feeding behaviour, and the nutritive factors essential for an adequate diet. Most of the reviews on the nutritional requirements of phytophagous insects (FRIEND, 1958; LEGAY, 1958; AUCLAIR, 1963) and of insects in general (TRAGER, 1953; LEVINSON, 1955; LIPKE and FRAENKEL, 1956; HOUSE, 1961, 1962) are more or less an accumulation of data on the importance of nutritive substances for growth. The information gained is unfortunately not always applicable to the development of artificial diets for many other phytophagous insects. Recently

a general concept for the development of artificial diets for phytophagous insects was presented by VANDERZANT (1966).

The Colorado potato beetle is highly selective in its choice of food. Its food plants are limited to some species belonging to the family *Solanaceae*, and as an agricultural pest it feeds mainly on *Solanum tuberosum* L. Other solanaceous species appear to have an adverse effect on larval growth (CHIN, 1950) as well as on egg production (DE WILDE, SLOOFF and BONGERS, 1960). However, under laboratory conditions this beetle has been reared on *Asclepias syriaca* (Asclepiadaceae) for 4 successive generations, and on *Lactuca sativa* var. *romana* (Compositae) for 8 generations (HSIAO and FRAENKEL, 1968d); both are non-solanaceous species.

Despite the high selectivity in choice of food, the larvae show a moderate rate of growth throughout the fourth larval instar when reared on an artificial diet without extraneous leaf material (HSIAO, 1966, cited in HSIAO and FRAENKEL, 1968c). In an earlier paper (Wardojo, 1967) it was reported that third instar larvae can complete their development on an artificial diet without plant material, although the larval growth as well as the weights of the emerging adults were suboptimal.

This paper deals with the development of an artificial diet for the larval growth of the Colorado potato beetle. The results are presented as a preliminary study of the nutritional requirements of this insect.

## 2. REARING OF STOCK MATERIAL

Adult Colorado potato beetles for continuous rearing on potato plants were obtained from the Entomological Laboratory of the Agricultural University of Wageningen. The rearing of stock material took place under the same environmental conditions prevailing during the experiments with artificial diets. Both were carried out in a phytotron at a temperature of  $25 \pm 0.5^{\circ}\text{C}$ . and a relative humidity around 50–70% under illumination of four Ekco fluorescent tubes of 80 Watt each for 18 hours a day. These 'long day conditions' were applied to prevent diapause (DE WILDE, 1957), in order to secure a continuous supply of test insects.

Twenty pairs of newly emerged Colorado potato beetles were divided equally in 4 rearing cages measuring  $20 \times 15 \times 30$  cm for oviposition. Fresh potato sprouts were supplied daily in a glass vial containing fresh water. Under these conditions they started to lay eggs after 5 to 6 days.

Every day the egg masses were carefully removed from the leaves with a spatula and spread over a sheet of filter paper. This was to prevent moulding and also to assure that the newly hatched larvae needed for the experiments had no previous contact with potato leaves. The egg masses were put in plastic boxes measuring  $18.5 \times 10 \times 7$  cm for incubation. At  $25^{\circ}\text{C}$  these eggs would hatch within 4 to 5 days. Although the eggs were more or less of the same age they never hatched simultaneously. Early hatched larvae and eggs containing hatching larvae were removed to prevent feeding of the larvae on the eggs, a phenomenon which was also reported by SWINGLE et al. (1941), CHIN (1950) and DE WILDE (1957). Cannibalistic larvae might have a different physiological condition and were not used for the experiments.

Larvae after hatching were reared in the same plastic boxes; after two days they were divided into several boxes of the same size, each of which contained no more than 20 larvae. Fresh potato leaves were supplied daily in these boxes. A number of these larvae were reared into adults. After passing the fourth instar, the larvae became full grown. Their colour changed to orange-yellow and they showed a strong positive geotactic response. About fifty of these larvae were then transferred to a zinc container measuring  $22.5 \times 22.5 \times 11$  cm containing moist sand. These larvae burrowed into sand and reached the prepupal stage. Adult Colorado potato beetles emerged 11 to 12 days later.

For oviposition the adults were kept for only two weeks in the rearing cages and were then replaced by a new batch, although oviposition took place daily for more than one month. This was done to obtain offspring from adults of comparable physiological condition.

The potato plants used for maintaining the culture were obtained the whole year round from the glass house of the Entomological Laboratory. Usually the potato variety 'Eigenheimer' was used, but during winter it was replaced by the variety 'Mult'a' or 'Voran', as the latter grew better during this season.

### 3. ARTIFICIAL DIET FOR PHYTOPHAGOUS INSECTS

#### 3.1. GENERAL REVIEW

The importance of artificial diets of known composition for laboratory mass rearing of insects has long been recognized. A continuous rearing of insects independent of the supply of plant material, which is determined by the season, is certainly the first immediate advantage of an artificial diet. Not less important is the availability of insects of known and reproducible nutritional condition for fundamental studies of physiological processes such as metabolism and nutrition itself.

While laboratory cultures of stored product infesting insects and many species of the order *Diptera* have been produced for many years on artificial media, rearing of phytophagous insects on an artificial diet has started only recently.

It was BOTTGER, who in 1942 for the first time introduced an artificial diet as a means for the nutritional study of the phytophagous European corn borer larvae, *Ostrinia nubilalis* (Hübner). The diet of BOTTGER consisted of casein, sugars, fat, vitamins, salts, cellulose, agar and water. Its chemical composition was partly based on the chemical composition of the host plant.

Already in this early stage of study it was concluded that phytophagous insects are able to accept and to utilize food that is totally foreign to their natural diet. In 1949, BECK et al. reported a successful rearing of the European corn borer on the same type of diet, to which was added crude material from the host plant. It was thought that this plant material contained unknown factors essential for larval growth, besides their contribution to inducing feeding.

Diets containing substances derived from the host plant were subsequently devised for the Asiatic rice borer, *Chilo suppressalis* (Walker) by ISHII in 1952, and for the Oriental fruit moth, *Grapholitha molesta* (Busck) by MATSUMOTO in 1954 (VANDERZANT, 1966). In 1956, VANDERZANT and REISER reported a successful rearing of the pink boll worm, *P. gossypiella*, on a diet without any substance from the host plant. From 1960 on, the number of phytophagous insects reared successfully on artificial diets is increasing steadily. From the compilation work of HOUSE (1967) and the Annotated Bibliography of GOODWIN (1967) it appears that work on the development of artificial diets for phytophagous insects has concentrated on the order of *Lepidoptera*. Up to the present time at least 50 phytophagous species of this order have been reared with partial or complete success on artificial diets ranging from those containing crude materials to highly defined diets. Of each of the following orders, i.e., *Hemiptera*, *Orthoptera* and *Coleoptera*, only a comparatively small number of phytophagous species has been included in the study.

In formulating diets for phytophagous insects the following factors should be taken into consideration. Firstly, the physical and chemical factors essential for the release and maintenance of normal feeding behaviour and secondly, the nutritive factors essential for an adequate diet (BECK, 1956). Most of the diets

that proved to be successful for the rearing of phytophagous insects for many generations contain crude materials from the host plant or from another plant species. Since a successful diet has to satisfy feeding requirements as well as nutritional requirements, the inclusion of crude plant material in the diet seems to be necessary for various reasons (BECK et al., 1968). Firstly, it might supply the feeding insects with a non-nutritive feeding stimulant. Thus, DAVID and GARDINER (1965) included cabbage leaf powder in the diet for *Pieris brassicae* L. The most important component contained in the leaf powder was *sinigrin* that promoted the food intake by the larvae (DAVID and GARDINER, 1966a). Secondly, crude plant materials might contain a trace nutrient substance, the so-called unidentified growth factor. For the successful rearing of the European corn borer, BECK et al. (1949) had to include corn leaf powder. The unknown growth factor proved to be ascorbic acid (CHIPPENDALE and BECK, 1964). Thirdly, the addition of crude plant material to the diet might be necessary to provide optimum concentration ratios among certain substances necessary for the release of normal feeding behaviour, or among certain nutrients necessary for optimal growth.

Diets containing crude plant material serve as well for practical purposes. Replacement of plant material of unknown chemical composition is the following step in devising diets for studies of the nutritional requirements of insects. This empirical method has been widely applied by investigators.

There are several diets that do not contain crude plant materials or plant extracts, but can support growth of several phytophagous insects for several or many generations (VANDERZANT and REISER, 1956; ROCK et al., 1964; BECK et al., 1968). Those diets contain macro molecular substances like casein. Many other diets of this type contain large molecule substances with different degrees of purity. Only a few species of phytophagous insects can be reared on artificial diets that are composed of small molecule substances of known purity and known chemical structure (FRIEND and PATTON, 1956; VANDERZANT, 1957, 1965; AUCLAIR and CARTIER, 1963; ROCK and KING, 1967; DADD and KRIEGER, 1967).

The last mentioned works show, at least for the species concerned, that the feeding requirements as well as the nutritional requirements can in principle be satisfied solely by the nutrients.

### 3.2. ARTIFICIAL DIET AS A MEANS FOR THE STUDY OF NUTRITIONAL REQUIREMENTS - DEFINITIONS

The question concerning the degree of purity and the chemical structure of the constituents that make up the diet seems to be the crucial point in the confusion that has arisen around the terminology used to describe diets for insects. Thus, diets containing large molecule substances with different degrees of purity are termed artificial, synthetic (BOTTGER, 1942), purified (BECK et al., 1949) or chemically defined (BECKMAN et al., 1953).

An account of the description of different types of diets and rearing methods

has been given by DOUGHERTY (1959). He suggested the term *holidic* for diets that are composed of pure chemicals with known chemical structure before compounding, and *meridic* for diets composed of a holidic base to which is added at least one substance or compound of unknown purity or unknown chemical structure. Since the presence of symbiotes and other micro-organisms in the insect body can mask the importance of certain nutritive substances for growth, he distinguished an *axenic culture*, i.e. a culture of one known organism, and a *xenic culture*, i.e. when the number of organisms present is unknown or when their identification is uncertain.

The terms proposed by DOUGHERTY have not been widely accepted. VANDERZANT (1966) disagreed with the idea of DOUGHERTY who considered diets containing agar to be meridic. Agar is of doubtful importance as a nutrient, and can be obtained in a sufficiently pure condition to permit the study of the requirements of amino acids, lipids and many other nutrients. Otherwise, by the same reasoning all diets, whether or not containing agar, should be classified as meridic, because they may contain trace contaminants, especially minerals.

VANDERZANT (1966) herself suggested the term *defined diet*, which in the broadest sense may be considered as one that contains chemically pure nutrient substances and one or more specially purified natural products. A consequence of this definition is the requirement that micro-organisms have to be excluded from the culture.

## 4. ARTIFICIAL DIET FOR THE COLORADO POTATO BEETLE

### 4.1. INTRODUCTION

The term artificial diet refers to any diet that is not the natural food of the insect. This term is used throughout this paper.

The oligophagous Colorado potato beetle is highly selective in its food choice. Its host plants are limited to some species of the family *Solanaceae*. In its natural habitat, it occurs on *Solanum (Androcera) rostratum* (Dunal), but as an agricultural pest it feeds mainly on the leaves of *Solanum tuberosum* L.

For several decades it has been assumed that the potato plant contains secondary plant substances that act as token feeding stimulants (RAUCOURT and TROUVELOT, 1933, 1936; CHAUVIN, 1945, 1952; HESSE and Meier, 1950; HSIAO and FRAENKEL, 1968b, c) which are of great importance in food plant selection by the Colorado potato beetle. RITTER (1967), however, is of the opinion that if there is any secondary plant substance acting as a feeding stimulant, it is of minor importance. Various combinations of different chemicals, nutritive and non-nutritive, if they present themselves in just the right concentrations, will release a feeding response in the larva of the Colorado potato beetle.

In an earlier paper (WARDOJO, 1967), it was reported that the larva as well as the adult of the Colorado potato beetle readily accepted a diet that did not contain any substance derived from the host plant. The above mentioned diet was a modification of the diet for locusts developed by DADD (1960). The larval growth on this diet was generally poor, and egg production was suboptimal.

### 4.2. BASIC DIET

Except for the general principles outlined by different investigators (FRIEND, 1955; BECK, 1956; VANDERZANT, 1966) there are no clear-cut directives for the formulation of an artificial diet for insects.

After further modifications of DADD's diet it was decided to start preliminary experiments with a pilot diet, from which all other diets were empirically derived. The composition of basic diet-1 is illustrated in table 1.

#### Preparation

Besides the solid dietary constituents, some nutrients were prepared in solutions, and appropriate aliquots were measured out as needed. The amino acids were dissolved in distilled water by addition of 0.4 g potassium hydroxide per 40 ml, followed by heating in a water bath at 100°C. B-vitamins, choline chloride, inositol and vitamin-A palmitate were separately dissolved in 1 ml distilled water. These stock solutions were kept in a refrigerator for no longer than three weeks. The sterols and  $\alpha$ -tocopherol acetate were dissolved in the mixture of oleic acid, linoleic acid and linolenic acid by heating in a water bath at 100°C.

TABLE 1. Composition of Basic Diet-1.

Constituents*	Quantity
Vitamin free casein	6.00 g
Egg-albumin	4.00 g
Amino acid mixture <sup>a</sup>	0.825 g
Sucrose	6.00 g
Vegetable-lecithin	1.00 g
Fatty acid mixture <sup>b</sup>	1.00 ml
Sterol mixture <sup>b</sup>	0.08 g
Wesson's salt mixture	0.50 g
Mixture of B-vitamins <sup>c</sup>	2.76 mg
Choline chloride	0.10 g
Meso-inositol	0.04 g
Ascorbic acid	0.10 g
Vitamin-A palmitate	0.002 g
$\alpha$ -tocopherol acetate <sup>b</sup>	0.016 g
Potassium hydroxide	0.56 g
Alphacel non-nutritive bulk	2.00 g
Agar (granulated)	2.50 g
Inhibitor <sup>d</sup>	0.27 g
Distilled water (to 100 g)	

\* All constituents were obtained from Nutritional Biochemical Corporation, Cleveland, Ohio, unless otherwise indicated.

<sup>a</sup> Consists of (in mg): Glycine 25; L-alanine, 50; L-serine 75; L-proline, 50; L-valine, 75;  $\gamma$ -aminobutyric acid, 200; DL- $\alpha$ -aminobutyric acid, 100; L-glutamic acid, 125; L-aspartic acid, 125.

<sup>b</sup> Consists of (in ml): Oleic acid, 0.6; Linolenic acid 55%, 0.3; and Linoleic acid 75%, 0.1. In this mixture are dissolved (in mg) Cholesterol, 40;  $\beta$ -sitosterol, 40; and  $\alpha$ -tocopherol acetate, 16.

<sup>c</sup> Consists of (in mg): Niacinamide, 1; Ca-pantothenate, 0.5; Pyridoxine-HCl, 0.25; Thiamine-HCl, 0.25; Riboflavin, 0.25; Folic acid, 0.25; Biotin, 0.01; and p-aminobenzoic acid, 0.25.

<sup>d</sup> Consists of (in mg): Sorbic acid, 150; Methyl p-hydroxybenzoate, 100; Streptomycine sulphate, 20.

The ascorbic acid was dissolved in 1 ml distilled water each time before preparing the diet.

Preparation of 100 g of diet was as follows. The casein was suspended in distilled water containing 0.16 g potassium hydroxide in a glass vial of 375 ml. The other solid ingredients and the nutrient solutions were added and blended to make a paste. The agar was dissolved in 20 ml distilled water by heating together with the amino acid solution to 90–95°C and poured into the glass vial, followed by a thorough mixing by means of a Philips' electric hand mixer. When the temperature dropped to about 60°C the ascorbic acid solution was added and the mixture was again stirred. The hot mass was then poured out into a petridish of 9 cm diameter and cooled to room temperature to form a gel. The diet was kept in a refrigerator for no longer than one week, and each time the amount needed was cut out.

#### 4.3. FEEDING RESPONSE OF THE DIFFERENT LARVAL INSTARS

The fact that several phytophagous insects can be reared during many generations on diets without any substance derived from the host plant (VANDERZANT, 1957, 1965; ROCK et al., 1964; BECK et al., 1968) indicates that those diets satisfy the feeding requirements of the insects concerned. Certain combinations of nutrients and non-nutritive carriers such as cellulose, agar and water seem to be sufficient to elicit optimal biting and feeding response.

In recent years, investigations concerning the role of nutrients at the chemosensory level and their effect on the total feeding behaviour of insects have attracted a great deal of attention. In general, nutrient substances are divided into five categories, (1) proteins and amino acids, (2) carbohydrates, (3) lipids and sterols, (4) vitamins and (5) minerals. The agar-gel method developed by HSIAO and FRAENKEL (1968a) made it possible to distinguish the biting from the feeding response of the Colorado potato beetle larvae. No biting was observed on the 'neutral' substrate consisting of 4% agar and 4% cellulose. Cholesterol or  $\beta$ -sitosterol included in the gel, evoked biting, but this process was not followed by a feeding action. Several members of each category of nutrients have proved to be phagostimulatory to different phytophagous insects.

From the overall feeding responses of the insects studied, there emerge some general rules. In the first place, there exists an optimum concentration of the particular nutrient tested at which the stimulating effect is at a maximum. RITTER (1967) worked out the effect of different concentrations of sucrose on the feeding of Colorado potato beetle larvae and found an increasing effect at the beginning till a maximum was reached, followed by a decline of activity at higher concentrations. In the second place, there are nutrients which can operate individually, while the effect of other nutrients is obvious only when they are offered in combination with phagostimulatory nutrients. Thus, cholesterol is found to be inactive in evoking feeding responses in the Colorado potato beetle larva (RITTER, 1967; HSIAO and FRAENKEL, 1968a). A combination of sucrose, L-alanine, stearic acid and cholesterol, however, appeared to be more active than the individual components alone (RITTER, 1967). There is no indication that inorganic salts stimulate feeding by the larva of *L. decemlineata*. However, HSIAO and FRAENKEL (1968a) showed that the effect of combinations of various salts and sucrose on the feeding of Colorado potato beetle larvae was higher than the stimulative effect of sucrose alone. The effect of KCl in combination with sucrose, L-alanine or  $\gamma$ -aminobutyric acid was far beyond the effect of the last mentioned nutrients individually.

The above mentioned examples indicate that the effect of a nutrient mixture on the feeding behaviour of an insect is determined by three distinctive types of joint actions of the components (viz. additive, synergistic and antagonistic effects), which might operate at the same time (HSIAO and FRAENKEL, 1968a). In other words, the degree of feeding response is determined by the action that is prevailing.

An artificial diet consisting of non-nutritive bulk materials and nutrients can

be considered as a neutral substrate in which is included a large number of nutrients which act jointly on the gustatory senses of the insect. It seems that there is no sufficient theoretical background available as yet indicating how to achieve a maximum effect from the joint action of a large number of components.

Many experiments on the feeding response of phytophagous insects by means of artificial substrates have been conducted by using larvae of the last instar as test insects (ITO, 1960; RITTER, 1967; HSIAO and FRAENKEL, 1968a). As reported in an earlier paper (WARDOJO, 1967) growth of larvae of the first instars reared on an artificial diet was fairly poor when compared with growth of larvae of the fourth instar. This phenomenon might be the result of a difference in feeding response between the different larval instars.

### Method

The basic diet was cut into rod-shaped pieces of approximately 1 g. A piece of diet was put in a glass tube of 2 cm diameter with a capacity of 17.5 ml. Newly hatched larvae and newly moulted larvae of successive instars were used for the experiment, each group consisting of 20 individuals. The larvae of the second, third and fourth instar were starved for 3 to 4 hours. The ranges of the weights of the larvae used for the experiments were respectively: I: 0.45–0.60 mg; II: 2.60–3.60 mg; III: 10.0–20.0 mg; IV: 45.0–65.0 mg. Each larva was put in the glass tube containing diet, which was then stoppered with a cellulose stopper. The glass tubes were put in a metal rack. Since the larvae showed a strong positive phototactic response, the racks were placed in a black plastic bag. Every two hours each tube was examined under a dissection microscope and the faecal pellets produced were counted. The number of larvae producing pellets and the number of pellets produced were used as criteria for the feeding response of the Colorado potato beetle larvae to the artificial diet. This observation during each two hour interval was terminated after 30 hours, but the same larvae were further reared on the diet. Every day they were transferred to clean glass tubes containing fresh diet.

### Results

The results shown in table 2 and fig. 1, indicate that there is a difference in the feeding response between the different instars. The fact that the fourth instar larvae produced excrement earlier indicates that continuous feeding must have taken place earlier than in the case of the younger instars. After 12 hours 85% of the fourth instar larvae showed a feeding response, while of the first instar larvae only 35% did so. When the number of larvae reacting is used as a criterion, there is no great difference in the feeding response between the second, third and fourth instar. From the second day on all larvae of the different instars appeared to be attached to the diet, and no positive phototaxis was observed. From the presence of excrement after the transfer to a fresh diet it was concluded that continuous feeding took place.

The results of this experiment suggest that there is an increasing degree of tolerance towards foreign food in successive instars. Further, larvae of the

TABLE 2. Feeding response of *Leptinotarsa* larvae of different instars to Basic Diet-1. E = total number of faecal pellets produced up to the hour indicated. N = total number of larvae excreting.

Instar		0	2	4	6	8	10	12	24	26	28	30
I	E	0	0	0	0	4	5	10	70	—	—	—
	N	0	0	0	0	3	4	7	17	18	20	—
II	E	0	0	1	11	32	51	61	154	—	—	—
	N	0	0	1	5	12	14	15	18	18	19	20
III	E	0	0	10	22	51	80	100	247	—	—	—
	N	0	0	6	8	11	15	15	20	—	—	—
IV	E	0	1	12	36	106	168	210	609	—	—	—
	N	0	1	6	10	14	16	17	20	—	—	—

Colorado potato beetle have a capacity for adaptation, which also increases in consecutive instars.

#### 4.4 GROWTH RESPONSE OF THE DIFFERENT LARVAL INSTARS

##### 4.4.1. Introduction

Growth of living organisms implies the accumulation of materials assimilated from the food in the body that leads to an increase of body weight and size. In general growth is a discontinuous process, periods of activity alternating with periods of standstill. This process is very evident in insects and arthropods in general.

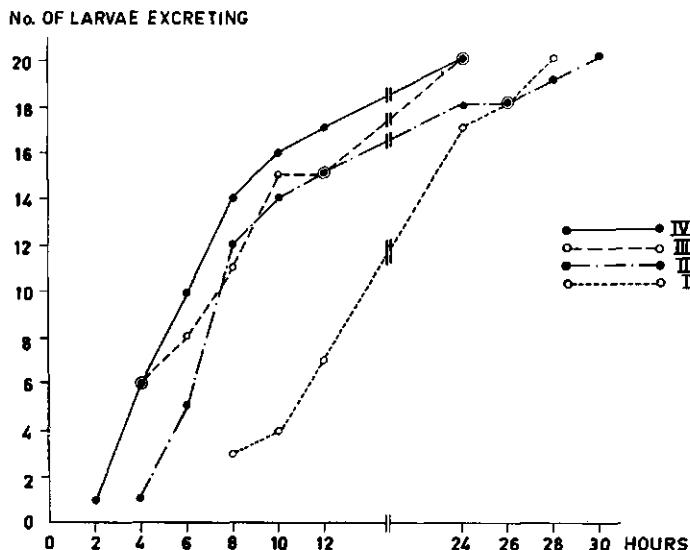


FIG. 1. Feeding response of the first (I), second (II), third (III) and fourth (IV) instar larvae of *L. decemlineata* to Basic Diet-1 (see Table 1).

During each period the insect undergoes the process of moulting or ecdisis, by which the larval stage is divided into a series of instars. The instars are generally considered to represent the biological age of an insect which implies the degree to which the insect has achieved its development. For each species there is a temperature at which growth is optimal.

Under optimal external conditions the quality and quantity of the food may influence the growth rate, the number of instars, the duration of each instar and the linear growth. The adverse effect of various *Solanum* species on larval growth of the Colorado potato beetle has been shown by CHIN (1950).

In the autumn of 1965, 20 newly hatched larvae of the Colorado potato beetle were reared individually upon leaves of *Solanum tuberosum* var. *Eigenheimer*. Each was kept in a glass tube of 2 cm diameter with a capacity of 17.5 ml, provided with a cellulose stopper and placed in the phytotron. The temperature was kept at  $25 \pm 0.5^\circ\text{C}$  and the daylength 18.00 hours. The leaves were changed daily and the body weight of each larva was determined by means of a torsion balance. At the end of the fourth instar when the larvae stopped feeding and started bending the body axis, they were transferred to glass tubes containing moist sand.

Under the experimental conditions all the 20 larvae completed their development. The results are illustrated in figs. 2 and 3 and in table 3.

As shown by CHIN (1950), the larvae of the Colorado potato beetle, like most insects, show an S-shaped growth curve, when growth is expressed in terms of body weight. The comparatively slow rate of growth before the second moult is easy to distinguish from the rapid weight increase thereafter (fig. 2). The change in growth rate seems to be the result of a shift from protein synthesis at a certain point of development to a preponderant fat synthesis. According to BUSNEL (1939) accumulation of a large amount of fat in the larval body starts in the third instar and intensifies in the fourth instar. The accumulation of a large quantity of body reserves results in a tremendous increase in body weight up to the end of the last instar. At that moment feeding is terminated and loss of water results in a slight decline of body weight. The larvae enter the prepupal stage and are ready for the pupal moult. The maximum weight is attained two to three days after the third moult with a mean value of  $191.7 \pm 4.0$  mg.

Colorado potato beetle larvae reared according to the above mentioned method at different times of the year show the same growth pattern with a slight variation, which might be due to a variation in the quality of the potato leaves.

#### 4.4.2. Growth response

Adverse effects of different foodplants on the growth of the Colorado potato beetle usually express themselves as a slow growth rate of the larvae, and the prolonged duration of the larval instars (CHIN, 1950). These phenomena correspond with the following processes. On unsuitable plants delayed feeding occurs, the amount consumed is usually small, and a reduced percentage of the

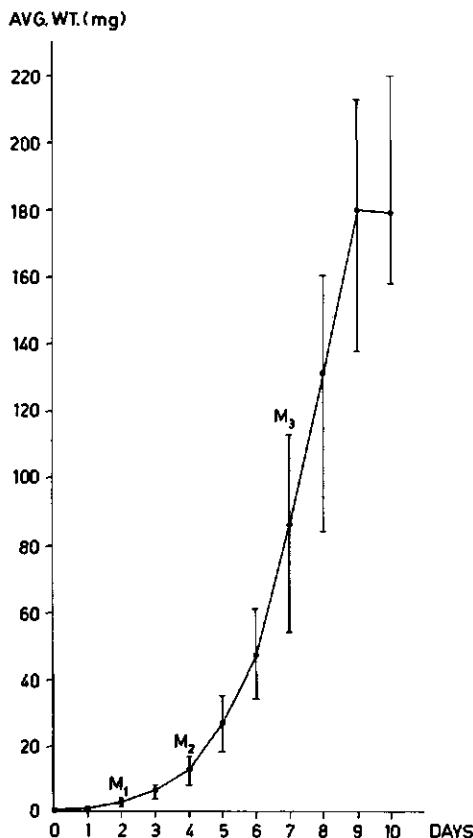


FIG. 2. Larval growth on potato leaves. Average weight of 20 larvae. Vertical bars indicate the ranges of the weights.  $T = 25 \pm 0.5^{\circ}\text{C}$ . DL = 18.00 hours.  $M_1$ ,  $M_2$  and  $M_3$  indicate first, second and third moult respectively.

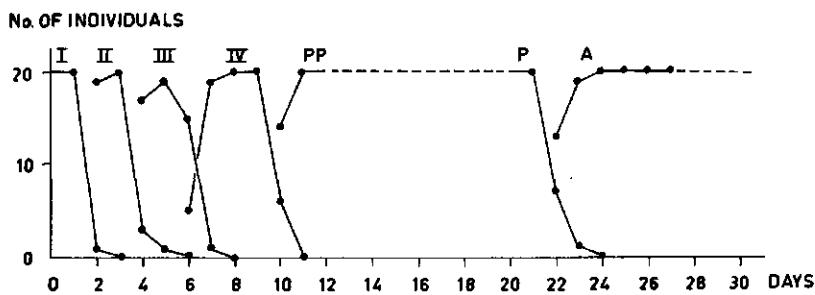


FIG. 3. Succession of the instars of the instars of *L. decemlineata* reared on potato leaves. I, II, III and IV indicate first, second, third and fourth instar. PP, P and A indicate prepupal, pupal and adult stage respectively.

TABLE 3. Growth of *Leptinotarsa* larvae reared on potato leaves. Mean duration of the different instars (Days  $\pm$  S.E.) and mean weight gained during each instar (mg  $\pm$  S.E.).

	Instar			
	I	II	III	IV
Mean duration (Days) of each instar	2.1 $\pm$ 0.1	2.1 $\pm$ 0.1	2.6 $\pm$ 0.1	2.6 $\pm$ 0.1
Mean weight gained (mg) during each instar	2.8 $\pm$ 0.1	9.8 $\pm$ 0.7	59.6 $\pm$ 4.5	118.9 $\pm$ 4.7

food is utilized. This is very pronounced with *Solanum demissum*. A small percentage of the survivors on this plant can reach the maximum weight prior to pupation, but the average maximum weight attained is lower than when the larvae are reared on potato leaves.

Larvae of the Colorado potato beetle reared on the artificial diet show a delayed feeding response (section 4.3.), especially those of the first instar.

### Method

The same larvae used for the experiments on the feeding response were transferred every day to fresh diet and the weights were determined by means of a torsion balance. When a larva stopped feeding and showed a decline in body weight, it was transferred to moist sand to determine whether it would show a positive geotactic response. The number of larvae burrowing into sand was recorded.

### Results

From table 4a it is seen that newly hatched larvae were not able to complete larval development on the artificial diet. In the course of the experiment they reached a maximum average weight of 7 mg on the ninth day. Only 8 larvae

TABLE 4a. Growth of larvae of the successive instars on Basic Diet-1. Mean maximum weight attained prior to pupation and mean duration of larval development. S.E. = Standard Error. PP = prepupae. (a) = growth on potato leaves. (b) = growth on diet.

Instar	PP (No.)	Mean max. wt mg $\pm$ S.E.	Larval development Days $\pm$ S.E.
Ia	20	185.4 $\pm$ 4.9	9.5 $\pm$ 0.3
Ib	—	—	—
IIa	19	179.1 $\pm$ 5.0	7.4 $\pm$ 0.3
IIb	13	131.0 $\pm$ 6.1	15.6 $\pm$ 0.9
IIIa	20	186.5 $\pm$ 4.4	5.2 $\pm$ 0.2
IIIb	19	144.4 $\pm$ 4.8	12.2 $\pm$ 0.8
IVa	20	173.2 $\pm$ 4.6	2.4 $\pm$ 0.4
IVb	20	182.3 $\pm$ 4.2	2.9 $\pm$ 0.4

out of 20 reached the second instar, and all larvae died after 16 days.

Of the second instar larvae reared on the artificial diet 13 individuals completed their development, while the rest died in the course of the experiment. The time required to reach the maximum weight was, however, twice as long as when they were reared on potato leaves. Moreover, the mean maximum weight attained was significantly lower than that of the control group ( $P < 0.05$ ). The mean maximum weight attained by the third instar larvae on the diet was also much lower than that of the control group ( $P < 0.05$ ), although almost all larvae completed their development.

No significant difference was found between fourth instar larvae reared on diet and on potato leaves with respect to the duration of growth, the average maximum weight attained and the number of individuals surviving. To confirm this finding, in ten replicates, 10 fourth instar larvae were reared in a petridish provided with 10 g of diet. Every day they were transferred to fresh diet and after completing their development they were allowed to burrow into sand in a zinc container, 50 individuals per container. All of them showed a positive geotactic response, indicating that they had reached the prepupal stage. The adults were weighed immediately after emergence. It appeared that 96% of the adults emerged with a normal appearance, except for the colour of the body which was slightly more pale than that of the individuals reared on potato leaves. A difference was found in the weights, especially of the females ( $P < 0.05$ ) (table 4b).

TABLE 4b. Mass rearing of larvae of the fourth instar on Basic Diet-1. Mean weight of emerging adults. S.E. = Standard Error. Initial number 100 larvae.

Food	♀♀		♂♂		♀♀ + ♂♂	
	No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.
Potato leaves	49	142.8 $\pm$ 2.2	51	119.9 $\pm$ 1.9	100	131.1 $\pm$ 2.1
Artificial diet	44	131.8 $\pm$ 1.8	52	118.2 $\pm$ 1.3	96	124.4 $\pm$ 1.4

The results of this experiment indicate that the ability of the Colorado potato beetle larvae to utilize an artificial diet increases during successive instars.

From the fact that the average maximum weight attained by the second instar is lower than that of the third instar, which in its turn is lower than the average maximum weight attained by the fourth instar, it may be concluded that the amount of diet assimilated was limited. The ability of the older larvae to complete their development on the diet seems to be related to the presence of reserve materials accumulated during their stay on potato leaves. But there is another factor which is not less important. Postembryonic growth is always preceded by food intake. The reactions of the gustatory senses towards foreign food determines whether feeding will take place or not. The first instar larvae of the Colorado potato beetle appear to be the least tolerant towards unspecific food (section 4.3.), and consequently no growth takes place on the diet.

It seems that there are two main aspects which are closely related to each other, i.e. the physical and chemical properties of the diet to satisfy the feeding requirements on one hand, and the adequacy of the diet to obtain optimal growth on the other hand. In cases where an artificial diet can support growth of an insect for many generations, it may be taken for granted that the diet satisfies the requirements for optimal feeding.

#### 4.5. OVIPOSITION

Adults of some insect species do not require food for reproduction, since they are able to utilize materials accumulated during the pre-imaginal stage for yolk synthesis and the formation of accessory gland products. In other species food intake by adults is obligatory for reproduction. The quality and quantity of the food may influence the fecundity and fertility of the female insect. The reproductive processes in insects in relation to feeding and nutrition have been treated recently by JOHANSSON (1964).

In order to reproduce, adults of the Colorado potato beetle require the same food as the larvae, viz. potato leaves. At 25°C they start to copulate three to four days after emergence and oviposition starts on the fifth or sixth day. The maximum number of eggs is laid on the ninth or tenth day, but oviposition takes place regularly for more than one month. A continuous egg production is only possible when there is a constant access to the source of food.

Different *Solanum* species appear to have an adverse effect on egg production by the Colorado potato beetle (DE WILDE et al., 1960). Females fed on *S. dulcamara*, *S. esculentum* and *S. luteum* appeared to be less fecund than those fed on *S. tuberosum*.

Newly emerged adults of the Colorado potato beetle which were reared as larvae on potato leaves, readily accepted the artificial diet. The quality of the artificial diet can be measured by its effect on the fecundity of the females.

#### Method

Newly emerged adults from the stock culture were divided in petridishes of 9 cm diameter, one pair per petridish. Per 24 hours cycle they were allowed to stay (1) 24 hours on potato leaves, (2) 21 hours on diet and 3 hours on potato leaves, (3) 24 hours on diet, (4) 3 hours on potato leaves. (5) Adults emerged from larvae, which had been reared on the diet beginning with the fourth instar, were allowed to feed on the same diet 24 hours per day. Every day they were transferred to a clean petridish containing fresh food. The eggs deposited were collected every day two to three times, to prevent the beetles from feeding on their own eggs. The experiment was conducted in 5 replicates.

#### Results

From table 5 it is seen that the artificial diet is able to support egg production by the female Colorado potato beetle (treatments 3 and 5), although only at a rate of 14.1 resp. 20.4% of the value obtained with beetles fed on potato leaves.

TABLE 5. Oviposition under influence of Basic Diet-1.  $X_f$ ,  $X_m$  = weight of resp. female and male (mg) at emergence.  $Y_{14}$  = number of eggs laid during the first 14 days.

Treatment	Pair	$X_f$	$X_m$	$Y_{14}$	Total (%)
1. Larvae on potato leaves, adults on potato leaves 24 hours per day	1	157.0	145.0	494	
	2	124.5	126.0	795	
	3	132.0	112.5	756	
	4	128.5	141.0	724	
	5	134.0	108.0	738	3507 (100.0)
2. Larvae on potato leaves, adults 21 hours on diet and 3 hours per day on potato leaves	1	137.0	144.0	413	
	2	120.0	110.0	278	
	3	128.0	116.0	265	
	4	124.0	123.5	402	
	5	134.0	112.5	339	1697 (45.5)
3. Larvae on potato leaves, adults on diet 24 hours per day	1	142.0	133.0	64	
	2	140.5	122.0	235	
	3	121.5	121.0	121	
	4	125.0	127.5	0	
	5	117.0	136.0	76	496 (14.1)
4. Larvae on potato leaves, adults on potato leaves 3 hours per day	1	140.0	121.0	0	
	2	151.0	123.5	0	
	3	158.5	141.0	0	
	4	149.0	137.5	0	
	5	135.5	115.0	0	0 (0.0)
5. Larvae from the fourth instar on diet, adults on diet 24 hours per day	1	111.0	107.0	197	
	2	125.0	124.0	90	
	3	144.0	115.0	164	
	4	131.0	111.0	112	
	5	120.0	118.0	140	703 (20.4)

Oviposition on the diet appeared to be delayed for two to three days.

Beetles which were allowed to feed on potato leaves for only three hours a day produced no eggs at all (treatment 4). Alternate feeding, 21 hours on diet and 3 hours on potato leaves, appeared to increase the egg production to 45.5% of the above standard value (treatment 2). It seems that the amount of potato leaves consumed during a period of three hours per day could compensate for the deficiency of the diet to a certain extent.

Eggs, laid at later dates by the females fed on diet, showed a pale colour in contrast to the normal orange colour at the beginning. All of them were, however, viable. At 25°C they hatched after four to five days and yielded normal larvae. It may be concluded that there is no adverse effect of the diet on female fertility.

## 5. EFFECT OF SOME DIETARY CONSTITUENTS ON THE GROWTH OF THE THIRD INSTAR LARVA

### 5.1. INTRODUCTION

Complete information on the qualitative and quantitative chemical composition of a host plant is seldom available. Hence the composition of most of the artificial diets for insects is only partially based on the composition of the host plant. Different natural products of plant and animal origin constitute diets for phytophagous insects. Nutrient substances used in insect diets have been classified and tabulated by VANDERZANT (1966).

An adequate assortment of nutrients does not necessarily support growth, if the diet lacks the stimuli that initiate and maintain feeding (VANDERZANT, 1966). Since many phytophagous insect species can be reared successfully on diets without any specific plant chemicals, it is believed that these chemicals are not necessary for most insects, provided that all other feeding requirements, including the physical ones, are met. From the study of insect feeding behaviour, it appears that the degree of feeding is determined by the relative concentration ratios of the nutrients tested (RITTER, 1967; HSIAO and FRAENKEL, 1968a). Given an adequate assortment of nutrients, it is also the relative concentration ratios of the dietary constituents which determine the degree of growth (HOUSE, 1962, 1966; BECK et al., 1968).

The failure of the artificial diet to support growth of the younger larval instars of the Colorado potato beetle might be due to improper concentration ratios of the dietary constituents necessary for optimal feeding and growth. But the possibility that complete lack of certain nutrients is the major cause of the poor larval growth cannot be excluded.

Since there are no clear cut directives for the development of an artificial diet for a particular insect species, improvement of the artificial medium for the Colorado potato beetle was done empirically by testing the effect of different substances included in the diet. The experiments were carried out with newly moulted third instar larvae. These larvae had to pass through the fourth instar before reaching the prepupal and pupal stage. The information gained seemed therefore more valuable than when fourth instar larvae were used, which proved to be highly tolerant in their feeding and growth response towards different unnatural food, indicating that minimal utilizable nutrients may suffice for the completion of the last larval instar.

### 5.2. METHOD

Newly hatched larvae reared on potato leaves at  $25 \pm 0.5^{\circ}\text{C}$  will undergo a second moult after four days and enter the prepupal stage six days after that moult (fig. 3). From the second moult till the termination of feeding at the end of the fourth instar there is an average weight increase of  $178.5 \pm 4.7$  mg.

Newly moulted third instar larvae were collected from the stock culture and

starved in a petridish for three to four hours to empty the gut in order to standardize the physiological condition. Larvae with an average body weight of 10–20 mg were used. One larva of known weight was placed in a glass tube containing approximately 1 g of medium. The tube was then stoppered with a cellulose stopper. Every day the larva was transferred into a clean glass tube containing fresh medium. On the sixth day the body weight of the larva was determined by means of a torsion balance. The mean weight gained during this period was the criterion for the adequacy of the diet. The above mentioned period of six days was adopted, since there were indications that on further rearing the difference in the mean maximum weight gained, i.e. the mean maximum weights prior to pupation minus initial weight (table 7), as well as the difference in the mean weight of the emerging adults (table 8) remained more or less constant. In each experiment 20 larvae were used.

The effect of various substances on the growth of the third instar larva was investigated by means of basic diets 1 to 6 (table 6), viz. by addition, substitution or deletion. Each basic diet was a modification of the foregoing one, and its composition was based on the results obtained according to the aforementioned procedure.

### 5.3. CRUDE PLANT MATERIALS

BECK et al. (1949) were the first who successfully applied lyophilized leaves of the host plant to improve the adequacy of the diet for larvae of the European corn borer, *O. nubilalis*. The importance of crude plant materials might lie in the supply of specific substances necessary for optimal feeding, or in improving the concentration ratios of the dietary constituents necessary for optimal feeding, or optimal growth, or in the supply of one or more nutrients previously lacking but necessary for optimal growth. The last two possibilities appeared to be the case since by modifying the diet and including ascorbic acid in it, the host plant material could be omitted (CHIPPENDALE and BECK, 1964).

In the case of the diet for *P. brassicae* (DAVID and GARDINER, 1965) the major role of the cabbage leaf powder added seems to be in the supply of 'secondary plant substances' necessary for optimal feeding (DAVID and GARDINER, 1966a, b).

The fourth instar larva of the Colorado potato beetle grows better when potato leaf powder or fresh leaf homogenate is added to the basic diet (HSIAO and FRAENKEL, 1968c). These authors believe that a phenolic flavonoid or related compound contained in potato leaves acts as a feeding stimulant.

#### Method

Potato leaf powder was prepared by drying fresh leaves in a ventilated room at 30°C and then pulverizing them by means of a Philips' blender at the highest speed and sifting through a 40-mesh screen. The powder was stored in a brown bottle at room temperature.

The amount of leaf powder added to basic diet-1 replaced the same amount

TABLE 6. Composition of Basic Diets 1 to 6.

Constituents	Quantity					
	B.D.-1	B.D.-2	B.D.-3	B.D.-4	B.D.-5	B.D.-6
Vitamin free casein	6.00 g	6.00 g	3.00 g	3.00 g	3.00 g	3.00 g
Egg-albumin	4.00 g	4.00 g	4.00 g	4.00 g	4.00 g	4.00 g
Amino acid mixture <sup>a</sup>	0.825 g	—	—	—	—	—
Amino acid mixture <sup>a*</sup>	—	1.06 g	1.06 g	1.06 g	—	—
Amino acid mixture <sup>a**</sup>	—	—	—	—	—	0.22 g
Sucrose	6.00 g	6.00 g	6.00 g	60.0 g	6.00 g	6.00 g
Vegetable-lecithin	1.00 g	1.00 g	1.00 g	0.50 g	0.50 g	0.50 g
Fatty acid mixture <sup>b</sup>	1.00 ml	1.00 ml	1.00 ml	—	—	—
Sterol mixture <sup>b</sup>	0.08 g	0.08 g	0.08 g	—	—	—
Fatty acid mixture <sup>b*</sup>	—	—	—	0.75 ml	0.75 ml	0.75 ml
Sterol mixture <sup>b**</sup>	—	—	—	0.072 g	0.072 g	0.072 g
Wesson's salt mixture	0.50 g	0.50 g	0.50 g	—	—	—
Salt mixture after Beck et al. 1968	—	—	—	0.50 g	0.50 g	0.50 g
Mixture of B-vitamins <sup>c</sup>	2.76 mg	2.76 mg	2.76 mg	2.76 mg	2.76 mg	2.76 mg
Choline chloride	0.10 g	0.10 g	0.10 g	0.10 g	0.10 g	0.10 g
Meso-inositol	0.04 g	0.04 g	0.04 g	0.04 g	0.04 g	0.04 g
Ascorbic acid	0.10 g	0.10 g	0.10 g	0.10 g	0.10 g	0.10 g
Vitamin-A palmitate	0.002 g	0.002 g	0.002 g	0.002 g	0.002 g	0.002 g
$\alpha$ -tocopherol acetate <sup>b, b*</sup>	0.016 g	0.016 g	0.016 g	0.016 g	0.016 g	0.016 g
Potassium hydroxide	0.56 g	0.56 g	0.56 g	0.56 g	0.56 g	0.56 g
Alphacel non-nutritive bulk	2.00 g	2.00 g	2.00 g	2.00 g	2.00 g	2.00 g
Agar (granulated)	2.50 g	2.50 g	2.50 g	2.50 g	2.50 g	2.50 g
Inhibitor <sup>d</sup>	0.27 g	0.27 g	0.27 g	0.27 g	0.27 g	0.27 g
Distilled water (to 100 g)						

<sup>a, b, c, d</sup> Table 1.<sup>a\*</sup> Consists of (in mg):

I. L-arginine	60	II. L-alanine	60	III. L-aspartic acid	60
L-histidine	60	L-cystine	40	L-asparagine	80
L-isoleucine	15	Glycine	40	L-glutamic acid	180
L-leucine	25	L-proline	40	Total	320
L-lysine	80	L-serine	80		
L-methionine	20	L-tyrosine	40		
L-phenylalanine	20	$\gamma$ -aminobutyric acid	40		
L-threonine	40	DL- $\alpha$ -aminobutyric			
L-valine	40	acid	20		
L-tryptophane	20	Total	360		
Total	380				

Total I, II and III = 1060

<sup>a\*\*</sup> Consists of (in mg):

L-alanine	60
Glycine	40
L-proline	40
L-serine	80
Total	220

<sup>b\*</sup> Consists of (in ml): Oleic acid, 0.45; Linolenic acid 55%, 0.225; and Linoleic acid 75%, 0.075. In this mixture are dissolved (in mg)  $\beta$ -sitosterol, 36; Stigmasterol, 27; Cholesterol, 9; and  $\alpha$ -tocopherol acetate, 16.

of alphacel. When the amount of leaf powder exceeded 2 g, the amount of water was adjusted to keep the total weight of the diet constant.

Lyophilized potato leaves were powdered and stored in the same way as mentioned above. One gram of powdered lyophilized leaves substituted the same amount of alphacel in basic diet-3.

Ten grams of fresh leaf homogenate were added to basic diet-3. The amount of alphacel was reduced to 1 g and the water contents of the diet was adjusted.

## Results

From table 7 it is seen that feeding during the first 24 hours is improved by the addition of potato leaf powder to basic diet-1. But leaf powder has practically no effect on the growth of the third instar larvae. All larvae moulted to the fourth instar and reached a maximum weight prior to pupation. But in general growth on these diets was suboptimal in terms of final weight and rate of increase. No significant difference was found between the mean weights gained within six days or between the mean maximum weights attained.

TABLE 7. Effect of potato leaf powder in Basic Diet-1 on the weight gain of the third instar larva and the time required for the completion of the larval development. E = total number of faecal pellets produced within the first 24 hours. N = number of larvae excreting.  $W_6$  = mean weight gained (mg) within 6 days on diet.  $W_{max.}$  = mean maximum weight gained (mg) prior to pupation. S.E. = Standard Error.

Leaf powder g/100 g	E	N	$W_6$ mg $\pm$ S.E.	$W_{max.}$ mg $\pm$ S.E.	Prepupae	
					No.	Days $\pm$ S.E.
0.0	417	20	48.0 $\pm$ 5.5	118.7 $\pm$ 3.1	19	13.2 $\pm$ 0.5
0.5	582	20	50.6 $\pm$ 5.3	123.0 $\pm$ 4.6	19	12.6 $\pm$ 0.7
1.0	565	20	45.3 $\pm$ 4.4	125.8 $\pm$ 4.1	19	12.8 $\pm$ 0.7
2.0	636	20	54.0 $\pm$ 4.7	124.7 $\pm$ 3.3	18	11.5 $\pm$ 0.6
4.0	601	20	50.3 $\pm$ 4.8	120.6 $\pm$ 3.0	18	11.7 $\pm$ 0.7

Basic diet-3 is a modification of the previous basic diets. Growth of the third instar larva on this diet is significantly better than on basic diet-1 (table 7, 8). Addition of 10 g fresh leaf homogenate or 1 g lyophilized leaves to basic diet-3 does not promote larval growth (table 8). No significant difference was found between the mean weights of the emerging females. The mean weight of the males from diet containing lyophilized leaves is significantly lower than that of those reared on the other two diets.

The results of the experiments indicate that crude material from the host plant has no growth promoting effect, nor does it have an adverse effect on larval growth. There are indications that the relative concentration ratio of the dietary constituents is the crucial point, since by the inclusion of crude plant material the composition of the diet should have been improved qualitatively. Compared to basic diet-1, basic diet-3 contains half the concentration of vitamin free casein. Especially when the lipid fraction, i.e. vegetable-lecithin and fatty

TABLE 8. Effect of homogenized fresh potato leaves and lyophylized potato leaves in Basic Diet-3\* on the growth of the third instar larva.  $W_6$  = mean weight gained (mg) within 6 days on diet. S.E. = Standard Error. PP = prepupae. Initial number of larvae 20 per treatment. ♀♀ and ♂♂ = emerging adults, females and males.

Crude plant material	g/100 g of diet	$W_6$ mg $\pm$ S.E.	PP (No.)	♀♀		♂♂		♀♀ + ♂♂	
				No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.
Control	-	108.9 $\pm$ 4.3	19	11	91.5 $\pm$ 3.5	6	89.8 $\pm$ 5.0	17	90.9 $\pm$ 2.8
Homogenized fresh leaves	10.0	110.8 $\pm$ 9.3	16	6	96.3 $\pm$ 3.9	6	94.7 $\pm$ 4.0	12	95.5 $\pm$ 2.7
Lyophylized leaves	1.0	113.7 $\pm$ 3.8	19	9	87.7 $\pm$ 3.7	7	78.7 $\pm$ 4.9	16	83.7 $\pm$ 3.1

\* Contains 0.5 g lecithin and 0.5 ml fatty acid-sterol mixture.

acid-sterol mixture were also halved, growth on basic diet-3 was better than on basic diet-1.

#### 5.4. PROTEINS AND AMINO ACIDS

##### 5.4.1. Proteins

Among different proteins, casein has been used most widely in artificial diets for leaf feeders and borers. In some cases, egg-albumin has been successfully used, viz. for the rearing of the pink boll worm, *P. gossypiella* (VANDERZANT and REISER, 1956) and in combination with casein and peptone in the diet for locusts and grasshoppers, *Locusta migratoria* L. and *Schistocerca gregaria* Forskål (DADD, 1960, 1961c). Protein of plant origin, i.e. soybean protein or soybean casein has been used in the diets for respectively larvae of the boll weevil, *A. grandis* (VANDERZANT and DAVICH, 1958) and the silkworm, *Bombyx mori* L. (ITO, 1960a; ITO and HORIE, 1962).

In the present study the artificial diet for the Colorado potato beetle was derived from the diet of DADD (1960). In the course of its modifications it appeared that pepton can be omitted from the diet, while casein and albumin have to be present in combination.

The fact that feeding by the larvae on basic diet-1 took place, although moderately, and inclusion of potato leaf powder did not improve larval growth (table 7), indicates that the quantitative aspect, i.e. the concentration ratio of the dietary components may be the most important factor.

The following experiments were designed to examine the effect of several proteins on larval growth.

#### Method

In cases where the amount of casein plus albumin, or one of the two was replaced by the same amount of another protein, the concentrations of all other components were kept constant. When the concentration of the proteins

was varied, the amount of water was adjusted to keep the total weight of the diet constant.

## Results

From table 9 it is seen that reduction of vitamin free casein in basic diet-2 from 6.0 g to 3.0 g/100 g of diet has no adverse effect on larval growth. The latter amount of casein cannot be replaced by the same amount of casein hydrolyzate. At the levels of 3.0 g and 1.5 g/100 g of diet, casein hydrolyzate has an adverse effect on larval growth.

TABLE 9. Effect of vitamin free casein and casein hydrolyzate on the weight gain of the third instar larva. Substitution of vitamin free casein in Basic Diet-2 by casein hydrolyzate (acid).  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Constituents tested	g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
Vit. free casein	6.00	19	65.2 $\pm$ 4.0
Vit. free casein	3.00	20	80.3 $\pm$ 7.9
Vit. free casein	0.00	19	11.8 $\pm$ 1.2
Casein hydrolyzate	3.00		
Vit. free casein	0.00	19	32.2 $\pm$ 4.2
Casein hydrolyzate	1.50		

TABLE 10. Effect of different levels of egg-albumin in Basic Diet-3 on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Egg-albumin g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
4.00	18	84.8 $\pm$ 6.9
2.00	14	56.4 $\pm$ 7.5
0.00	11	30.2 $\pm$ 4.3

Table 10 shows that reduction of the amount of egg-albumin or deletion of it from basic diet-3 results in poor larval growth.

Egg-albumin in basic diet-3 cannot be replaced by vitamin free casein (table 11). Although the mean weight gained on this diet does not differ significantly from that on the basic diet, larval mortality is very high. On the other hand, egg-albumin cannot replace vitamin free casein.

The difference in weight gained between the larvae reared on basic diet-4 and those, reared on the diet in which soybean protein is the only protein source, is not significant (table 12). But substitution of either casein or albumin in basic diet-4 by soybean protein resulted in poor larval growth.

The results of these experiments indicate that the use of either casein or egg-albumin, or soybean protein as the only protein source does not improve the adequacy of the artificial diet so far modified.

TABLE 11. Effect of vitamin free casein and egg-albumin on the weight gain of the third instar larva. Substitution of egg-albumin in Basic Diet-3\* by vitamin free casein and the reverse.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Constituents tested	g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
Vit. free casein	3.00	19	103.7 $\pm$ 7.9
Egg-albumin	4.00		
Vit. free casein	7.00	11	87.5 $\pm$ 8.7
Egg-albumin	0.00		
Vit. free casein	0.00	12	65.0 $\pm$ 6.2
Egg-albumin	7.00		

\* Contains 0.5 g lecithin and 0.5 ml fatty acid-sterol mixture.

TABLE 12. Effect of soybean-protein on the weight gain of the third instar larva. Substitution of casein and albumin in Basic Diet-4 by soybean-protein.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Constituents tested	g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
Vit. free casein	3.00	20	124.7 $\pm$ 3.4
Egg-albumin	4.00		
Soybean-protein	0.00		
Vit. free casein	0.00	20	81.2 $\pm$ 5.6
Egg-albumin	4.00		
Soybean-protein	3.00		
Vit. free casein	3.00	19	78.4 $\pm$ 5.8
Egg-albumin	0.00		
Soybean-protein	4.00		
Vit. free casein	0.00	20	111.8 $\pm$ 5.9
Egg-albumin	0.00		
Soybean-protein	7.00		

#### 5.4.2. Amino acids

Up to the present time only a small number of phytophagous insect species has been reared successfully on amino acid diets, i.e. the onion maggots, *Hylemya antiqua* Meigen (FRIEND and PATTON, 1956), the pink boll worm, *P. gossypiella* (VANDERZANT, 1957), the red-banded leaf roller, *Argyrotaenia velutinana* Walker (ROCK and KING, 1967), the boll weevil, *A. grandis* (VANDERZANT, 1965), the pea aphid, *Acyrtosiphon pisum* Harris (AUCLAIR and CARTIER, 1963), the potato aphid, *Macrosiphum euphorbiae* Thomas (CARTIER and MORIN, 1965), the green peach aphid, *Myzus persicae* Sulzer (DADD and MITTLER, 1966), the bean aphid, *Aphis fabae* Scopoli (DADD and KRIEGER, 1967) and the cotton aphid, *Aphis gossypii* Glover (AUCLAIR, 1967).

The amino acid requirements have been determined for the first mentioned four species (FRIEND et al., 1957; VANDERZANT, 1958, 1965; ROCK and KING, 1967) and also for the Asiatic rice borer, *C. suppressalis* and the silkworm, *B. mori* (ISHII and HIRANO, 1955; ARAI and ITO, 1964, cited in VANDERZANT,

1966). These species require the ten indispensable amino acids for growth, i.e. arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Under non-aseptic conditions, *M. persicae* requires only methionine, histidine and isoleucine. Growth ceased in one generation on omission of methionine, and in two generations on deletion of histidine or leucine. Apparently the extraordinary versatility of this species is a function of its possession of profuse intracellular symbionts (DADD and KRIEGER, 1968).

In several cases, one or more amino acids are supplemented in a protein diet. Cysteine has been supplemented in the casein diet for *Heliothis zea* Boddie and *A. grandis* (VANDERZANT, POOL and RICHARDSON, 1962) and cystine in the casein diet for *P. gossypiella* (VANDERZANT, KERUR and REISER, 1957). The reason is that casein has a very low content of the above mentioned sulphur containing amino acids. ROCK et al. (1964) included cystine, glycine, arginine and lysine in the casein diet for *A. velutinana*.

Several amino acids show a phagostimulatory effect on the fourth instar larva of the Colorado potato beetle (HSIAO and FRAENKEL, 1968a; RITTER, 1967) and on the larva of the European corn borer, *O. nubilalis* (BECK and HANEC, 1958), while other amino acids cause a negative feeding response.

The present experiments are to examine whether various amino acids included in the artificial diet will promote larval growth of the Colorado potato beetle.

### Method

The amino acids were dissolved either in distilled water or in a KOH-solution. Mixtures containing cystine, tyrosine or glutamic acid were dissolved in alkali. The total amount of KOH used in the diet was 0.56 g/100 g of diet. The pH-value of the diet varied between 5.5 to 6.5.

### Results

Deletion of amino acid supplement in basic diet-2 (table 13) has no adverse effect on larval growth. The possible explanation is that there is already an excess of proteins in this basic diet. As shown before the concentration of casein in the above mentioned basic diet can be reduced to half without any adverse effect on larval growth (table 9). The composition of amino acid mixture

TABLE 13. Effect of different levels of amino acid mixtures in Basic Diet-2 on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Amino acid mixture ** g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
1.060	19	65.2 $\pm$ 4.0
0.530	19	70.3 $\pm$ 6.0
0.000	18	66.1 $\pm$ 5.8

\*\* Table 6.

(a\*, table 6) was based on the relative concentration ratios of amino acids in young potato leaves found by CIBULA et al. (1967).

The same amino acid mixture (a\*, table 6) was used to replace the total amount of protein in basic diet-3 at two different levels, viz. 6.360 g and 7.420 g/100 g of diet. On these two amino acid diets, no feeding took place at all. After several bites the larvae avoided any contact with the diet and tried to creep along the wall of the glass tube. After three days the experiment was stopped, and there was a loss of weight of 4-9 mg per larva.

In another experiment cystine was added to basic diet-5 at different levels, viz. 25, 50, 100 and 150 mg/100 g of diet. It was found that larval growth on basic diet-5 was not improved by the addition of L-cystine.

The same basic diet-5 was also not improved by the addition of different mixtures of non-essential amino acids (table 14a, b).

TABLE 14a. Different mixtures of amino acids to be supplemented to Basic Diet-5. Quantities in mg.

Amino acids	A	B	C	D
L-alanine	60	60	60	60
Glycine	40	40	40	—
L-cystine	40	—	—	—
L-proline	40	40	40	—
L-serine	80	80	80	80
L-tyrosine	60	—	—	—
γ-aminobutyric acid	60	40	—	40
DL-α-aminobutyric acid	20	20	—	—
L-aspartic acid	60	—	—	60
L-glutamic acid	80	—	—	80
Total (mg)	540	280	220	320

TABLE 14b. Effect of different mixtures of amino acids supplemented to Basic Diet-5 on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Amino acid mixture*	mg/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
None	—	18	118.3 $\pm$ 5.9
A	540	19	103.0 $\pm$ 5.2
	1080	20	106.7 $\pm$ 6.7
B	280	20	119.6 $\pm$ 3.8
	560	19	121.3 $\pm$ 6.4
C	220	20	125.5 $\pm$ 3.7
	440	20	116.6 $\pm$ 4.0
None	—	18	117.5 $\pm$ 4.5
D	160	19	120.0 $\pm$ 4.7
D	320	20	125.7 $\pm$ 5.2
D	640	18	109.6 $\pm$ 4.5

\* Table 14a.

The fact that amino acid supplement neither improves nor effects larval growth adversely (tables 13, 14a, b) may indicate that the relative concentration ratios of the dietary constituents of the basic diets concerned are still far from being optimal.

### 5.5. CARBOHYDRATES

All diets for phytophagous insects are provided with carbohydrates. The most widely used among them are glucose and sucrose, solely or in combination. The diet for locusts and grasshoppers (DADD, 1960a, c) contains sucrose and dextrin as a carbohydrate source. ITO (1960a) included sucrose and potato starch in the diet for the silkworm, *B. mori*.

Among different nutrients that stimulate feeding of phytophagous insects sugars are most important. BECK (1956) found that glucose has a marked phagostimulatory effect on the larvae of *O. nubilalis*. Sucrose stimulates feeding of the Colorado potato beetle larvae (RITTER, 1967; HSIAO and FRAENKEL, 1968a) and many other insects.

The artificial diet for the larvae of the Colorado potato beetle, which was derived from the above mentioned diet of DADD (1960a), previously contained sucrose and dextrin, but the latter could be omitted in the course of the modifications of the diet. In the following experiments the effect of several carbohydrates on larval growth was examined. The results are presented in tables 15, 16, 17 and 18.

### Results

From table 15 it is seen that the disaccharide sucrose in basic diet-6 cannot be substituted by its components glucose and fructose. Substitution for sucrose by glucose resulted in high larval mortality and reduced weight gain markedly. This adverse effect was brought about by fructose to a lesser degree.

Reduction of the amount of sucrose in basic diet-6 to a level of 4.5 g/100 g of diet has no adverse effect on larval growth (table 16). Partial substitution for

TABLE 15. Effect of glucose and fructose on the weight gain of the third instar larva. Substitution of sucrose in Basic Diet-6 by glucose and fructose.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Constituents tested	g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
D-sucrose	6.0	20	120.0 $\pm$ 6.5
D-sucrose	0.0	8	66.4 $\pm$ 4.3
D-glucose	6.0		
D-sucrose	0.0	14	78.2 $\pm$ 9.5
D-fructose	6.0		
D-glucose	3.0	15	89.7 $\pm$ 9.1
D-fructose	3.0		

TABLE 16. Effect of different sugars on the weight gain of the third instar larva. Partial substitution of sucrose in Basic Diet-6 by several hexoses.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Constituents tested	g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
D-sucrose	6.0	10	119.3 $\pm$ 7.2
D-sucrose	4.5	19	115.0 $\pm$ 5.9
D-sucrose	4.5	18	110.2 $\pm$ 5.7
D-glucose	1.5		
D-sucrose	4.5	20	112.5 $\pm$ 4.8
D-fructose	1.5		
D-sucrose	4.5	19	116.6 $\pm$ 5.4
D-mannose	1.5		
D-sucrose	4.5	18	108.7 $\pm$ 5.9
L-sorbose	1.5		

sucrose by glucose, fructose, mannose or sorbose does not improve the quality of the diet (table 16).

Substitution for 1.5 g sucrose by trehalose has a marked effect on larval growth (table 17). This insect blood-sugar seems to have a promoting effect on key processes, the rate of which sets limitations to growth.

The trisaccharide melezitose that proved to be phagostimulatory (HSIAO and FRAENKEL, 1968a) does not improve larval growth when it substitutes 1.5 g of sucrose (table 17).

Among the polysaccharides tested (table 17) dextrin or glycogen at a level of 1.5 g/100 g of diet has no effect on larval growth, while potato starch does. A combination of 1.5 g sucrose and 4.5 g potato starch per 100 g of diet improves larval growth significantly; but potato starch alone has an adverse effect (table 18). Besides its nutritive value, potato starch might improve the consistency of the diet, but the latter is a property that is difficult to express quantitatively.

TABLE 17. Effect of different carbohydrates on the weight gain of the third instar larva. Partial substitution of sucrose in Basic Diet-6 by several di-, tri- and polysaccharides.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Constituents tested	g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
D-sucrose	6.0	20	120.8 $\pm$ 4.1
D-sucrose	4.5	17	142.7 $\pm$ 5.4
D-therhalose	1.5		
D-sucrose	4.5	19	121.1 $\pm$ 4.8
D-melezitose	1.5		
D-sucrose	4.5	20	122.6 $\pm$ 3.9
Dextrin	1.5		
D-sucrose	4.5	19	133.4 $\pm$ 4.4
Potato starch	1.5		
D-sucrose	4.5	20	121.8 $\pm$ 3.8
Glycogen	1.5		

TABLE 18. Effect of potato starch on the weight gain of the third instar larva. Substitution of sucrose in Basic Diet-6 by potato starch.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Constituents tested	g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
D-sucrose	6.0	20	120.4 $\pm$ 4.5
Potato starch	0.0		
D-sucrose	1.5	17	137.8 $\pm$ 4.2
Potato starch	4.5		
D-sucrose	0.0	16	103.1 $\pm$ 9.5
Potato starch	6.0		

### 5.6. LIPIDS AND STEROLS

Tests with *Lepidoptera* species have revealed that they require fat in their diet. The fat requirements can be satisfied by either linoleic or linolenic acid. These unsaturated fatty acids are necessary for certain physiological processes, viz. growth, adult emergence and wing development. VANDERZANT (1966) found that linolenic acid promotes growth and adult emergence of *H. zea*, while linoleic acid only promotes growth. CHIPPENDALE et al. (1964, 1965) obtained similar results for the cabbage looper, *Trichoplusia ni* Hübner. For normal emergence of *A. velutinana* linoleic acid has the same effect as linolenic acid (ROCK et al., 1965). Linolenic acid is more effective for promoting emergence of adults of *P. gossypiella* than linoleic acid (VANDERZANT et al., 1957).

Linoleic acid is necessary for growth as well as moulting and normal wing development of *L. migratoria* and *S. gregaria* (DADD, 1960a) and for normal wing development of the emerging adults of *Melanoplus bivittatus* Say (NAYAR, 1964).

Sterol requirements of most insects tested can be satisfied by including cholesterol in the diet. Several other sterols appeared to be as effective as cholesterol, e.g. the plant sterols sitosterol and stigmasterol for the larvae of *A. grandis* (VANDERZANT, 1963) and ergosterol, sitosterol and stigmasterol for the pink boll worm, *P. gossypiella* (VANDERZANT and REISER, 1956b). As compared to  $\beta$ -sitosterol and stigmasterol, cholesterol causes retarded growth in the silkworm, *B. mori* (ITO, 1961). This inferior growth seems mainly due to the lack of feeding stimulation by cholesterol, since the two other sterols were found to be phagostimulatory (ITO et al., 1964). *L. migratoria* and *S. gregaria* are able to utilize cholesterol and its derivatives cholestryl acetate and dihydrocholesterol as well as  $\beta$ -sitosterol (DADD, 1960b).

According to BARANOWA and STEPANOWA (SCHREIBER, 1961), the dry weight fraction of potato leaves contains 7% lipids.

GRISON (1957, 1958) found that application of lecithin to old potato leaves increased the fecundity of the Colorado potato beetle. Lecithin was always used in the artificial diet for the Colorado potato beetle, and its effect on larval growth is the subject of the present experiment. HSIAO and FRAENKEL (1968a) found that lecithin stimulates feeding of the potato beetle larva.

The haemolymph of adult Colorado potato beetles contains palmitic, stearic, oleic, linoleic and linolenic acid (DE KORT, 1967). A mixture of the last three mentioned fatty acids was used in the diet for the Colorado potato beetle instead of only one of them. Cholesterol and  $\beta$ -sitosterol, which are found to be the principle sterols of the potato beetle (VON ARDENNE et al., 1965b), were included in the diet. According to VON ARDENNE et al. (1965a), the potato leaves contain at least four sterols, viz.  $\beta$ -sitosterol, stigmasterol, cholesterol and campesterol, in a ratio 16:12:4:1. The effect of the first three sterols is the subject of the present experiments.

### Method

The effect of lecithin on larval growth was examined by varying its amount in basic diet-3 (table 6) and keeping the fatty acid-sterol mixture constant.

When testing the effect of fatty acids and sterols, the amount of lecithin in basic diet-4 was kept constant at a level of 0.5 g/100 g of diet. When fatty acids were not included in the diet, the sterols were suspended in distilled water containing Tween 80, after being dissolved in hot alcohol (ROCK et al., 1964), to obtain equal distribution in the diet. In other cases, the sterols were dissolved in the fatty acid mixture as described in section 4.2.

### Results

From table 19 it is seen that deletion of lecithin results in retarded growth of the larvae. A maximum effect of lecithin was found at a level of 0.75 g/100 g of diet.

Deletion of fatty acids, sterols, or both from basic diet-4 resulted in poor larval growth (table 20). The average weight gained was reduced significantly when the concentration of the fatty acid-sterol mixture was reduced to 1/3 and 2/3 or increased to 4/3 times the standard concentration in basic diet-4 (table 20).

By testing the effect of the individual sterols at a level of 72 mg/100 g of diet it appeared that cholesterol was the least effective, while  $\beta$ -sitosterol and stigmasterol had an equal effect (table 21). Nevertheless, a maximum effect was obtained only when these sterols were included together in the diet according to the relative concentration ratio found by VON ARDENNE et al. (1965a).

No tests were conducted with the individual fatty acids.

TABLE 19. Effect of different levels of lecithin in Basic Diet-3 on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Vegetable-lecithin g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
0.00	13	21.2 $\pm$ 1.3
0.25	16	56.5 $\pm$ 3.9
0.50	20	87.9 $\pm$ 6.9
0.75	20	100.1 $\pm$ 7.3
1.00	18	84.8 $\pm$ 6.8

TABLE 20. Effect of different levels of fatty acid- and sterol mixtures in Basic Diet-4 on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Fatty acid mixture (ml)	Sterol mixture (mg)	$N_6$	Wt gained mg $\pm$ S.E.
0.00	0	18	94.7 $\pm$ 7.2
0.50	0	17	72.9 $\pm$ 8.1
0.00	48	18	107.1 $\pm$ 5.6
0.50	48	14	112.3 $\pm$ 8.5
0.25	24	16	119.1 $\pm$ 9.8
0.50	48	14	112.3 $\pm$ 8.5
0.75	72	19	145.5 $\pm$ 3.8
1.00	96	20	116.2 $\pm$ 5.4

TABLE 21. Effect of the individual sterols in Basic Diet-3\* on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

mg sterol/100 g of diet			$N_6$	Wt gained mg $\pm$ S.E.
$\beta$ -sitosterol	Stigmasterol	Cholesterol		
36	27	9	19	139.1 $\pm$ 5.3
72	0	0	20	119.5 $\pm$ 4.9
0	72	0	19	120.2 $\pm$ 5.8
0	0	72	19	99.4 $\pm$ 5.4

\* Contains 0.5 g lecithin and 0.75 ml fatty acid mixture.

### 5.7. VITAMINS

Phytophagous insects in general require only the hydrophilic vitamins in their diets, especially B-vitamins and in many cases also ascorbic acid.

Tests under aseptic conditions have revealed that among the B-vitamins nicotinamide, pantothenic acid, riboflavin, pyridoxine, folic acid, and thiamine are required by *C. suppressalis* (ISHII and URUSHIBARA, 1954, cited in VANDERZANT, 1966), *H. antiqua* (FRIEND and PATTON, 1956), *L. migratoria* and *S. gregaria* (DADD, 1961b) and *A. grandis* (VANDERZANT, 1963). Vitamin-B12 is usually included in the diet, but its requirement by insects is not known. It improves larval growth of *H. antiqua* (FRIEND and PATTON, 1956), but it is not indispensable. DADD (1961b) included p-hydroxybenzoic acid in the diet for locusts and grasshoppers. Biotin is included in almost all defined diets.

Many insects do not require ascorbic acid because they can synthesize their own needs. Among phytophagous insects *H. antiqua* and *P. gossypiella* can grow without dietary ascorbic acid (FRIEND and PATTON, 1956; VANDERZANT, 1957). The majority of plant feeders seem, however, to require it, e.g. *L. migratoria*, *S. gregaria* (DADD, 1957, 1960d), *A. grandis*, *H. zea*, *Estigmene acrea* Drury (VANDERZANT et al., 1962), *O. nubilalis* (CHIPPENDALE and BECK, 1964), *T. ni* (CHIPPENDALE et al., 1965) and *B. mori* (ITO, 1961).

Ascorbic acid is essential for the production of fertile eggs by *A. grandis* (VANDERZANT and DAVICH, 1961), but it is not required for larval growth (VANDERZANT et al., 1962).

Choline has been found to be an indispensable nutrient for *O. nubilalis* (BECK et al., 1949). Since VANDERZANT (1959) found that inositol is indispensable for *A. grandis*, this substance is included in many diets for phytophagous insects.

Phytophagous insects in general do not require hydrophobic vitamins in their diet. However, DADD (1957, 1961a) found that omission of  $\beta$ -carotene from the diet of *S. gregaria* resulted in retardation of growth and reduced melanization. Inclusion of vitamin-A acetate improved growth but not pigmentation. This indicates that the growth promoting effect of  $\beta$ -carotene is independent of the effect on pigment formation.

The importance of other fat-soluble vitamins is not known as yet, although several diets for phytophagous insects contain vitamin-D, vitamin-E or vitamin-K (BECKMAN et al., 1953; VANDERZANT et al., 1962; VANDERZANT, 1968).

The artificial diet for the Colorado potato beetle as used in the present study was provided with the vitamin mixture of DADD (1960a). This mixture was included in basic diet-2, at different levels so as to examine the importance of the B-vitamins. It was also the subject of the present experiments to examine the importance of vitamin-A palmitate, which was always included in the diet.

## Results

From table 22 it is seen that variation of vitamin level does not effect larval

TABLE 22. Effect of different levels of B-vitamin mixture in Basic Diet-2 on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Vitamin mixture mg/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
1.38	20	53.1 $\pm$ 6.4
2.76	20	58.6 $\pm$ 6.6
11.04	20	53.8 $\pm$ 5.8

TABLE 23. Effect of  $\beta$ -carotene on the weight gain of the third instar larva. Substitution of vitamin-A palmitate in Basic Diet-3\* by  $\beta$ -carotene.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Constituents tested	mg/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
Vitamin-A palmitate	0	10	98.5 $\pm$ 5.4
	2	20	109.9 $\pm$ 5.4
	10	19	98.6 $\pm$ 6.4
Vitamin-A palmitate $\beta$ -carotene	0	20	94.8 $\pm$ 4.6
	2		
Vitamin-A palmitate $\beta$ -carotene	0	19	90.1 $\pm$ 3.5
	10		

\* Contains 0.5 g lecithin and 0.5 ml fatty acid-sterol mixture.

growth. The result indicates that the concentration ratios of the dietary constituents in basic diet-2 is far from being optimal.

There are indications that basic diet-3 is also still suboptimal. No significant effect of vitamin-A palmitate or of  $\beta$ -carotene is observed on larval weight and on appearance (table 23).

No experiment with ascorbic acid was conducted. This vitamin was always included in the diet.

### 5.8. SALTS

Different commercial salt mixtures which originally were formulated for vertebrates, have been used successfully in insect diets. Among them WESSON'S salt mixture (WESSON, 1932) has been most widely used. Salt mixtures of a more simple composition have been included in defined diets for *P. gossypiella* (VANDERZANT, 1957), *A. grandis* (VANDERZANT, 1965), *O. nubilalis* (BECK et al., 1968) and many other insects.

In the present experiments the effect of salt mixtures after WESSON, U.S.P.-II and that after BECK et al. (1968) was examined.

TABLE 24. Effect of several salt mixtures on the weight gain of the third instar larva. Substitution of Wesson's salt mixture in Basic Diet-2.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Salt mixture	g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
Wesson	0.50	20	53.8 $\pm$ 5.8
U.S.P.-II	0.50	19	51.0 $\pm$ 5.9
Beck et al.	0.50	20	61.4 $\pm$ 5.4

### Results

From table 24 it is seen that substitution of WESSON's salt mixture by salt mixture U.S.P.-II, or salt mixture after BECK et al. (1968) at a level of 0.5 g/100 g of diet does not improve larval growth on basic diet-2. The reason is that the concentration ratios of the dietary constituents are not optimal. Adjustments of the concentration of casein (basic diet-3, table 6) and lipids revealed that minerals are required for larval growth (table 25, 26). A maximum effect was found at a level of 0.5 g/100 g of diet for both WESSON's salt mixture and salt mixture after BECK et al. (1968). The last mentioned mixture seems to be slightly better than WESSON's salts.

### 5.9. DISCUSSION

The aim of the foregoing experiments was to select different possible nutrients to improve the artificial diet. Since the fourth instar larvae are highly tolerant of the artificial diet in terms of food acceptance as well as food utilization, the

TABLE 25. Effect of different levels of Wesson's salt mixture in Basic Diet-3\* on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Salt mixture g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
0.00	19	50.9 $\pm$ 2.9
0.10	19	91.4 $\pm$ 4.1
0.20	18	93.1 $\pm$ 6.1
0.40	20	103.2 $\pm$ 6.7
0.50	19	97.7 $\pm$ 3.9
0.60	20	102.2 $\pm$ 5.7

\* Contains 0.5 g lecithin and 0.5 ml fatty acid-sterol mixture.

TABLE 26. Effect of different levels of salt mixture after Beck et al. in Basic Diet-3\* on the weight gain of the third instar larva.  $N_6$  = number of larvae (out of 20) surviving on the 6th day.

Salt mixture g/100 g of diet	$N_6$	Wt gained mg $\pm$ S.E.
0.00	18	60.5 $\pm$ 5.2
0.10	19	94.5 $\pm$ 9.2
0.30	17	109.9 $\pm$ 7.0
0.40	18	120.9 $\pm$ 6.0
0.50	17	120.8 $\pm$ 8.3
0.60	17	96.9 $\pm$ 8.9

\* Contains 0.5 g lecithin and 0.5 ml fatty acid-sterol mixture.

experiments were conducted with the third instar larvae. Results of experiments with first instar larvae will be presented in the next chapter.

Newly moulted third instar larvae when further reared on potato leaves under the present experimental conditions showed a mean weight gain of  $178.5 \pm 4.7$  mg at the end of the fourth instar. This weight increase was attained within 6 days. The highest weight increase during the same period on an artificial diet was  $145.5 \pm 3.8$  mg.

Undoubtedly suboptimal growth is primarily a result of suboptimal feeding. As mentioned in section 4.3., there is no general rule as yet how to achieve optimal concentration ratios for optimal palatability of a medium. In one of the foregoing experiments an extreme case was observed, i.e. that a diet which otherwise would be accepted was completely rejected by the larvae when all proteins were replaced by pure amino acids (section 5.4.2.). Between this extreme case and the best results so far obtained feeding intensity varied, depending on the qualitative and quantitative composition of the diets.

The pH-values of the diets in the foregoing experiments varied between 5.5 to 7.0, in most cases between 6.0 and 6.5. The effect of pH variations on feeding by this beetle was not studied.

Generally speaking, the concentration ratios of the dietary constituents determine both the degree of feeding and growth. Excess of one or more components, i.e. casein and lipids in the present study, could have been the major cause of the ineffectiveness of potato leaf powder in basic diet-1 (table 7). The qualitative composition of the basic diets 2 and 3 are basically the same (table 6). By reducing the casein concentration in basic diet-2, larval growth was slightly improved (table 9). Thus, there was an excess of casein. The amount of 3.0 g casein per 100 g of diet was then adopted in basic diet-3. When the concentrations of lecithin and fatty acid-sterol mixture were halved, larval growth on the diet containing 3.0 g casein and 4.0 g albumin per 100 g of diet was further improved (table 11).

However, the concentration ratios of the dietary components of basic diet-3 were still far from being optimal.

Adjustments of casein and lipid concentrations in basic diet-3 brought to light that the level of salt mixtures, i.e. 0.5 g per 100 g of diet, was about optimal (tables 25 and 26).

Within the prevailing concentration ratios, the growth promoting effect of amino acids might be reduced by their adverse effect on the palatability of the diet. Anyhow, the use of macro-molecular substances such as casein and albumin as a protein source was desirable with regards to food acceptance.

Also in the case of carbohydrates it appeared to be necessary to include a macro-molecular compound. When 4.5 g of sucrose was substituted by the same amount of potato starch, the consistency of the diet and consequently larval growth were improved. Potato starch is always used by Japanese investigators in developing artificial diets for the silkworm, *B. mori* (HOUSE, 1967).

Results of the experiments with sterols (table 21) indicated that the plant sterols,  $\beta$ -sitosterol or stigmasterol, were more effective than cholesterol. The same phenomenon has been observed by ITO (1961) on *B. mori*. HSIAO and FRAENKEL (1968c) included cholesterol in an artificial diet that supports growth of the fourth instar larvae of the Colorado potato beetle.

The results of the experiments with third instar larvae of the Colorado potato beetle indicate that this beetle apparently requires an extensive assortment of nutrients. However, since there is still a disparity between larval growth on an artificial diet and on potato leaves no conclusion as yet can be drawn. Moreover, not all nutrients known have been tested, and the number of combinations of nutrients offered to the larvae was just a sample chosen from a large number of possible combinations.

## 6. FACTORS RELATED TO FEEDING AND GROWTH OF THE FIRST INSTAR LARVA

### 6.1. INTRODUCTION

The aim of the experiments reported in the foregoing chapter was to search for the best combinations of dietary constituents which, being presented to newly hatched larvae, were expected to be suitable for growth. However, in no case was good growth of the first instar larvae observed. Total larval mortality of more than 90% was a common occurrence, the surviving individuals required more than twice the time they did on potato leaves to complete larval development, while the emerging adults were very small. Since the number of individuals attaining maturity was always small and moreover not constant, the occurrence of adults could be considered as being exceptional.

It was also obvious that newly hatched larvae would not immediately attach themselves to the diet, but just wandered on the surface and frequently left it. Continuous feeding on the diet started on the second day. To prevent the larvae from leaving the diet indefinitely, because of phototaxis, it was necessary to use a black plastic bag during the first 24 hours to enclose the glass tubes in which the larvae were confined.

Furthermore it was observed that on average 20% of the individuals died in the first instar, 20% in the second instar and 50% in the third instar, immediately after the second moult. Most of the surviving individuals felt soft when pinched, indicating that they were not healthy. Despite the addition of anti microbial agents, an unpleasant smell emanating from one day old diet was often detected.

It is clear that the requirements for feeding and growth of the newly hatched larvae are more difficult to satisfy than those of the older larvae. Newly hatched larvae could develop only to the third instar on diet, while third instar larvae from the stock culture could attain maturity when reared on the same artificial diet. This indicates that the digestibility of the diet might be a critical factor. Reserve material carried over from the younger instars during feeding on potato leaves enables the older instars to utilize the artificial diet. Newly hatched larvae, however, can utilize the diet only to a very limited extent.

Because of the above mentioned reason, another method of preparing an artificial diet has been developed (section 6.3.).

### 6.2. FEEDING AND GROWTH ON POTATO LEAVES

The embryonic development of the Colorado potato beetle at 25°C requires four days. Under a dissection microscope a dark colour of the lateral stigmata is seen on the third day. A hatching larva has the same colour as the egg, viz. orange and it is very feeble. The cuticle becomes hard within three hours, while the colour becomes dark. Especially the head-capsule, the legs and the lateral stigmata become black and hard. Immediately the larva starts feeding on the empty egg-shell.

When a larva is released on a potato leaf it walks and finds its way with difficulty because of the leaf hairs. Within no longer than ten seconds, however, it stops and starts moving its head up and down rhythmically indicating that continuous feeding is taking place. Observations on ten young larvae showed that the duration of the first continuous feeding varied between 3'5" to 8'48". When feeding ceases, the larva usually rests on the same spot. In other cases it walks, but not more than a few millimeters away and immediately it stands quietly for several minutes. Examination of the small hole made by the larva revealed that the larva had pierced a hole right through the leaf tissue. In other cases it had eaten only the epidermal and the upper mesophyll tissue of the leaf, leaving the lower parts untouched. Continuous feeding starts again after fifteen to twenty minutes. The already existing hole is broadened or a new hole is made. The average number of feeds of ten larvae during the first hour was three. All larvae started excreting within six hours.

Larvae of the second instar showed a similar pattern of feeding activity and were also more or less sessile.

According to CHLODNY (1967) the total amount of fresh leaf material consumed by one individual larva of the Colorado potato beetle during the whole period of larval development is 1.2719. g. The portions consumed by each of the consecutive instars are respectively I = 2.8%, II = 6.3%, III = 20.8% and IV = 70.1%. However, the 'voracity coefficient', i.e. the ratio of the amount of food consumed per hour and the weight of the larva at the end of the instar, decreases with the succession of the instars. The values of the voracity coefficients are respectively I = 3.00, II = 1.62, III = 0.94 and IV = 1.02. These values indicate that feeding activity of the larvae of the first two instars is relatively very high.

It has been mentioned that young larvae will feed on their own empty egg shells immediately after hatching. The following experiment was conducted to examine whether feeding on an egg-shell is merely a habit or whether it has a certain importance for larval growth.

### Method

Newly deposited eggs were separated from each other and placed on the bottom of a petridish at distances of about 5 mm. After hatching the larvae were reared individually on potato leaves in glass tubes of 2 cm diameter with a capacity of 17.5 m<sup>1</sup>, plugged with a cellulose stopper and incubated at 25 ± 0.5 °C. Every day they were transferred to fresh potato leaves and the weights were determined by means of a torsion balance. After completing larval development they were allowed to pupate in moist sand and the weights of the emerging adults were determined. A record was made of the growth of larvae which were transferred to potato leaves without having the opportunity to feed on the egg shells, larvae which were transferred to potato leaves resp. 6 hours and 24 hours after hatching. The last two mentioned groups of larvae had ample time to feed on their own egg shells. In each test 20 individuals were used.

TABLE 27. Growth of newly hatched larvae reared on fresh potato leaves. Larvae were released on potato leaves resp. (A) 0 hour, (B) 6 hours and (C) 24 hours after hatching. ♀♀ and ♂♂ = emerging adults, females and males. Initial number of larvae 20 per treatment.

Treatment	Mean max. wt.*		Larval period		♀♀		♂♂	
	No.	mg $\pm$ S.E.	No.	Days $\pm$ S.E.	No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.
A	18	183.2 $\pm$ 6.2	18	11.2 $\pm$ 0.2	6	162.2 $\pm$ 5.6	10	138.4 $\pm$ 8.1
B	18	225.1 $\pm$ 4.7	18	10.0 $\pm$ 0.0	9	176.8 $\pm$ 4.4	8	155.4 $\pm$ 5.2
C	19	208.5 $\pm$ 7.2	19	10.7 $\pm$ 0.2	7	170.7 $\pm$ 9.7	8	132.1 $\pm$ 8.1

\* Maximum weight attained prior to pupation.

## Results

The results (table 27) indicate that there is a significant difference in the mean maximum weight of the larvae which had not fed on egg shells and those which were allowed to do so. Moreover the first mentioned group of larvae showed a slower growth rate. On the other hand there is no difference in the mean weight between the newly emerged adults, especially between the females of the three different groups. It can be concluded that the course of larval development is influenced by feeding on egg shells, but the ultimate result expressed in the weights of emerging adults is independent of it.

For the experiments with artificial diets larvae of 6 to 8 hours old were used.

### 6.3. ALTERNATIVE METHOD OF PREPARATION OF ARTIFICIAL DIETS

#### 6.3.1. *Introduction*

The ordinary method of preparing artificial diets containing agar for a non-aseptic rearing of many phytophagous species is in principle the mixing of a nutrient mixture with boiled agar. Usually microbial growth inhibitors are added to the diet. In the case of an aseptic rearing, the diet has first been prepared by the same method without the addition of growth inhibitors. After being cooled, it is autoclaved at 121°C for 15 minutes. To prevent deficiency of the diet because of destruction of several nutrients, excess amounts of heat-sensitive substances are usually added.

Vitamins may also be sterilized by filtration and added to other sterile constituents of the diet at lower temperatures (VANDERZANT, 1962). Although little is known about changes of the chemical characteristics of nutrients during the mixing and heating, successful growth of many species on such diets has been observed.

Artificial diets prepared according to the ordinary method (section 4.2) did not support growth of the first instar larva of the Colorado potato beetle satisfactorily. Nothing is known about the cause of failure of growth. The application of a high temperature treatment was then introduced to the preparation of the diet for a non-aseptic rearing of first instar larvae of this beetle.

TABLE 28. Composition of Basic Diets 7 to 9.

Constituents	Quantity		
	Basic Diet-7	Basic Diet-8	Basic Diet-9
Vitamin free casein	3.00 g	3.00 g	3.00 g
Egg-albumin	4.00 g	4.00 g	4.00 g
L-alanine	0.06 g	0.06 g	0.06 g
Glycine	0.04 g	0.04 g	0.04 g
L-proline	0.04 g	0.04 g	0.04 g
L-serine	0.08 g	0.08 g	0.08 g
Sucrose	6.00 g	2.00 g	2.00 g
Potato starch <sup>a</sup>	—	5.00 g	5.00 g
Vegetable-lecithin	0.50 g	0.50 g	0.50 g
Oleic acid	0.450 ml	0.450 ml	—
Linolenic acid	0.225 ml	0.225 ml	—
Linoleic acid	0.075 ml	0.075 ml	0.75 ml
β-sitosterol	0.036 g	0.036 g	0.10 g
Stigmasterol	0.027 g	0.027 g	—
Cholesterol	0.009 g	0.009 g	—
Mixture of B-vitamins <sup>b</sup>	2.76 mg	2.76 mg	2.76 mg
Choline chloride	0.10 g	0.10 g	0.10 g
Meso-inositol	0.04 g	0.04 g	0.04 g
Ascorbic acid	0.40 g	0.40 g	0.40 g
Vitamin-A palmitate	0.01 g	0.01 g	0.01 g
Menadione	0.005 g	0.005 g	0.005 g
Wesson's salt mixture	0.50 g	0.50 g	0.50 g
Agar, granulated	2.50 g	2.50 g	2.50 g
Alphacel non-nutritive bulk	2.00 g	2.00 g	2.00 g
Potassium hydroxide	0.56 g	0.56 g	0.56 g
Inhibitor <sup>b</sup>	0.27 g	0.27 g	0.27 g
Distilled water (to 100 g)			

<sup>a</sup> Scholten, Foxhol, Holland.<sup>b</sup> Table 1.

### 6.3.2. Method: High temperature treatment

The composition of basic diets no. 7, 8 and 9 is presented in table 28. The casein was dissolved in distilled water containing 0.56 g potassium hydroxide in a glass vial of 375 ml. The other solid ingredients were weighed and added to the casein solution and thoroughly stirred. The fatty acid mixture, the amino acid solution and the vitamin solutions were added to the other dietary components and the whole mixture was again stirred. The vial was then covered with a piece of aluminium foil and autoclaved at 121 °C under 15 pounds pressure for 15 minutes. By this treatment a viscous mass of distinctive layers was obtained. The uppermost part consisted of a thin brown layer. This browning did not occur on omission of ascorbic acid. The hot mass was stirred continuously by means of a Philips' electric hand mixer till the temperature dropped to about 60 °C, and poured out in a petridish. After being cooled to room temperature the diet was stored in a refrigerator.

#### 6.4. REARING PROCEDURE

It was observed in some preliminary experiments that larvae reared in a group in a petridish tended to be cannibalistic when the food was of inferior quality. To prevent them from eating each other, newly hatched larvae were reared individually in glass tubes of 2 cm diameter with a capacity of 17.5 ml.

The diet was cut into rectangular pieces of about 1 g and each piece was put in the glass tube. Six to eight hour old larvae, which had only eaten their own egg shells, were used for the experiments. After a larva was released on the diet by means of a fine brush, the glass tube was plugged with a non-absorbent cellulose stopper. This kind of stopper is suitable for preventing desiccation and allowing ventilation. Especially young larvae are sensitive to desiccation. On the other hand bad results were obtained when newly hatched larvae were reared in a non-ventilated space, viz. when an ordinary cork was used to plug the glass tube.

The glass tubes were placed in a metal rack and the larvae were incubated at  $25 \pm 0.5$  °C. During the first 24 hours they were enclosed in a black plastic bag to prevent them from leaving the diet because of phototaxis. This treatment has later been found to be unnecessary when the quality of the diet was improved. Nevertheless this procedure was applied throughout the present study. After one day the plastic bag was removed. The larvae were reared in a phytotron under the same conditions as the stock material (chapter 2).

Although microbial growth inhibitors were always included in the diet, the larvae were kept for no longer than one day on the diet. They were transferred to clean glass tubes containing fresh diet every day. After completing the larval development they were allowed to burrow into moist sand in plastic boxes to pupate. Newly emerged adults were weighed on a torsion balance.

The initial number of larvae used per experiment was 20.

#### 6.5. EFFECT OF HIGH TEMPERATURE TREATMENT OF ARTIFICIAL DIETS ON FEEDING AND GROWTH

##### 6.5.1. Feeding

It has been observed that delayed feeding on artificial diets by first instar larvae occurred. When a newly hatched larva is released on a piece of diet it immediately presses its mouth and palpi against the diet. This position will be maintained for several seconds. Then it shows biting movements several times, but this activity is seldom followed by continuous feeding as it is on potato leaves. Instead the larva wanders on the surface of the diet and from time to time inserts its mouth parts into the diet. Under the influence of light it may leave the diet. In other cases it will stay quietly for several minutes on the diet, and then it starts moving restlessly. Usually 50% of the individuals will produce excrement only after 12 hours on the diet. Examination after 24 hours showed that all 20 larvae had produced excrement which was spread over the surface of the diet. This indicates that the larvae did not stay in one position. Excavation

of the diet was observed only occasionally, indicating that during the first 24 hours continuous feeding rarely took place.

Consequently growth on artificial diets of different compositions was very poor.

In the present experiment a comparison is made on the feeding of young larvae on two diets of the same composition, but prepared in a different way. The first diet (basic diet-8, table 28), was prepared according to the method described in section 4.2. and the second one by autoclaving (section 6.3.3.).

### Method

The diet in the petridish which was 15 mm thick was cut into rectangular pieces measuring  $5 \times 5 \times 15$  mm and each piece was put on one of its sides on the bottom of a glass tube, while the position of the bottom and the top of the diet was marked.

One newly hatched larva was put in a tube which was then stoppered. The tubes were kept in a black plastic bag. Every day the larvae were transferred to clean tubes containing a piece of fresh diet.

The number of faecal pellets produced within the first and the second 24 hours served as a criterion of feeding activity.

When a larva is adapted to the diet it feeds continuously on the same spot and several faecal pellets are excreted upon each other to form a pile. Usually an excavation of the diet in the immediate vicinity of such an accumulation can be seen. As a criterion of adaptation the number of piles of faecal pellets was used. A group of faecal pellets was denoted as a pile when at least 5 pellets were excreted close to each other and when an excavation of the diet in an adjacent place was observed.

The position of the piles was recorded so as to examine whether there was a preference for a special feeding site. For this purpose imaginary boundaries were made to divide the area of the diet into three equal parts, respectively the basal, middle and top areas.

To differentiate growth from feeding, the larvae were further reared and the number of individuals attaining the adult stage was recorded. From the third day on no observation on feeding activity was conducted.

TABLE 29. Feeding of newly hatched larvae of the Colorado potato beetle on autoclaved and non-autoclaved diet of identical composition (Basic Diet-8, Table 28). E = total number of faecal pellets produced. N = number of larvae (out of 20) excreting. S = total number of faecal piles spread over the basal (B), middle (M) and top (T) parts of the diet. I = number of larvae producing no faecal piles.

Diet	Feeding period	E	N	S	B	M	T	I
Non-autoclaved	1st 24 hours	185	19	8	2	2	4	12
	2nd 24 hours	755	20	25	6	8	11	4
Autoclaved	1st 24 hours	179	16	3	1	0	3	17
	2nd 24 hours	723	20	24	10	4	10	6

## Results

The results presented in table 29 indicate that feeding by newly hatched larvae on the two diets is more or less identical.

All larvae showed a feeding activity only in the course of the second day on diet. Several individuals, respectively 4 and 6, did not adapt to the diet. These larvae did not show continuous feeding, and they died early in the course of the larval period.

It is possible that during the process of cooling of the diet, substances with high specific gravities sink to the bottom of the petridish, while light substances move to the upper surface. If this occurs, it could have a direct bearing either on feeding or larval growth, or on both processes. From the distribution of the faecal piles it was concluded that there was a preference for the upper part (T) of the non-autoclaved diet. However, was this not the case with the autoclaved diet (table 29).

### 6.5.2. Growth

#### Method

The same larvae from the feeding experiment (section 6.5.1.) were reared further on the same diets. Every day they were transferred to clean glass tubes containing fresh diet. Records were made of the number of larvae which died, the number of individuals which completed their larval development and the weights of the emerging adults.

## Results

Although no difference in palatability was observed growth on both diets differed markedly (table 30). On the non-autoclaved diet, larval growth was retarded and after the second moult they could not proceed with development

TABLE 30. Growth of newly hatched larvae of the Colorado potato beetle on autoclaved and non-autoclaved diet of identical composition. Initial number of larvae 20 per treatment.

Criteria	Basic Diet-8*	
	Non-autoclaved	Autoclaved
Larval mortality (No.)		
I	-	1
II	5	2
III	15	-
IV	-	1
Prepupae (No.)	-	16
50% prepupae (Days)	-	19
Pupal mortality (No.)	-	3
Adults, ♀♂/♂♂ (No.)	-	9/4
Mean weight ♀ (mg $\pm$ S.E.)	-	128.4 $\pm$ 6.5
Mean weight ♂ (mg $\pm$ S.E.)	-	97.5 $\pm$ 3.2
♀ ovipositing (No.)	-	2

\* Table 28.

and died immediately. On the autoclaved diet, however, 65% of the individuals reached maturity.

High temperature treatment by means of autoclaving thus improved the quality of the diet.

However, the rate of growth on the diet was comparatively slow. Fifty percent of the individuals reached the prepupal stage after 19 days on diet, while larvae reared on potato leaves under the same experimental conditions completed their development within 11 days at the most.

It is noteworthy that autoclaved diet of one day old remained fresh and did not have an unpleasant odour, whereas such an odour was detected in a number of tubes containing non-autoclaved diet of one day old.

#### 6.5.2.1. Ascorbic acid and B-vitamins

Ascorbic acid and B-vitamins are heat-sensitive substances which are assumed to be destroyed upon heating. In preparing artificial diets for phytophagous species these substances are usually added to other dietary constituents at lower temperatures (VANDERZANT, 1962).

High temperature treatment of the diet for the Colorado potato beetle gives a survival rate of 65% adults (section 6.4.2.). Larval mortality of 35% may partly be attributed to a partial destruction of ascorbic acid and B-vitamins. Therefore excess amounts of these substances in the diet ought to improve larval growth.

The effect of different levels of ascorbic acid and B-vitamins is the subject of the present experiments.

### Results

The results presented in table 31 indicate that ascorbic acid is required for larval growth. Without this vitamin (diet 8-1) no larva could attain maturity. In fact all of them died during the first and second instar. A level of 0.2 g/100 g of diet (diet 8-2) resulted in a slow growth rate, but the emerging adults had similar weights to those which were obtained from the basic diet (diet 8-0). Larval growth rate was not improved markedly when the concentration of ascorbic acid was doubled (diet 8-3). Moreover the number and the average weight of the emerging adults were not increased by increasing the amount of ascorbic acid from 0.4 g to 0.8 g/100 g of diet.

TABLE 31. High temperature treatment of the diet and the effect of ascorbic acid on larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Ascorbic acid g/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	♀		♂	
					No. mg ± S.E.			
8-1	0.0	—	—	—	—	—	—	—
8-2	0.2	30	10	8	3 117.0 ± 4.3	5 90.6 ± 8.1	5 91.6 ± 5.4	4 93.0 ± 7.9
8-0	0.4	22	11	10	5 112.0 ± 5.2	—	—	—
8-3	0.8	20	12	9	9 117.6 ± 3.8	—	—	—

\* The percentage is calculated on the initial number of larvae.

TABLE 32. High temperature treatment and the effect of B-vitamins on larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	B-vitamins mg/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	♀		♂♂	
					No. mg $\pm$ S.E.	No. mg $\pm$ S.E.	No. mg $\pm$ S.E.	
8-11	0.0	—	—	—	—	—	—	—
8-0	2.76	19	14	9	4 127.8 $\pm$ 8.4	5 110.8 $\pm$ 7.2	—	—
8-5	13.80	20	13	11	6 129.2 $\pm$ 5.6	5 113.4 $\pm$ 8.3	—	—

\* See footnote to Table 31.

The same applies to B-vitamins (table 32). Increasing the concentration five-fold does not improve the larval growth rate, number of adults obtained and average weight of adults.

The results of these experiments indicate that the critical factor for optimal larval growth might lie in the overall concentration ratios of the dietary constituents.

#### 6.5.2.2. Albumin and potato starch

Egg-albumin coagulates upon heating. Because of this property dietary nutrients may be improperly mixed when the diet is prepared according to the ordinary method (section 4.2.). Autoclaving at 121 °C under 15 pounds pressure may alter the physical and chemical properties of albumin, which is finely distributed throughout the medium upon stirring.

The subject of the present study is to find out whether the positive effect of autoclaving involves albumin. For this purpose a non-autoclaved diet was developed in which albumin was replaced by the same amount of casein.

Addition of potato starch improves the consistency of the diet. Moreover high temperature treatment may result in the hydrolysis of potato starch, therefore this process promotes digestion. To investigate this possibility potato starch was substituted by soluble starch in a non-autoclaved diet.

#### Results

From table 33 it is concluded that feeding activity of newly hatched larvae is reduced when albumin is omitted from the diet or when potato starch is substituted by soluble starch.

Reduced feeding activity seemed partly due to reduced palatability but partly also to some physical factors. It was observed that a film of water was present on the surface of the above mentioned diets. Moreover diets with soluble starch were sticky. These two factors hampered feeding of the larvae markedly. Observation of the behaviour revealed that the larvae tended to avoid frequent contact with the sticky diet the surface of which was covered with a film of water.

TABLE 33. The importance of albumin and potato starch for larval growth. CA = casein, AL = albumin. PO = potato starch. SO = soluble starch. E = number of faecal pellets produced. N = number of larvae (out of 20) producing faecal pellets.

Diet	CA	AL	PO	SO	1st 24 hrs		2nd 24 hrs		Larval mortality				(No.)
					E	N	E	N	I	II	III	IV	
8-0*	3	4	5	—	179	18	742	20	2	4	14	—	20
8-6*	7**	—	5	—	25	7	211	19	6	7	7	—	20
8-7*	3	4	—	5	8	3	113	15	8	10	2	—	20
8-8*	7**	—	—	5	0	0	97	14	10	10	—	—	20

\* Non-autoclaved.

\*\* Supplemented with L-cystine 50 mg/100 g of diet.

On all non-autoclaved diets no single larva could develop beyond the third instar.

The results of the above mentioned experiments are not conclusive and do not exclude the possibility that hydrolysis of certain nutrients may occur by autoclaving.

#### 6.5.3. Discussion

High temperature treatment of artificial diets is usually applied by investigators to sterilize the diet for aseptic rearing of insects. The results of the present study indicate that autoclaving does not improve the acceptability of the diet. But an autoclaved diet supports growth markedly better than a non-autoclaved diet of the same composition.

The positive effect of autoclaving may lie in the improved digestibility of the diet. Undoubtedly hydrolysis of certain nutrients and destruction of undesirable substances occur during autoclaving, thus promoting digestibility. Moreover, the application of high pressure may improve the dispersal of nutritive substances throughout the whole medium. Hence, all categories of nutrients are proportionally represented in each bite taken by the larva.

The fact that on average 50% of the individuals become adults indicates that the qualitative composition of the diet satisfies the requirements for growth to a great extent. The average mortality of 50% may be then attributed to the presence of undesirable substances in the diet and/or the improper concentration ratios of the dietary constituents. The possible negative effects of growth inhibitors on the metabolism of the larva are not known yet. The effects of these can be studied by omitting them from the diet in an aseptic culture of the insect.

Destruction of nutritive substances, which is not necessarily limited to ascorbic acid and B-vitamins (section 6.5.2.1.), and various other chemical processes may occur during the autoclaving of the diet, thus possibly leading to the formation of undesirable substances which may hamper digestion.

The concentration ratios are usually the ultimate determinant for optimal growth when all other requirements have been met.

Despite some uncertain factors, autoclaving of artificial diets may be sug-

gested as an alternative method of preparing diet. In the case of the diet for the Colorado potato beetle autoclaving leads to the improvement of the physical and chemical properties.

### 6.6. PROTEINS AND AMINO ACIDS

In the course of development of an artificial diet for the Colorado potato beetle, 3% casein plus 4% albumin as a protein source has been found to be the most suitable (basic diet-3, table 6). Subsequently the concentrations of these nutrients were kept constant, while those of the other dietary constituents were varied.

It has been shown that in diets for third instar larvae prepared according to the ordinary method, substitution of casein by albumin and the reverse did not improve larval growth (table 11). The importance of these nutrients for growth of newly hatched larvae reared on an autoclaved diet is the subject of the present experiment. Another test was also conducted to examine the effect of the amino acid mixture, which was always included in the diet.

### Results

The results presented in table 34 indicate that vitamin free casein and egg-albumin could not substitute for each other and at the present state of diet development both should be included in the diet.

TABLE 34. Effect of vitamin free casein and egg-albumin on larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Casein	Albumin	50% PP*	PP	Adults	♀♀		♂♂	
	g/100 g	g/100 g	(Days)	(No.)	(No.)	No. mg ± S.E.	No. mg ± S.E.		
8-0	3	4	20	12	10	6 118.7 ± 3.9		4 102.4 ± 7.6	
8-9	7	—	—	—	—	—	—	—	—
8-10	7 <sup>a</sup>	—	—	1	—	—	—	—	—
8-11	7 <sup>b</sup>	—	—	—	—	—	—	—	—
8-12	—	7	—	4	1	1 106.0		—	—

\* See footnote to Table 31.

<sup>a</sup> Supplemented with L-cystine 50 mg/100 g.

<sup>b</sup> Supplemented with L-cystine 100 mg/100 g.

TABLE 35. Effect of amino acid mixture on larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Mixture	50% PP*	PP	Adults	♀♀		♂♂	
	mg/100 g	(Days)	(No.)	(No.)	No. mg ± S.E.	No. mg ± S.E.		
8-0	220	20	14	11	6 126.3 ± 4.5		5 106.1 ± 5.8	
8-13	0	20	10	7	4 131.2 ± 3.4		3 103.4 ± 6.7	

\* See footnote to Table 31.

From the chemical point of view, the concentration ratios of amino acids in the proteins, which were changed by the substitution of one for the other protein, might be the determining factor. Supplementation of L-cystine to the casein media did not promote larval growth. On the other hand it was observed that the diets without albumin had a different consistency. They were soft and the surface exposed after cutting was wet. It was observed that feeding on diets with these physical properties was far from being optimal.

In the case of the albumin medium, which was hard and had a dry surface, poor larval growth might be due mainly to the absorptivity of the protein and the concentration ratios of the amino acids contained in it.

From table 35 it is seen that the number of emerging adults declined upon deletion of amino acids from the diet. However, the weights of the surviving individuals were similar to the weights of adults from the basic diet. The time required by 50% of the individuals to complete larval development was also the same.

The results of the above mentioned experiments indicate that amino acid supplement is required.

## 6.7. CARBOHYDRATES

In the course of the development of a diet for third instar larvae, a satisfactory result was obtained when 1.5% sucrose and 4.5% potato starch were added to the diet (table 18). Newly hatched larvae grew as well on an autoclaved diet (basic diet-8), which contained 2% sucrose and 5% potato starch.

To examine the effect of potato starch, this dietary constituent was omitted from basic diet-8 and substituted by alphacel or tapioca starch.

### Results

The results presented in table 36 indicate that unless potato starch is added to the diet larval development cannot be completed. Diet 8-14 especially was sticky, and the young larvae could not feed properly on it. Substitution for potato starch by alphacel (diets 8-15, 8-16) appear to have an adverse effect on larval growth. This indicates that potato starch is required with regard to the physical properties of the diet as well as to its nutritive value.

TABLE 36. Effect of potato starch on larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Potato	Alpha-	50% PP*	PP	Adults	♀		♂	
	starch	cel				(No.)	(No.)	mg ± S.E.	mg ± S.E.
8-0	5.0	2.0	19	14	12	7	124.2 ± 6.3	5	100.7 ± 3.4
8-14	0.0	2.0	—	—	—	—	—	—	—
8-15	0.0	3.5	—	2	—	—	—	—	—
8-16	0.0	7.0	—	—	—	—	—	—	—

\* See footnote to Table 31.

TABLE 37. Effect of tapioca starch on larval growth. Substitution of potato starch by tapioca starch. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	5 g/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	♀♀		♂♂	
					No.	mg ± S.E.	No.	mg ± S.E.
8-0	Potato starch	19	14	12	7	124.2 ± 6.3	5	100.7 ± 3.4
8-17	Tapioca starch	22	13	8	4	117.0 ± 8.4	4	94.0 ± 2.5

\* See footnote to Table 31.

However, these two functions can be fulfilled to a limited extent by tapioca starch (table 37). On the diet with tapioca starch the length of the larval period was longer and the number of individuals attaining maturity was less than on the basic diet, but the weights of the adults emerging from both diets were similar.

#### 6.8. LIPIDS AND STEROLS

The lipid fraction of the diet for the Colorado potato beetle consisted of lecithin, oleic acid, linoleic acid and linolenic acid. Besides these substances three kinds of sterols were added to the diet, i.e.  $\beta$ -sitosterol, stigmasterol and cholesterol. The lipid and sterol requirements of many phytophagous larvae of *Lepidoptera* can be satisfied by one kind of fatty acid and one kind of sterol (section 5.6.).

The successful method of preparation of diet by means of autoclaving prompted the examination of the effect of the individual fatty acids and sterols on the larval growth of the Colorado potato beetle. This is the subject of the following experiment.

#### Results

The results presented in table 38 indicate that lecithin is required for larval growth. Growth in terms of number of individuals completing larval develop-

TABLE 38. Effect of different levels of lecithin on larval growth. Fatty acid mixture 0.75 ml and sterol mixture 0.072 g/100 g resp. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Lecithin g/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	♀♀		♂♂	
					No.	mg ± S.E.	No.	mg ± S.E.
8-18	0.0	—	6	1	—	—	1	104.0
8-19	0.25	23	13	5	2	115.5	3	108.0 ± 3.5
8-0	0.50	17	14	11	6	120.7 ± 3.6	5	97.6 ± 3.5
8-20	0.75	21	13	9	5	124.2 ± 6.1	4	88.3 ± 7.3
8-21	1.00	16	15	12	7	113.9 ± 3.1	5	97.8 ± 3.3

\* See footnote to Table 31.

TABLE 39. The importance of lecithin for larval growth. Substitution of lecithin by fatty acid-sterol mixtures. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Lecithin	Fatty	Sterols	50% PP*	PP	Adults	♀	♂
	g/100 g	acids ml/100 g	g/100 g	(Days)	(No.)	(No.)	No. mg $\pm$ S.E.	No. mg $\pm$ S.E.
8-0	0.5	0.75	0.072	19	13	9	5 116.2 $\pm$ 8.4	4 109.5 $\pm$ 7.1
8-22	0.0	0.75	0.072	—	2	1	—	1 98.0
8-23	0.0	1.50	0.144	18	16	11	5 110.3 $\pm$ 5.7	6 107.2 $\pm$ 6.1
8-24	0.0	3.00	0.288	—	—	—	—	—

\* See footnote to Table 31.

ment, number of adults obtained and weights of the emerging adults did not increase by increasing lecithin level from 0.5 g to 1.0 g/100 g of diet.

The lecithin could be omitted from the diet when the amounts of fatty acid mixture and sterol mixture were doubled (table 39). On further increase of the fatty acid and sterol concentrations growth was very poor (diet 8-24). It was concluded that this was mainly due to a reduction in the palatability of the diet because of an excess of fatty acids.

With the presence of 0.5 g lecithin 0.75 ml of linoleic acid/100 g of diet (diet 8-26) could replace the fatty acid mixture in the basic diet (table 40). Among the three unsaturated fatty acids, oleic acid appeared to be the least effective

TABLE 40. Effect of individual fatty acids on larval growth. Lecithin 0.5 g and sterols 0.072 g/100 g resp. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	0.75 ml/100 g	50% PP*	PP	Adults	♀	♂
		(Days)	(No.)	(No.)	No. mg $\pm$ S.E.	No. mg $\pm$ S.E.
8-0	Mixture**	19	14	9	4 117.8 $\pm$ 9.4	5 108.2 $\pm$ 7.2
8-25	Oleic acid	—	4	2	—	2 88.5
8-26	Linoleic acid	19	14	14	6 111.7 $\pm$ 5.2	8 93.5 $\pm$ 5.1
8-27	Linolenic acid	(29)	9	7	3 120.0 $\pm$ 9.7	4 88.8 $\pm$ 10.5

\* See footnote to Table 31.

\*\* Composed of 0.450 ml oleic acid, 0.075 ml linoleic acid and 0.225 ml linolenic acid.

TABLE 41. Effect of different levels of  $\beta$ -sitosterol on larval growth. Lecithin 0.5 g and linoleic acid 0.75 ml/100 g resp. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	$\beta$ -sitosterol g/100 g	50% PP*	PP (No.)	Adults (No.)	♀	♂
					No. mg $\pm$ S.E.	No. mg $\pm$ S.E.
9-1	0.0	—	2	—	—	—
9-0	0.10	19	13	12	5 120.1 $\pm$ 6.2	7 102.1 $\pm$ 7.4
9-2	0.20	17	14	13	7 119.1 $\pm$ 5.6	6 96.2 $\pm$ 6.7
9-3	0.40	20	10	10	4 114.7 $\pm$ 5.8	6 93.8 $\pm$ 5.2
9-4	0.60	20	12	9	4 112.0 $\pm$ 5.2	5 91.2 $\pm$ 4.3

\* See footnote to Table 31.

(diet 8-25). Larval growth rate on diet with linolenic acid (diet 8-27) was significantly slower than on the basic diet, but the weights of the emerging adults were similar.

The results presented in table 41 indicate that the lipid and sterol fractions of the diet can be reduced to lecithin, linoleic acid and  $\beta$ -sitosterol. The diet containing 0.5 g lecithin, 0.75 ml linoleic acid and 0.1 g  $\beta$ -sitosterol was designated as basic diet-9. Deletion of  $\beta$ -sitosterol from the diet resulted in poor larval growth (diet 9-1). However, growth was not promoted by increasing the concentration of  $\beta$ -sitosterol from 0.1 g to 0.6 g/100 g of diet.

$\beta$ -sitosterol could be substituted by the same amount of stigmasterol, cholesterol or ergosterol (table 42). These sterols were found to be equally effective.

TABLE 42. Effect of different sterols on larval growth. Lecithin 0.5 g and linoleic acid 0.75 ml/100 g resp. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	0.1 g/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	$\text{♀}$		$\text{♂}$	
					No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.
9-0	$\beta$ -sitosterol	(20)	9	9	5	117.2 $\pm$ 4.3	4	99.4 $\pm$ 6.8
9-5	Stigmasterol	21	11	10	6	106.7 $\pm$ 5.4	4	94.7 $\pm$ 6.6
9-6	Cholesterol	19	11	11	6	115.8 $\pm$ 4.1	5	98.2 $\pm$ 5.2
9-7	Ergosterol	19	12	11	4	114.3 $\pm$ 3.7	7	95.1 $\pm$ 5.9

\* See footnote to Table 31.

### 6.9. FAT SOLUBLE VITAMINS

In the course of diet development vitamin-A palmitate and menadione have been included. The effect of these substances on larval growth was examined in the present study by deleting them from the standard diet. The effect of  $\alpha$ -tocopherol acetate, the function of which is to protect certain substances against oxidation, was studied as well.

TABLE 43. The importance of vitamin-A palmitate,  $\alpha$ -tocopherol acetate and menadione for larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Treatment	50% PP* (Days)	PP (No.)	Adults (No.)	$\text{♀}$		$\text{♂}$	
					No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.
8-0	Basic Diet	20	13	9	4	126.7 $\pm$ 8.4	5	108.2 $\pm$ 7.2
8-28	B.D. + 10 mg $\alpha$ -tocoph. ac.	20	12	10	6	128.1 $\pm$ 9.2	4	104.6 $\pm$ 6.1
8-29	B.D. minus vit. A palm.	19	12	12	6	125.7 $\pm$ 6.6	6	101.0 $\pm$ 6.4
8-30	B.D. minus menadione	21	11	9	3	124.3 $\pm$ 3.2	6	95.5 $\pm$ 5.7

\* See footnote to Table 31.

## Results

The results presented in table 43 indicate that none of the above mentioned vitamins has a positive effect on larval growth. A diet without vitamin-A or menadione supports larval growth as well. Addition of  $\alpha$ -tocopherol acetate does not improve the quality of the diet.

### 6.10. SALTS

The optimum concentration of WESSON's salt mixture in the diet for third instar larvae was between 0.4–0.6 g/100 g of diet (table 25). The importance of the same salt mixture is the subject of the present experiment. No experiments were conducted on the effect of the individual salts constituting the mixture.

## Results

From table 44 it is seen that no single larva attained the adult stage on a diet without salts. On the other hand an increase of concentration to 1.0 g salt mixture/100 g of diet resulted in a slow growth rate and a higher pupal mortality. The surviving individuals, however, attained similar weights to those reared on the basic diet.

TABLE 44. The importance of Wesson's salt mixture for larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Salt mix.-W. g/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	♀		♂	
					No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.
8-31	0.0	—	—	—	—	—	—	—
8-0	0.5	18	14	12	8	127.4 $\pm$ 6.5	4	98.5 $\pm$ 3.2
8-32	1.0	24	13	7	4	124.5 $\pm$ 6.7	3	106.0 $\pm$ 5.3

\* See footnote to Table 31.

### 6.11. CRUDE PLANT MATERIALS

Diets containing crude plant materials from the host plant have been developed successfully for various phytophagous insects. The role of host plant material seems to be the supply of certain substances essential for promoting feeding and/or growth (DAVID and GARDINER, 1966). Crude material from other plant species, e.g. wheat germ, has been included in the diets for many phytophagous species. It has been shown by BECK et al. (1968) that the primary role of wheat germ in the diet for *O. nubilalis* is improving the concentration ratios of the mineral fraction of the diet.

The effect of crude plant materials on growth of *Leptinotarsa* larvae is the subject of the following experiments.

#### 6.11.1. Crude material from the host plant

In the present study the effect of fresh potato leaves and potato leaf powder

TABLE 45. Effect of fresh potato leaves on larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Leaves g/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	♀♀		♂♂	
					No. mg ± S.E.	No. mg ± S.E.	No. mg ± S.E.	
7-0	0	(29)	4	3	1 108.5		2 96.7	
7-1	5	25	12	11	4 116.0 ± 6.2		7 102.8 ± 3.6	
7-2	10	17	12	10	6 140.7 ± 7.2		4 106.5 ± 8.9	
7-3	25	18	15	11	6 136.7 ± 8.4		5 116.4 ± 6.4	

\* See footnote to Table 31.

was examined. Fresh potato leaves were autoclaved at 121 °C for 15 minutes in a glass vial containing water and then pulverized. Different amounts of fresh potato leaves treated in this way were added to basic diet-7 (table 28). This diet has been derived from basid diet-4 (table 6).

The effect of potato leaf powder was examined by means of basic diet-8 (table 28).

## Results

The results presented in table 45 indicate that fresh potato leaves promote larval growth markedly. Increasing the amount of potato leaves from 5.0 g to 10.0 g/100 g of diet resulted in faster growth. But growth was not improved by increasing the amount of potato leaves to 25.0 g.

The effect of potato leaves on larval growth rate was, however, less significant, when basic diet-8 was used (table 46a). But the positive effect of potato leaves was manifested in the high number of emerging adults (diet 8-33). Potato leaf powder had practically no effect on the growth of the Colorado potato beetle.

Although the ultimate result, when expressed as the weights of the emerging adults, is similar, differences have been observed in feeding response (table 46b). Feeding of newly hatched larvae on the diets containing plant material was markedly better than on the basic diet. The results indicate that feeding during

TABLE 46a. Effect of fresh potato leaves and potato leaf powder on larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	Crude material	g/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	♀♀		♂♂	
						No. mg ± S.E.	No. mg ± S.E.	No. mg ± S.E.	
8-0	—	—	18	13	12	6 135.7 ± 6.5		6 111.0 ± 6.4	
8-33	Fresh leaves	10.0	16	16	16	9 130.8 ± 3.3		7 113.3 ± 4.7	
8-34	Leaf powder	1.0	—	8	8	3 142.7 ± 4.5		5 110.2 ± 6.6	

\* See footnote to Table 31.

TABLE 46b. Effect of fresh potato leaves and potato leaf powder on larval feeding. E = number of faecal pellets produced. N = number of larvae (out of 20) excreting.

Diet	Crude material	g/100 g	1st 24 hours		2nd 24 hours	
			E	N	E	N
8-0	—	—	98	16	378	20
8-33	Fresh leaves	10.0	265	20	548	20
8-34	Leaf powder	1.0	256	20	392	20

the initial stage may have a direct bearing on the growth and development of the larvae.

Further it was observed that the larvae reared on the basic diet and on the diet containing leaf powder were white as compared to those reared on the diet with fresh potato leaves, which were yellow-orange. The same colour was also to be seen on the eggs of the next generation, when the emerging adults were reared on the same diet. These results indicate that certain substances contained in potato leaves, which are responsible for the coloration of the larval body and the eggs become ineffective when the leaves are dried. But coloration and growth seem to be determined by two mechanisms, which are independent of each other.

#### 6.11.2. Crude material from other plant species

##### 1. Lettuce

As shown in table 45, larval growth was improved when fresh potato leaves were added to basic diet-7. To examine whether fresh potato leaves contain specific substances required for growth, these leaves were substituted by leaves of cut lettuce, *Lactuca sativa* L.

##### Results

The results indicate that the same amount of fresh lettuce can replace potato leaves to a certain extent (table 47a). Growth on diet containing lettuce is slower, but the weights of the emerging adults on both diets are similar. The primary effect of potato leaves seems to lie in the promotion of feeding of the young larvae (table 47b).

TABLE 47a. Effect of fresh lettuce leaves on larval growth. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	15 g/100 g	50% PP* (Days)	PP (No.)	Adults (No.)	♀		♂	
					No.	mg ± S.E.	No.	mg ± S.E.
7-4	Fresh potato leaves	21	11	10	3	126.6 ± 4.0	7	96.4 ± 7.2
7-5	Lettuce leaves	25	9	9	6	123.5 ± 3.9	3	108.0 ± 4.6

\* See footnote to Table 31.

TABLE 47b. Effect of fresh lettuce leaves on larval feeding. E = number of faecal pellets produced. N = number of larvae (out of 20) excreting.

Diet	15 g/100 g	1st 24 hours		2nd 24 hours	
		E	N	E	N
7-4	Fresh potato leaves	193	20	617	20
7-5	Lettuce leaves	27	10	101	19

## 2. Wheat germ

Wheat germ has been used in diets for several phytophagous species. The effect of this crude plant material on the growth of *Leptinotarsa* larvae is the subject of the present study. In the following experiments wheat germ was added to basic diet-8, either or not replacing casein and albumin.

## Results

It was observed that the feeding responses on the above mentioned diets were more or less identical. However, diets containing wheat germ did not support larval growth satisfactorily (table 48).

The addition of wheat germ may upset the balance of the dietary nutrients. This could explain the poor growth of the larvae.

TABLE 48. Effect of wheat germ on larval growth. CA = vitamin free casein. AL = egg-albumin. WH = wheat germ. PP = prepupae. Initial number of larvae 20 per treatment.

Diet	g/100 g			50% PP* (Days)	PP (No.)	Adults (No.)	♀		♂	
	CA	AL	WH				No.	mg±S.E.	No.	mg±S.E.
8-0	3.0	4.0	—	20	11	9	5	125.2±6.1	4	99.2±7.4
8-35	3.0	4.0	3.00	—	5	3	1	130.0	2	90.0
8-36	—	4.0	3.00	—	—	—	—	—	—	—
8-37	3.0	—	4.00	—	—	—	—	—	—	—

\* See footnote to Table 31.

## 6.12. INHIBITORS AND ASEPTIC REARING TECHNIQUES

For non-aseptic rearing of phytophagous insects on an agar based medium it is necessary to include microbial growth inhibitors in the diet. In the present study sorbic acid, methyl p-hydroxybenzoate and streptomycine sulfate were used. Conceivably, these substances exert an adverse effect on the metabolism of *Leptinotarsa*.

Therefore we have tried to develop an aseptic rearing method in order to examine the importance of the above mentioned inhibitors as well as to devise a technique for studies of nutritional requirements.

The results obtained so far are not satisfactory. In no case could larvae of the

Colorado potato beetle attain maturity when reared aseptically according to the present method. Some data obtained from the experiments are reported here to illustrate these preliminary studies.

### Method

Two diets of the same composition, viz. basic diet-9 (table 28), from which the inhibitors were omitted, were prepared respectively according to the ordinary method (section 4.2.) and the autoclaving method (section 6.3.2.). After being cooled the diets were cut into pieces of approximately 5 grams each, which were then put in glass tubes of 2 cm diameter with a capacity of 17.5 ml. These tubes were tightly plugged with cotton wool and put in a glass vial which was then covered with a glass lid for autoclaving. They were autoclaved at 121°C under 15 pounds pressure for 15 minutes. The tubes were then kept overnight in a metal rack at room temperature. After 24 hours condensed water inside the glass tube had disappeared.

A piece of stainless wire was fixed at one end to a metal holder and the other end was bent to make a noose. Eggs of three days old were carefully separated from each other by means of the noose and washed for 5 minutes in a mercuric chloride solution according to VANDERZANT and DAVICH (1958). This solution consists of 0.25 g mercuric chloride, 0.5 g sodium glycocholate, 6.5 g sodium chloride, 1.25 ml concentrated hydrochloric acid, dissolved in 250 ml ethyl alcohol and 750 ml distilled water. The eggs were rinsed once in sterile distilled water in a petridish and transferred aseptically to the diet by means of the above mentioned noose.

### Results

The results presented in table 49 indicate that under aseptic rearing conditions no larva completed its development on the diet, which otherwise supported growth (compare to diet 9-0, table 41). Desiccation of the diet was observed, but this seemed not to be the major cause of poor larval growth, since transferring the larvae aseptically to fresh diet every 5 days did not improve the result. Experiments with other diets gave similar results.

The possible adverse effect of autoclaving the diet twice could not be demonstrated (table 49).

TABLE 49. Growth of *Leptinotarsa* larvae under aseptic conditions. N = initial number of larvae.

Diet	Autoclaving	N	Larval mortality (No.)				
			I	II	III	IV	Total
9-0	Once	19	3	1	11	4	19
9-0	Twice	18	2	2	6	8	18
9-0*	Once	18	3	2	5	2	12**

\* Larvae were transferred aseptically to fresh diet every 5 days.

\*\* Six tubes were contaminated and discarded.

### 6.13. MASS REARING

The adverse effect of the diet prepared according to the ordinary method (section 4.2.) was revealed by the occurrence of cannibalism among the larvae reared in large numbers. The positive effect of autoclaving prompted the test for the quality of the diet in connection with the above mentioned interaction between larvae.

#### Method

Five pieces of autoclaved diet of 1 g each were put on a filter paper disk in a petridish of 9 cm diameter. Ten newly hatched larvae were released on the filter paper and incubated in the phytotron at  $25 \pm 0.5^\circ\text{C}$ ., without being enclosed in a black plastic bag. The larvae were transferred to fresh diet in a clean petridish every day. For this experiment, which was conducted in 10 replicates, diets 8-0 and 8-33 (table 46a) were used.

#### Results

It was observed that the larvae immediately after their release in the petridish moved straight to the diet and showed biting responses. After several minutes they dispersed again. Six hours later most of them remained quietly on the diet. The next day they had a swollen abdomen, indicating that food intake had taken place. The presence of excrement confirmed this view.

No case of cannibalism was recorded in the course of this experiment. On both diets more than 50% of individuals attained maturity (table 50) which corresponds with the results obtained when the larvae were reared individually (compare table 46a).

TABLE 50. Mass rearing of first instar larvae. PP = prepupae. Initial number of larvae 100 per treatment.

Diet	50% PP* (Days)	PP (No.)	Adults (No.)	♀		♂	
				No.	mg $\pm$ S.E.	No.	mg $\pm$ S.E.
8-0	16	75	53	23	124.3 $\pm$ 5.4	30	109.5 $\pm$ 5.8
8-33	16	78	62	34	122.8 $\pm$ 4.6	28	98.2 $\pm$ 4.9

\* The percentage is calculated on the initial number of larvae.

### 6.14. OVIPOSITION

Adults of the Colorado potato beetle require food for reproduction. As long as there is constant access to the food supply, females in confinement will deposit their eggs on different substrates, e.g. potato leaves, filter paper, plastic or glass. This indicates that under certain conditions the nature of the substrate does not play a decisive role in the choice of oviposition site.

Adults obtained from the culture on an artificial diet were reared on the same diet for oviposition. It was observed that males on the fourth day after emerg-

ence started to copulate. Mating behaviour of the males reared on artificial diets did not differ from those reared on potato leaves. However, most of the females were uncooperative. Usually the females which accepted males would lay eggs. Dissection of non-laying females of different ages revealed that they had varying degrees of development of the ovaries. In many cases the ovaries of three weeks old females were undeveloped. These females were never cooperative. The physiological condition of the females, which were undoubtedly influenced by the quality of the food, determined their mating behaviour.

Variations in the longevity of adults, number of females laying eggs, pre-oviposition period, number of eggs laid and percentage of viable eggs were observed.

Some data obtained from experiments with diets 8-0, 8-33 and 8-34 (see table 46a) are presented to illustrate the effect of the artificial diet on oviposition.

#### Rearing procedure

Adults of the Colorado potato beetle were put in pairs in petridishes of 9 cm diameter provided with a filter paper disk and a piece of diet of approximately 5 grams. When the number of males was less than the number of females, they were transferred daily from one female to the other. The beetles were transferred every day to clean petridishes containing fresh diet.

Eggs were collected twice daily to prevent them from being eaten by the adults. They were separated carefully by means of a spatula and put on the bottom of a petridish at distances of approximately 5 mm from each other. The number of hatched larvae was recorded.

#### Results

From table 51 it may be seen that not all females produced eggs. Moreover oviposition started only after three weeks, while beetles reared on potato leaves laid eggs within less than one week after emergence. The average number of eggs laid by the diet-grown females ranged from 10 to 20 % of the value obtained from beetles reared on potato leaves (for comparison see table 5). The percentage of viable eggs laid on the experimental diets ranged from 70 to 90 %.

Similar results have been obtained in experiments with other diets.

### 6.15. GROWTH OF THE SECOND GENERATION

In general the growth of the second generation of insects reared on the same diet was very poor as compared to the growth of the first generation. As an illustration data obtained from the experiments with the diets 8-0, 8-33 and 8-34 are presented in table 51. The larvae were reared according to the procedure described in section 6.4.

#### Results

It is seen from table 51 that larval mortality is very high. Similar results have

TABLE 51. Oviposition of first generation females and growth of the second generation on the same artificial diet.

Criteria	Diet		
	8-0	8-33	8-34
<i>First generation*</i>			
♀♀ (No.)	6	9	3
♀♀ ovipositing (No.)	2	2	2
Avg. preoviposition period (Days)	23	19	20
<i>Second generation</i>			
Eggs laid on the first 14 days (No.)	284	171	253
Viable eggs (No.)	206	142	227
Initial number	20	20	20
Prepupae (No.)	4	4	2
Adults, ♀♀ (No.)	1	—	—
Adults, ♂♂ (No.)	—	2	1
Mean weight ♀♀ (mg)	126	—	—
Mean weight ♂♂ (mg)	—	103	84

\* Table 46a.

been obtained in experiments with other diets. On average 5 to 10% of the hatchlings gave rise to adults. However, in general the number of adults in the second generation was significantly lower than in the first generation.

## 7. DISCUSSION

The Colorado potato beetle is an oligophagous insect. However, the chemical basis of host plant selection in this beetle has not yet been identified in terms of specific 'feeding stimulants' (RITTER, 1967; HSIAO and FRAENKEL, 1968b, c).

Despite its oligophagous character, successive instars of this beetle show an increasing degree of tolerance towards foreign food such as artificial diets. It has been shown in chapter 4 that larvae of the last instars and adults accepted an artificial diet more readily than larvae of the younger instars did. This diet did not contain crude material or any specific substance from the host plant. In view of this property diets that support growth of the third instar larva have been developed (chapter 5). The development of the artificial diet was conducted empirically by 'trial and error', since there were no clear cut directives. It appeared that unless macro-molecular substances were included (sections 5.4. and 5.5.) larval growth was far from being optimal. These macro-molecular substances, including casein, albumin and potato starch, seem to be essential for the construction of the physical properties of the diet. The terminology used for describing the physical properties of artificial diets does not cover all aspects. Consistency is a conception for which no exact quantitative description is available, but the term is commonly used. It is associated in some way with the ratio of the solid components and the water content of the diet. Casein, albumin and potato starch improve the consistency of the diet for the Colorado potato beetle. However, the physical requirements for feeding are closely related to the chemical requirements. When the protein fraction of the diet was replaced by amino acids (section 5.4.) the physical properties of the diet including the consistency changed. Consequently, feeding did not take place. The joint effects of the various components of the food on the gustatory senses of the insect determined the degree of feeding. Depending on the qualitative and quantitative composition of the food additive, synergistic and antagonistic effects may play a role (RITTER, 1967; HSIAO and FRAENKEL, 1968a). Amino acids as a substitute of casein and albumin may change the physical properties of the diet and at the same time the balance of nutritive substances. Antagonistic effects now may become dominant and feeding is prevented.

Besides the physico-chemical requirements for feeding there are chemical requirements for growth. The general procedure for the development of the diet (chapter 5) implies the testing and discarding of various possible nutrients and at the same time selecting substances which are neutral or active when tested individually and which sustain feeding and promote growth in combination with other substances. The degree of growth was determined by the qualitative and quantitative composition of the food. Growth of the third instar larvae, for instance, was poor when lecithin was omitted from the diet (table 19). This applied also to sterols (table 20) and salts (tables 25 and 26). Reduction of the amount of casein from 6.0 g to 3.0 g/100 g of diet improved larval growth (table 9). For each substance there is a concentration at which growth is optimal.

Increasing the amount of one of the dietary constituents does not necessarily result in improved larval growth, since other substances may become the limiting factor. This applies to lecithin (table 19) and salts (tables 25 and 26).

In the course of the development of the diet it appeared that the best diets for third instar larvae did not support growth of first instar larvae satisfactorily. Newly hatched larvae were unable to complete larval development on these diets. This might be due to the following reasons:

Assuming that the qualitative composition of the diet was correct, poor larval growth may be the result of improper concentration ratios of the dietary constituents. This imbalance of the nutrients may either be the result of 1. inadequacy of the formulation of the diet, 2. the indigestibility of certain nutrients, 3. the presence of unfavourable substances which prevent the digestion of certain nutrients or 4. the presence of some substances which act as antimetabolites. Several of these possibilities may occur simultaneously.

The significantly improved larval growth on a diet which was autoclaved confirmed the assumption that the qualitative composition of the diet was more or less correct (table 30). The positive effects of autoclaving may be: 1. the destruction of unfavourable substances, 2. the hydrolysis of certain nutrients and 3. an improved dispersal of nutrients throughout the whole medium.

It is known from literature that autoclaving is usually applied to sterilize media for aseptic rearing of insects. In the present study autoclaving was introduced in order to improve the quality of the diet. It has been shown that this also applies to a diet formulated for *P. brassicae* (WARDJO, 1969).

The improved method of preparing an artificial diet by means of autoclaving made it possible to determine to some extent the importance of several substances for larval growth. Lecithin, which was always included in the diet appeared to be required. Deletion of this phospholipid from the diet resulted in poor larval growth (table 38). However, a mixture of unsaturated fatty acids may replace lecithin (table 39). Among the fatty acids tested linoleic acid appeared to be the most effective (table 40). Beta-sitosterol, stigmasterol, cholesterol and ergosterol could substitute each other (table 42). Absence of ascorbic acid resulted in poor larval growth, indicating that this vitamin is required (table 31).

It should be noted that throughout the present study, the experimental insects taken at random from the stock culture showed a certain pattern of response. Firstly, there was always a small fraction out of the total of 20 larvae which could not adapt to the artificial diet at all (table 29). Secondly, the weights of the emerging adults after different treatments in one experiment usually did not show significant differences. Differences were found in the duration of the larval stage, the numbers of individuals completing larval development and the numbers of emerging adults. These factors should be taken into consideration in the interpretation of the results. For instance, the omission of amino acids decreased the number of emerging adults by 36% (table 35). Increased concentration of WESSON's salt mixture prolonged the duration of the larval stage by 6 days and reduced the number of emerging adults by 33%, but no significant

difference was observed in the average weight of the emerging adults. Detailed studies of the effects of the individual compounds are necessary.

The results of the present study indicate that the Colorado potato beetle can be reared in principle on an artificial diet composed of non-specific dietary constituents. This finding agrees with the statement by VANDERZANT (1966) that most phytophagous insects can be reared in principle on an artificial diet without specific plant substances, when all other requirements are met. Moreover, the qualitative composition of the diet for *Leptinotarsa* does not differ essentially from diets for many other phytophagous insects. This indicates that the present results support the general opinion that the nutritional requirements of various insect species are qualitatively more or less similar, but from species to species differ a great deal quantitatively.

Larval growth on the best diets developed in the present investigation was, however, still suboptimal, even when crude material from the host plant was added to the diet (tables 45, 46a, b). This was deduced from the slow growth rate, the reduced percentage of individuals attaining maturity, the low percentage of females laying eggs and the increased larval mortality in the second generation (tables 46a, 51).

The results of the present study should therefore be considered as a basis for further study which may be conducted now more systematically. Several aspects which are to be considered may be summarized as follows:

The suboptimal growth of *Leptinotarsa* larvae may be caused by the absence of some non-essential nutritive substances in the diet, or of an improper concentration ratios of the dietary nutrients. These non-essential nutritive substances may be amino acids or other chemicals which are important for inducing feeding activity or for procuring a short-cut in the metabolic processes of the insect. VANDERZANT (1958, 1965) showed that growth of the pink boll worm and the boll weevil was suboptimal when the diet only contained the ten essential amino acids. Addition of non-essential amino acids resulted in the acceleration of growth and increase of body weight. In this context it is interesting to note that several non-essential amino acids appeared to be phagostimulatory in Colorado potato beetle larva (RITTER, 1967; HSIAO and FHAENKEL, 1968a).

An improper concentration ratio of dietary constituents may be the result of heat treatment of the diet. Presumably autoclaving may cause the loss of some heat-sensitive substances, which then may become the limiting factor. Addition of extra amounts of these substances may alleviate the deficiency. It is known that heat treatment of meat and fish beyond 100°C may result in loss of lysine and histidine (DEN HARTOG, 1963).

Modifications in the quality and quantity of the different nutrient categories of the diet should as much as possible be based on their corresponding compositions in the host plant. MEDICI and TAYLOR (1966) based the salt formulation of the artificial diet for *Tribolium confusum* (Duval) on elemental analyses of the insect's natural food and the beetles themselves. Applying this principle to different categories of nutrients may lead to the formulation of an artificial diet that supports optimal growth of the Colorado potato beetle.

Our findings indicate that specific substances from the host plant are not required for successful rearing of the Colorado potato beetle on an artificial diet. From the point of view of insect-host plant relationship these results should not be interpreted as indicating that secondary plant substances do not play a role in the aforesaid relationship. Growth of an insect reared under artificial conditions may follow other principles than in nature. Confinement in a no-choice situation may result in starvation, and this may lead to a decrease of the threshold value above which the artificial diet becomes acceptable. When the diet does not contain any deterrent substance, food intake occurs, and the amount eaten depends on the physical and chemical properties of the diet. The rate of growth is determined by the qualitative and quantitative composition of the diet. In fact a diet of known chemical composition which is suitable for continuous rearing of an insect provides us with a means for the study of some aspects of ecology (YAMAMOTO, 1969) or for the analysis of the nutritional requirements of the insect species (HOUSE, 1969).

The results of the present study may be of some help in the development of a diet for insect mass rearing, a diet for studies concerning the physiology and the ecology of this species, as well as a diet for studies of its nutritional requirements.

## 8. SUMMARY

A brief account of the history of the development of artificial diets for phytophagous insects is given. Some conceptions with regard to terminology are discussed (chapter 3).

Artificial diets for the larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, were developed and in addition some factors determining the feeding and rate of growth of the larvae have been brought to light.

Larvae of the four consecutive instars reared on a diet were compared concerning their feeding and growth response (chapter 4). From the increased feeding activity of the larvae of the successive instars it appeared that an advance in tolerance towards the diet was observed as the larvae grew older. In addition it was noticed that as being in an advanced instar, the larvae were better able to accomplish their development on the diet.

Because of the above mentioned phenomenon diets for the third instar larvae were based on the results of tests on the effects of various substances on larval growth (chapter 5). The nature of the substances tested as well as the concentration ratios of the dietary constituents appeared to be the most critical factors. Macro-molecular substances had to be included in the diet in view of the physico-chemical requirements for feeding and growth.

First instar larvae reared on the best diets formulated so far for the third instar, could only attain the third instar.

Non-aseptic rearing of newly hatched larvae on an autoclaved diet revealed that although palatability remained unchanged, the nutritive value of the diet was increased remarkably by heat treatment (chapter 6, section 6.5.). Further experiments, however, are required to determine the factors causing this positive effect of autoclaving.

In many cases during subsequent experiments 50% or more of the individuals reared on an autoclaved diet from the time of hatching attained maturity. Addition of potato leaf powder appeared to promote feeding; however, it has no effect on growth. Inclusion of fresh potato leaves in the diet at a level of 10% led to an increased feeding activity as well as an improved growth rate. However, growth ceased after two generations on diets with or without crude material from the host plant.

In the present study it has been shown that ascorbic acid is required for larval growth. Commercial  $\beta$ -sitosterol, stigmasterol, cholesterol and ergosterol proved to be equally effective as sterol sources.

An aseptic rearing technique for studies of the nutritional requirements was also developed, however, without satisfactory results.

The improved quality of the diet by means of autoclaving enabled a mass rearing of larvae under non-aseptic conditions.

In the general discussion (chapter 7) it was concluded that in principle the Colorado potato beetle could be reared on an artificial diet without addition of specific substances from the host plant. The results of the present study support the general opinion that the nutritional requirements for phytophagous insects are qualitatively more or less similar, but differ to a great extent quantitatively between species.

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## 10. SAMENVATTING

De geschiedenis der ontwikkeling van kunstmatige diëten voor fytofage insecten wordt in het kort weergegeven. Enige begrippen in verband met de gebezigde terminologie worden besproken (hfdst. 3).

Voor de larven van de Coloradokever, *Leptinotarsa decemlineata* Say, werden kunstmatige diëten ontwikkeld, waarbij enige factoren die bepalend zijn voor voeding en groei van de larven aan het licht zijn gekomen.

Van larven van de vier verschillende stadia, voortgekweekt op een dieet, werden vraat en groeiresultaten vergeleken (hfdst. 4). Uit de verhoogde vraat-activiteit van de larven van de opeenvolgende stadia bleek een stijging in tolerantie t.o.v. het dieet waarneembaar te zijn, naarmate de larven ouder waren. Daarbij werd opgemerkt dat, naarmate de larven zich in een ouder stadium bevonden, zij beter in staat waren hun ontwikkeling op het dieet te voltooien.

Met inachtneming van bovengenoemd verschijnsel werden er voor larven van het derde stadium diëten ontwikkeld, gebaseerd op de resultaten van proeven die gedaan werden om het effect van verschillende stoffen op de groei van de larven na te gaan (hfdst. 5). De belangrijkste bepalende factoren bleken zowel de aard der onderzochte stoffen als de concentratieverhoudingen van de dieet-bestanddelen te zijn. Met het oog op de physisch-chemische voorwaarden die aan vraat en groei verbonden zijn, moesten er aan het dieet macro-moleculaire stoffen toegevoegd worden.

Larven van het eerste stadium die voortgekweekt werden op de beste diëten, tot dusver samengesteld voor het derde stadium, waren slechts in staat zich tot aan het derde stadium te ontwikkelen.

Een niet-aseptische kweek van eilarven op een geautoclaveerd dieet wees uit dat, hoewel de smaak van het dieet onveranderd was gebleven, de voedingswaarde door de warmtebehandeling sterk was toegenomen (hfdst. 6, sectie 6.5.). Maar er zijn meer experimenten nodig om te kunnen vaststellen welke factoren dit positief resultaat van het autoclaveren hebben bewerkstelligd.

In daarop volgende proeven werd geconstateerd dat in vele gevallen bij het kweken op een geautoclaveerd dieet 50% van de eilarven of een hoger percentage het imaginale stadium bereikten. Toevoeging van aardappelbladpoeder bleek de vraat wel te stimuleren, maar heeft geen effect op de groeiresultaten. Toediening van verse aardappelbladeren tot een waarde van 10% van het totale dieetgewicht leidde zowel tot een verhoogde vraatactiviteit als tot betere groeiresultaten. Op diëten met of zonder ruwe stoffen uit de waardplant hield de groei in beide gevallen echter op na twee generaties.

In dit onderzoek werd aangetoond dat de aanwezigheid van ascorbinezuur noodzakelijk is voor de groei van de larven. Als sterolbron bleken  $\beta$ -sitosterol, stigmasterol, cholesterol en ergosterol, uit de handel verkregen, in dezelfde mate effect te hebben.

Ter bestudering van de voedingsbehoeften werd ook een aseptische kweektechniek ontwikkeld, die echter geen bevredigende resultaten opleverde.

Door de verbeterde kwaliteit van het dieet dankzij het autoclaveren werd een massale kweek van larven onder niet-aseptische condities mogelijk.

In de algemene discussie (hfdst. 7) concluderen wij, dat het in principe wel mogelijk is de Coloradokever op een kunstmatig dieet zonder toevoeging van specifieke stoffen uit de waardplant te kweken. De resultaten van dit onderzoek sluiten aan bij de algemene opvatting, dat de voedingsbehoeften van fytofage insecten in kwalitatieve zin min of meer gelijk zijn, maar dat er in quantitatief opzicht grote verschillen bestaan tussen de soorten onderling.

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Successive instars of larvae of *L. decemlineata* from the time of hatching reared on an artificial diet without any specific substance from the host plant.

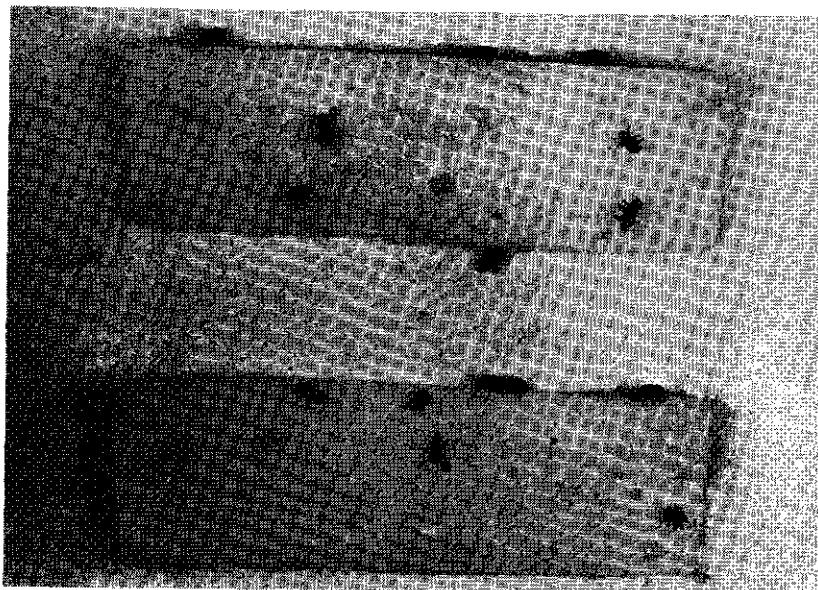
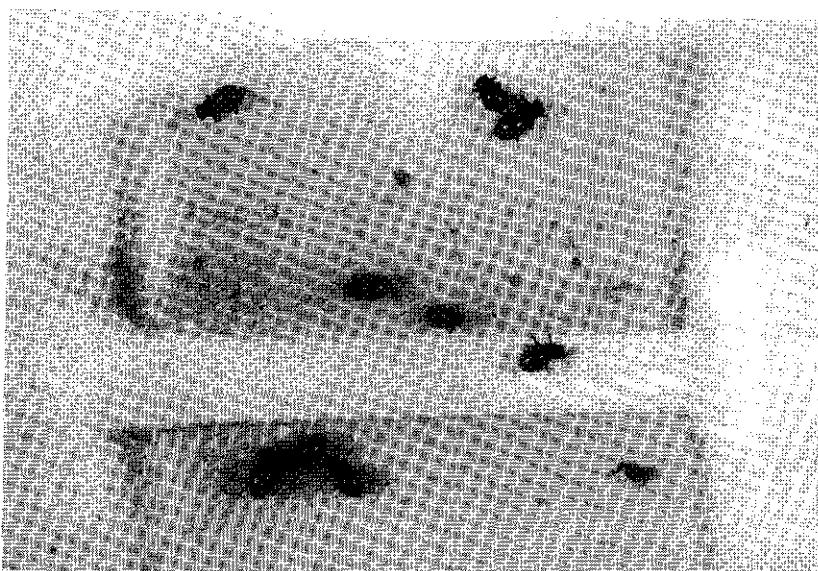


PHOTO 1. Newly hatched larvae, a few seconds after release on diet.

PHOTO 2. First instar larvae after two days on diet.



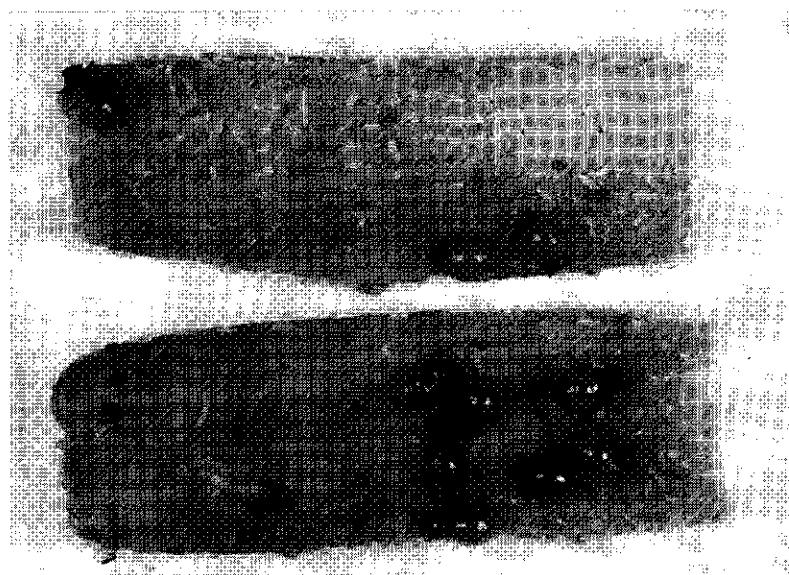


PHOTO 3. Second instar larvae.

PHOTO 4. Third instar larvae.

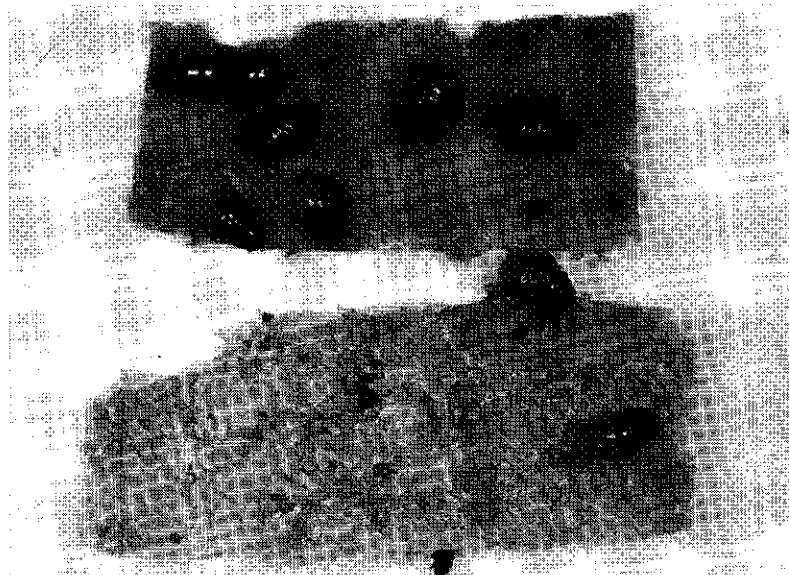




PHOTO 5. Fourth instar larvae.

PHOTO 6. Fourth instar larvae after cessation of feeding (prepupae).

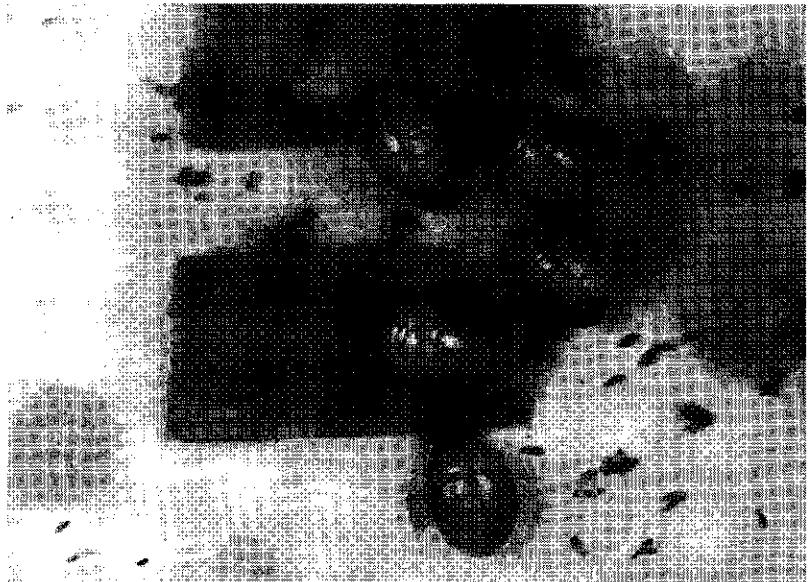




PHOTO 7. Adult Colorado potato beetles.