TIJDSCHRIFT OVER PLANTENZIEKTEN

Onder redactie van Mej. H. L. G. DE BRUYN; Dr J. A. A. M. H. GOOSSENS;
Ir P. HUS; Prof. Dr A. J. P. OORT en Dr H. J. DE FLUITER (Secretaris)

Redactie-adres: Lab. voor Entomologie, Rijksstraatweg 37, Wageningen
Drukkers-Uitgevers: H. Veenman & Zonen - Wageningen

56e JAARGANG (1950) 1e AFLEVERING JAN.-FEBR.

STUDIES ON THE PHYSIOLOGICAL RELATIONS BETWEEN THE LARVAE OF *LEPTINOTARSA DECEMLINEATA* SAY AND SOME SOLANACEOUS PLANTS

Met een samenvatting: Onderzoekingen over de physiologische betrekkingen tussen de larven van de Coloradokever (Leptinotarsa decemlineata Say) en enkele Solanaceae

BY

CHUN-TEH CHIN

CONTENTS

CHAPTER I

INTRODUCTION

On the basis of the diversity in feeding habits, phytophagous insects are generally classified as monophagous, oligophagous, and polyphagous. Monophagous and oligophagous insects can feed on only one and a few species of food plants respectively, while polyphagous insects feed indiscriminately on a large number of plants. Though this arbitrary division is universely used to indicate the dietary habits of this vast horde of hexapods, the difference generally appears to be one of degree. A monophagous insect would rather starve to death in the absence of the proper food plant; this is exemplified by the larva of *Vanessa urticae*, which, in Holland, feeds only on *Urtica dioica*. Not to mention the strictness in the host selection of many hymenopterous entomophagous parasites, the specialization of the feeding habits of these monophagous insects can only be compared with that of some helminthes among the invertebrates. Many parasitic cestodes are characterized by their unique host relationships and the nematode worms of the genera *Heterodera* and *Tylenchus* may occur as strains that attack the species of host plant in which their parents lived (STEINER, 1925). Polyphagous insects may feed and grow on numerous species of plants belonging to very diverse taxonomic groups, but they always show some preference for one or at most a few species. Thus the caterpillars of the gypsy moth, *Lymantria dispar*, prefer oak trees to a large number of other broad-leaf trees, on which they may also feed. These instances indicate that food selection in plant-feeding insects is a very wide-spread phenomenon.

Food plant selection involves the instinct of locating the specific food plants. In phytophagous insects this capacity is very highly developed, and the unerring accuracy with which they find their food plants caused the earlier naturalists to assume that they were possessed of a kind of botanical instinct which enabled them to distinguish the systematic relations of plants. The Dutch botanist VERSCHAFFELT (1910) did very important pioneer work concerning the mechanism of food selection. His study on the response of the larvae of cabbage butterflies, *Pieris rapae*, to the mustard oils contained in their food plants initiated the idea that some plants are subject to the attack of certain insect pests because of the presence of some chemicals such as essential oils and glucosides which are attractive to the pests. At the present time, host plant recognition is generally attributed to their visual, tactile, and chemical senses (PAINTER, 1943). Chemical senses, under the terms of olfaction and gustation, are in most cases far more important than the others, especially in the larvae of holometabolous insects whose visual organs are poorly developed. The relationships between insects and their food plants were established through a long period of association and under the constant action of natural selection. As a result, the physiological constitutions of the insects were adjusted to the chemical characters of these plants and the insects now have to depend upon them as the sole means of maintaining their existence. Since the food plants are by no means always aggregated in certain areas, the insects must provide some way to find them. Visual organs in some insects are important in food plant recognition, but in most phytophagous insects the olfactory and gustatory senses are more relied on for this purpose. The odour and taste of plants, which may be caused by the presence of some volatile substances in small quantities, are universely employed as the tracers by these food hunters.

Here we must not mix up the problems of food plant selection with those of nutrition. Food selection seems essentially connected with insect sense organ physiology and orientation, while nutrition has to do with the proper utilization of the food after ingestion and the adequacy of all required nutrients and accessory dietary factors such as vitamins and minerals. Two species of plants of the same nutritive value and containing the same quality and quantity of accessory factors may meet very different responses from an insect. One is chosen as the food on account of the presence of a small quantity of substance which is responsible for the preferred odour or taste of the insect but has nothing to do with nutrition. The other is rejected because it has a repulsive odour or taste which is produced by chemicals which may not be really poisonous to the insect. An insect may also feed on a „wrong" plant with low nutritive value or even containing poisons simply because it contains chemicals on which the insect relies in locating its „right" food plants. Any maladjustment of the insect to its food plants may cause adverse effects in its life; and economic entomologists usually seek for knowledge in this respect in order to provide a basis for effective means in preventing pest ravages.

The Colorado beetle is an oligophagous insect whose food plants are limited to some species of the family Solanaceae. It has become a menacing pest in agriculture after adopting the habit of feeding on the cultivated potato, *Solanum tuberosum*, about a century ago. An account of the change of its food habit was given by BRUES (1940).

Solanaceous plants are rich in alkaloids, which make them as a group „notorious hallucinants, the drugs of fanaticism" (GILMAN 1938). The Colorado beetle can feed and grow on a small number of them, and the food relation is determined by some physiological bases involving the following factors. Firstly the insect can only live on those plants whose alkaloids and other chemical substances will not cause adverse effects in its life process. Secondly the response of the insect towards the food plants is apparently conditioned by the presence or absence of some chemical constituents which have either attractive or repulsive effects on the insect. MCINDOO (1935) demonstrated with his olfactometer the attractiveness of the odours of some food plants to the adult beetle. RAUCOURT and TROUVELOT (1936) pointed out that the feeding of Colorado beetle larvae on potato leaves was conditioned by the presence of some attractive chemical constituents in the leaves and they have determined many properties of the chemicals by studying the feeding action of the larvae. Whether these constituents are responsible for the odours of the food plants shown by MCINDOO to be attractive, is not known, but the chemical stimulations exerted on the sense organs by these constituents appear important in food selection. Thirdly the insect can only live on those plants whose nutritive level is high enough to maintain its development and existence. TROUVELOT and his collaborators found that Colorado beetles could feed well on *Solanum hendersonii*, but they failed to grow. The adverse effect may be attributed to the inadequate nutritive value of that plant (TRAGER, 1947).

In recent years in Europe, efforts have been made to find some tuberiferous solanaceous plants on which the insect fails to grow or to flourish. Such plants are referred to as resistant to the Colorado beetle. *Solanum demissum* is an example and it can be interbred with *Solanum tuberosum* to form interspecific hybrids (TROUVELOT and MÜLLER-BÖHME, 1938). These hybrids are potentially of economic value in the production of potato varieties immune to the notorious ravages of this pest. The adult may lay eggs upon these plants, but the adverse effects in the developmental stages of the insect will prevent the pest from causing any damage. The study of the adverse effects in the larval stage will throw light on the value of these plants.

The Colorado beetle larvae can feed on some varieties of the garden tomato and on *Petunia hybrida*, a common ornamental plant in Europe. The poisonous effect of *Petunia* is very apparent and the larvae will always perish when they „wrongly" feed on this plant. They can, however, eat a considerable quantity of the leaves which perhaps contain some attractive substances. Though this insect cannot flourish on tomato plants, female beetles were observed to lay eggs upon them (BRUES, 1940), and in some regions damage has been recorded (TOWER, 1906).

The present paper deals with the results of a series of laboratory studies carried out in 1948 and 1949 concerning the physiological relationships between the Colorado beetle larvae and some of their food plants, accompanied by a search of the literature relating to the subject. The aim of the studies is to clarify the feeding habits of the larvae, their behaviour in the presence of food plants involving the sensory mechanisms concerned in food selection and orientation, and the influence of the food plants on various physiological processes, such as survival and growth, digestion, circulation, and respiration.

This investigation was aided in part by a grant from the "INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK" for which the writer wishes to express his sincere gratitude. Special acknowledgements are made to Prof. Dr J. TEN CATE and Dr J. DE WILDE for their guidance in the course of the work. The writer is deeply indebted to Mr C. T. GIMINGHAM who has kindly read and corrected the manuscript.

CHAPTER II

MATERIALS AND BREEDING METHODS

The adult Colorado beetles used for oviposition were obtained from the Entomological Laboratory of the University of Amsterdam. They were the offspring of a group of beetles collected from potato fields in 1946. In the autumn of 1948, beetles previously kept in the shade on a minimum amount of potato leaves were stimulated to commence oviposition by transfering them to a temperature of 25 °C and under the illumination of a 100-watt electric lamp during the night. When enough leaves were given, these beetles would lay eggs about one week after being placed under such conditions. A temperature around 25 °C and an adequate supply of good fresh potato leaves seemed to be the most important requirements to keep the oviposition process continuing. Prolonged illumination might be important in inducing oviposition, but when oviposition had started it appeared to be less important. An account of a method for rearing Colorado beetles throughout the season was given by SWINGLE and his collaborators (SWINGLE et al. 1941).

About twenty beetles (females and males approximately equal in number) were constantly kept in a rearing cage of the size 30 × 45 × 60 cm. for oviposition. Fresh potato plants were supplied almost daily and care was taken to maintain the temperature always between 20 and 27 °C.

The egg masses clipped from the leaves were kept separately in glass vials and these vials were put in hygrostats with suitable humidities, i.e. around 60–70 % R.H. At 25 °C these eggs would hatch in about four days. Egg masses were also kept in rearing dishes with moistened filter paper at their bottoms. Since the newly hatched larvae were apt to feed on the incubating eggs, the egg masses in one dish should be of the same age and separated at proper distances. The prompt removal of the hatched larvae or eggs containing hatching larvae provided the best means of preventing cannibalism.

A newly hatched larva usually has a body weight of about 0,5 mg. Larvae after feeding on eggs may show a considerable gain in their body weight. Since these larvae might have quite different physiological status, they were never used in the growth and mortality experiments.

Larvae after hatching were reared in glass dishes with diameters of 11 cm and 9 cm and depths of 6,5 cm and 5 cm respectively. Potato leaves were changed almost daily in these dishes. When the larvae had reached the fourth instar they were transferred into a breeding cage with moistened soil at the bottom; and potato leaves were supplied daily in the cage. Some larvae were reared into adults. The full-grown larvae usually became orange in color and showed a strong positively geotactic response; they would burrow into the soil and reach the pre-pupal stage. Adults emerged about two weeks later.

The normal food plants used for maintaining the culture were potatoes of several commercial varieties including "Noorderling", "Koopman's Blauwe", "Eerstelingen", and "Bintje". In late autumn and winter, potato sprouts were secured from cool-stored old potato tubers sent from the Centraal Instituut voor Landbouwkundig Onderzoek in Wageningen. They were planted in the greenhouses in the Hortus Botanicus of Amsterdam.

Solanum demissum and *Petunia hybrida* were also cultivated in the Hortus Botanicus. The *Solanum demissum* used in the experiments consisted of several "lines", including "line 23", "line 48", and the hybrid of "line 34" and "line 48". The two former „lines" were regarded as resistant to the Colorado beetle and the hybrid as less resistant. All other plants used in the experiments were obtained from the Hortus.

CHAPTER III

THE FOOD RANGE AND FEEDING HABITS OF THE LARVAE

1. THE LARVAL FOOD RANGE

The larval food of *Leptinotarsa decemlineata* SAY is essentially decided at the time when the gravid females lay their eggs. The ovipositing beetles normally deposit the egg masses on the leaves of the plants they themselves feed upon and the taste of the hatched larvae is generally in agreement with that of their parents. Though differences may be found in the food preference of the adult and the larva, or even of the different stages of the larva (TROUVELOT, et al., 1933*b*), the food range of the larva in general reflects that of the adult in this species.

According to various authors, the original food plant of the Colorado beetle was *Solanum* (*Androcera*) *rostratum*. About the middle of the nineteenth century, it found that potatoes were a suitable food plant, and the shift of the feeding habits, which greatly helped the insect in its dispersal, was no doubt due to the introduction of potatoes into the region where the insect occured. For detailed earlier accounts concerning the food plants, reference is made to the work of TOWER (1906) and BRUES (1940).

This insect only grows on some species of plants belonging to the genus *Solanum*. Its oligophagous habit was first noticed in the more or less frequent attacks on several plants belonging to other genera in Solanaceae such as tomatoes (*Lycopersicum esculentum*), ground cherry (*Physalis* spp.), and certain varieties of cultivated tobacco (*Nicotiana* spp.) that developed a low nicotine content in the leaves. In his field experiment from 1932 to 1935, BRUES in America used more than twenty-four species in the genus *Solanum* for testing the food preferences of this beetle. He found that the insect would greedily accept and voraciously feed upon *Solanum rostratum* , *S. dulcamara*, *S. melongena*, *S. marginatum*, *S. subinerme*, and wonder-berry (an unidentified species), eat with a lessened appetite *Solanum pyracanthum* and *S. atropurpureum*, and refuse *Solanum nigrum*, *S. pseudocapsicum*, *S. barbisetum* and *S. granulosa-leprosum*. His results also showed that though both the adult and the larva had a fairly wide range of food plants, the insect could only occur continually on *Solanum rostratum* and *S. melongena* in addition to potato; the female beetles might deposit eggs on tomato, but the adverse effect due to the food plant usually killed the larvae before pupation (BRUES, 1940). Since 1931, very thorough studies on the food range and feeding habits of the Colorado beetle have been carried out in France by TROUVELOT and his collaborators, with a view to finding varieties of potato less liable to the ravages of the pest than those then cultivated. Among contemporary workers, KOZLOVSKY reared the insect from egg to adult on four varieties of tomato, *Hyoscyamus niger*, and flowering plants of *Atropa belladonna*, though young plants of the last species were poisonous to the larvae (FEYTAUD, 1932). FEYTAUD in 1933 found that the larvae could feed on *Petunia* and *Salpiglossis* which, however, were toxic to them (GUILLAUME, 1934). TROUVELOT and THENARD (1931) observed the reaction of the young larvae towards *Lycopersicum esculentum*, *Nicotiana*, *Phaseolus vulgaris*, etc., and noticed the influence of pilosity of these plants on the feeding and movement of the larvae. TROUVELOT et al. (1933*a*, 1933*b*) also artificially induced the larvae to feed on various Solanaceous plants and found that *Solanum marginatum* and *S. stramonifolium* were the most attractive and that the rate of growth on these plants was the greatest. *Solanum cornutum*, *S. dulcamara*, and *S. rostratum* were the next most successful, *Solanum tuberosum*, *S. lacinatum*, and *S. etuberosum* were less attractive, and *Solanum pyracanthum*, *S. halbisii*, and *S. heterodoxum* were only able to maintain prolonged larval development. On *Solanum atropurpureum* larval development was short, but mortality was high. *Solanum ciliatum* and *S. caldasii* were not attractive. In addition to these species of *Solanum*, *Datura ferox* was greedily attacked but not the double *Datura* of Egypt. On *Nicotiana affinis*, *N. sanderae*, and *N. polydiclia* there was complete development, but *N. rustica saufi* was little attacked and the Paraguay variety of *N. tabacum*, *N. sylvestris*, *N. glaucea*, and *N. paniculata* were completely rejected. Their results showed that there was a continuous gradation from the plants preferred by the larvae to those refused. They attributed this to differences in the pilosity of the foliage and in the chemical constitution of the plants. Thus the young larvae would cease to feed on *Solanum insulaepaschalis*, *S. pseudocapsicum*, and *S. nigrum* after a few bites and keep on feeding on *S. tuberosum* and *S. dulcamara*. On *Solanum hendersoni* and *S. capsicastrum* they would feed but did not grow and death ensued four or five days later. These investigators also noticed that there was no constant agreement in the larval and adult tastes and a species such as *Solanum ciliatum* which was definitely rejected by the adults might be suitable for the larvae to complete their development. There was not even constant agreement in the behaviour of the larvae in the same developmental stage towards the same species of plant and the partial or total immunity of a plant to their attack therefore did not depend upon a single character only.

Further observations in France and Germany revealed that there was a group of Solanaceous plants, including *Solanum demissum*, *S. polyadenum*, *S. henryi*, *S. jamesii*, and *S. chacoense*, which could be attacked by this insect to a greater or less extent, but the adverse effect of these plants on the insects made them very resistant (BUSNEL and CHEVALIER, 1938; SCHAPER, 1938). The interesting point discovered is that the characters causing resistance to attack by the insect in these plants can be preserved in the hybrids between potato and some of these species, especially *Solanum demissum* and *S. chacoense* (TROUVELOT and MÜLLER-BÖHME, 1938; TOXOPEUS, 1949).

It is not proposed here to make a complete list of the accepted or rejected species in the family Solanaceae in reference to the feeding of the Colorado beetle. The ecological value of such observations will be limited because there may be a number of potentially acceptable plants with which the insect still has no chance to come in contact. This is exemplified by *Solanum sisymbriifolium*, which, when available in the field, may provide a highly acceptable food plant for the insect (BARBER, 1933). On the other hand, however, the long list of accepted Solanaceous plants will give invaluable information concerning the common characters which make species susceptible to attack, as pointed out by TROUVELOT and his co-workers. With this idea in mind, the writer carried out observations concerning the food range of the young larvae by using the materials that were available.

In the summer of 1949, about fifty-five species of Solanaceous plants belonging to nineteen genera were tested with second and third instar larvae of the Colorado beetle. The leaves of the plants were separately placed in rearing dishes with moistened filter paper at the bottom to maintain a suitable humidity. Into each dish five well-fed larvae reared on potato (two second and three third instar) were introduced and observations were made two, twenty-four, and fourty-eighth hours respectively after the larvae were put into the dishes. Any occurrence of damage on the leaves due to feeding was noticed.

It was found that on the following twenty-two species of plants, feeding occurred within two hours after the larvae could reach the leaves. They were regarded as attractive to the young larvae when feeding took place almost at once.

1. *Hyoscyamus niger pallidus*
2. *H. albus major*
3. *Datura stramonium tatula*
4. *D. meteloides*
5. *D. fastuosa*
6. *Nicotiana sanderae*
7. *N. alata*
8. *Lycopersicum esculentum*
9. *L. racemigerum*
10. *Atropa belladonna*
11. *Atrophanthe sinensis*
12. *Solanum dulcamara*
13. *S. jasminoides*
14. *S. oblongum*
15. *S. nigrum*
16. *S. nigrum luteum*
17. *S. nigrum ochroteuca*
18. *S. nigrum villosus*
19. *S. simplicifolium*
20. *S. polyadenium*
21. *Schizanthus pinnatus*
22. *Lycium halimifolium*

Delayed attack occurred on the following thirteen species which were regarded as less attractive to the young larvae. Marks of attack were found only at the second observation and it was assumed that the larvae would feed on them only when starved.

1. *Datura stramonium*
2. *D. innoxia*
3. *D. gigantea*
4. *Lycium chinense*
5. *Petunia hybrida*
6. *P. violacea*
7. *P. nyctaginiflora*
8. *Scopolia lurida*
9. *Solanum capsicastrum*
10. *S. pseudocapsicum*
11. *Schizanthus wisetonensis*
12. *Mandragora officinarum*
13. *Nolana prostrata*

Feeding was still further delayed on the following seven species and was noted only at the third observation. They are assumed to be very slightly attractive to the young larvae.

1. *Datura inermis*
2. *D. quercifolia*
3. *D. ferox*
4. *Nicandra violacea*
5. *Nicotiana paniculata*
6. *N. glutinosa*
7. *Physochlania orientalis*

No feeding was observed on the following thirteen species. They were definitely rejected by the young larvae even under long starvation and the early death of the larvae on the species of *Nicotiana* and *Iochroma coccina* showed that these plants had a poisonous effect.

1. *Datura arborea*
2. *Nicandra physaloides*
3. *Nicotiana suaveoleus*
4. *N. glauca*
5. *N. rustica*
6. *N. tabacum*
7. *Scopolia carniolica*
8. *Physalis Alkekengi*
9. *Cestrum tomentosus*
10. *C. parqi*
11. *C. aurantiacum*
12. *Fabiana imbricata*
13. *Iochroma coccinea*

At the end of forty-eight hours, it was seen that a comparatively large amount of foliage had been consumed on the species nearly all of which the larvae attacked within two hours after they came into contact. Though the Colorado beetle can attack a fairly large number of the species, there are six or seven among them on which the larvae are known to be able to complete development and give rise to adult beetles. These are *Hyoscyamus niger*, *Nicotiana sanderae*, *Lycopersicum esculentum*, *Atropa belladonna*, *Solanum dulcamara*, and *Lycium halimifolium* (FEYTAUD, 1932; TROUVELOT et al., 1933*a*, 1933*b*; DE WILDE, 1946). It seems that no common physical character such as pilosity or the thickness of the epidermal cuticle in these plants can account for their susceptibility to attack; the only plausible explanation is the particular chemical constitution of these plants which is able to fulfill the physiological requirements of ingestion and digestion. In the field, the beetle cannot flourish on *Lycopersicum esculentum* (BRUES, 1940), indicating that this species is at the limit of suitable food plants; and undoubtedly there are many plants which bear a resemblance to this species with regard to suitability as food plants for this insect.

It was also observed that fourth instar larvae are less fastidious in their feeding habits than the young larvae. The young larvae completely rejected *Datura arborea*, *Nicandra physaloides*, and even *Petunia hybrida*, but fourth instar larvae could feed on them under certain circumstances. They could feed on *Petunia hybrida* when well fed, on young foliage of *Datura arborea* when starved, and on *Nicandra physaloides* when much starved.

2. THE INFLUENCE OF TEMPERATURE

The rate of larval growth of the Colorado beetle, as well as that of other insects, is regulated on the one hand by the availability of an adequate amount of the necessary food, and on the other hand by the physical conditions in which the insect is living. Among the physical factors that affect the physiological processes of an insect, temperature is the most important. It is known that increase of temperature to a certain point reacts favourably on the life processes of an organism because the chemical reactions on which the retention and release of energy depends are accelerated by a rise in temperature. Between the thermal threshold and the optimal temperature for the development of a given species, therefore, growth is quickened by high temperatures and retarded by low ones. Since organic growth denotes the accumulation of materials assimilated from food in the organism under consideration, the rate of ingestion of food will be largely dependent on the temperature. In the Colorado beetle, the total amount of potato foliage needed to complete larval development may not be vastly different under differing conditions (TROUVELOT and MÜLLER-BÖHME, 1938), but the speed in utilising them is greatly influenced by temperature. Moreover, a high temperature can affect the activity of the larva and thus influence its feeding habits. The following two experiments were carried out in 1949 with the aim of clarifying to what extent the rate of food consumption and the food range are changed at different temperatures.

a. Influence on the rate of food consumption.

Method. For the determination of the influence of temperature on the rate of food consumption, a serial thermal cabinet was used. The temperatures chosen were 16, 19, 22, 26, 32 and 36° Centigrade. In one series of experiments, five third instar larvae of comparable size were kept in a rearing dish with a diameter of 10 cm together with the food plant leaves. In another series five fourth instar larvae were used. A moistened filter paper was put at the bottom of the dish in order to maintain a suitable humidity. The dish was kept for twenty-four hours at the respective temperature in the cabinet, six dishes being used for each determination. Before being put into the cabinet, the outlines of the leaves were taken down by imposing them on a sheet of „Ozalid" blue-print paper and exposing them to a light intensity of 3,000 lux for five minutes. This sheet of paper, on which the outlines of the leaves appeared after treatment with ammonia vapour, was compared with a second sheet bearing the outlines of the corresponding leaves after experiment. The parts of the leaves consumed could thus be exactly determined by means of a tracing apparatus provided that no shrinkage occurred in the leaves after the experiment. This could be prevented by maintaining a suitable humidity in the dishes throughout the experiment. The consumed areas shown on the second sheet of paper, after being traced, were cut out with a pair of scissors and weighed on a torsion balance. The „Ozalid" blue-print paper purchased in the Amsterdam market was found to be of even thickness throughout and equal areas almost always had the same weight, with a difference of less than 5 per cent. The areas of the parts consumed could therefore conveniently be calculated by comparing the weights of their counter parts on the blue-printpaper with the weight of the paper of a known area.

Result. Tables 1 and 2 give figures of the quantities of different foliage consumed by the third and fourth instar larvae respectively at different temperatures. Figures 1 and 2, based on the figures in these tables, show that the rate of feeding increases with rising temperature and reaches a maximum at 32°, above which it decreases. This phenomenon is very pronounced when the larvae feed on potato leaves. In the case of less preferred plants it still exists, except with *Lycium chinense*, which has a maximum consumption at 36° by fourth instar larvae. The optimum temperature for larval development in the Colorado beetle was found to be 25°, at which rapid growth was accompanied by relatively low larval mortality (DE WILDE, 1948). Above this temperature development could be completed even in a much shorter time, but more larvae perished because of the exceedingly high metabolic rate. In the present experiment, the larvae were previously reared at a temperature about 25°, and the experimental time was relatively short. Therefore the larvae could still show greatest physiological activities at 32°. At 36° most larvae would still live after the experiment, except those feeding on *Petunia hybrida*. The poisonous effect of that plant was intensified by high temperature and most of the larvae died before twenty-four hours. At that temperature undoubtedly the physiological processes of the insects were disturbed and the adverse effect was manifested by the slowing down of movement

TABLE 1. Food consumption by the third instar larvae at various temperatures in 24 hours
(Hoeveelheid voedsel, opgenomen door de larve van het 3e stadium, gedurende 24 uur, in verband met de voedselplant en de temperatuur)

Plant	Temperature °C	Quantity of foliage consumed by 5 larvae (mm²)	Quantity of foliage eaten by each larva (mm²)	Relative quantity as compared with potato in %
Solanum tuberosum	16°	166	33,2	100
	19°	589	117,8	100
	22°	753	150,6	100
	26°	1016	203,2	100
	32°	1264	272,8	100
	36°	701	140,2	100
Solanum demissum	16°	26	5,2	16
	19°	77	15,4	13
	22°	76	15,2	10
	26°	157	31,4	15
	32°	219	43,8	17
	36°	111	22,2	16
Lycopersicum esculentum	16°	27	5,4	16
	19°	42	8,4	7
	22°	123	24,6	18
	26°	132	26,4	13
	32°	243	48,6	19
	36°	83	16,6	12
Petunia hybrida	16°	12	2,4	7
	19°	61	12,2	10
	22°	42	8,4	6
	26°	22	4,4	2
	32°	242	48,4	19
	36°	35	7,0	5

and the low rate of food consumption. It is also seen that food consumption will proceed with a greater speed, as the temperature rises on a preferred plant than on a less preferred plant. This fact implies that on a preferred plant the insect can derive more profit from high temperatures in completing its development. As shown in the fifth column of Table 1, the quantities of less preferred plants consumed by the third instar larvae bear rather constant ratios to that of potato at the corresponding temperatures. In the fourth instar larvae the variations in the ratios appear to be greater; and in the cases of *Datura arborea* and *Lycium chinense*, the least preferred plants in the list, considerable quantities of foliage were only consumed at high temperatures.

b. Influence on the food range

In the above-mentioned experiments it was seen that the fourth instar larvae reacted in a quite different way towards *Datura arborea* and *Lycium chinense* when compared with *Solanum tuberosum*, *S. demissum*, *Petunia hybrida*, and *Lycopersicum esculentum*. In the four last mentioned species the quantities of foliage sonsumed by the larvae increased rather gradually with the rise in temperature, while in the first two species the increase of food consumption at high temperatures was rather sudden. This fact leads one to think that the larval

TABLE 2. Food consumption by the fourth instar larvae at various temperatures in 24 hours
(*Als tabel 1. Larve van het 4e stadium*)

Plant	Temperature °C	Quantity of foliage consumed bij 5 larvae (mm²)	Quantity of foliage eaten by each larva (mm²)	Relative quantity as compared with potato in %
Solanum tuberosum	16°	1076	215,2	100
	19°	1147	229,4	100
	22°	1983	396,6	100
	26°	2925	585,0	100
	32°	4505	901,0	100
	36°	3194	638,8	100
Solanum demissum	16°	291	58,2	27
	19°	170	34,0	14
	22°	432	86,4	22
	26°	253	50,6	9
	32°	670	134,0	15
	36°	476	95,2	15
Lycopersicum esculentum	16°	184	36,8	17
	19°	415	83,0	36
	22°	339	67,8	18
	26°	869	173,8	20
	32°	1019	203,8	23
	36°	570	114,0	18
Petunia hybrida	16°	0	0	0
	19°	59	11,8	5
	22°	29	5,8	2
	26°	82	16,4	3
	32°	216	43,2	5
	36°	99	19,8	3
Datura arborea	16°	4	0,8	0
	19°	34	6,8	3
	22°	0	0	0
	26°	55	11,0	2
	32°	642	128,4	14
	36°	23	4,6	1
Lycium chinense	16°	0	0	0
	19°	0	0	0
	22°	0	0	0
	26°	49	9,8	2
	32°	12	2,4	0
	36°	658	131,6	21

food range depends on thermal conditions; plants that the larvae will not attack at low temperatures can be fed upon at high temperatures. The bearing of this fact on the diet of the insect may be of great importance. Therefore the following experiments were carried out to study this point further.

Method. The serial cabinet was again used and the six temperatures chosen were the same as in the previous experiments, i.e. 16, 19, 22, 26, 32 and 36° C. In each determination six rearing dishes each of which contained five third instar larvae in equal growth and six blocks of foliage were used. The six blocks of

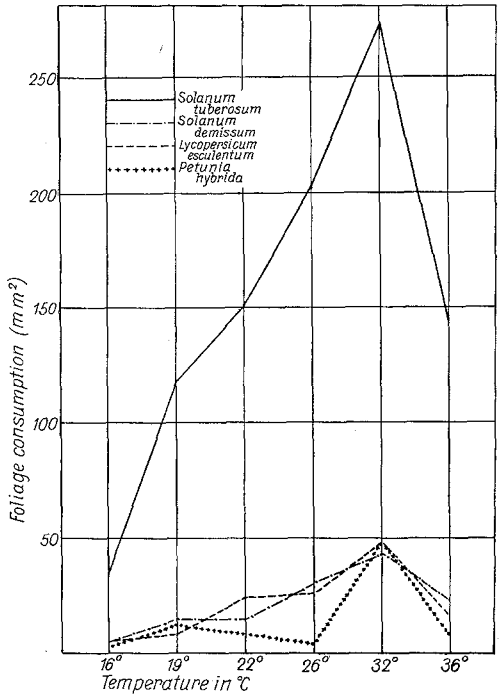


Fig. 1. The influence of temperature on the amount of foliage consumed by each third instar larva in 24 hours.

(*Invloed van de temperatuur op de hoeveelheid blad die door de larve van het 3e stadium in 24 uur wordt opgenomen.*)

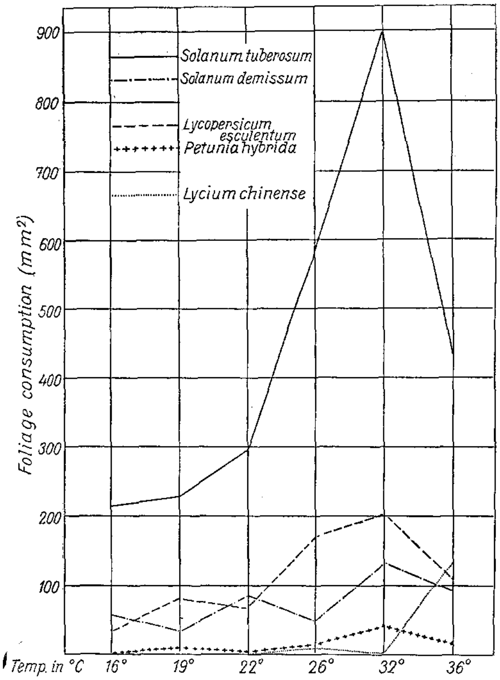


Fig. 2. The influence of temperature on the amount of foliage consumed by each fourth instar larve in 24 hours.

(*Als fig. 1; larve van het 4e stadium.*)

foliage were stamped out with a square die of 225 mm^2 in area and they were from three species of plants each of which contributed two blocks. At the bottom of each dish a piece of rough glass cut just to fit the dish was placed so that the larvae could crawl more easily. Upon this glass the six blocks of leaves were alternatively arranged in a circular order and a piece of cotton soaked with water was put at the center of the dish in order to maintain suitable humidity and prevent shrinkage of the leaf blocks. Observations were made one and eighteen hours after the dishes were placed at their respective temperatures in the cabinet. The areas of the parts of the blocks consumed were determined at each observation by comparing the blocks with a square of 225 mm^2 on a graph paper.

Results. The results of twelve determinations are shown in Tables 3 and 4. The figures in Table 3 are from two kinds of tests; three tests with *Solanum tuberosum*, *S. demissum* and *Datura arborea* and three with *Solanum tuberosum*, *S. demissum* and *Lycium chinense*. Since the third instar larvae would never attack *Datura arborea* and *Lycium chinense* in the presence of *Solanum tuberosum* and *S. demissum*, it seemed convenient to regard the former as similarly unat-

TABLE 3. The quantities of different foliage consumed by third instar larvae at various temperatures with the possibility of food selection
(*Voedselkeuze van de larve van het 3e stadium, uitgedrukt in mm² opgenomen blad, bij verschillende temperaturen*)

Temperature	Plant	Quantity of foliage consumed by 30 larvae (mm²)	Relative food consumption on different plants
16°	*Solanum tuberosum*	650	100,0
	Solanum demissum	154	23,7
	Datura arborea or *Lycium chinense*	0	0,0
19°	*Solanum tuberosum*	843	100,0
	Solanum demissum	92	10,9
	Datura arborea or *Lycium chinense*	0	0,0
22°	*Solanum tuberosum*	906	100,0
	Solanum demissum	248	27,3
	Datura arborea or *Lycium chinense*	0	0,0
26°	*Solanum tuberosum*	1419	100,0
	Solanum demissum	380	26,7
	Datura arborea or *Lycium chinense*	0	0,0
32°	*Solanum tuberosum*	1906	100,0
	Solanum demissum	672	35,2
	Datura arborea or *Lycium chinense*	0	0,0
36°	*Solanum tuberosum*	1141	100,0
	Solanum demissum	511	44,7
	Datura arborea or *Lycium chinense*	0	0,0

tractive plants. The quantities of potato and *demissum* foliage consumed increase with rise in temperature until a maximum point is reached after which the extent of consumption declines. The maximum food consumption occurred at 32°, in agreement with that found in the preceding experiments. The quantities of tomato foliage eaten increased with the increase in temperature and maximum consumption occurred at 36°, as shown in Table 4. This might be due to the different reaction of the larvae towards a less preferred plant at high temperatures, as previously observed. If the quantities of potato leaf are taken as the standard at each temperature, it is seen that the relative quantities of *demissum* leaf consumed from 16 to 26° do not increase in a regular way but they do increase at 32 and 36°. Table 4 also shows that the relative quantities of tomato leaf consumed increase with the increase in temperature, especially at 32 and 36°. Perhaps these relatively high temperatures enable the larvae to consume greater quantities of less preferred plants and thus alter their feeding habits. However, we must not exclude the possible influence of temperature on the food plants such as a change in the vapour tension of volatile substances which are attractive to the larvae.

On the whole, under our experimental conditions, the food range of the larvae seems reasonably stable at temperatures below the optimum for development. It is, however, extended at super-optimum temperatures, at which both the physiological activities of the larvae and the properties of the food plants are changed. Experiments under natural conditions will be necessary to show the ecological importance of these points.

TABLE 4. The quantities of different foliage consumed by third instar larvae at various temperatures with the possibility of food selection
(*Als tabel 3, doch met Lycopersicum esculentum*)

Temperature	Plant	Quantity of foliage consumed by 30 larvae (mm²)	Relative food consumption on different plants
16°	*Solanum tuberosum*	396	100,0
	Solanum demissum	91	25,0
	Lycopersicum esculentum.	6	1,5
19°	*Solanum tuberosum*	698	100,0
	Solanum demissum	123	17,6
	Lycopersicum esculentum.	24	3,4
22°	*Solanum tuberosum*	837	100,0
	Solanum demissum	136	16,1
	Lycopersicum esculentum.	30	3,6
26°	*Solanum tuberosum*	1094	100,0
	Solanum demissum	276	25,2
	Lycopersicum esculentum.	39	3,5
32°	*Solanum tuberosum*	1246	100,0
	Solanum demissum	500	40,2
	Lycopersicum esculentum.	143	11,5
36°	*Solanum tuberosum*	638	100,0
	Solanum demissum	375	58,7
	Lycopersicum esculentum.	209	32,8

CHAPTER IV

THE SENSORY MECHANISMS INVOLVED IN SELECTION OF FOOD PLANTS

1. THE PERCEPTION OF THE FOOD PLANTS BY THE LARVAE

The perception of food plants by phytophagous insects at a distance is generally effected through two sensory routes, i.e. the visual and the chemical. The visual organs of the Colorado beetle larva are the six pairs of stemmata or lateral ocelli, which, similar to the compound eyes of the adult, are innervated from the optic lobes of the brain (SNODGRASS, 1935). As pointed out by WIGGLESWORTH (1939), there is no doubt that in the larvae of holometabolous insects and the adults of Apterygota and Siphonaptera the stemmata are organs for the perception of light; but in some insects, such as the larvae of *Vanessa* and *Lymantria*, in spite of their very simple structure, they also subserve colour vision and a rudimentary perception of form (GÖTZ, 1936; DE LEPINEY, 1928).

In insects as in vertebrates, besides the common chemical sense which is concerned with stimulation by irritant chemical substances in comparatively large dosages, there has been a differentiation of the chemical sense into taste and smell. In terrestrial insects, taste is the perception of liquid or dissolved substances in relatively high concentrations, while smell is concerned with the perception of substances in the gaseous phase, usually in comparatively small quantities and from a distance (DETHIER, 1948). Smell plays a very important part in the detection of food at a distance in some insects, and the site of this sense is believed to be on the antennae and the maxillary and labial palpi. Thus *Periplaneta* was observed to locate its food at a distance by means of the antennae and at close quarters by the olfactory apparatus on the palpi (GLASER, 1927). The sense of smell was demonstrated to be present in the larvae of *Pieris brassicae*, which, in their gregarious behaviour, were guided by the „species odour''. The odour could be detected by older larvae at a distance of 5 cm (WOJTUSIAK, 1932). Larvae of *Danais plexippus* separated by a wire screen from leaves of *Asclepias* and also those that the larvae would not eat made zigzag paths and exploratory movements of the head when passing the leaves of *Asclepias* but moved straight ahead over the leaves from other plants (DETHIER, 1937). This behaviour indicated that the larvae were able to recognize the leaves of *Asclepias* by their odour.

In the field the egg masses of the Colorado beetle are deposited on the food plants; the larvae after hatching do not need to locate the plants themselves. Yet the capacity of food perception still exists, and the larvae are able to locate the potato leaves from a distance by smell and possibly by vision. The following experiments were carried out to ascertain the distance of perception, the capacity in discriminating plant odours, and the role played by the antennae, palpi, and ocelli in the perception.

a. The distance of perception

For testing the distance of perception of the food plants by the larvae, two series of experiments were carried out.

Experiment 1

Method. At the center of a rearing dish with a diameter of 10 cm and having a piece of moistened filter paper at the bottom, a square of potato leaf was placed. A second or a third instar larva starved for three hours was introduced into the dish, at a distance of about 3 cm from the leaf. A glass plate was added to the dish as a cover. The observations were carried out at a place where the light was very even from different directions, in order to avoid phototactic movements of the larva. When the larva was crawling in the dish, its pathway was plotted on a cellophane paper on the glass cover.

Observations and results. It was observed that when there was no leaf present, the paths of the larva were rather evenly dsitributed over the bottom of the dish and abrupt turnings were rather rare (Fig. 3). The paths of a larva in the presence of a square of potato leaf are shown in Fig. 4. The left track shows that a second instar larva nearing a square of potato leaf has an abrupt turning point about 2 mm from the leaf. The other track shows that the larva does not come to that square when it is about 5 mm away. The observations indicated that the second instar larva can detect the presence of a piece of potato leaf at a distance of 2 mm, but not at 5 mm.

Experiment 2

Method. At the bottom of a rearing dish with a diameter of 10 cm, a piece of moistened graph paper cut to fit the dish was placed. A second or a third instar larva starved for six to eight hours was introduced. When the larva was crawling

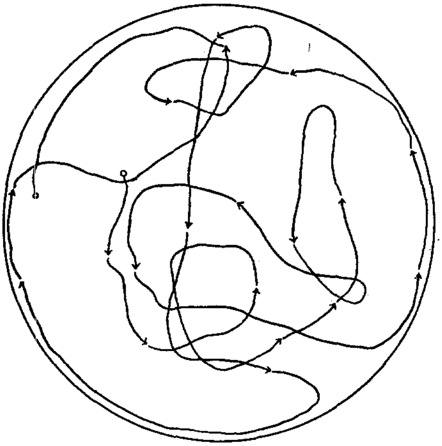


Fig. 3. The track of a third instar larva in a hygrostat dish without leaf. The time was marked at the intervals of 30 seconds.
(*Loopspoor van een larve van het 3e stadium in een ledige hygrostaatschaal. Tijdsinterval tussen twee opeenvolgende pijlen 30 sec.*)

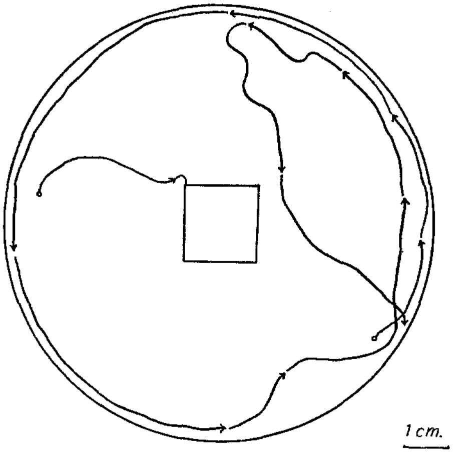


Fig. 4. The tracks of a third instar larva in a hygrostat dish, with a piece of potato leaf.
(*Loopspoor van een larve van het 3e stadium in een hygrostaatschaal met aardappelblad*)

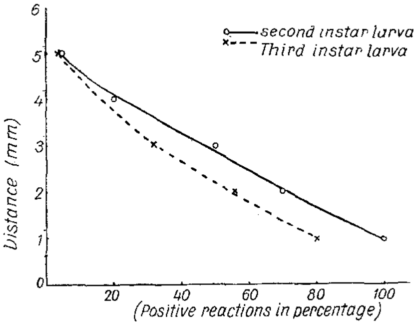


Fig. 5. The perception of potato leaf at a distance by the second and third instar larvae. (*Percentage positieve reacties van de larven van het 3e en 4e stadium t.o.v. aardappelblad, op verschillende afstand van de kop geplaatst.*)

on the graph paper, a small piece of potato leaf held by means of a pair of forceps was offered at various distances from the larva at right angles to its path. If the larva reacted in a short time by bending its head and moving to reach the leaf, the reaction was counted as positive; if not, the reaction was counted as negative. The distances of the leaf from the insect could be determined by looking at the graph paper. The distances chosen for test were from 1 to 5 mm from the head, the midleg, and the last abdominal segment respectively and twenty-five determinations were made for each distance.

Observations and results. When the potato leaf was held more than 5 mm away from the head, no positive reactions were seen, even if the leaf was very close to the legs and the abdominal segment. Table 5 and Fig. 5 indicate that 5 mm is the maximum distance of perception. The larvae showed a high percentage of positive reactions when the leaf was 1 mm away from the head; it decreased as the distance increased. Therefore the head seemed to be the essential site of perception. No great difference in the perceptive capacity could be found in different instars. The difference between the second and third instar larvae that appears in Table 5 may be due to the individual variation of the two larvae used in the experiment.

TABLE 5. The reactions of starved larvae at a distance from a piece of potato leaf. (25 determinations for each distance from the head, diffuse daylight, 20°) (*Percentage positieve reacties van de hongerende larve van het 3e stadium t.o.v. aardappelblad, op verschillende afstand van de kop geplaatst*)

Larva	Distance (mm)	Positive reactions (in %)
Second instar	1	100
	2	70
	3	50
	4	20
	5	5
Third instar	1	80
	2	56
	3	32
	5	4

b. The capacity for discriminating plant odours

The above experiment has proved that the larvae possess a short-range perceptive capacity. Therefore the olfactory sense must also have a short range. On account of this, the olfactometer designed by McIndoo for testing the adult beetle was not suitable for the larvae; and the construction of another kind of apparatus with the source of odour close to the insects was necessary.

The construction of a simple apparatus used in my experiment was based on the same idea as the „screen test" for *Danais plexippus* (DETHIER, 1937), which showed that the larvae would remain longer over the leaves they preferred than over other leaves or odourless paper. This reaction will be of value in the study of the attractiveness of some food plants to an oligophagous insect with a short-ranged olfactory sense. In addition to the test concerning smell, the reaction of the larva toward colours and different intensities of light can also be determined.

This simple apparatus (Fig. 6) was made in the following way. Into a glass rearing container with a diameter of 9.5 cm and a depth of 4 cm, another glass container with a smaller diameter and a depth of 2 cm was placed, with the bottom upwards. On the bottom of the smaller container a layer of moistened filter paper was placed, on which there was a cross made of two pieces of wood to divide the bigger container into four equal quarters at that region. Into the two opposite quarters potato leaves were added in adequate quantity so that, when a piece of wire screen cut to fit the container was placed flat upon the wooden cross, the leaves came into direct contact with the screen on the under surface. The other two opposite quarters remained blank. In control experiments the four quarters were all blank or the two opposite quarters contained green or black papers instead of leaves. During the experiments this apparatus was placed on a table illuminated evenly with an intensity of 3000 lux; and after each determination it was turned a 45° angle in order to eliminate any influence resulting from slightly unequal illumination at the different sides. In each determination ten young fourth instar larvae starved for three to four hours were placed on the center of the container on the screen by means of a glass vial. After one minute the distribution of the larvae over different quarters was recorded. For each experiment fifty determinations were made and the total number of larvae over the quarters containing leaves in these fifty determinations was divided by 50. This quotient or the arithmetic mean thus obtained was regarded as the index of distribution. The standard error of this mean was calculated from

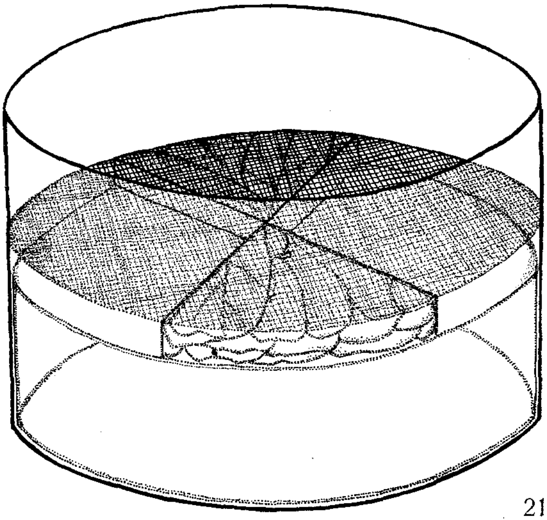


Fig. 6. The apparatus for the "screen test".
(*Het keuzeapparaat voor de „screen test"*)

the formula $\delta = \sqrt{\frac{\Sigma(d^2)}{n(n-1)}}$, where n = the number of determinations, and d = variation from the mean in each determination.

Results. It was seen that the index number of distribution in two opposite quarters was 5.02 (4.98) ± 0.28 when the four quarters contained nothing except the moistened white filter paper at the bottom. It suggests that under such a condition distribution was practically equal in these two areas each of which occupied half of the surface of the screen. The larvae are well known to be positively phototactic. Determinations were also made concerning the reaction of the larvae when two opposite quarters were occupied by green papers whose tint was practically the counterpart of that of potato leaf. The index number of distribution over the green quarters was found to be 4.68 ± 0.22, indicating positive phototaxis. Then the green papers were replaced by the leaves of *Solanum tuberosum*, *S. demissum*, and *Petunia hybrida* in succession, and the index numbers over the quarters with leaves were found to be 5.82 ± 0.21, 5.92 ± 0.25, and 5.72 ± 0.24 respectively. When these indices were compared with that from green paper, viz. 4.68 ± 0.22, the t values were found to be greater than 3; therefore the difference was significant. [1]) When the two opposite quarters contained the leaves of *Doronicum pardalianches* (Compositae), a plant that is never attacked by the Colorado beetle, the index was 4.92 ± 0.24, which when compared with the index 4.68 ± 0.22 from the green paper, showed no significance.

These experiments indicated that the larvae were not attracted by the green tint of their food plants, but by their odours.

c. The role played by the antennae, palpi, and stemmata in the perception of food plants

In the above-mentioned screen tests we were not able to prove any food perception through the photoreceptors. Opposite quarters in the apparatus containing black paper were found to have an index of 4.48 ± 0.24, which, when compared with 4.68 ± 0.22 from the green papers and 5.02 ± 0.28 from the white paper, suggested that the stemmata are only concerned in the perception of light intensity.

The following experiments were carried out to test the significance of the antennae and palpi in olfactory perception.

Method. It was found that to cauterize the antennae and palpi of the larva after narcotization with CO_2 gas by means of an electrically heated cauterizer would injury the animal too much for recovery. The method adopted by the writer was to amputate these parts during narcosis under a binocular microscope. The arrangement for holding the larva in a narcotizing apparatus is shown in Fig. 7. In four sets of fourth instarl arvae, each of which contained ten animals, the following parts were amputated: *a.* the right antenna, *b.* both antennae, *c.* maxillary and labial palpi, and *d.* the antennae and maxillary and labial palpi. The larvae after operation were reared in a glass dish with potato leaves for one day; the larvae that did not recover in that period were rejected. The recovered

---

[1]) Calculated from the formula reliability $t = \frac{M_a - M_b}{\sqrt{(\delta_a)^2 + (\delta_b)^2}}$, where M = the index, δ = standard error (Simpson & Roe, 1939).

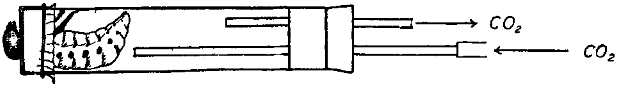


Fig. 7. The arrangement for holding the larva during narcotization and operation. (*Buis voor fixatie en narcose gedurende de amputaties.*)

larvae, which could feed on potato leaves, were starved for three hours before the experiment. Two opposite quarters in the apparatus for screen test were always filled with potato leaves; the experiments were performed under the same conditions as in the previous experiments.

Result. The indices of distribution of the operated larvae are shown in Table 6. The larvae deprived of the right antenna did not have any definite circulatory movement.

TABLE 6. The indices of distribution of the operated larvae over the quarters containing potato leaves
(*Verdelingsindices van larven met verschillende amputaties boven de quadranten met aardappelblad; blanco = wit filtreerpapier*)

Larva	Operation	Index
Set 1	amputation of the right antennae	5,24 ± 0,24
Set 2	amputation of both antennae	5,10 ± 0,25
Set 3	amputation of maxillary and labial palpi	5,60 ± 0,25
Set 4	amputation of antennae and palpi	4,88 ± 0,26

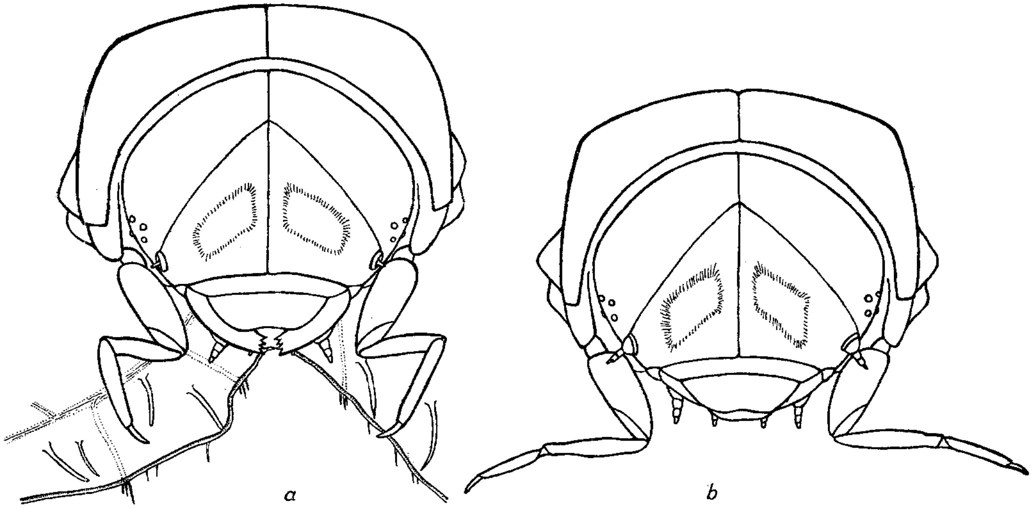


Fig. 8. Fourth instar larvae showing the antennae. a. during feeding; b. when searching for food.
(*Positie van de antennen bij de larve van het 4e stadium. a. gedurende de voedselopname; b. op zoek naar voedsel.*)

The experiments have proved that the antennae and palpi are the essential sites of olfactory perception; and the partial elimination of these sites leads to various degrees of incompetence in olfaction. The antennae seem to be more important than the palpi; the part played by the maxillary and labial palpi is far less than that of one antenna. Therefore, it seems that the antennae are the most important areas concerned with the perception of odours, especially when the sources are at a distance.

It has been observed that the larval antenna is composed of three segments which can be telescoped and retracted into the antennal socket. When the larva is searching for food the antennae protrude prominently, but they cannot move laterally. During feeding they are usually retracted (Fig. 8). The palpi cannot be retracted but the maxillary palpi are able to move laterally. During feeding they are usually in contact with the leaf. These facts suggest that the antennae are concerned with relatively „long-ranged" (though always proximate) perception while the palpi are very short-ranged in function.

2. AN ANALYSIS OF THE INGESTIVE PROCESS OF THE LARVAE

The ingestion of food by the Colorado beetle larva is typical of phytophagous insects with biting and chewing mouthparts. The process is divisible into operative steps and punctuated by periods of resting and wandering. The operative steps are the successive bites on the leaf, followed by a masticatory action rather suggestive of that of mammals, and the swallowing of the food fragments after being cut into suitable size and arranged in proper order. To swallow the food from the mouth cavity is a reflex effected by a complex series of coordinated movements in the mouth cavity and in the oesophagus. It is the final step in the ingestive process which scarcely can be altered if once set in action. During biting and mastication, however, the chemical character of the food is censored by the chemical senses located in the head region, especially on the mouthparts. As a consequence of this censorship, the insect may accept the food with "greediness" or refuse it with "disgust". Though this is the reaction of the larva towards more or less preferred plants, there are many plants whose disagreeable nature the larva seems to recognize at the very first encounter. The larva would keep its mouth tightly closed without any attempt to ingest the slightest quantity of the foliage. This is exemplified by its reaction towards *Doronicum pardalianches* which has been used in the screen test. When deeply starved, the larva would bite on most plants that in normal condition it would not eat; and this is especially marked in the fourth instar larvae. Though the bites were started, continuous feeding would not follow; the unfavourable reaction may be attributed to the chemical censorship carried out in the mouth cavity. Anyhow the biting action is the prelude to the relation between the larva and the plant concerned; therefore, the following studies aim to clarify the factors responsible in initiating the biting action and those relating to continuous feeding.

a. The biting response

In general the biting response of the larva, as well as of other insects, may be initiated by a variety of stimulations including both internal physiological conditions and external sensory factors. It seems that the internal physiological conditions are usually complementary to the external stimulations in their operation; thus a starved larva generally will try bites on a plant to which it will not pay the slightest attention when well-fed. The following is a tentative list of the factors that can bring forth the biting response.

A. Internal physiological factors.
   I. Hunger-stimulation, caused primarily by the lack of food in the digestive tract, lowering the threshold of the biting response.
   II. Impulses from the higher nerve centers, causing "spontaneous" bites, even if the mouthparts are not in contact with any subject.

B. External stimulations.
   I. Strong stimulations from the outside, regarded by the larva as ,,hostile'', causing defensive bites.
   II. Sensory stimulations from the outside causing the insect to react but not related to nutrition, bringing forth offensive bites.
   III. Sensory stimulations related to the nutrition of the insect such as the odours, tastes, humidity, and the tactile nature of the food plants, causing ingestive bites.

As regards the second category of external stimulations, insects usually respond with bites on some substances "for reasons which have nothing to do with nutrition, e.g. burrows made in wood, or even in metal (lead, larvae of *Sirex*)" (UVAROV, 1928). In the Colorado beetle larva the bites on the wall of a glass container may be regarded as belonging to this category.

Where the ingestive bites are concerned, the sensory stimulations may be either physical, or chemical, or the combined action of both. Among phytophagous insects, the physical nature of the substratum such as the roughness and hardness may determine whether a plant will be accepted as food and the moisture stimuli together with the odours from the leaves may play a very important part in causing the feeding action. It was observed that in the Colorado beetle larva instant biting and feeding would occur when the plant that the larva encountered was a preferred one and delayed feeding was always the case when the plant was unsuitable. In the former case, a large quantity of foliage might be consumed and normal growth and development would follow, as shown in the previous chapter. Therefore, the readiness to bite and feed is usually an indication of the attractiveness of the substratum.

Experiment 1

Method. Following the principles explained above, experiments were carried out by introducing larvae of similar physiological status on substrata of various characters and recording the time elapsed before any biting response was observed. The time thus recorded would serve as a measurement of the readiness to bite. The substrata chosen were different in their hardness, roughness, water content, and odours; the following is an account of their characters.

I. Dry, hard, smooth, and odourless substratum ..... a clean, dry glass Petri dish cover.
II. Dry, hard, rough, and odourless substratum .... a disk of dry, clean, white filter paper.
III. Humid, soft, rough, and odourless substratum .... a disk of clean white filter paper, with a diameter of 10 cm and wetted with 1 cc of distilled water.

iv. Substrata afforded by plants that the larva would never attack ..... leaves of *Doronicum pardalianches* and the common geranium, *Pelargonium zonale.*
v. Substrata afforded by plants that the larva would attack ..... leaves of *Petunia hybrida*, *Solanum demissum*, and *S. tuberosum.*

For testing the last four sorts of substrata, the paper or the leaves were placed at the bottom of a Petri dish cover with a diameter of 10 cm. A well-fed fourth instar larva was introduced on the substratum concerned and each observation was carried out within a period of half an hour at temperatures from 22° to 23° C, under natural daylight. If no bites were observed within this period, the reaction was regarded as negative. If bites occurred within one minute, between one and two minutes, and after two minutes, the reactions were arbitrarily classified as strongly positive, positive, and slightly positive respectively. The corresponding substrata were referred to as strongly stimulating, stimulating, and slightly stimulating in inducing the biting response. Fourth instar larvae starved from 4.5 to 6 hours were tested in the same way.

Results. The results shown in Table 7 indicate that the dry, hard, smooth, and odourless substratum is scarcely stimulating to a well-fed larva. A starved larva might bite on such a substratum instantly, as in the case of the larva No 4. But the response perhaps could not be regarded as ingestive, as already explained. A dry, hard, rough, and odourless substratum is entirely unstimulating, both to the well-fed and the starved larvae; and the humid, soft, rough, and odourless substratum is practically the same. On the latter substratum a slightly positive reaction might occur; and this indicated that water vapour emanating from a substratum might play a minor part in inducing the biting response. The larva

TABLE 7. The biting response of fourth instar larvae of *Leptinotarsa decemlineata* on various substrata, all sense organs intact
(Optreden van de bijtreactie bij normale larven van het 4de stadium op verschillend substraat)

Substrata	Larvae after feeding					Larvae starved from 4.5 to 6 hrs				
	1	2	3	4	5	1	2	3	4	5
Glass Petri dish cover	–	–	–	+	–	+	–	–	+ + +	–
Dry filter paper . . .	–	–	–	–	–	–	–	–	–	–
Wetted filter paper . .	+	–	–	+	–	–	–	–	–	+
Doronicum leaf . . .	+	+	+	–	–	+ + +	+	+ + +	+ + +	+
Geranium leaf	+ +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Petunia leaf	+ + +	+ +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Demissum leaf	+ + +	+	+ +	+	+ + +	+ + +	+ + +	+ + +	+ +	+ + +
Tuberosum leaf . . .	+ + +	+ + +	+ + +	+	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

– 30 minutes elapsed without any biting action.
\+ + + biting action less than one minute after the larva was introduced on the substratum.
\+ + biting action between one and two minutes after the larva was introduced on the substratum.
\+ biting action more than two minutes after the larva was introduced on the substratum.
\- *geen bijtreactie binnen 30 minuten*
\+ + + *bijtreactie binnen 1 minuut*
\+ + *bijtreactie in de 2e minuut van waarneming*
\+ *bijtreactie in de 3e–30e minuut van waarneming)*

would never feed on the leaf of *Doronicum* though its physical properties bore a resemblance to those of *Petunia*. The leaf of this plant, however, was very slightly stimulating when the larva was well-fed, and stimulating when starved. The geranium leaf also resembles that of *Petunia* in texture and pilosity and it was very stimulating to the well-fed and starved larva. In spite of this, prolonged feeding did not follow on geranium. Therefore, the leaf of geranium is regarded as unattractive to the larva. The leaves of *Petunia hybrida*, *Solanum demissum*, and *S. tuberosum* were practically equal in their effect in inducing the biting response, though the detailed events following the bites were quite different.

The difference in the readiness of the well-fed larva to bite on the *Doronicum* leaf, on the one hand, and on those of other four species, on the other, suggests that the odours from these two groups of plants have different sensory effects on the larva. The starved larva is generally deprived of such sensory discrimination in regard to biting but not to that of continued feeding.

Experiment 2

This experiment was carried out to ascertain whether the odours of the plants are the most important factor in eliciting the biting response. It was proved in the above experiment that the antennae and the maxillary and labial palpi were the essential sites of olfaction. In the present experiment the antennae and palpi were amputated to eliminate the capacity of odour perception.

Method. The antennae and the maxillary and labial palpi of a number of young fourth instar larvae were amputated in the way described on page 22. Five larvae after feeding were used to test the attractiveness of the substrata as described in the above experiment. On the next day, the larvae were starved from 4.5 to 6 hours before the experiment.

Results. Table 8 gives the results of this experiment. It was seen that the operated larvae showed slightly positive reactions on the dry, hard, smooth, and odourless substratum, either in well-fed or starved condition. Positive reactions occurred on the dry, hard, rough, and odourless substratum. When compared with the results in the above experiment, the larvae bit on these substrata with more readiness. The antennae and palpi, therefore, seem to have an inhibitory effect on the biting response. In Table 8 it is seen that there are instant biting responses on the wetted filter paper, and the leaves of *Doronicum*, geranium, and the two species of *Solanum*. The results were contrary to what was to be expected, because the larvae deprived of antennae and palpi, which contain the olfactory receptors, could bite on the leaves as readily as the normal larvae. Perhaps these larvae then had to rely on their tactile and humidity senses to locate their food plants. That these senses are still present in the operated larvae is clearly indicated in Table 8, which shows that these larvae could distinguish between glass and filter paper and between dry and wetted filter paper. The humidity and tactile senses must at least partly be located beyond the antennae and palpi.

It was found in the above experiment that the wetted filter paper, the foliage of *Doronicum*, and the foliage of geranium, *Petunia*, *demissum*, and potato met with different reactions by the normal well-fed larvae. The operated larvae in this experiment were observed to bite with the same readiness on these substrata. Though the loss of discrimination may be partly attributed to the removal of the inhibitory influence derived from the antennae and palpi which has lowered

TABLE 8. The biting response of fourth instar larvae of *Leptinotarsa decemlineata* on various substrata, with antennae and palpi amputated
(Optreden van de bijtreactie bij larven van het 4e stadium; antennen en palpen geamputeerd; voor verklaring der tekens zie tabel 7)

Substrata	Larvae after feeding					Larvae starved from 4.5 to 6 hrs				
	1	2	3	4	5	1	2	3	4	5
Glass Petri dish cover	+	+	–	+	–	+	+	+	–	+ + +
Dry filter paper . . .	+ +	+ + +	–	+ +	+	+ + +	+	+ + +	+ + +	+
Wetted filter paper . .	+ +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Doronicum leaf . . .	+ + +	+ + +	+ +	+	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Geranium leaf	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
Petunia leaf	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Demissum leaf	+ +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+
Tuberosum leaf . . .	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +

– 30 minutes elapsed without any biting action on the substratum
+ + + biting action less than one minute after the larva was introduced on the sustratum
+ + biting response between one and two minutes after the larva was introduced on the substratum
+ biting action more than two minutes after the larva was introduced on the substratum.

the threshold of the biting response, there is reason to believe that the elimination of the olfactory sense is the cause of this phenomenon. In Table 7 it is shown that the reactions of the larvae towards dry and wetted filter papers are less different than those shown in Table 8. Therefore the lowering of the threshold of the biting response does not entirely abolish the capacity of discrimination.

As a whole, the results in these two experiments show that the odours of the plants are the most important factor in eliciting the biting response, the moisture and the texture of the substrata play a minor part in this reaction, and the physiological conditions of the larvae generally influence the threshold of the required stimulations.

b. The feeding action

To bite on a substratum is only an attempt at ingestion. The continuous feeding on a plant, however, undoubtedly depends upon its taste which can only be perceived after the insect has come into contact with the plant and fragments of food have been taken into the mouth cavity. Since attractive smell does not always go hand in hand with agreeable taste, to nibble on a plant does not mean that continuous feeding certainly will follow. Thus the larva would nibble very readily on the geranium leaf, but no considerable quantity of the foliage was ever seen to be consumed. Moreover, the feeding of the larva is not a continuous process; it is interrupted by periods of resting and wandering (BUSNEL, 1939). It was thought that the relative lengths of the time spent in feeding, resting, and wandering gave the indication whether the plant under attack was preferred.

It was noticed that plants unsuitable for the larvae could be distinguished by the numerous small holes made in the leaves (TROUVELOT, MÜLLER-BÖHME and LACOTTE, 1938). This is especially marked in the case of young larvae feeding on such resistant plants as *Solanum demissum* and *Solanum nigrum* (Fig. 9, a-b). Preferred plants such as *Solanum tuberosum*, *S. dulcamara*, and *Atropa belladonna* were greedily attacked and the parts of the leaves consumed were usually big areas reaching the midribs (Fig. 9, d, e). Intermediate types of attack occurred on less preferred plants such as the hybrids between *Solanum tuberosum* and *S. demissum*, and *Atrophanthe sinensis* (Fig. 9, c). In the latter case the consumed parts were small areas reaching the secondary veins but not the midribs.

These differences in the signs of attack are directly concerned with the feeding action. They may be produced by each of the following causes: *a.* the small number of successive bites before the food is swallowed, *b.* the small quantity of leaf taken into the mouth at each bite, and *c.* the short duration of feeding at one place followed by periods of resting and wandering and then feeding at another place. In the plants these differences can partly be attributed to the physical properties of the leaves such as the thickness of the epidermal cuticle, the water content, and the pilosity. The variation in the chemical constitution of the leaves, however, has been proved by many workers to be the main cause of the difference (RAUCOURT and TROUVELOT, 1936; KUHN and GAUHE, 1947). The

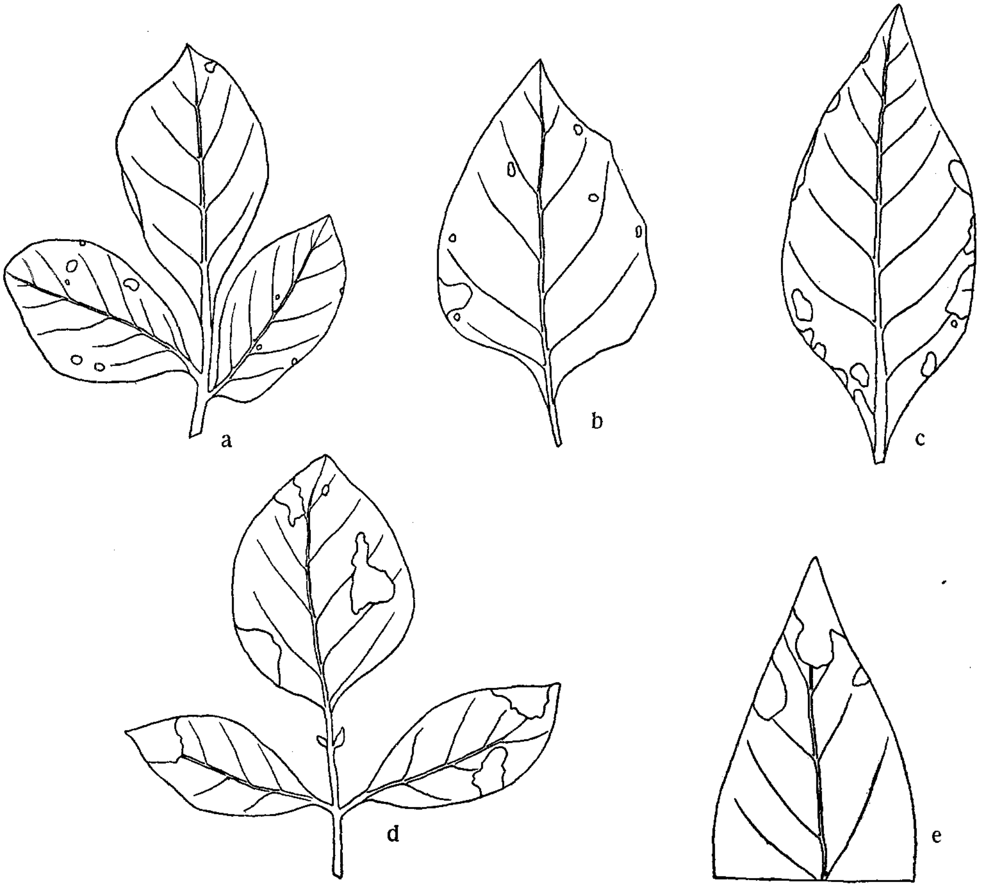
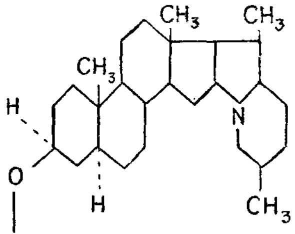


Fig. 9. The types of attack by the third instar larva. a. Solanum demissum; b. Solanum nigrum; c. Atrophanthe sinensis; d. Solanum tuberosum; e. Atropa belladonna. (*Vraatbeelden veroorzaakt door de larve van het 3e stadium op verschillende Solanaceae.*)

preferred plants seem to contain certain "active principles" which are attractive to the insect in taste and thus responsible for the prolonged feeding. It was supposed that these "active principles" are superimposed on nutritive material like a condiment on insipid food. On the other hand the rejected plants appear to contain "repellent principles" which induce abandonment after the insect has tasted the food. In the case of *Solanum demissum*, the "repellent principle" seems to be the alkaloidglycoside demissin or solanin d, which has the structural formula



tetrasaccharide
(1 xylose + 1 galactose + 2 glucose)

(Kuhn and Löw, 1947). These chemical differences in the leaves of Solanaceous plants, therefore, seem to be the most important factors leading to different types of attack.

During feeding, the Colorado beetle larva, like caterpillars, starts the bites on the leaf with the head stretched forward. The feeding may be initiated on the edge or on the surface of the leaf. As the bites proceed the larva changes the inclination of the head axis in such a way that the lower part of the head moves toward the body of the larva. At the limit of the inclination, mastication and swallowing will occur. The number of bites at each time before swallowing is not consistent, varying from two to twenty, most frequently about ten. Like the feeding action of *Danais plexippus* larva (Mayer and Soule, 1906), the feeding of the larva once set in motion on its food plant will continue "as if it possesses momentum". The ten bites occur in rather rapid succession; they are followed by a short period of mastication and finally the swallowing of the contents in the mouth cavity. In the last two steps the larva on unsuitable plants may move its head repeatedly up and down, perhaps indicating the unfavourable gustatory reaction. On the other hand, no difference in the number of bites in each feeding action can be distinguished when the plant is *Solanum tuberosum*, or *S. demissum*, or *Petunia hybrida*. Therefore, the different types of attack are not caused by the difference in the number of bites before the food is swallowed.

It has been found that the protraction of larval development on *Solanum demissum* was due to insufficient feeding, since the amount of foliage consumed was only 2.5 percent of that on potato (Busnel and Chevalier, 1938). Periods of feeding on *S. demissum* are short and frequently interrupted by periods of resting and wandering. Thus the periods of feeding, resting, and wandering of the larva on *Solanum tuberosum* were observed in one case to be 42, 42, and 5 minutes respectively; on *S. demissum* they were 20, 53, and 17 minutes. In the former case the leaf consumed was 22 mm^2; in the latter it was 13 mm^2 (Busnel, 1939). In my experiments the feeding of three slightly starved fourth

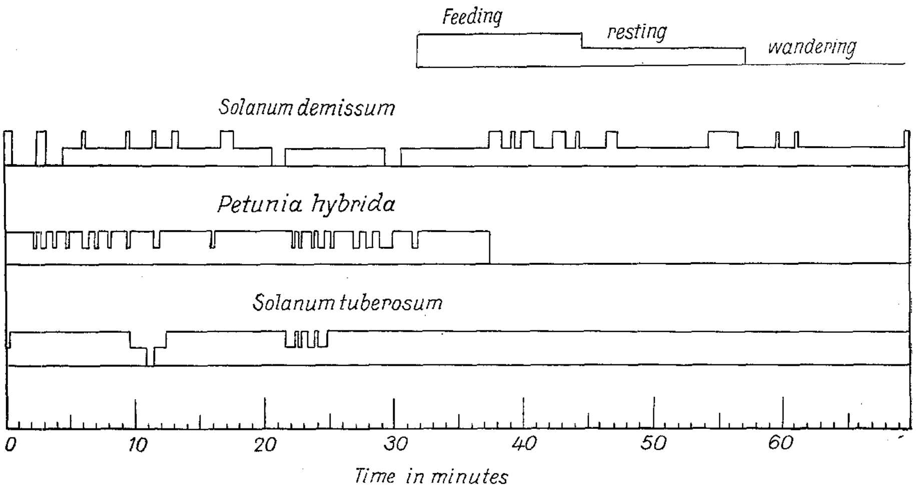


Fig. 10. The activities of the fourth instar larvae on the leaves of *Solanum tuberosum, Solanum demissum* and *Petunia hybrida.*
(*Schema van de activiteit van de larve van het 4e stadium op de bladeren van Solanum tuberosum, Solanum demissum* en *Petunia hybrida.*)

instar larvae on *Solanum tuberosum*, *S. demissum*, and *Petunia hybrida* was observed with the help of a kymograph and the periods of feeding, resting, and wandering were recorded in each case continuously on a smoked drum for 70 minutes. Fig. 10 and Table 9 describe the observed activities of these larvae.

In this experiment the larva on *Solanum demissum* was observed to change its feeding place and this behaviour seemed to be the principal cause of the production of small holes on the leaf surface. The larva feeding on *Petunia* spent half the time in wandering which occurred in the latter half of the observation period, indicating the poisonous effect of the plant. The quantity of foliage consumed in this experiment is already more than the lethal dosage to some susceptible larvae.

The results of this experiment have shown the difference in the feeding action of the larvae towards the preferred, less preferred, and rather preferred but toxic plants. It is manifested in the times and total duration of the feeding action and the extent of food consumption.

TABLE 9. The periods of activities of fourth instar larvae on the leaves of some Solanaceous plants (temperature = 24 °C, observation period = 70 minutes)
(*Duur van de perioden van voedselopname, rust en rondlopen bij de larven van het 4e stadium, op verschillende Nachtschaden. Totale waarnemingsperiode 70 minuten*)

Plant	Feeding period		Resting period		Wandering period		Foliage consumption (mm^2)
	number	total duration (minutes)	number	total duration (minutes)	number	total duration (minutes)	
Solanum tuberosum .	6	65	7	4	1	1	52
Solanum demissum . .	18	11	17	50	5	9	10
Petunia hybrida . . .	20	30	19	7	1	33	17

TABLE 10. Food consumption of normal and operated fourth instar larvae on various plants for two hours
(*Hoeveelheid voedsel opgenomen door normale en geopereerde larven op verschillende voedselplanten gedurende 2 uur bij kamertemperatuur*)

Plant	Body weight (mg)		Foliage consumed (mm²)	
	operated larva	normal larva	operated larva	normal larva
Solanum tuberosum . .	158,5	137,0	9	80
	156,5	202,0	93	91
	176,5	153,5	26	35
	326,5	245,5	19	14
Solanum demissum	235,5	224,0	30	22
	184,0	214,5	6	39
	159,0	270,0	14	3
	285,0	229,5	45	13
Petunia hybrida	251,5	259,5	3	58
	343,0	309,0	52	26
	219,5	247,5	38	54
	188,0	153,0	30	37
Total				
Solanum tuberosum . .	1018,0	738,0	147	220
Solanum demissum . .	863,5	938,0	95	77
Petunia hybrida	1002,0	969,0	123	175

The following experiment was carried out to determine whether the antennae and palpi, which were known to contain the olfactory organs, could be responsible for this difference.

Method. A number of young fourth instar larvae were operated on to remove the antennae and palpi in the way already described. The recovered larvae were starved for one hour and each of them was then transferred into a separate container with the leaf of either *Solanum tuberosum*, or *S. demissum*, or *Petunia hybrida*. The larvae were allowed to feed for two hours. The parts of the leaves consumed were determined on graph papers. Normal fourth instar larvae starved for the same period were selected and used for the control. The experiment was performed at a room temperature of 23 °C.

Results. Table 10 gives the results. It is seen that the amounts of foliage ingested by these larvae show considerable individual variation; the order of the total quantities of foliage consumed either by the normal or by the operated larvae on these three species is: *Solanum tuberosum*, *Petunia hybrida*, and *S. demissum*, and there seems no apparent correlation between the amount of foliage consumed and the presence or absence of the antennae and palpi.

The difference in feeding on the three named plants must be attributed to the gustatory sense which is not located in the antennae and palpi. Amputation of legs does not result in any deviation from the normal feeding. Gustatory organs, in the larva, therefore, are assumed to be situated in the mouth cavity. Since the operation to remove the labrum and labium usually causes serious injury to the feeding mechanism of the larva, the result does not yield any conclusion. At the present time it is not certain whether the loss of gustatory receptors would lead to the elimination of the feeding difference.

3. THE SELECTION OF THE FOOD PLANTS

Much work has been done concerning the feeding preference and selection of food plants of the Colorado beetle in the field and in laboratories (TROUVELOT, LACOTTE, DUSSY, and THENARD, 1933; MCINDOO, 1935; BRUES, 1940). In the natural condition it can select only from among those plants that are available to it and undoubtedly the feeding preference is subject to ecological and geographical limitations. In the presence of several kinds of Solanaceous plants, however, there is always a food selection which leads to the difference in the quantities of foliage consumed on each plant, as discussed in Chapter II. We have seen that the activity of the larva is divided into periods of feeding, resting and wandering, and the relative lengths of time spent in these actions are influenced by sensory reactions. The following experiments were performed to study the relation of this behaviour to the selection of food plants.

Experiment 1

Method. In a hygrostat dish with a diameter of 11 cm nine blocks of leaves stamped out with a square die (225 mm^2) from *Solanum tuberosum*, *S. demissum* and *Petunia hybrida* were arranged as shown in Fig. 11. Nine third instar larvae were dispersed in the dish and observations were made one hour and one day afterwards. In each observation the number of larvae present on each block was counted and the areas of the consumed parts were drawn on a graphic paper.

Observations and results. The results of this experiment are shown in Table 11 and Fig. 11. It is seen that the larvae usually remained longest on the blocks of the most preferred leaf, resulting in the greater consumption of the foliage.

TABLE 11. The food selection of nine third instar larvae
(*De voedselkeuze van 9 larven van het 3e stadium, uitgedrukt in de verdeling van de larven en de oppervlakte gegeten blad na 1 en 24 uur*)

Observation	Distribution of the larvae				Foliage consumed in proportion (potato: demissum: Pet.)
	on potato leaf	on *demissum* leaf	on *Petunia* leaf	on paper	
After one hour	5	4	0	0	5 : 1 : 0
After 24 hours	4	1	0	4	5 : 1 : 0

Experiment 2

Method. A young fourth instar larva deprived of antennae and palpi was introduced into a hygrostat dish where eight blocks of leaves of potato, *Petunia hybrida*, geranium and *Doronicum* were arranged in the order shown in Fig. 12. The larva was left there for 17 hours. In another dish, a normal fourth instar larva of comparable growth was used as the control. The parts of the blocks consumed were determined on a graph paper.

Result. In the case of the operated animal, the leaves of potato, geranium and *Petunia* were partially consumed; the order of the consumed quantities on these leaves was: potato, *Petunia*, and geranium. The normal larva consumed partially the leaves of potato and *Petunia*, and the area consumed on potato leaf was slightly greater than that on *Petunia* leaf (Fig. 12). Therefore, the larva deprived of antennae and palpi which contain the olfactory receptors still

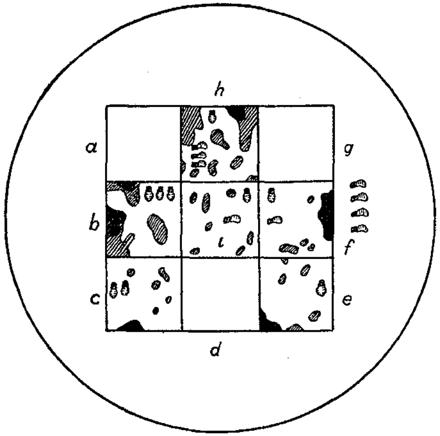


Fig. 11. Food selection by third instar larvae.
(*Voedselkeuze door de larven van het 3e stadium.*)
Solanam tuberosum: b-f-h.
Solanum demissum: c-e-i.
Petunia hybrida: a-d-g.

▨ Areas consumed in the 2nd observation.
(*Gegeten oppervlakte blad na 24 uur.*)
■ Areas consumed in the 1st observation.
(*Gegeten oppervlakte blad na 1 uur.*)
● Distribution of larvae in the 1st observation.
(*Verdeling v. d. larven over de bladfragmenten na 1 uur.*)
● Distribution of larvae in the 2nd observation.
(*Zelfde na 24 uur.*)

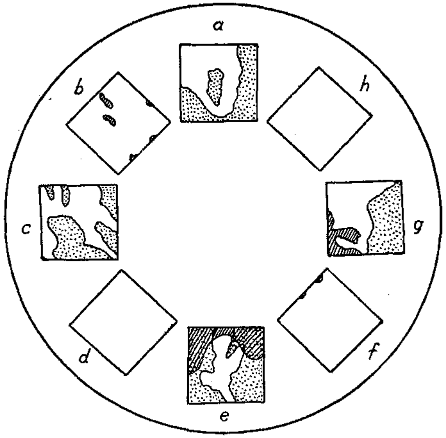


Fig. 12. Food selection of a fourth instar larva deprived of antennae and palpi and a normal fourth instar larva.
(*Voedselkeuze door een larve van het 4e stadium en een dito larve, waarvan antennen en palpen waren geamputeerd.*)
Solanum tuberosum a-e
Petunia hybrida c-g
Geranium: b-f
Doronicum d-h

▨ Areas consumed by an operated larva.
(*Oppervlakte blad gegeten door een geopereerde larve.*)
░ Areas consumed by a normal larva (the control).
(*Oppervlakte blad gegeten door een normale larve.*)

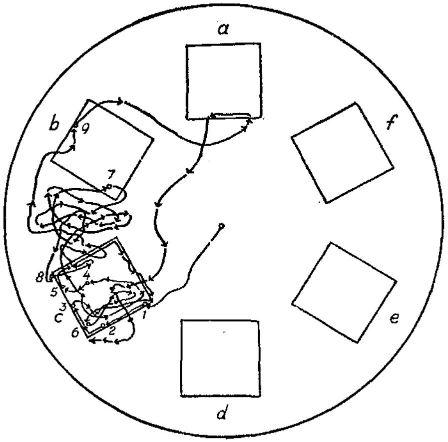


Fig. 13. Food selection of a third instar larva. The time was marked at the intervals of 30 seconds in the track.
Solanum tuberosum: c, f
Solanum demissum b, e
Petunia hybrida a, b
1, 2. feeding for 2,5 min.
3. feeding for 4 min.
4. feeding for 8 min.
5. feeding for 2,5 min.
6. resting for 24,5 min.
7, 8, 9. feeding for 1 min
(*Loopspoor van een larve va het 3e stadium gedurende een keuzeproef. Tijdsinterval tussen twee opeenvolgende pijlen: 30 sec.*
c, f Solanum tuberosum
b, e Solanum demissum
a, b Petunia hybrida
1, 2. *Voedselopname ged. 2,5 min.*
3. *Voedselopname ged. 4 min.*
4. *Voedselopname ged. 8 min.*
5. *Voedselopname ged. 2,5 min.*
6. *Immobiliteit ged. 24,5 min.*
7, 8, 9. *Voedselopname ged. 1 min.*

retains the behaviour of food selection. The operated larva could partially consume the geranium leaf which was rejected by the normal larva. This may be due to the lowering of threshold of the biting response, as discussed above. The result of this experiment is also in agreement with that of the feeding experiment, where we have seen that larvae deprived of antennae and palpi are still able to distinguish tastes. Therefore, the selection among these three species, at least, is influenced by the gustatory sense.

Experiment 3

Method. A slightly starved third instar larva was liberated at the center of a hygrostat dish which contained six blocks of leaves of potato, *demissum* and *Petunia* (Fig. 14). The activity of the larva in the dish was observed for eighty minutes and its path was plotted on a piece of paper. The time was noted at intervals of 30 seconds.

Observations and results. Fig. 13 gives an example of the observations. The klinokinetic movement was represented by the tracks in the dish when the blocks were beyond the range of olfactory perception. The total duration of feeding that occurred on the block of the potato leaf amounted to about 17 minutes. It spent two minutes feeding on the *demissum* leaf and none on the *Petunia* leaf. In addition, it rested for a total period of about 29 minutes on the potato leaf. The observation indicates that most of the activities involving feeding, resting and wandering are confined to the block of potato leaf which apparently induced favourable sensory reactions.

4. THE PERCEPTIVE ORGANS

The sensory receptors of insects are of various types but all of them are anatomically evolved from the sensillum, which is formed of the cuticula, the sense cell, and the associated chitinogenous cells. There is a group of widespread organs, which have thick cuticular walls and are innervated by singular sense cells. They are only able to receive mechanical stimuli and therefore regarded as tangoreceptors. In contrast to this, sensilla are present in certain areas of the body which are innervated by groups of sense cells and whose thin and non-sclerotized walls suggest that they may be pervious to odour and taste substances. They are the chemoreceptors (SNODGRASS, 1935).

In the above-mentioned experiments, it was found that the head of the larva contained the organs of food perception. The seat of the olfactory sense was essentially on the antennae and the palpi, while that of gustation seemed to be located in the mouth cavity. The olfactory and gustatory organs are the most important receptors concerned with the localization and selection of food plants. In addition to these, the visual, tactile, and humidity receptors also play a certain part in larval orientation and feeding behaviour. The present section aims at an account of the structure, localization and significance of these receptors.

a. Technique

I. Maceration method

The heads of fourth instar larvae were severed and boiled in 90 % alcohol for 20 minutes. Then they were transferred into a glass vial containing 75 %

lactic acid and heated on a water bath for 30 minutes. They were again transferred to another vial containing chloralhydrate/phenol and heated for about 30 minutes on a water bath. When the maceration had been completed, the objects were transferred into a shallow dish containing 80 % alcohol and dissected with the help of a binocular microscope. The objects were dehydrated and mounted in Canada balsam.

II. Paraffin method

The heads of the fourth instar larvae were severed and the mandibles removed. They were fixed in Carnoy's solution for one day and imbedded in paraffin after treatment with absolute alcohol and 1 % and 3 % methylbenzoate celloidine (ROMEIS, 1948). They were sectioned at the thickness of 5 μ, stained with HEIDENHAIM haemotoxylin and eosin, and mounted in Canada balsam.

b. The olfactory and gustatory organs and their significance

I. Organs on the antennae

It is found that only the membranous portion at the tip of the third segment and that distal to the second segment possess sensilla with very thin cuticular walls (Fig. 14a). At the tip of the third segment there are five sensilla styloconica and one big sensillum basiconicum arranged in a circle. The sensillum basiconicum is oriented from the vertex of the head and is approximately twice the size of the sensilla styloconica. All the sensilla have sharply pointed tips.

On the membranous portion distal to the second segment there are four sensilla styloconica and one stout sensillum basiconicum. The sensilla styloconica are of the same type as those on the third segment, but the sensillum basiconicum is much stouter and has a very blunt termination. This sensillum is also oriented from the vertex. The closer proximity to the substratum of these two sensilla basiconica suggests their important part in olfaction.

II. Organs at the inner surface of the labrum

About twenty-one sensilla placodea are present on the middle portion of the inner surface of the labrum (Fig. 14*b*). They are divided into five groups: two groups near the distal margin of the labrum, each of which is composed of three sensilla; one group of five sensilla arranged in two rows on the middle portion; and two groups near the proximal margin of the labrum, each of which is composed of five sensilla. Lateral to the last two groups, there are four minute sensilla trichodea, two at each side. Numerous cuticular projections are present at the middle portion and the two inner corners of the labrum, but an examination of cross sections of the labrum has revealed that they are not innervated and therefore are not sense organs. Four thickened areas are present in the middle portion of the labrum; the distal pair are lateral to the two groups of sensilla placodea near the distal margin, and the proximal pair are smaller and close to the two groups of sensilla placodea on the inner sides.

Since these organs are situated in the mouth cavity they are unlikely to be olfactory receptors. They are perhaps gustatory in function.

III. Organs on the maxillary and labial palpi

On the membranous portion of the basal segment of the maxillary palpus there are numerous cuticular projections. Like those on the inner surface of the

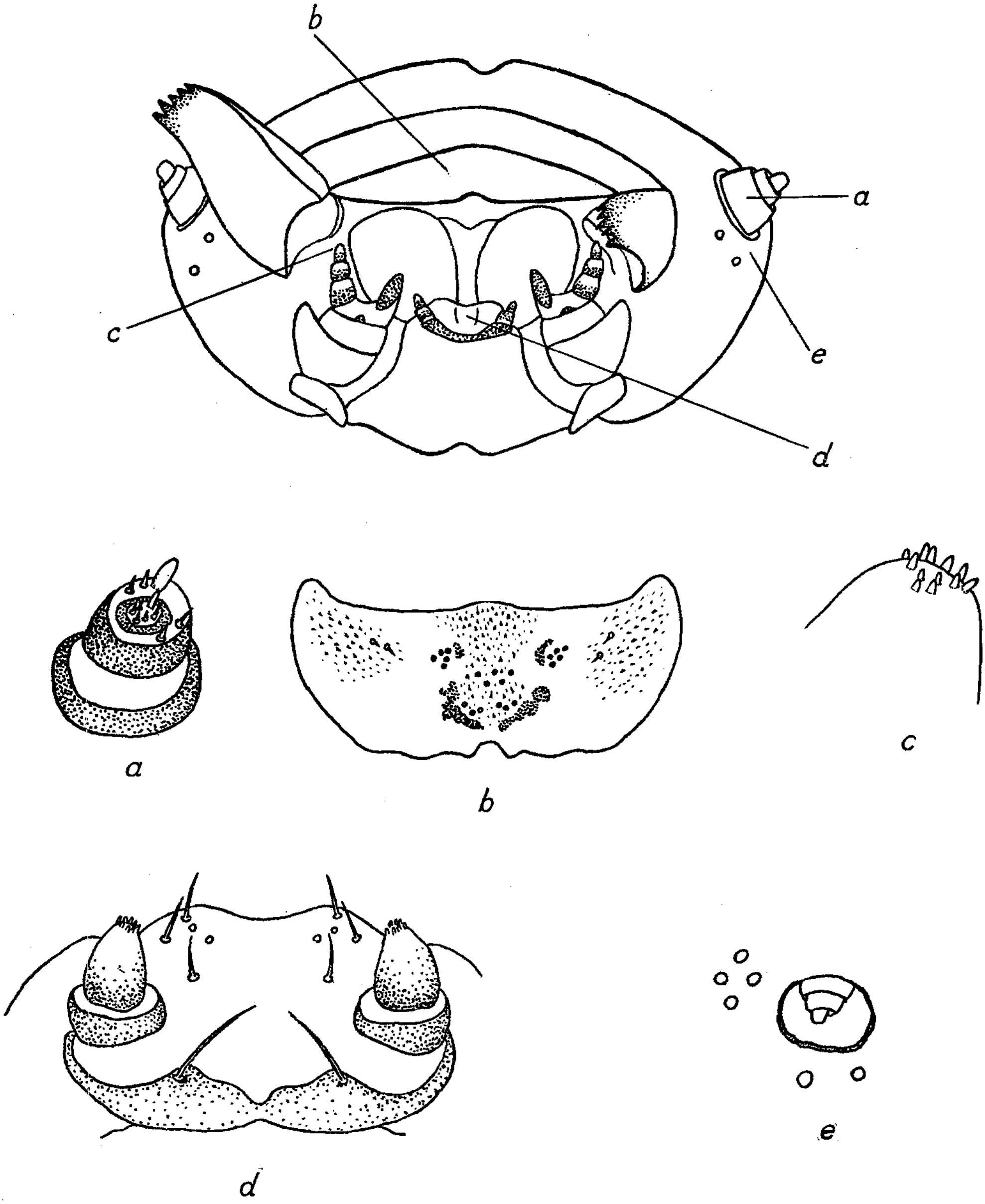


Fig. 14. The position of the essential chemoreceptive organs and the stemmata on the head of a fourth instar larva.
a. Antenna; b. Inner surface of the labrum; c. The terminal segment of maxillary palpus; d. Labium; e. Stemmata near the antenna. For explanation see the text.
(*Ligging van de chemoreceptoren en stemmata, die belangrijk zijn bij de voedselkeuze, larve in 4e stadium*).
a. Antenne; b. Binnenoppervlakte van het labrum; c. Eindlid van de maxillaire palp; d. Labium met labiale palpen; e. Ventrale stemmata. Verklaring in de tekst.)

labrum they are not innervated. Only at the tips of the terminal segments of the maxillary and labial palpi are there sensilla with very thin cuticular walls (Fig. 14*c*, *d*). They are small, delicate sensilla basiconica, sixteen on the maxillary palpus and eleven on the labial palpus. Since the palpi have not been shown in my experiments to be concerned with gustation, these sensilla must be olfactory in fuction. Their effectiveness in perceiving odours is less than those on the antennae.

IV. Organs on the labium

On the membranous portion of labium at the entrance to the mouth cavity there are two groups of sensilla placodea and sensilla trichodea with comparatively thin cuticular walls. (Fig. 14 *d*). They are situated at the right and left sides; each contains three sensilla trichodea and two sensilla placodea. The former are lateral to the latter.

Numerous cuticular projections are arranged in rows on the hypopharynx but no sense organs can be found.

The essential chemoreceptors and their localization are shown in Fig. 15.

c. Other organs related to the perception of food plants

I. The stemmata or lateral ocelli

The ocelli of the larva, as shown in the experiments in this chapter, are the organs chiefly concerned with the perception to light intensity. There are six pairs, situated proximal to the base of the antennae (Fig. 14 *e*). The six ocelli at each side are divided into two groups, four above the antenna and two below the antenna. It seems that the latter are more important in perceiving the light reflected from the substratum.

The ocelli play an important role in the orientation of the larva, which is positively phototactic and guided by light in its klinokinetic movement.

II. The tactile hairs

A large number of sensilla chaetica and sensilla trichodea innervated by single sense cells are present in various areas of the head capsule, the mouthparts, the legs, and other parts of the body. The short hairs with thick walls in the notch of the labrum and on the lacinia are very striking features. These tangoreceptors are concerned with the detection of the physical nature of the substratum.

III. The humidity receptors

The work of GEERTSEMA (1) indicates that larvae of the Colorado beetle possess a humidity sense, which guides the animals in their reactions towards the water vapour tension of the substratum. The water evaporated from the substratum may induce the biting response, as seen in the above experiments. But the effective receptors are not yet located.

---

(1) DE WILDE & GEERTSEMA, in press.

CHAPTER V

INFLUENCE OF THE FOOD PLANTS ON LARVAL MORTALITY

1. THE LETHAL EFFECTS OF SOME SOLANACEOUS PLANTS

The most obvious manifestations of the adverse effect of food plants on Colorado beetle larvae are the protraction of development and the untimely death of individuals. In general, the latter event may be attributed to the exhaustion of nutritive substances which are required to maintain a minimum activity of the life process, or to the poisonous effect of some chemicals contained in the plants. In the young larvae the physical structure of the food plants may greatly influence locomotion and lead to death by exhaustion. Thus the larvae after hatching are weakened on *Lycopersicum*, where the young leaf stalks are generally covered with long, stiff hairs, mixed with shorter glandular ones (TROUVELOT and THENARD, 1931). Sometimes the physical handicap, nutritive deficiency and toxic effect may act together in one plant and it is difficult to ascertain which factor is the real lethal agent. Therefore we shall only consider here the lethal effect in general as caused by different Solanaceous plants.

a. Petunia hybrida

Death may follow shortly after the ingestion of a certain amount of foliage in the case of a larva feeding on *Petunia hybrida*. The symptoms are vomiting of liquids and loss of the power of locomotion. The vomiting of liquids may result in a shrinkage of the abdominal segments. If forced to move, the newly poisoned larva may crawl, but only with steps characterized by incoordination. After a short distance the larva may fall on its side, with the legs and maxillary palpi trembling. Slightly intoxicated larvae usually show some other characteristics. They tend to wander aimlessly, as shown in the case of the larva on *Petunia hybrida* in the feeding experiments. After a time they may hide themselves under the leaves so as to avoid the light. These individuals may not vomit, but a considerable quantity of liquid will be excreted with the faeces.

It has been observed that in general only the third and fourth instar larvae would feed on *Petunia hybrida*; larvae in the first two instars showed scarcely any preference for this plant. Resistance to the toxic effect varies greatly with individuals, but the maximum lengths of time that the larva is able to survive on this plant has been found to be about four days. Individuals that can consume a comparatively large quantity of the foliage at the first encounter are usually those with high resistance. On the other hand, some larvae are extremely susceptible; they will succumb almost immediately after the ingestion of a small quantity of the foliage.

In my experiments a number of young fourth instar larvae were used for the determination of their tolerance for *Petunia hybrida*. Each was kept in a small rearing dish and the areas on the leaves consumed were determined on graph papers. Observations were made daily at ten o'clock in the morning and four o'clock in the afternoon. Table 12 gives the results.

TABLE 12. The tolerance of fourth instar larvae for *Petunia hybrida*
(*De tolerantie van de larve van het 4e stadium t.o.v. Petunia hybrida, uitgedrukt in de overlevingstijd en de oppervlakte van het gegeten blad*)

Larva	Time of survival (in days)	Foliage consumed (mm^2)
a	less than 1 day	10
b	3	138
c	3	137
d	1	56
e	4	100

Mortality = 33 %

It was found that slightly intoxicated larvae might be saved by offering only potato foliage to them. A number of larvae were induced to take a meal of *Petunia* leaves, after which they were transferred to potato leaves. The areas on the *Petunia* leaves consumed were determined on graph papers. The larvae were reared seperately in rearing dishes with potato foliage and observations made daily. Table 13 records these observations.

TABLE 13. The events following a meal on *Petunia hybrida* by fourth instar larvae afterwards fed on potato foliage
(*Gevolgen van het opnemen van een hoeveelheid Petunia-blad; larven 4e stadium na het maal van Petunia op aardappelblad geplaatst*)

Larva	Petunia foliage consumed (mm^2)	Consequence
a	2. . .	died shortly after the meal
b	7. . .	died afterwards
c	2. . .	normal growth, transformed into pupa and emerged as adult
d	13. . .	normal growth, transformed into pupa and emerged as adult
e	8. . .	normal growth, but died three days later
f	10	normal growth, transformed into pupae and emerged as adults
g	10	
h	4	
i	8	

In the rearing experiments in 1948, larvae just after hatching and moulting were divided into lots according to their age and each larva was reared in a glass vial kept in a hygrostat with 100 % R. H. and at a constant temperature of 27 °C. The larvae were supplied daily with *Petunia* leaves. Lots of larvae in the same stages of growth were used as the control but without food. The mortality was observed daily. The results are shown in Fig. 15. It is seen that the larvae which feed on *Petunia* leaves have a much shorter life span than those starved, indicating the toxic action of *Petunia*.

The chemical compounds in *Petunia* that are responsible for the lethal effect are not yet known. The glandular hairs on the leaves and stems secrete an oily substance which is volatile and soluble in water. In France, experiments to determine whether nicotine was present in *Petunia* and might account for poisoning the larvae gave negative results (GUILLAUME, 1934).

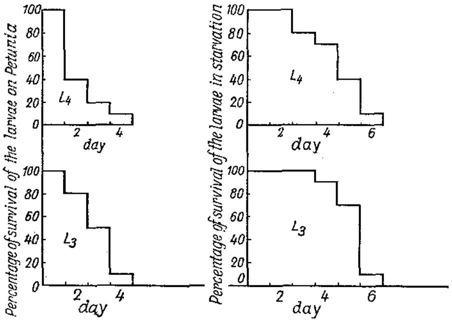


Fig. 15. The percentage of survival of the Colorado beetle larvae on *Petunia hybrida* and in starvation. L_3 = third instar; L_4 = fourth instar.
(*Verloop van het overlevingspercentage van larven die gevoed zijn met Petunia hybrida en van hongerdieren.*
L 3 = 3e larvestadium; L 4 = 4e larvestadium.)

b. *Solanum demissum*

It has been stated that in *Solanum demissum* the factor causing resistance to the attack of Colorado beetle larvae seems to be the presence of demissin in the leaves (KUHN and GRAUHE, 1947). At the present time it is still difficult to decide whether this alkaloidglycoside is poisonous to the larvae. If it is so, its effect must be slowly cumulative. On the other hand, sufficient evidence has been accumulated to show that the mortality of larvae on this plant is principally due to inanition and that the aversion to feeding is caused by an unfavourable sensory reaction. No conspicuous symptoms of intoxication such as those observed with *Petunia hybrida* can be detected, though in France it has been noticed that the larvae avoid light and shelter from it on the lower surfaces of the leaves (BUSNEL and CHEVALIER, 1938). The larvae that show aversion to feeding on *Solanum demissum* may wander aimlessly over the leaves and finally abandon them. But these phenomena are still not enough to justify the view that the mortality is due to intoxication, because a larva kept starved in a glass vial with a piece of moistened filter paper may behave similarly. Larvae that die on *Solanum demissum* may turn dark in color, but this may also happen to those that die on *Solanum tuberosum.*

It has been shown that larvae reared for one day on *Solanum demissum* and then transferred to feed on *Solanum tuberosum* may have a low mortality of 10 % (DE WILDE, 1948*a*). Therefore, the adverse effect that may be caused by a fairly large dose of this foliage is not maintained when the food plant has been changed into a suitable one.

In 1948 several lots of larvae just after hatching or after moulting were reared on *Solanum demissum* at a temperature of 27 °C till they were ready to form the prepupae. They were kept individually in glass vials and the leaves were changed daily. Mortality and development were observed every day. A lot of newly hatched larvae was reared on potato and used as the control. The results are presented in Fig. 16. An interesting point is that under such laboratory conditions, a small percentage, usually about 20 %, of the newly hatched larvae can mature on a resistant plant such as *Solanum demissum* "line 23". The rearing experiments in the summer of 1949 further revealed that about 25 % of the newly hatched larvae could grow into adult beetle on *Solanum demissum* "line 23" with body weights ranging from 69 to 123 mg. In the experiments in 1948, only the lot composed of larvae after the first moult failed to give rise to mature larvae. The results also indicate that first and second instar larvae are least able to grow

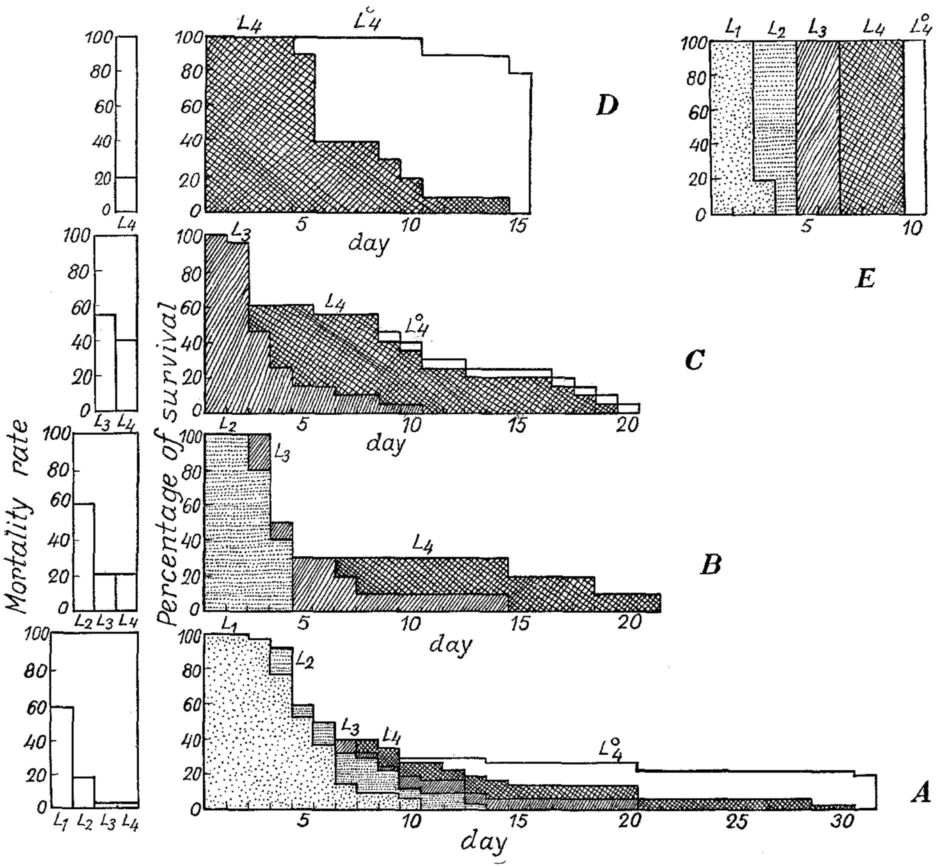


Fig. 16. Mortality rate and development of the Colorado beetle larvae reared on *Solanum demissum* (line 23) at 27° C. A = after hatching; B = after the first moult; C = after the second moult; D = after the third moult; E = control, reared on potato after hatching. L_1 = first instar; L_2 = second instar; L_3 = third instar; L_4 = fourth instar; L_4° = mature larva.
(*Verloop van het overlevingspercentage van larven, die gevoed zijn met Solanum demissum stam 23, bij 27 °C. A. Ab ovo op Solanum demissum gekweekt; B. Tot de eerste vervelling op Solanum tuberosum; C. Tot de tweede vervelling op Solanum tuberosum; D. Tot de derde vervelling op Solanum tuberosum; E. Controle, gevoed met Solanum tuberosum. De sterfte in ieder stadium in % is links aangegeven.* L_1, L_2 *etc. = 1e, 2e etc. larvestadium;* L_4° *= praepupa*)

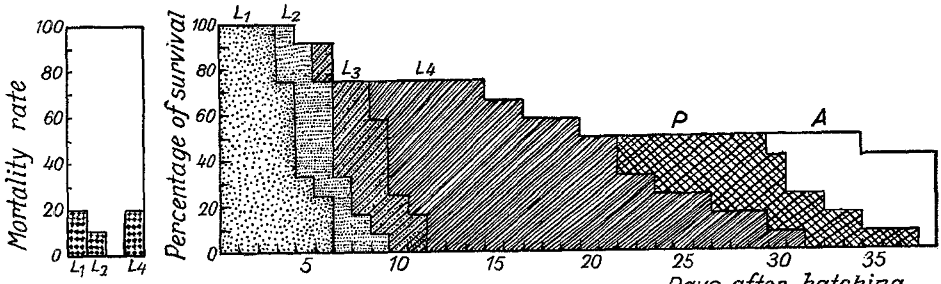
on this plant; high mortality usually occurs when the larvae are induced to feed on the foliage. The larvae that can survive the first period after being reared on this plant are usually those which are tough and able to reach maturity. When compared with the larvae reared on *Solanum tuberosum*, development was slow and irregular, indicating the profound adverse influences.

c. *Atropa belladonna*

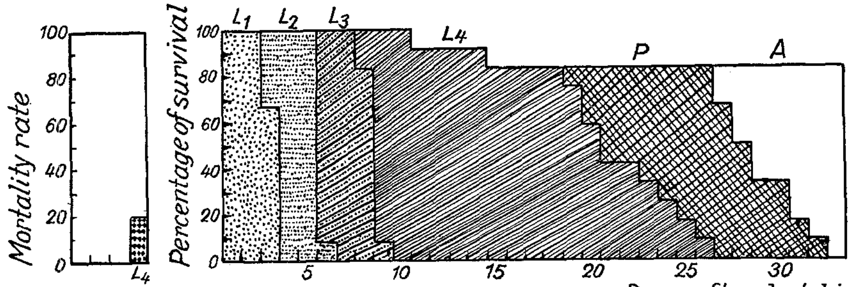
It is known that the leaf of *Atropa belladonna* contains atropine, $C_{17}H_{23}O_3N$, which can influence the beat of the isolated heart of some insects (DAVENPORT, 1949). As has been mentioned in Chapter III, young plants of this species are toxic to the larvae while on flowering plants development can be completed. This difference between young and old plants seems to be principally due to a quantitative difference in the chemical composition of the leaves.

In texture the *Atropa* leaf is different from that of potato; it is devoid of hairs and has a smooth surface. The larvae will feed on the leaf greedily, resulting in rapid growth; those that died on this plant usually turned dark in color.

In the summer of 1949, twelve newly hatched larvae were reared at room temperature on *Atropa belladonna*. Twelve larvae hatched from the same batch of eggs were reared on potato as the control. The individuals were kept separately in vials which were placed in a hygrostat dish and the leaves from the flowering plants were changed daily. The results are presented in Fig. 17. It is seen that development on *Atropa* is slightly prolonged and a higher mortality occurs in the first and fourth instars. In the control, death of the larvae only occurs in the fourth instar. The mortality on *Atropa* has been found to be 50 %; that on potato about 20 %. Therefore the rate of development on the flowering plant of *Atropa belladonna* may be normal but the mortality is high. This fact recalls the relationship between the larvae and *Solanum atropurpureum* (TROUVELOT et al., 1933).



Atropa belladonna



Solanum tuberosum

Fig. 17. Mortality rate and development of the Colorado beetle larvae reared on *Atropa belladonna* and *Solanum tuberosum*. L_1 = first instar; L_2 = second instar; L_3 = third instar; L_4 = fourth instar. P = pupal stage; A = adult.
(*Verloop van het overlevingspercentage van larven die ab ovo op Atropa belladonna en Solanum tuberosum zijn gekweekt. Het sterfte-percentage in ieder stadium is links aangegeven.* L_1, L_2 *etc.* = *1e, 2e etc. larvestadium; P = Pupa; A = imago.*)

2. THE EFFECT OF ALTERNATIVE FEEDING

Under natural conditions larvae of the Colorado beetle may abandon an unsuitable plant and seek to feed on a preferred one. But they are able to travel only for several yards (FEYTAUD, 1931) and such movement is perhaps not very common. In the laboratory, alternative feeding may be artificially induced. The result of alternative feeding may indicate whether a plant is really toxic or only repellent. With larvae feeding alternatively on a suitable and a toxic plant mortality would be expected to be higher than that in larvae feeding alternatively on a suitable and a repellent but non-toxic plant. The following two experiments were performed to test the effects of alternative feeding on *Solanum tuberosum* and *Solanum demissum*, and *Solanum tuberosum* and *Petunia hybrida.*

a. Solanum tuberosum and *Solanum demissum*

Larvae just after hatching and moulting were divided into several lots, according to their age. Each larva was separately reared in a glass vial kept in a hygrostat with 100 % R. H. and at a constant temperature of 27 °C. They were supplied on alternate days with potato leaves and with *demissum* leaves and mortality and development were observed daily.

The result of this experiment is shown in Fig. 18. It is seen that 70 % of the newly hatched larvae receiving this diet can reach maturity; mortality only occurs in the first and fourth instars. On the other hand a relatively high mortality occurred in the lot where the alternative feeding started after the first moult. This is in agreement with the result of the above mentioned experiments where very high mortality occurred in second instar larvae that were then induced to feed continuously on *Solanum demissum* (cf. Fig. 16). It seems difficult for second

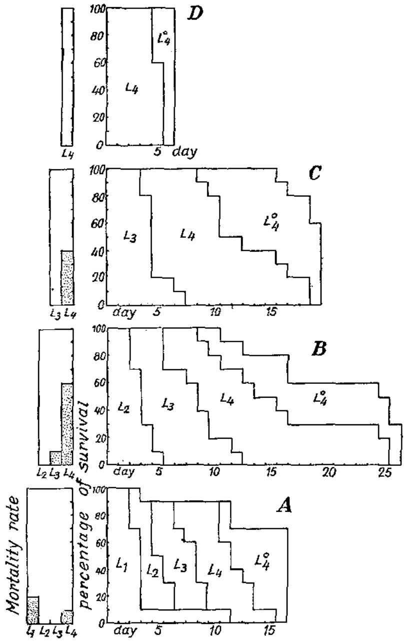


Fig. 18. Mortality rate and development of the Colorado beetle larvae feeding alternatively on potato and *Solanum demissum* (line 23). A = after hatching; B = after the first moult; C = after the second moult; D = after the third moult; L_1 = first instar; L_2 = second instar; L_3 = third instar; L_4 = fourth instar; L_4° = mature larva.
(*Verloop van het overlevingspercentage van larven, die afwisselend gedurende 24 uur met Solanum tuberosum en met Solanum demissum, stam 23, zijn gevoed. Het sterftepercentage in ieder stadium is links aangegeven.*
L_1, L_2 = *1e, 2e larvenstadium,* L_4° = *praepupa.*
A. Ab ovo afwisselend gevoed.
B. Tot 1e vervelling op Solanum tuberosum. C. Tot 2e vervelling op Solanum tuberosum. D. Tot 3e vervelling op Solanum tuberosum.)

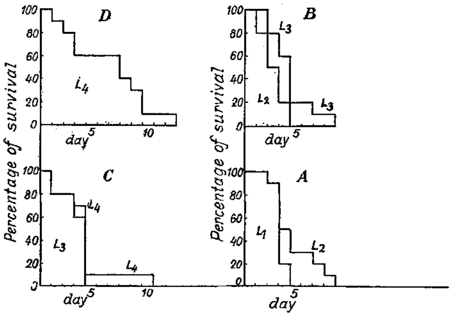


Fig. 19. Mortality rate and development of the Colorado beetle larvae feeding alternatively on potato and *Petunia hybrida*. For description, same as in Fig. 18. (*Verloop van het overlevingspercentage van larven, die afwisselend.*)

instar larvae to adjust themselves on this plant. As a whole the result indicates that mortality can be partially avoided when a preferred plant is added to a diet of *Solanum demissum*, suggesting that mortality on *Solanum demissum* is due to insufficient feeding.

b. Solanum tuberosum and Petunia hybrida

All the conditions in this experiment were the same as in the above experiment except that leaves of *Petunia hybrida* were used instead of *Solanum demissum*. The result is shown in Fig. 19. As already mentioned, in general only the third and fourth instar larvae would attack *Petunia hybrida*. In this experiment the first and second instar larvae were observed also to eat the *Petunia* leaves but they were extremely vulnerable and succumbed within 24 hours after ingestion of a small quantity of the foliage. Some of them avoided feeding on this plant, but death still occurred, perhaps caused by the volatile substance from the glandular hairs on the leaves. As compared with the larvae continuously feeding on *Petunia hybrida*, the lives of fourth instar but not of third instar larvae were prolonged (cf. Fig. 15). Therefore fourth instar larvae possess greater tolerance than third instar larvae though none can reach maturity on this plant.

3. TEMPERATURE AS A FACTOR INFLUENCING LARVAL MORTALITY

The importance of temperature in affecting the life processes of insects has been mentioned in Chapter III. For thermal influences on the embryonic and postembryonic development of the Colorado beetle, reference is made to the work of DE WILDE (1948*b*).

In the field the adverse effect of food plants is usually enhanced by unfavourable climatic conditions so that the tolerance of an insect for a resistant plant is always lower in the field than in the laboratory (RAUCOURT and TROUVELOT, 1936; SCHWARTZ, 1948). Thus it was observed that at low temperatures the development of Colorado beetle larvae on some resistant hybrids between potato and other tuberiferous species of *Solanum* ceased although it proceeded normally on potato (ANONYMOUS, 1937). Moreover, as has been mentioned in Chapter III, a rise of temperature may alter to a certain extent the properties of the food plants such as the vapour tension of the volatile substances in the leaves which affect the feeding habits of the larvae. The following experiment was performed to test the effect of different temperatures in the developmental range on the tolerance of Colorado beetle larvae for *Solanum demissum*.

Larvae after hatching were divided into several lots each of fifty individuals.

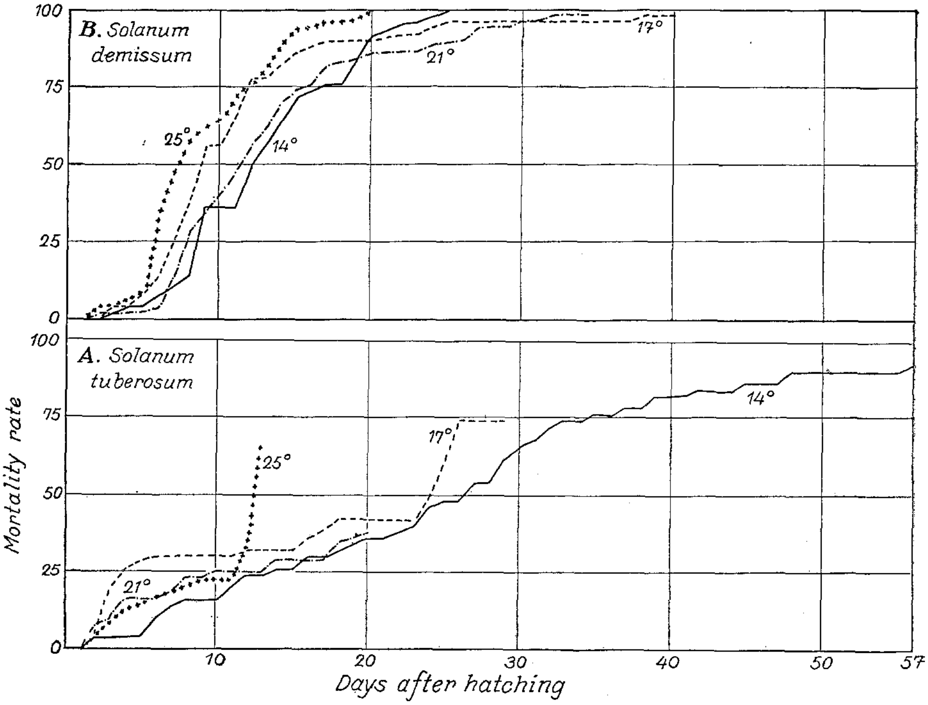


Fig. 20. The influence of temperature on the mortality rate of the Colorado beetle larvae reared on *Solanum tuberosum* and *Solanum demissum* (line 48).
(*Invloed van de temperatuur op het verloop van het sterftepercentage van larven, gekweekt op Solanum tuberosum en Solanum demissum, stam 48*)

Each lot was reared on the leaves of *Solanum demissum* "line 48" in a rearing container. The lots of larvae were kept separately in a thermal cabinet at the following temperatures: 14, 17, 21, and 25 °C. Equal numbers of larvae were reared on potato leaves at the same temperatures as controls. Observations were made daily.

The mortality rate of the larvae in this experiment is shown in Fig. 20. It is seen that the mortality of the larvae reared on *Solanum demissum* at 14 and 25° is 100 %; that at 17 and 21 ° is 98 %. At 14 ° all larvae died before the second moult; at 17 ° more than 90 % of the larvae died before the third moult; and at 21 and 25 ° the mortality rate before the third moult was lessened. On *Solanum tuberosum* larvae matured at all temperatures. The survival periods of the larvae feeding on *Solanum demissum* at different temperatures used in this experiment were in general shorter than those on *Solanum tuberosum*. The result indicates that larvae feeding on a resistant plant can be much weakened by an unfavourable temperature.

As a whole the results from the above experiments demonstrate that *Petunia hybrida* is definitely toxic; besides many signs of intoxication, the larvae feeding on this plant die earlier than when starved. The larvae feeding on flowering plants of *Atropa belladonna* may have a relatively high mortality rate. The death of larvae feeding on *Solanum demissum* seems to be essentially due to inanition and the adverse effect of this plant is enhanced by low temperatures.

CHAPTER VI

INFLUENCE OF THE FOOD PLANTS ON THE GROWTH OF THE LARVAE

1. INFLUENCE ON THE INCREASE OF THE BODY WEIGHT

Organic growth denotes the accumulation of the materials assimilated from the food in the body. In general, the quality and quantity of the diet greatly influence the growth rate, which is manifested in the body weight and the size of the animal. It is difficult, however, to indicate the increase of energy content in the body of an insect solely by the increasing body weight during growth, because a shift from protein to fat synthesis may obscure the relations (MAYER, 1949). Thus in first and second instar larvae of the Colorado beetle protein synthesis seems to be the most important process while in the third and fourth larval stages there is a rapid increase in fat storage.

With the increase in body weight as a criterion, it has been found by many authors that most insects show an S-shaped growth curve (PRZIBRAM, 1927; PRZIBRAM and MEGUSAR, 1912; YAGI, 1926; TEISSIER, 1931, 1936; HODGE, 1933). This means that under normal conditions, an insect shows a slow gain in body weight in the early part of development, followed by a steep rise in the growth rate, and finally a slight decline in weight due to the termination of feeding and loss of water, indicating preparation for transformation. The final decline in body weight shows as a characteristic peak in the curve. It is interesting to see that in such an insect as *Melanoplus differentialis*, the absence of the preadult peak from the curve for individuals on an unsatisfactory diet prophesies death before maturity (HODGE, 1933). Moreover, the curve indicates the general tendency of growth, and any adverse influence from the diet or other factors can be detected by the curve showing zigzag inconsistency.

In the summer of 1949, four lots of 12 newly hatched larvae were reared on several sorts of food plants in order to test the difference in growth. The food plants used were *Solanum tuberosum* (Noorderling), *Solanum demissum* "line 23", the hybrid of *Solanum demissum* "line 34" and "line 48", and the flowering plant of *Atropa belladonna*. The larvae were separately kept in glass vials with a capacity of 14 cc, stoppered with cotton wool and placed in a large hygrostat container (100 % R. H.) at room temperature varying from 20° to 25 °C. The leaves were changed daily and the body weight of each larva was determined on a torsion balance.

Most of these larvae completed their development on *Solanum tuberosum*, a large proportion on *Atropa belladonna*, and a small proportion on the two sorts of *Solanum demissum*. The change in body weight of five individuals in each lot is shown in Fig. 21–24.

On *Solanum tuberosum*, the larvae showed the typical S-shaped growth curve (Fig. 21). It is seen that in the first two instars growth is rather moderate in rate though the body weight at the second moult mostly doubles that at the first

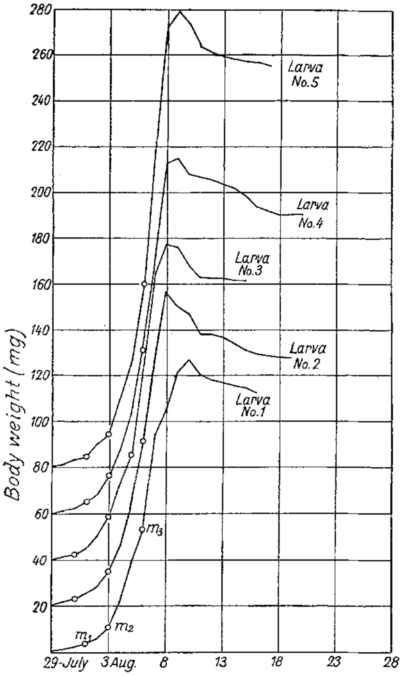


Fig. 21. Growth curves of the Colorado beetle larvae reared on potato leaves; m = moult.
(Groeicurven van larven gekweekt op Solanum tuberosum; m = vervelling)

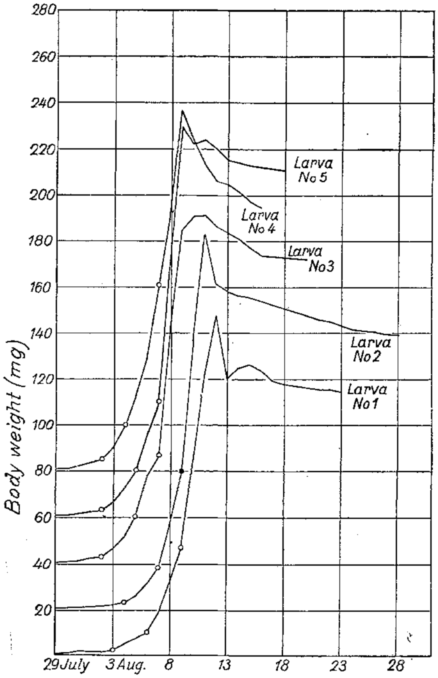


Fig. 22. Growth curves of the Colorado beetle larvae reared on the flowering plant of *Atropa belladonna.*
(Groeicurven van larven gekweekt op Atropa belladonna)

moult. Tremendous growth has taken place in the third and fourth instars, in which, as has been mentioned above, the process of fat synthesis is prevailing. The fourth instar larvae attained the maximum body weight (average: 155.5 ± 6.2 mg) about three or four days after the third moult. Then the larva showed a decrease in appetite, usually accompanied by a change of colour to orange. At that stage the larva was mature and showed the tendency to bury itself in the soil.

The larvae reared on *Atropa belladonna* have almost the same rate of growth as those on potato; the only difference seems to be in the first instar, where the increase of the body weight is relatively slow (Fig. 22). The maximum body weights of the fourth instar larvae have an average value of 155.2 ± 4.4 mg, indicating that the larvae can grow on the leaf of *Atropa* just as well as on the potato leaf.

The larvae reared on *Solanum demissum* "line 23" may be divided into two groups. A small number can use the leaves rather successfully; and in these cases the S-shaped growth curve is more or less retained (Fig. 23, larvae No 3–5). Growth in the first instar is comparatively slow and the average maximum body weight in the fourth instar is 141.4 ± 20.4 mg, which suggests that this plant is slightly inferior to potato and *Atropa* for growth. Sometimes the larva may decrease in body weight before becoming mature, as in the case of larva No 3. This phenomenon is also noticed in the larvae feeding on potato. A majority of the larvae cannot reach maturity and the manner in which they grow is represented by larvae No 1–2. In these cases either there is no considerable gain in body weight or the increase is rather irregular so that the growth curve is characterized by a change in direction.

The larvae reared on the hybrid of *Solanum demissum* "line 34" and "line 48" have in general the same features in their growth as those on "line 23". A small number can mature on this plant under laboratory conditions. They still show more or less S-shaped growth curves, with an average maximum body weight in the fourth instar of 114.7 ± 10.4 mg, which demonstrates inferior growth. The larvae that succumb before maturity have the same growth curves as those reared on "line 23" (Fig. 24).

The results of this experiment indicate that potato is the most suitable food plant among the species used; on it normal growth is accompanied by a low mortality rate. On the flowering plant of *Atropa belladonna*, larval mortality is high, but growth is normal, indicating that the death of larvae is not due to inanition but to the presence of some special chemicals in the leaves. The two sorts of *Solanum demissum* provide poor food for the larvae; mortality on them

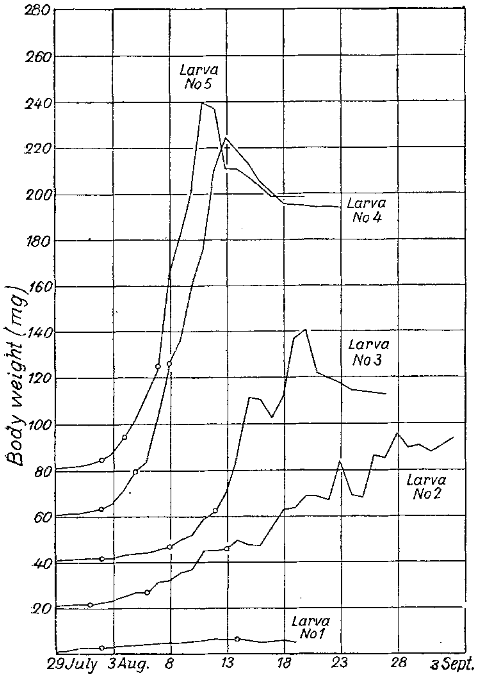


Fig. 23. Growth curves of the Colorado beetle larvae reared on *Solanum demissum* (line 23).
(*Groeicurven van larven gekweekt op Solanum demissum, stam 23*)

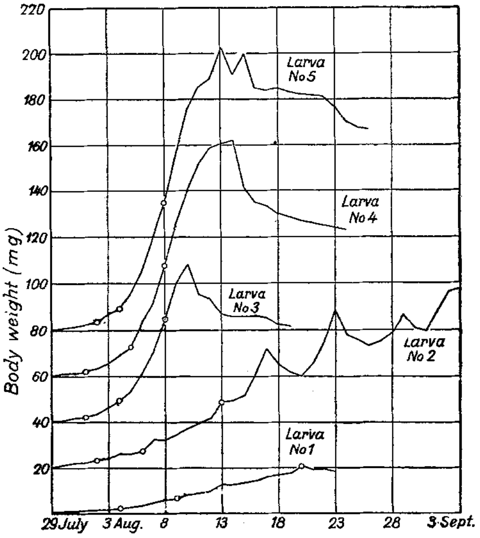


Fig. 24. Growth curves of the Colorado beetle larvae reared on *Solanum demissum* (hybrid of "line 48 and 34").
(*Groeicurven van larven gekweekt op Solanum demissum, 48 × 34*)

is very high and the growth of most of the animals is abnormal or subnormal. Apart from insufficient feeding caused by unfavourable sensory reaction as discussed in the above chapters, there is some indication that *Solanum demissum* is inferior to potato in nutritive value.

2. INFLUENCE ON THE LARVAL INSTARS

The growth of the larvae of the Colorado beetle, as well as other arthropods, is not a continuous process but is characterized by periods of activity alternating with periods of quiescence. In the periods of quiescence, the insect undergoes the process of moulting or ecdysis which divides the larval life into a series of instars. The instars are generally regarded as indicating the biological age of an insect which implies the degree to which the insect has achieved its development. A deficiency in nutrition, which affects larval growth as revealed in the change of body weight, will also adversely influence the larval instars, and can be detected in the number and duration of the instars and the linear growth of various parts of the animal.

a. The number of instars

It is known that inadequacy of nutrients, leading to a prolonged larval life, may increase the frequency of ecdyses. An extreme case is afforded by the clothes moth *Tineola*, which, when kept on a rich or poor medium, may have a larval period varying from 26 days with 4 moults to 900 days with 40 moults (TITSCHACK, 1926). Colorado beetle larvae have four instars. In my experiments and the rearing work carried out in 1948 and 1949, excessive ecdysis in the larvae has never been observed; but in France it was reported that larvae reared on *Solanum nigrum* underwent an additional moult (KOZLOVSKY, 1937).

b. The lengths of instars

In holometabolous insects, the later instars generally have a longer duration, and if the diet is not satisfactory all the stages may be prolonged. The lengths of instars of the Colorado beetle larvae on different food plants have already partly been shown in the graphs in the preceding chapter (cf. Fig. 16, 17, 18). The larvae reared on *Solanum tuberosum*, *Solanum demissum* "line 23", the hybrid of *Solanum demissum* "line 34" and "line 48", and *Atropa belladonna* showed some difference in the durations of each instar. The lengths of the stages of the larvae which completed their development are presented in Table 14. The fourth larval stage is regarded as the period from the third moult to the cessation of feeding.

TABLE 14. The lengths of the instars (in days) as influenced by food plants
(*Duur van de larvenstadia (in dagen) op verschillende voedselplanten*)

Plant	First instar	Second instar	Third instar	Fourth instar
Solanum tuberosum	2,2 ±0,2*	2,6 ±0,2	2,8 ±0,2	4,2 ±0,2
Atropa belladonna	4,0 ±0,3	2,6 ±0,2	2,7 ±0,2	3,6 ±0,2
Solanum demissum „line 23"	3,8 ±0,3	4,0 ±0,9	4,2 ±0,9	9,0 ±2,4
Solanum demissum . . . . „342" × „48" F_1	4,0 ±0,3	3,2 ±0,5	4,4 ±0,7	10,0 ±3,0

* standard error

With the lengths of the larval instars on *Solanum tuberosum* as the criterion, it is seen that only the first instar on *Atropa belladonna* is prolonged, the second, third and fourth being normal. Perhaps the young larvae are not accustomed to the effect of the alkoloids contained in the leaves but in the older larvae the effect is diminished. It has already been mentioned that the mortality rate on this plant is relatively high in the first instar (cf. Fig. 17). For the larvae feeding on *Solanum demissum* the adverse influence from the diet is more profound in the fourth instar, which usually requires more than twice the time of those feeding on *Solanum tuberosum* and *Atropa belladonna*. The larvae cannot adapt themselves to growing on this plant as they do on *Atropa belladonna*. Therefore the adverse effects of *Atropa* and of *Solanum demissum* are of different kinds. Because of the prolonged later instars, the result of this experiment confirms that the adverse influence of *Solanum demissum* is caused by an inadequate supply of nutrients.

c. Linear growth in each instar

The linear growth of insects has attracted the attention of many authors and attempts have been made to correlate the growth in each instar with theoretical considerations. PRZIBRAM (1927) expressed the view that the increase in size of various parts is caused by cellular multiplication rather than by cellular growth. Thus the weight is doubled during each instar, and at each moult the linear dimensions of the various parts are increased by the ratio 1.26 or 23, indicating the „division step" or *Teilungsschritt*. DYAR showed that the head capsule of caterpillars grows in geometrical progression, increasing in width at each moult by a factor of about 1.4 (TEISSER, 1931, 1936). This rule is sometimes helpful in deducing from incomplete series of exuviae the actual number of moults.

An insect feeding on a deficient diet may moult without growth. In the case of *Tineola*, the insect may even grow gradually smaller after the moults (TITSCHACK 1926). Therefore linear growth may give an indication of the general nutritional situation.

Differences in the body size of the larvae reared on *Solanum tuberosum*, *Atropa belladonna*, and the two strains of *Solanum demissum* from the above experiment were noticed. The exuviae after the moults were preserved and the widths of the

TABLE 15. The widths of the head capsules and the lengths of the mandibles of the larvae reared on different plants
(*Breedte van het kopkapsel en lengte van de mandibulae van larven, gekweekt op verschillende voedselplanten*)

Plant	Measurement (mm)	First instar	Second instar	Third instar	Fourth instar
Solanum tuberosum	head	0,72 ±0	1,04 ±0,00	1,52 ±0,00	2,26 ±0,02*
	mandible	0,27 ±0	0,38 ±0,00	0,58 ±0,00	0,89 ±0,01
Atropa belladonna	head	0,72 ±0	1,04 ±0,00	1,52 ±0,00	2,28 ±0,02
	mandible	0,27 ±0	0,38 ±0,00	0,58 ±0,00	0,91 ±0,01
Solanum demissum „line 23"	head	0,72 ±0	1,04 ±0,00	1,38 ±0,07	2,13 ±0,11
	mandible	0,27 ±0	0,38 ±0,00	0,55 ±0,02	0,84 ±0,09
Solanum demissum „34" × „48" F_1	head	0,72 ±0	1,03 ±0,01	1,47 ±0,03	2,06 ±0,06
	mandible	0,27 ±0	0,38 ±0,00	0,57 ±0,01	0,80 ±0,02

* standard error

head capsules and the lengths of the mandibles were measured as accurately as possible under a binocular microscope with the help of an ocular micrometer. The numbers of exuviae from the larvae reared on potato, *Atropa belladonna*, *Solanum demissum* "line 23", and the hybrid were 47, 34, 21 and 34 respectively. The average measurements are shown in Table 15.

It is found that regarding the widths of the head capsules at successive moults of the larvae reared on *Solanum tuberosum*, the factor for linear growth has the value of 1.45, in quite close accordance with DYAR's rule. Thisv alue applies also to the mandibles. The larvae reared on *Atropa belladonna* have approximately the same factor, but those feeding on the two strains of *Solanum demissum* show deviations which are especially conspicuous in the two later instars.

CHAPTER VII

THE DIGESTION OF THE DIFFERENT PLANTS

1. THE PASSAGE OF THE FOOD THROUGH THE DIGESTIVE TRACT

All nutrients required by phytophagous insects for maintaining their growth and development may be derived from one single plant. This is so in the case of the Colorado beetle. Success in development depends on the one hand upon the availability of all the required or potentially convertible substances in the plant and on the other hand upon the ability of the insect to utilize them. Digestion is directly concerned with food utilization, the extent of which can be shown from the proportion of the ingested food retained in the insect body. Moreover the rate of passage of the food through the digestive tract and the composition of the excrement may also give indications concerning the normal functioning of the digestive system.

In order to study the functioning of the digestive system, it is necessary first to understand the anatomical features. As indicated by their embryological origin, the anterior and posterior parts of the tract are derived from the ectoderm and the middle part from the entoderm. The anterior portion is the stomodeum, which gives rise to the foregut including the mouth cavity, pharynx, crop and proventriculus. The middle portion or the mesenteron in the larva constitutes the long ventriculus or stomach (midgut) and is the essential seat of digestion. The posterior portion is called the proctodeum, which forms the hindgut subdivided into an anterior colon and posterior rectum (SNODGRASS, 1935). In the fourth instar larva of the Colorado beetle, the lengths of the pharynx, crop, proventriculus, ventriculus and the combined portion of colon and rectum are 1, 1.5, 1, 10 and 7 mm respectively (BUSNEL, 1939). Morphologically the digestive tract of the larva is a tube of columnar epithelium invested by a thin layer of muscular coat, the length slightly exceeding that of the body. The greater part of the tube remains straight; convolution only occurs at the region where the mesenteron joins the proctodeum.

The body wall of the fourth instar larva is usually thin enough to reveal on the dorsal side the heart, the fat bodies and the digestive tract. Under a binocular microscope, the digestive tract of a well-fed larva can be seen in frequent peristaltic and churning movements. These movements lead to the mixing of the ingested food with digestive juices and also carry the contents along. The speed of the food current is influenced by various factors.

After one day of starvation, most of the digestive tract of the fourth instar larva becomes empty, only the rectum being filled with a mass of whitish uric acid. In my experiments, determinations were made of the rate of filling of the empty digestive tract with potato leaves. It was found that after 15 minutes of feeding the crop was filled with food. The anterior two thirds of the ventriculus was filled after 30 minutes and the entire ventriculus after one hour.

Determinations were also made of the periods required for the different foods to pass through the empty digestive tract of the fourth instar larvae. The experiment was performed in the following way: Larvae after the third moult were kept starved for one day. They were then divided into three groups and allowed to feed for 30 minutes on the leaves of *Solanum tuberosum*, *Solanum demissum* "line 23" and *Petunia hybrida.* At the beginning of feeding the time was recorded. When 30 minutes had elapsed, the larvae were separately transferred into hygrostat dishes provided only with filter paper and the time at which defecation occurred was recorded. The excreta were examined under a binocular microscope in order to make sure that fresh leaf fragments were present. The results are shown in Table 16.

TABLE 16. The rate of passage of food through the empty digestive tract in the fourth instar larva
(Aantal uren, verlopende tussen ingestie en defaecatie van het voedsel bij verschillende voedselplanten, nadat de darm tevoren was geledigd (Larve 4e stadium, hongerdieren).

Food plant	Larva	Hours required for passage of food
Solanum tuberosum	a	3,0
	b	4,5
	c	more than 5
	d	more than 5
Solanum demissum	e	3,7
	f	4,2
	g	more than 5
Petunia hybrida	h	3,5

It is seen that the rate of passage of the different food plants in the starved fourth instar larvae is nearly the same.

In well-fed individuals of such insects as the fifth and sixth instars of the American Southern army worm *Prodenia eridania,* food requires 3¼ hours to pass through the digestive tract (CROWELL, 1943). The digestive tract of the Colorado beetle larva is much shorter than that of a caterpillar and the rate of passage of food in a well-fed larva is found to be greater than in a starved larva.

Experiments were carried out to test the rate of passage of food through the digestive tract of the fourth instar larvae fed on different food plants. A number of young fourth instar larvae were fed on *Solanum tuberosum*, *Solanum demissum* "line 23" and *Petunia hybrida* for one or two days. Then each larva was placed on a piece of fresh leaf under a binocular microscope. When feeding was in good progress, a piece of colored wax was placed at the place where the leaf would be consumed. As the wax was ingested, the time was recorded and the larva was transferred into a rearing dish supplied with the same sort of leaf. When defecation occurred in that dish, the time was again recorded and the excreta were examined under a binocular microscope. The presence of the colored wax in the excreta indicated the length of time that the food had been in the digestive tract. The results are shown in Table 17.

The larvae reared on *Petunia hybrida* for two days were usually weakened and not suitable for this study. The three individuals listed in the table were

TABLE 17. The rate of passage of food through the digestive tract of well-fed larvae reared on different plants
(Als Tab. 16, doch darm niet eerst geledigd (larve 4e stadium, goed gevoede dieren).

Plant	Larva	Hours required for passage of food
Solanum tuberosum	a	1,3
	b	2,0
	c	2,0
Solanum demissum	d	4,7
	e	3,0
	f	3,7
Petunia hybrida	g	1,8
	h	2,1
	i	1,5

chosen out from a number of larvae previously fed on potato leaves; they showed greater tolerance for *Petunia*, after being introduced to *Petunia* leaves for a time. The colored wax was ingested after a considerable amount of *Petunia* leaf had been consumed.

Though the number of individuals used in this experiment is small, it is seen that the time required for the passage of food in a well-fed larva on *Solanum tuberosum* is about two hours. The food will pass with a lessened speed through the digestive tract of larvae reared on *Solanum demissum*, but this is not so in larvae fed for a time on *Petunia hybrida*. It has been shown that food will stay longer in the digestive tract of a starved larva. In the case of *Solanum demissum*, the lessened speed in the passage of food indicates either that the digestive tract is partially empty because of insufficient feeding, or that the digestive process has become abnormal.

2. THE RETENTION OF THE INGESTED FOOD IN THE INSECT BODY

As has been mentioned above, the extent of retention of the ingested food in the body usually indicates food utilization. It has been shown that in the adult beetles of *Leptinotarsa decemlineata*, newly transformed individuals and those after diapause may exhibit differences in food retention (GRISON and ROEHRICH, 1947). The larval instars are the stage of growth and the sole work in which the animal is engaged is to create a constant food current in the digestive tract and derive from it as much profit as possible. When feeding on an unsuitable plant, the adverse effect may be partly due to failure to digest and absorb the ingested food. The following experiments were carried out to determine the differences in food utilization having regard to the age of the larvae and the different food plants.

Method. The experiments were performed at room temperature, varying from 20 to 25 °C. The larvae were selected shortly after a moult and kept in rearing dishes for about one day so as to empty the guts. The second instar larvae were more vulnerable to starvation, and were therefore starved for a shorter period, about six hours. After starvation, their body weights were determined. Each of them was then reared in a glass vial on leaves of *Solanum tuberosum* (Noorderling)

TABLE 18. The retention of potato leaves by second instar larvae of *Leptinotarsa decemlineata*
(*Retentiepercentage van de droge substantie van aardappelblad door de larve van het tweede stadium*)

Larva no.	Body weights before experiment (mg)	Body weights after experiment (mg)	Dry weights of leaf eaten (mg)	Dry wt. of excreta (mg)	Percentage retention
1	3,9	11,4	4,4	1,1	75,0
2	5,0	13,5	3,6	0,9	75,0
3	4,7	7,1	1,4	0,3	78,6
4	3,8	5,5	1,0	0,3	70,0
5	3,7	7,6	2,7	0,7	73,7
6	2,3	7,7	2,6	1,0	61,5
7	3,3	8,7	2,9	0,7	75,8
8	4,9	7,8	1,5	0,3	80,0
9	3,9	8,5	3,6	0,9	75,0
10	5,7	10,3	2,3	1,0	56,5
11	4,3	8,9	2,8	1,0	64,3

Percentage of average retention = 71.4±2.3*

TABLE 19. The retention of potato leaves by third instar larvae of *Leptinotarsa decemlineata*
(*Retentiepercentage van de droge substantie van aardappelblad door de larve van het derde stadium*)

Larva no.	Body weights before experiment (mg)	Body weights after experiment (mg)	Dry weights of leaf eaten (mg)	Dry weight of excreta (mg)	Percentage retention
1	10,6	16,0	6,2	1,6	74,2
2	10,0	18,8	8,1	1,7	79,7
3	9,8	16,6	4,9	1,5	69,4
4	9,9	12,5	4,9	1,0	79,6
5	9,0	14,8	8,5	2,3	72,9
6	10,7	18,0	5,5	1,8	67,3
7	8,5	15,7	5,4	1,1	79,6
8	9,7	20,7	9,3	1,8	80,6
9	8,0	11,4	5,3	1,3	75,5
10	9,9	26,3	7,1	1,9	73,2
11	8,0	15,5	6,8	1,3	72,1
12	13,2	29,7	10,3	2,3	77,6

Percentage of average retention = 75,1 ±1,3*

in one series of experiments and on those of *Solanum demissum* "line 23" in another. The feeding period was two days, after which they were transferred separately into empty vials where they remained for 24 hours to empty the guts. Their body weights were again determined. All the vials were kept in a large hygrostat container. The outlines of the total leaves and the parts of the leaves consumed were determined with the „Ozalid" blue-print paper technique as described in Chapter III. After one day of feeding, the leaves were changed. All the excrement from each larva in the experiment was collected on dried filter paper of known weight. The remaining parts of the leaves after feeding and the papers containing the excrement were dried in an oven, (temperature 90 ° C, reduced pressure) and their dry weights were determined. If the remaining

* standard error

TABLE 20. The retention of demissum leaves (line 23) by third instar larvae of *Leptinotarsa decemlineata*
(*Retentie van de droge substantie van het blad van Solanum demissum (stam 23) door de larve van het derde stadium*)

Larva no.	Body weight before experiment (mg)	Body weight after experiment (mg)	Dry weight of leaf eaten (mg)	Dry weight of excreta (mg)	Percentage retention
1	15,3	23,2	4,8	1,1	77,1
2	9,2	16,8	5,8	1,3	77,6
3	10,2	9,7	1,7	0,8	53,0
4	9,3	10,6	1,8	0,8	55,5
5	11,4	12,2	1,7	0,9	47,0
6	9,1	7,1	1,0	0,4	40,0

Percentage of average retention = 58,4 ±5,5*

TABLE 21. The retention of potato leaves by fourth instar larvae of *Leptinotarsa decemlineata*
(*Retentiepercentage van de droge substantie van aardappelblad door de larve van het vierde stadium*)

Larva no.	Body weights before experiment (mg)	Body weight after experiment (mg)	Dry weight of leaf eaten (mg)	Dry weight of excreta (mg)	Percentage retention
1	44,1	57,3	24,6	3,6	85,4
2	45,3	94,5	61,5	10,2	83,4
3	51,3	96,5	62,4	10,2	83,7
4	62,3	112,6	67,5	11,3	83,6
5	51,0	70,8	24,6	4,0	83,7
6	50,3	99,6	59,6	10,9	81,7
7	44,8	87,2	58,2	10,5	82,0
8	47,2	65,4	27,9	4,3	84,6
9	37,8	78,8	35,7	6,6	81,5
10	32,5	68,6	32,1	6,8	79,1

Percentage of average retention = 82,9 ±0,6*

part of the leaf after the experiment had a dry weight (W) and an area (A) (obtained from the blue-print paper), the dry weight (w) of the parts of the leaf consumed could be calculated from their area (a) by the formula $w = W \times \frac{a}{A}$.

The percentage of food retained was calculated from the dry weight of the leaf ingested and the dry weight of the excrement.

Results. The results of five experiments are shown in Tables 18–22. With the larvae feeding on *Solanum tuberosum* it is seen that the average percentage of food retention increases as the individuals grow older. This means that the older larvae are more competent in utilizing the ingested food. The second instar larvae show greater individual variations regarding food retention which gradually diminishes after the second and third moults. It is also seen that the amount of food ingested increases greatly after the third moult and that the individuals in each instar with a greater intake do not always show a higher percentage of food retention. When these figures are compared with those of food retention in adult beetles which have values around 57 % (GRISON and ROEHRICH, 1947),

---

* standard error

TABLE 22. The retention of demissum leaves (line 23) by fourth instar larvae of *Leptinotarsa decemlineata*
(*Retentie van de droge substantie van het blad van Solanum demissum (stam 23) door de larve van het vierde stadium*)

Larva no.	Body weight before experiment (mg)	Body weight after experiment (mg)	Dry weight of leaf eaten (mg)	Dry weight of excreta (mg)	Percentage retention
1	46,5	53,4	11,3	2,7	76,0
2	43,2	70,4	23,2	5,2	77,6
3	38,5	45,0	5,8	1,8	68,8
4	41,0	46,7	6,8	1,6	76,6
5	40,1	44,3	13,8	4,3	68,9
6	33,6	50,8	5,8	3,9	33,2
7	44,2	54,7	5,5	2,7	51,3
8	35,5	38,9	4,0	1,2	69,7
9	36,1	32,9	1,4	0,9	36,7
10	38,5	32,4	1,7	0,7	58,8

Percentage of average retention = 61,8 ±5,2*

the larvae show a high capacity for food retention, indicating that the stage is one of growth.

Taking the figures in Tables 19 and 21 as the criteria of food retention on a suitable plant, Tables 20 and 22 demonstrate poor utilization of food in larvae feeding on *Solanum demissum*. On this plant greater individual variations are found, in agreement with the observation in the mortality experiments that some individuals can use *Solanum demissum* more successfully than others.

The results from these experiments suggest that apart from the comparatively small amount of foliage ingested when feeding on *Solanum demissum*, the deficiency in nutrition may be at least partly attributed to the incompetence of the larvae in utilizing the ingested *demissum* leaves. Whether the unfavourable gustatory reaction in the insect diminishes the secretion of the digestive enzymes in the digestive tract, or the alkaloid demissin causes indigestion has yet to be ascertained; but the facts here noted prove that the digestive system of the larva cannot deal with this plant in the same way as with potato or with *Atropa belladonna*.

3. A MICROSCOPICAL EXAMINATION OF THE EXCREMENT

The excrement of phytophagous insects usually gives indications about what part of the ingested food is digested. In the faeces of the leaf-feeding caterpillars, it has been found that the cells of the leaf fragments often remain intact except those at the margins which had been cut open (ACQUA, 1921; PLATEAU, 1874). But the larva of *Gatropacha rubi* is able to digest the cellular contents without the breakdown of the cell walls (BIEDERMANN, 1919). It has also been found that enzymes capable of attacking cellulose are absent in phytophagous insects (SCHLOTTKE, 1937); therefore most of the vessels and other fibrous elements in the leaves are usually discarded unchanged in the faeces. Decomposition of chlorophylls occurs in the gut of the Colorado beetle larva as well as of many other leaf-feeders. For the evidence of the breakdown of chlorophylls in the guts of the larva and the adult beetle, reference is made to the work of BUSNEL (1939).

From the experiments described in the above section, it is seen that larvae

---

* standard error

of the same age show conspicuous differences in their capacity to digest the foliage of potato and that of *Solanum demissum*. Possibly a difference can also be found in the leaf fragments in the faeces of the larvae feeding on these two plants. Moreover, the sizes of fragments of the different foliage in the faeces may also demonstrate the size of the leaf fragments bitten off during feeding.

a. The excrement of the fourth instar larvae feeding on Solanum tuberosum

The excrement of the larvae is not in the form of pellets as with caterpillars, but generally in elongated continuous masses, rather wet when newly defecated and sometimes mixed with whitish particles of uric acid excreted from the Malpighian tubes. During continuous feeding, defecation occurs at intervals varying from about 10 minutes to one hour. When examined under a microscope, the elongated mass of excrement is composed of many fragments of the ingested foliage pressed together. In the teased faecal mass, the epidermal hairs of the leaf, the spiral fibers from the vessel walls, and other fibrous elements in the vascular bundles can be seen. A large number of bacteria in the rod-form are usually scattered among the leaf fragments (Fig. 25).

The cells of the inner parts of the leaf fragments remained intact; they still contained chloroplastids. The cells on the margins of the fragments were broken up and the cellular contents and the chloroplastids were absent. In the larvae of different instars, the ingested fragments of foliage seemed to be different in size and under a microscope, the measurements of the fragments in the faeces of the first and second instar larvae were slightly smaller than those of the third and fourth instar larvae. Thus the fragments in the faeces of the first instar larvae varied from 0.27 by 0.08 mm to 0.35 by 0.11 mm in size, those of the second instar larvae from 0.21 by 0.09 mm to 0.42 by 0.15 mm, those of the third instar larvae from 0.30 by 0.15 mm to 0.75 by 0.27 mm, and those from the fourth instar larvae from 0.26 by 0.11 mm to 0.83 by 0.15 mm.

b. The excrement of the fourth instar larvae feeding on Solanum demissum

No difference could be detected between the faeces of larvae feeding on *Solanum demissum* and those on *Solanum tuberosum*. The epidermal hairs and fibrous elements from the vascular bundles could be found among the leaf fragment, and a large number of bacteria was also present. The cells in the inner parts of the leaf fragments were intact; only the contents of the cells at the edges were gone. In the faeces of the larvae reared for a time on *demissum* leaves after the action of frost, transparent rhomboid crystals could be found.

The leaf fragments in the faeces of the third instar larvae feeding on *Solanum demissum* were found to be 0.24 by 0.23 mm to 0.75 by 0.27 mm in size; those of the fourth instar larvae were from 0.23 by 0.15 mm to 0.75 by 0.20 mm. Therefore no appreciable difference in the size of the leaf fragments in the faeces of the larvae feeding on potato and *Solanum demissum* could be found.

c. The excrement of the fourth instar larvae feeding on Petunia hybrida

No differences could be found in the faeces of the larvae feeding on *Petunia hybrida* and those on potato, except the botanical features characterizing these two species. The cellular contents at the cut edge of the leaf fragments were gone; those at the inner parts still remained intact. Epidermal hairs with enlarged terminations were defecated unchanged and numerous bacteria could be found among the leaf fragments. Red crystals were present in the cells.

CHAPTER VIII

INFLUENCE OF THE FOOD PLANTS ON THE DIGESTIVE TRACT

1. GENERAL CONSIDERATIONS

In insects, as well as in other animals, the nature of the diet and the general nutritional conditions may influence the metabolism of various tissues. The digestive tract possesses the most susceptible tissue which is liable to be modified by the dietary conditions. The epithelial lining of the digestive tract, which functions for secretion and absorption, is always in direct contact with the food and shows sensitive reactions. The cells are continuously subject to various degrees of disintegration and replacement. When the diet is unsuitable, the epithelial cells will disintegrate and be replaced more frequently; otherwise they can live and function longer. In the grasshopper *Melanoplus differentialis*, the rate of replacement in the gastric caeca on an unsatisfactory diet of oat leaves was noticed to be almost double that on the satisfactory diet of the leaves of barley or wheat (HODGE, 1944). In vertebrates during inanition, there may be an increased nucleoplasmic ratio in the epithelial cell (KREMER, 1932; TRUSZKOWSKI, 1927, 1928); and this phenomenon was also found in *Melanoplus*.

As has been mentioned before, the ventriculus or the midgut of the Colorado beetle larva occupies nearly half the length of the tract and provides the most important site of digestion and absorption. The epithelial wall of this region undoubtedly undergoes various histological changes during moulting, starvation, or other physiological processes. In other insects, this part has been the subject of much elaborate work regarding its structure and histo-physiology (FRENZEL, 1885; DEEGENER, 1909; BUCHMAN, 1928; TSCHANG, 1928, 1929; SHINODA, 1927). The following study is undertaken to ascertain the influence of several Solanaceous plants and of starvation on the histology of the midgut.

2. THE HISTOLOGY OF THE EPITHELIAL LINING OF THE NORMAL GUT AND OF THAT OF THE LARVAE FEEDING ON SOLANUM DEMISSUM AND PETUNIA HYBRIDA OR WHEN STARVED

Technique. Larvae after the third moult were divided into four groups and reared separately on *Solanum tuberosum*, *Solanum demissum* "line 23", *Petunia hybrida*, or kept starved. After a few days (3 days on *Solanum tuberosum*, 6, 12 and 13 days on *S. demissum*, 1 day on *Petunia hybrida*, and 2 and 3 days without food), the larvae were killed and the midguts dissected out and fixed in Carnoy's or Bouin's solution. When Carnoy's was used the material was treated with methylbenzoate celloidine, as in making preparations of the sense organs (cf. p. 36). In both cases, the material was dehydrated, imbedded in paraffin, sectioned at 5 μ, stained with Heidenhaim's or Erlich's haematoxylin and eosin, and mounted in Canada balsam.

The normal gut. In cross-section, the midgut is a tube of columnar epithelial cells invested by a thin muscular coat. The peritrophic membrane, which occurs as an envelope for the food in the midgut of many insects, is wanting in the Colorado beetle larva; the food comes into direct contact with the epithelial cells. The cells rest upon the basement membrane which is regarded as the product of the cell bases. The muscular coat or muscularis is poorly developed in the midgut; it consist of a principal inner circular layer and a number of outer longitudinal fibers which are widely spaced and small in diameter. External to the muscularis, small tracheae can be found.

The epithelium is composed of two kinds of cells: the large columnar digestive cells and the smaller regenerative cells. The latter usually assume various shapes, have a denser texture, and occur either singly or in groups between the bases of the large cells. The digestive cells are engaged in secretion and absorption; the regenerative cells propagate new cells to replace the digestive cells when the latter are exhausted by secretory activities or shed during ecdysis (SNODGRASS, 1935). When they occur in groups, the regenerative cells form distinct „nidi", which are in contact with the basement membrane and surrounded by the digestive cells towards the lumen. The nuclei of these cells are big when compared with the size of the cells and are generally round in shape. The digestive cells, usually with elongated nuclei in the middle portions, are columns with the distal margin edged by a zone known as the striated border. This is a continuous layer about 5 μ in thickness and in cross section it is differentiated into minute alternating dark and clear lines. In the normal larvae feeding on *Solanum tuberosum*, the epithelial lining is found to have a thickness of about 88 μ, and the digestive cells are of a rather uniform structure except for the difference in size, which results in the formation of hollows of various depths (Fig. 26). The nuclei of the digestive cells have an average length of 14.6 ± 0.3 μ and an average width of 8.1 ± 0.2 μ, as determined by 25 measurements. Small vacuoles may be found in the regions near the nuclei towards the lumen, where the cytoplasm stains weakly.

The gut of the starved larvae. In my preparations, the epithelial lining of the midgut of the larvae starved for two or three days has approximately the same thickness as the normal gut, i.e. about 88 μ. In cross section the lumen of the gut is almost deprived of any food fragments though it may be partially filled with liquids. The epithelial cells show several pathological changes (Fig. 27). Firstly, big vacuoles occur very frequently in the region between the nuclei and the lumen. They may be oval in shape, with a size of 18 by 8 μ, or nearly round, with a diameter of about 19 μ. They are not always empty; very commonly they contain the disintegrated part of the cytoplasm, sometimes even the nuclei. The thickness of the striated border does not change, but in some cases there are minute vesicles in the striated border which undoubtedly contain discarded substances. As a result of the increase in the vacuolar size, the distal portion of the cell ruptures and the cellular contents are discharged into the lumen of the gut. The regenerative cells show more activity in the starved larvae. Mitoses are very common in the nidi and a great number of new cells are formed which can be recognized by their rounded nuclei. The nuclei of the newly formed digestive cells are 13.5 ± 0.5 by 9.4 ± 0.4 μ in size (from 12 determinations).

The gut of the larvae feeding on Solanum demissum. The thickness of the epithelium of the gut of larvae reared on this plant is not very different from that of the normal gut. The lumen may be filled with food fragments, but not to the same

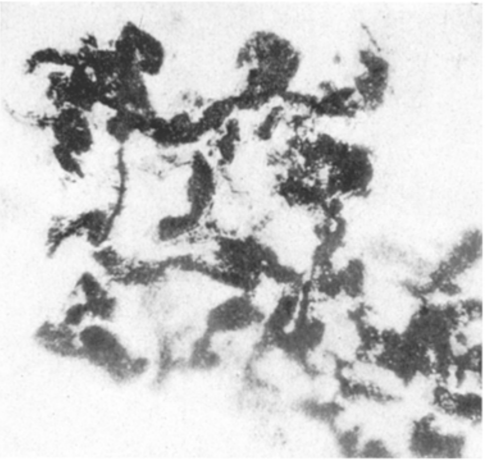


Fig. 25. The excrement of a fourth instar larva feeding on *Solanum tuberosum*, teased. Enl. 25 ×.
Excrementen van een larve van het vierde stadium, gevoed met Solanum tuberosum. Vergr. 25 ×.

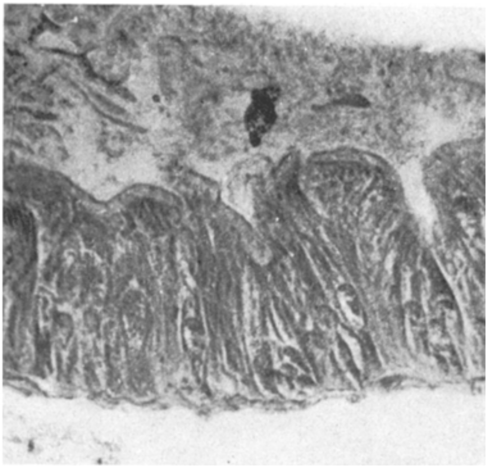


Fig. 26. The wall of the mid-gut of a fourth instar larva feeding on *Solanum tuberosum*. Cross section, enl. 430 ×. Ehrlich's haematoxylin-eosin.
Dwarscoupe door de wand van de middendarm van een larve van het vierde stadium, gevoed met Solanum tuberosum. Vergr. 430 ×. Ehrlich's haematoxyline-eosine.)

Fig. 27. The wall of the mid-gut of a fourth instar larva starved for two days. Cross section, enl. 430 ×. Heidenhain's haematoxylin-eosin.
(*Dwarscoupe door de wand van de middendarm van een hongerende larve van het vierde stadium. Vergr. 430 ×. Heidenhain's haematoxyline-eosine.*)

Fig. 28. The wall of the mid-gut of a fourth instar larva feeding on *Solanum demissum* (23) for 13 days. Cross section, enl. 430 ×. Ehrlich's haematoxylin-eosin.
(*Dwarscoupe door de wand van de middendarm van een larve van het vierde stadium, gedurende 13 dagen gevoed met Solanum demissum (23). Vergr. 430 × Ehrlich's haematoxyline-eosine.*)

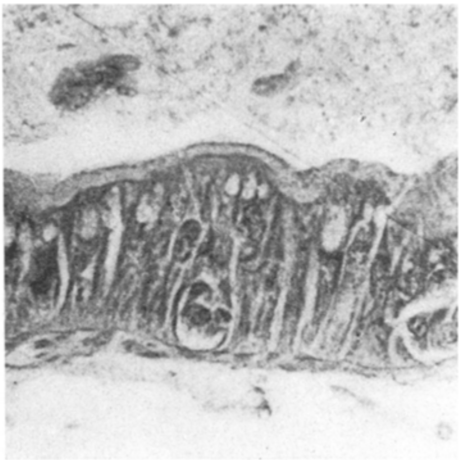


Fig. 29. The wall of the mid-gut of a fourth instar larva feeding on *Petunia hybrida* for 2 days. Cross section, enl. 430 ×. Ehrlich's haematoxylin-eosin.
(*Dwarscoupe door de wand van de middendarm van een larve van het vierde stadium, gedurende twee dagen gevoed met Petunia hybrida. Vergr. 430 ×. Ehrlich's haematoxyline-eosine.*

extent as in the case of *Solanum tuberosum.* In the digestive cells, vacuoles of various sizes may appear in the regions between the nuclei and the lumen, and many new cells may be produced by the regenerative cells (Fig. 28). The disintegrated parts of the cytoplasm are discharged into the lumen by means of „budding" which could lead to the false interpretation of holocrine secretion. Due to the prevailing process of cytoplasmic distintegration at the free ends of the digestive cells, the striated border in many places has disappeared, so that many cells become naked. The nuclei of the digestive cells have an average size of 12.0 ± 0.7 by 8.0 ± 0.3 (in 6 determinations).

All these pathological changes in the epithelial cells of the larvae feeding on this plant recall those of the starved larvae, though differing in degree.

The gut of the larvae feeding on Petunia hybrida. In well-fed larvae fed on this plant, the striated border of the epithelial lining at some places may disappear and the cytoplasm may show signs of disintegration (Fig. 29). The discharged substances are seen to be "budded off"; and small vacuoles may appear in several regions in a single cell. The other histological features of the epithelium of larvae newly induced to feed on this plant are quite similar to the normal, indicating that this tissue, at least in the midgut, is less influenced by the poisonous substances which may cause the death of the larva.

From this study it is seen that the histology of the epithelial lining of the midgut is definitely influenced by the dietary and general nutritional condition of the larvae. During starvation, the digestive cells disintegrate and are replaced by new cells more frequently and vacuoles that occur in the regions between the nuclei and lumen give the epithelium a very distinct appearance. When the diet is unsatisfactory, similar pathological features appear in the epithelium, as exemplified by the effects of feeding on *Solanum demissum* when the tissues of the larvae show the same changes as in starvation. On the other hand, if the toxic effect of the ingested plants can manifest itself in a relatively short time as in the case of *Petunia hybrida*, the epithelium of the midgut is very slightly influenced. This result suggests that the adverse effect of *Solanum demissum* is not due to the presence of poisonous substances, but to some factor which leads to insufficient feeding and inanition.

CHAPTER IX

INFLUENCE OF THE FOOD PLANTS ON THE HEART BEAT

The circulation of the haemolymph of the Colorado beetle larva is chiefly maintained by means of the dorsal vessel. This vessel, extending from the posterior end of the abdomen to the head, is differentiated into an anterior aorta and a long posterior heart which is slightly dilated in each body segment to form a series of chambers. These chambers are provided with ostia in their lateral walls for the admission of the haemolymph in the abdominal cavity. The haemolymph enters the heart chambers during their dilatation, and when the chambers are filled, a wave of contraction passes from behind forward so that the haemolymph is carried towards the head and discharged there through the aorta.

The rhythmical contraction and dilatation of the chambers, which characterize the cycle of the cardiac activity in three phases, viz. systole, diastole, and diastasis, can be discerned by examining the larva under a binocular microscope. The rate of the heart beat may be influenced by various conditions. It has been observed in other insects that the rate is increased with rising temperature and also with intensified exertion (CROZIER and FEDERIGHI, 1925). During the course of development it gradually decreases; thus in the larvae of *Sphinx ligustri*, the rate of heart beat is 82 per minute in the first instar, 89 in the second, 63 in the third, 45 in the fourth, and 39 shortly before maturity (NEWPORT, 1837). The rate of beat of the isolated heart of the sand cricket *Stenopelmatus* was observed to be influenced by various drugs such as nicotine and atropine (DAVENPORT, 1949).

It was noticed that the rate of the heart beat of the Colorado beetle larva was subject to individual variation and influenced by temperature and age. The ingestion of the leaves of *Solanum demissum* caused a drop in the rate of heart beat, the extent of wich depended upon the amount of foliage consumed. It has therefore been assumed that there were some "organic poisons" in the leaves which were responsible for this phenomenon (TROUVELOT and BUSNEL, 1937; BUSNEL, 1939).

In the present experiments, larvae after the second and third moults were reared separately in glass vials on various Solanaceous plants including *Solanum tuberosum*, *S. demissum* "line 23", *Atropa belladonna*, and *Petunia hybrida*, or kept starved. The rate of heart beat of each larva was determined daily at the same time under a binocular microscope. Before the determination the larvae were kept at a constant temperature of 25 °C more than 10 minutes and the number of contractions in each minute was counted at that temperature. The starved animals were usually restless and counting of the heart beats was made possible only by supplying potato leaves before the experiment.

The rate of heart beat of several individuals where the determinations were made after the second moult are shown in Fig. 30. The larva feeding on potato showed a drop in the rate in the later part of the third instar which perhaps indicated preparation for ecdysis. After the third moult and during the period

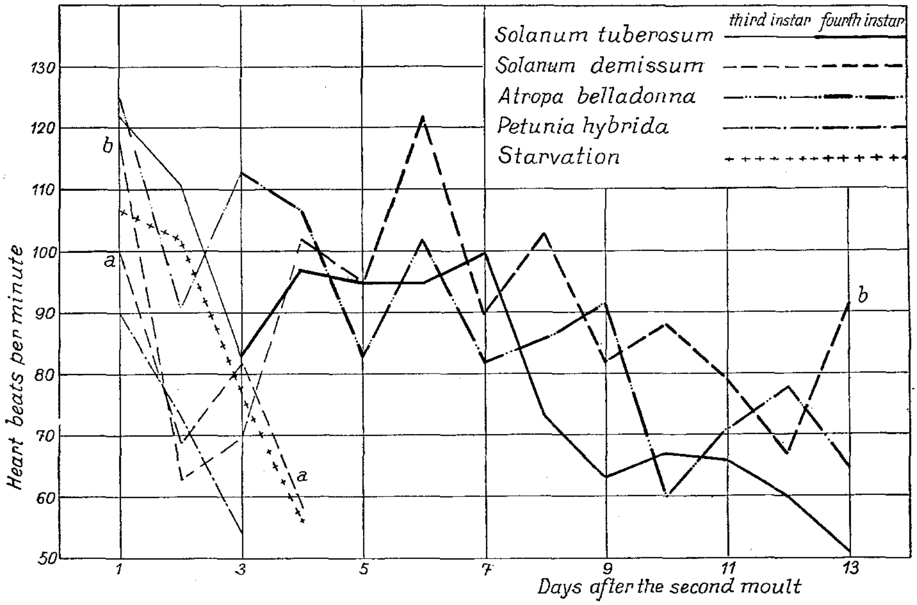


Fig. 30. The rates of heart beat of larvae (after the 2nd moult) feeding on different plants or when starved. Temperature = 25 °C.
(*Hartrhythme van larven, na de tweede vervelling, gevoed met verschillende gewassen en van hongerdieren. Temperatuur 25 °C.*)

of rapid growth of the fourth instar larva the rate might increase for several days. When the larva approached maturity the rate again decreased and reached a very low order on the thirtheenth day. The larvae feeding on *Atropa belladonna* showed similar cardiac activity: there was a drop towards the end of the third instar, a rise in the feeding period of the fourth instar, and finally again a drop when the larvae grew older. The larva which had eaten the foliage of *Petunia hybrida* was poisoned, its heart rate gradually decreased and death occurred on the third day after the second moult when the rate of heart beat was 54 per minute. The starved larva also showed a decline in the rate as starvation continued. The effect on larvae feeding on *Solanum demissum* differed according to whether the individual could survive on this plant for a longer or shorter period. The change in the rate of heart beat of larvae surviving for a longer period is represented by Larva *b* in Fig. 30; and of those surviving for a shorter period by Larva *a*. It is seen that if the larva could not grow on this plant, the rate dropped in a manner rather recalling that of the starved larvae. In the case of Larva *a*, death occurred on the fifth day and the rate at that time was 58 per minute. In the case of Larva b, which could grow on this plant rather successfully, the cardiac activity was similar to that of the larva feeding on *Solanum tuberosum*, or on *Atropa belladonna*.

The rates of heart beat of the fourth instar larvae under different dietary conditions are represented in Fig. 31. The four lots of larvae nearly all showed a decline in cardiac activity towards the end of the experiment. The larvae in the third lot were observed to have eaten a considerable quantity of *Petunia* foliage.

One fourth instar larva after ingestion of an amount of *Petunia* foliage was observed to have the heart chambers in a dilated condition for several hours. It died the next day.

In general the results of these experiments indicate that the rate of heart beat at constant temperature is conditioned by the metabolic rate of the animals. When the larva is inactive or grows old the metabolic rate seems to decrease (cf. the following chapter) and there is also a decline in the heart activity. There seems to be no reason to assume that the drop in the rate of heart of the larvae feeding on *Solanum demissum* is directly caused by the presence of some special organic substances in the ingested plant because the cardiac activity of the suspectible larvae resembles that of the starved larvae while that of the larvae able to grow on this plant is very similar to that of the larvae feeding on *Solanum tuberosum.*

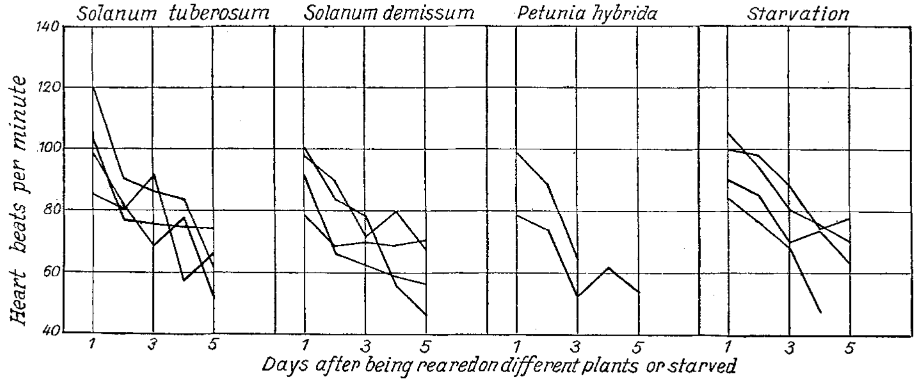


Fig. 31. The rates of heart beat of fourth instar larvae feeding on different plants or when starved. Temperature 25 °C.
(*Hartrhythme van larven van het vierde stadium, gevoed met verschillende gewassen en van hongerdieren. Temperatuur 25 °C.*)

CHAPTER X

INFLUENCE OF THE FOOD PLANTS ON RESPIRATION AND METABOLISM

1. THE INFLUENCE ON CO_2 PRODUCTION

The respiratory exchange, which may be measured by determining either the oxygen consumption or the carbon dioxide production, provides an important indication of the metabolic processes taking place in an insect or other organism. Since the observations of the French chemist VANQUELIN (1763–1829), much work has been done on insect respiration. TREVIRANUS (1831) noticed that CO_2 production increased with rising temperature and with voluntary movements. During the nymphal stage of the dragonfly *Aeschna umbrosa* the CO_2 output decreased progressively as the animals grew older. The CO_2 production of the starved nymphs decreased during the period of starvation, except in the second week when the metabolic rate was higher than the normal (SAYLE, 1928). For the respiratory exchange during embryonic and metamorphic development and as influenced by hibernation of the Colorado beetle, reference is made to the work of FINK (1925*a*, 1925*b*).

Investigations concerning the effects of feeding on respiratory exchange have been undertaken on some blood-sucking insects (PUNT, 1943); little work of the same kind has been done on phytophagous insects. Since the adverse influence of unsuitable food plants may finally alter the metabolic rate of the insect, such determinations are desirable.

Technique. For the determination of the gaseous exchange of the Colorado beetle larva, a diaferometer devised by NOYONS (NOYONS, 1938; MAAS, 1938) was used. The instrument has a sensitivity which causes movement of the image of the galvanometer needle on a screen over 24 cm during the passage of an air current containing 0.03 % CO_2. Thus the CO_2 output of a single fourth instar larva could be determined. A change of 0.10 % of oxygen contained in the passing air current free from CO_2 caused the image of the needle to shift for 10 cm. In the determination of O_2 consumption, which was undertaken in the following experiments, several larvae were used at the same time. During the experimental periods, several lots of fourth instar larvae were reared on potato, *Solanum demissum* "line 23", and *Petunia hybrida*, or kept starved. Determinations were made daily, usually at the same time for the same animal or group of animals. During the determinations the glass vial containing the larva was submerged in a water bath with a constant temperature of 25 °C. The percentage of CO_2 or O_2 in the air leading from the larva was measured for 10 minutes. After the measurement the body weight of the larva was determined on a torsion balance. The volume of the air passing through the apparatus in these 10 minutes was determined with the help of a spirometer. The volume of the CO_2 produced or the O_2 consumed in the 10 minutes was calculated by multiplying the volume of air which had passed through the apparatus in that period by the average increase

TABLE 23. CO_2 production of fourth instar larvae feeding on *Solanum tuberosum* Temperature 25 °C
(Productie van CO_2(mm^3/u.) door larven van het vierde stadium, gevoed met blad van Solanum tuberosum. Temperatuur 25 °C)

Larva	Days	Body weight (mg)	mm^3 of CO_2 produced per hour	mm^3 of CO_2 produced per gram larva per hour
No. 1	1	62,0	0,13	2,09
	2	84,0	0,17	2,02
	3	120,0	0,21	1,75
	4	161,0	0,22	1,36
	6	127,0	0,10	0,79
	7	125,0	0,07	0,56
	8	118,0	0,05	0,42
No. 2	1	49,0	0,07	1,43
	2	74,0	0,11	1,50
	3	101,0	0,19	1,88
	4	126,5	0,19	1,76
	6	181,5	0,20	1,10
	7	151,5	0,13	0,85
	8	150,5	0,08	0,53
No. 3	1	58,0	0,08	1,38
	2	72,0	0,11	1,50
	3	91,0	0,16	1,75
	4	111,5	0,14	1,25
	5	117,0	0,16	1,36
	7	166,0	0,20	1,21
	10	171,0	0,13	0,76
	11	156,0	0,07	0,45
No. 4	1	48,0	0,06	1,25
	2	69,0	0,08	1,16
	3	87,0	0,13	1,49
	4	100,0	0,14	1,40
	5	118,0	0,19	1,61
	10	143,0	0,13	0,91
	11	135,0	0,09	0,67
No. 5	1	56,0	0,10	1,78
	2	85,0	0,14	1,64
	3	119,0	0,18	1,51
	4	123,0	0,19	1,54
	5	145,0	0,21	1,44

or decrease in percentage of CO_2 or O_2 in the air, as recorded by the diaferometer.

Results. The results are shown in Tables 23–26 and Fig. 32.

From Table 23, it is seen that the total CO_2 output increases as the larva grows and that the maximum CO_2 production coincides with the maximum body weight except in the cases of larvae No 3 and 4, where maximum CO_2 production preceded maximum body weight. The amount of CO_2 produced gradually decreases after the maximum point is reached. If the volume of CO_2 produced in unit of time by one gram of larva is taken as the criterion, it is seen that the maximum CO_2 out-put always precedes the maximum body weight (cf. Table 23, last column); or in other words, after a certain stage of development the CO_2

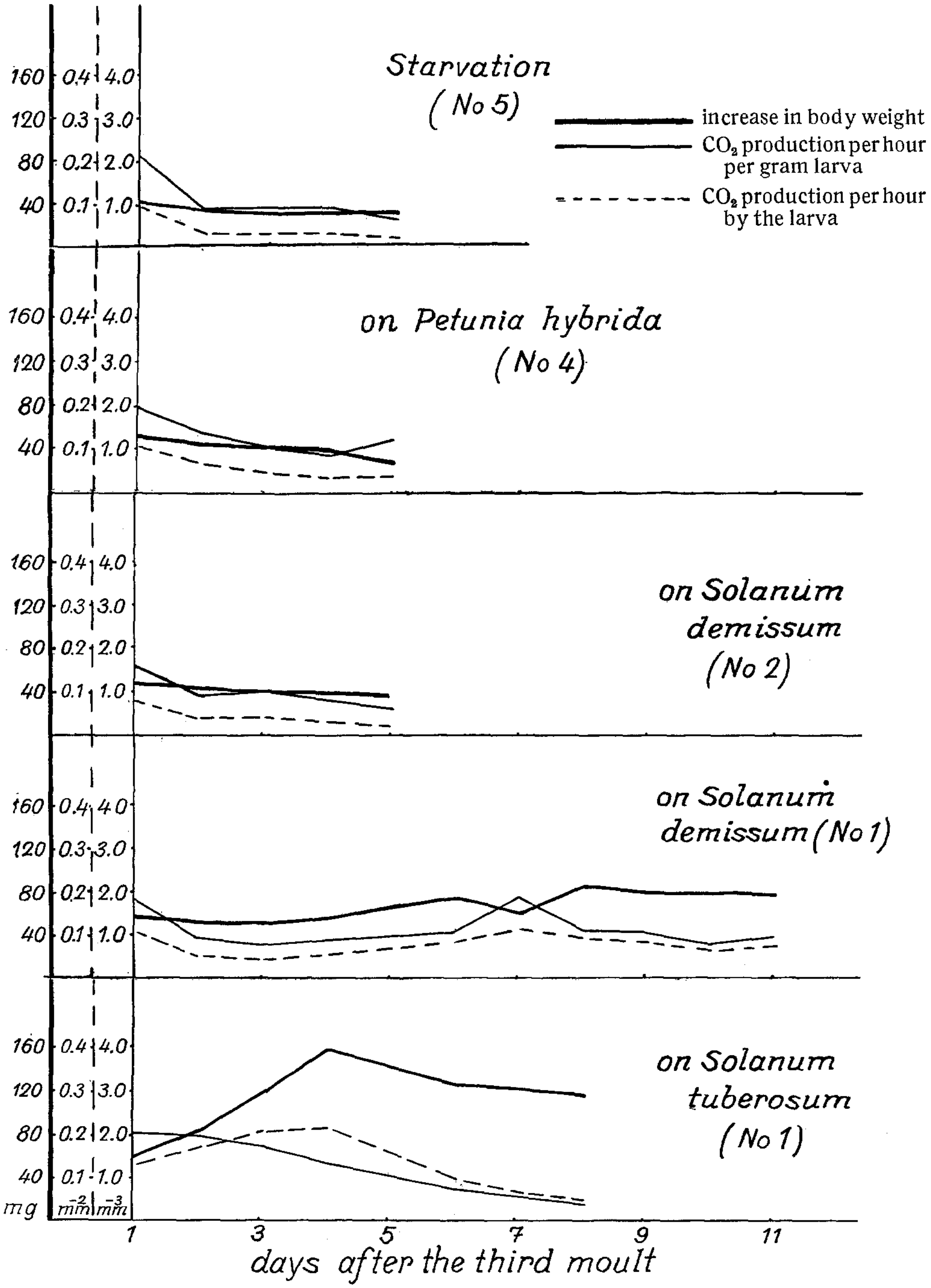


Fig. 32. CO_2 production of larvae feeding on different plants or when starved (fourth instar).
(CO_2 productie door larven van het vierde stadium, gevoed met verschillende planten of hongerende.)

produced decreases progressively as the larvae grow older, a fact recalling the respiratory exchange of the *Aeschna* nymphs. This perhaps indicates the decreasing metabolic rate of the animals after a certain stage in their growth. The CO_2 output decreases more quickly than the body weight, resulting in the divergent directons of these two curves as shown in Fig. 32.

The larvae feeding on *Solanum demissum* have other characteristics in their respiratory exchange. As mentioned in Chapter VI, individual larvae feeding on this plant are not consistent in their growth; the curves of their daily metabolic rate also show some irregularity. This is exemplified by Larva No 1 in Fig. 32 and Table 24. The total CO_2 output may drop, rise, and then drop again. Several other larvae on this plant failed to grow and their CO_2 output was simultaneously reduced, indicating the decreasing metabolic rate which usually characterizes inanition (Fig. 32, larva No 2). The divergent directions of the growth curve

TABLE 24. CO_2 production of the fourth instar larvae feeding on *Solanum demissum* Temperature 25 °C
(Productie van CO_2(mm³/u.) door larven van het vierde stadium, gevoed met blad van Solanum demissum (stam 23). Temperatuur 25 °C)

Larva	Days	Body weight (mg)	mm³ of CO_2 produced per hour	mm³ of CO_2 produced per gram larva per hour
No. 1	1	59,5	0,11	1,85
	2	53,0	0,05	0,94
	3	51,0	0,04	0,78
	4	56,0	0,05	0,89
	6	74,0	0,08	1,08
	7	60,0	0,11	1,83
	8	83,0	0,09	1,08
	9	78,0	0,08	1,02
	10	77,0	0,06	0,77
	11	76,0	0,07	0,92
No. 2	1	50,0	0,08	1,60
	2	43,0	0,04	0,93
	3	40,0	0,04	1,00
	4	38,0	0,03	0,79
	6	35,0	0,02	0,57
No. 3	1	49,0	0,08	1,63
	2	51,5	0,04	0,77
	3	49,5	0,04	0,81
	4	47,0	0,03	0,64
	5	46,0	0,04	0,87
No. 4	1	50,5	0,09	1,78
	2	45,0	0,04	0,89
	3	39,0	0,03	0,77
	4	37,0	0,02	0,54
	5	37,5	0,01	0,27
No. 5	1	62,0	0,10	1,61
	2	49,5	0,05	1,01
	3	50,0	0,05	1,00
	4	49,0	0,04	0,82
	5	46,5	0,04	0,86

TABLE 25. CO_2 production of the fourth instar larvae feeding on *Petunia hybrida* (*Productie van CO_2(mm^3/u.) door larven van het vierde stadium, gevoed met blad van Petunia hybrida. Temperatuur 25 °C*)

Larva	Days	Body weight (mg)	mm^3 of CO_2 produced per hour	mm^3 of CO_2 produced per gram larva per hour
No. 1	1	53,5	0,08	1,49
	2	40,0	0,05	1,25
	3	39,0	0,03	0,77
	4	31,0	0,02	0,64
No. 2	1	48,0	0,08	1,69
	2	34,0	0,03	0,88
	3	27,0	0,02	0,75
	4	18,0	0,02	1,11
No. 3	1	47,0	0,08	1,70
	2	39,5	0,05	1,26
	3	32,0	0,05	1,56
	4	26,0	0,02	0,77
No. 4	1	52,0	0,10	1,92
	2	44,5	0,06	1,34
	3	39,0	0,04	1,02
	4	36,0	0,03	0,83
	5	25,0	0,03	1,20

TABLE 26. CO_2 production of the fourth instar larvae when starved (*Productie van CO_2(mm^3/u.) door hongerende larven van het vierde stadium. Temperatuur 25 °C*)

Larva	Days	Body weight (mg)	mm^3 of CO_2 produced per hour	mm^3 of CO_2 produced per gram larva per hour
No. 1	1	56,0	0,09	1,60
	2	38,0	0,03	0,79
	3	37,0	0,03	0,79
	4	27,0	0,02	0,74
No. 2	1	60,0	0,11	1,83
	2	46,0	0,05	1,08
	3	40,5	0,04	0,98
	4	36,0	0,02	0,56
No. 3	1	48,0	0,07	1,44
	2	40,5	0,03	0,74
	3	40,0	0,03	0,75
	4	35,5	0,02	0,56
	5	34,0	0,02	0,59
No. 4	1	52,5	0,09	1,71
	2	50,0	0,05	1,00
	3	49,0	0,04	0,80
	4	44,0	0,03	0,68
	5	39,5	0,02	0,51
No. 5	1	43,0	0,09	2,08
	2	35,0	0,03	0,85
	3	33,0	0,03	0,90
	4	33,0	0,03	0,90
	5	31,0	0,02	0,64

and the CO_2 curve which we have seen in the larvae feeding on potato are not found in the larvae dying on *Solanum demissum*.

In the larvae feeding on *Petunia hybrida*, the body weight decreases, and so also does the total CO_2 output. From Table 25, it is seen that the quantity of CO_2 produced by one gram larva on this plant is slightly higher than that on *Solanum demissum*. Because of the small number of larvae used in this experiment, the evidence is not enough to prove that the larvae feeding on *Petunia hybrida* have a relatively higher metabolic rate.

By comparing the curves in Fig. 32 it is seen that starved larvae and those having failed to grow on *Solanum demissum* have approximately the same curves of growth and of CO_2 output. In both cases the metabolic rate declines and the animals suffer from inanition.

From these experiments, it is seen that the respiratory exchange of Colorado beetle larvae is rather low. Under normal conditions, the CO_2 output per unit body weight of the fourth instar larva decreases progressively as the animal grows older, thus demonstrating a declining metabolic rate. In the larva feeding on unsuitable food plants or suffering from inanition the CO_2 output gradually diminishes, but when compared with its growth curve, this decline in metabolic rate is not normal.

2. THE INFLUENCE ON THE RESPIRATORY QUOTIENT

The ratio of the volume of CO_2 produced to the volume of O_2 consumed during the same period, commonly referred to as the respiratory quotient or R. Q., usually gives some indication about the nature of the substances that are oxidized in the body of an animal for energy production. In the complete combustion of carbohydrates, the R. Q. is 1.0; of proteins, about 0.78; and of fats, 0.71. In omnivorous insects feeding on a mixed diet including carbohydrates, there is an increased production of CO_2, and the R. Q. indicates that carbohydrates are being used. The cockroach feeding on carbohydrates has a R. Q. of 1.01–1.07, on fat it is 0.78–0.83, and when starved it may fall to 0.65–0.85 (GOUREVITCH, 1928).

It is said that during starvation the relatively small amounts of carbohydrates are first consumed. Proteins may be used extensively as in *Dytiscus* or very slightly as in *Periplaneta* (PILEWICZOWNA, 1926). Fat is always the chief reserve substance to be drawn upon and Colorado beetle larvae in the third and fourth instars which had an increased fat store were observed to survive twice as long as those in the first and second instars during starvation. The following experiment was undertaken to determine the change in R. Q. of the fourth instar larvae feeding on different food plants or during starvation.

Technique. The volumes of CO_2 produced and O_2 consumed in 10 minutes were determined with the diaferometer as described above. The determination of the O_2 consumption was made immediately after the determination of the CO_2 output.

Results. The respiratory quotients of the fourth instar larvae feeding on *Solanum tuberosum*, *S. demissum* "line 23", and *Petunia hybrida*, and of those starved are shown in Table 27. The larvae feeding on potato leaves have an average value of about 1.00, which indicates that carbohydrates are the chief substances utilized in the body. The larvae in the first day after the third moult

TABLE 27. The respiratory quotients of fourth instar larvae feeding on different plants or when starved
(Respiratiequotient van de larve van het vierde stadium, gevoed met blad van verschillende planten of hongerende)

Plant	Set of larvae	Days after 3rd moult	Total body weight (mg)	Respiratory quotient
Solanum tuberosum	No. 1 (5 larvae)	1	273	1,19
		2	306	0,88
		3	376	0,95
		4	425	0,96
	No. 2 (2 orange-colored larvae)	10	314	0,93
Solanum demissum	No. 3 (5 larvae)	1	276	0,78
		2	295	0,91
		3	312	1,22
		4	317	1,11
Petunia hybrida	No. 4 (5 larvae)	1	200	0,88
when starved	No. 5 (9 larvae, 1 died on the 3rd day)	1	393	0,89
		2	344	1,25
		3	280	0,78
	No. 6 (6 larvae)	3	237	1,09
		4	221	0,81
	No. 7 (2 larvae)	1	107	0,79

have a value of 1.19; those on the tenth day, i.e. in the prepupal stage, have a value of 0.93, indicating that in the prepupal stage, fats or proteins are used in addition to carbohydrates.

The larvae reared on *Solanum demissum* in the first day have a low R. Q. value of 0.78, indicating that a very small amount of carbohydrates is used. In this lot the larvae grew on this plant rather successfully and their total body weight slightly increased. There is also an increase in the R. Q., implying that the larvae can derive some carbohydrates from the food plant for energy production.

The larvae feeding on *Petunia hybrida* have a R. Q. of 0.88 which is lower than the normal.

The starved larvae usually show a decrease in the R. Q. values. Therefore, as starvation goes on, the carbohydrates are consumed and the larvae have to draw upon their stored fat and proteins for energy production.

CHAPTER XI

DISCUSSION AND CONCLUSIONS

We have seen that although the larval food plants of *Leptinotarsa decemlineata* Say are essentially chosen by the female beetle during oviposition, the larvae do show restrictions in their feeding preferences. In agreement with the feeding habits of the adult beetles, they feed only upon plants confined to the family Solanaceae, especially the species in the genus *Solanum*. When a great variety of herbaceous plants is available, the larvae will feed on only a definite number of them. At normal temperatures this food range is reasonably stable.

The capacity to discriminate between different species of plants is essentially due to the chemical senses of the larvae. Two groups of stimuli, olfactory and gustatory, are in the main responsible for eliciting the biting response and maintaining the feeding action. A microscopical examination revealed the presence of chemoreceptors which are mostly confined to the head region, especially to the antennae and the mouthparts. The "botanical instinct" of phytophagous insects, as assumed by earlier naturalists, can at least partly be explained by these mechanisms. In Colorado beetle larvae, the olfactory sense is short-ranged, and accordingly the larvae in the field show little power of locating their food plants at a distance. The larvae, however, are capable of gustatory discrimination to a degree which can be compared with that of the adult. It is interesting to notice that a plant like *Solanum ciliatum* which is rejected by the adult beetle may be used by the larva to complete its development. This difference in feeding preferences may be caused by the different olfactory and gustatory reactions of these two stages.

Though the larva relies on its chemical senses in selecting the food plants, it seems that the attractiveness of a plant is more or less independent of its nutritive or toxic properties. Any substance bearing the specific smell and taste of its normal food plants may be greedily ingested provided that its physical properties do not go beyond certain limits. The ingestion of elder pith blocks impregnated with potato leaf juice provides a good example. The larvae may also feed greedily on potato tubers which apparently possess similar "active principles" to those found in the leaves, but they fail to mature on such a diet. On the other hand, the larvae will not ingest elder pith impregnated with the juices of geranium and *Doronicum* leaves, where the "active principles" seem to be lacking.

There are at least four requirements in the chemical properties of plants which determine whether they can be used as normal food plants for the larva. These are their smell, their taste, the lethal effects caused by a group of chemicals such as alkaloids, and the nutritive value for promoting growth. Undoubtedly a group of Solanaceous plants, including *Solanum tuberosum*, *S. marginatum*, *S. rostratum*, and *S. melongena*, can fulfill all the requirements perfectly well and thus provide the best food plants for the Colorado beetle. On them the larvae can grow rapidly and death seldom occurs. On the other hand, there are many Solanaceous plants whose chemical properties can only partially fulfill these requirements and thus form a group of plants which will cause adverse effects in various degrees.

Several examples from the present studies or from the work of other authors will explain the latter cases more clearly. *Solanum demissum* is a plant whose adverse effect on larval development has drawn the attention of many workers. As demonstrated by the screen test in Chapter IV, the leaves of this plant possess an odour attractive to the larvae to the same extent as potato leaves. A well-fed larva previously reared on potato will exhibit a biting response shortly after coming into contact with the leaves. But the feeding action is different from that on potato; the larva will spend more time in resting and wandering than in continuous feeding. This reaction seems to be caused by the presence of some chemicals which the larva can only detect after the leaf is ingested, because the leaf of *Solanum demissum* has about the same texture and pilosity as that of *Solanum tuberosum*. The unfavourable gustatory reaction from this plant will usually result in insufficient feeding leading to the death of the larva. Therefore *Solanum demissum* cannot fulfill the second chemical requirement of the normal food plants: i.e. an acceptable taste. In the second place, the leaf of *Solanum demissum* is not simply identical with the leaf of *Solanum tuberosum* plus an unfavourable taste, as can be shown by the reaction of a small proportion of larvae which can adapt themselves to the taste of *Solanum demissum*. As shown in the rearing work in Chapter VI, a small proportion of larvae after hatching could grow on this plant into adult beetles under laboratory conditions, but they showed inferior growth. Furthermore, in Chapter VII, it is seen that larvae feeding on *Solanum demissum* show a lower percentage of food retention than those feeding on *Solanum tuberosum*. These facts indicate that the larva cannot utilize this plant to the same extent as *Solanum tuberosum*. The cause may be attributed either to the lower nutritive value of this plant, or to the presence of certain toxic substances in the leaves which interfere with the digestive process. Though the opinion of the writer is in favour of the first idea, he would not like to exclude the possibility of a chronic toxic effect caused by such chemicals as the alkaloidglycoside demissin. The problem can only be solved by a comparative chemical analysis of the nutrients and intoxicants.

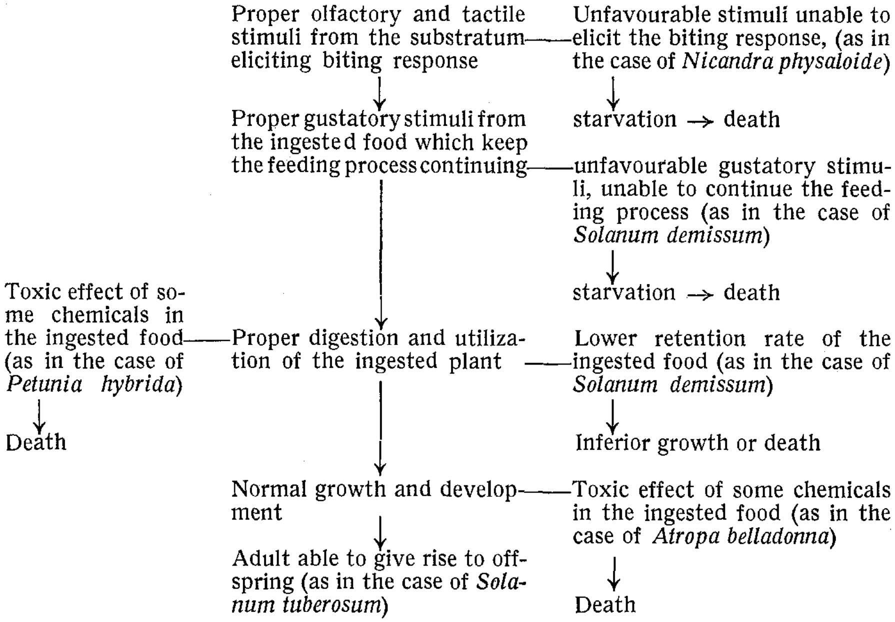
Petunia hybrida and *Atropa belladonna* provide two examples of plants containing toxic substances. From the screen test, it is known that the larva is attracted to the odour of *Petunia* leaves, and when it meets with such leaves, a biting action soon takes place. A relatively large amount of the foliage can be consumed by the third and fourth instars and the "active principles" found in potato leaves seem also to be present in *Petunia*. But poisonous chemicals in the leaves at a dosage which cannot be detected by the chemoreceptors in the head region will kill the larva. *Atropa belladonna* possesses poisonous properties with a milder character; the lethal dose seems to be greater. The plant has an odour and taste attractive to the larva and contains nutrients able to maintain normal growth. But some larvae cannot reach maturity simply because they are susceptible to the toxic effect of the special chemicals in the leaves. If the Colorado beetle can develop a slightly higher tolerance to the special chemicals in *Atropa belladonna*, this species undoubtedly could play the part of a normal food plant just like potato.

With regard to the chemical properties of the Solanaceous plants which principally decide whether the plants are "correct" food plants of the Colorado beetle, the species may be classified as satisfactory or unsatisfactory according to their olfactory, gustatory, toxic, and nutritive properties. An attractive smell and taste, absence of poisons, and high nutritive contents by no means always go hand in hand together; they seem to combine just by chance and into sixteen possibilities. Maybe all these combinations exist. It is not attempted here to give an example for each of them, but a description of the characters of some common Solanaceous plants according to the principles discussed above will be necessary. Let the capital letters O, G, T, and N signify the favourable reactions or situations in olfaction, gustation, tolerance to (or absence of) toxic substances, and nutrition respectively and the small letters o, g, t, and n the same for unfavourable situations, some of the species may be classified as follows.

1. The O – G – T – N group: *a. Solanum tuberosum*
   b. Solanum marginatum
   c. Solanum rostratum
2. The O – G – T – n group: *a.* tuber of *Solanum tuberosum*
   b. Solanum hendersonii (?)
3. The O – G – t – N group: *a. Atropa belladonna*
   b. Solanum atropurpureum
4. The O – g – T – n group: *a. Solanum demissum*
   b. Solanum nigrum
5. The o – G – T – N group *Solanum polyadenium* (?)

Among these five groups only the first one can provide an ideal food plant for the insect; all the others will produce adverse effects.

The feeding and development of Colorado beetle larvae may be regarded as a chain process. The action undertaken at a certain moment will usually have a decisive influence on that which follows. Viewing the larval life as a whole, these stages or phases of the process are generally interrelated and may be described as "nourishing steps". These involve the reception of the chemical and physical stimuli from the substratum causing a biting response; the feeding action which is maintained by an acceptable taste due to the presence of so-called "active substances"; the proper digestion and utilization of the ingested food; the maintenance of a normal growth; and a release of the energy thus accumulated for morphogenesis and transformation into the reproductive stage. If unfortunately a toxic plant is ingested, a disturbance in the process can set in immediately after the feeding action. The insect, though there is individual variation, can be beaten at any one of these „nourishing steps" and the result may be the incompetence in reproduction of the adult or more commonly, an untimely death of the individual. Most of the causal relations described are of a purely physiological nature, but the internal mechanism that causes the insect to react in its stereotyped way towards certain chemicals, especially the olfactory and gustatory substances, as such can only be attributed to its inherent characters or genetic constitution. In the reactions of the larva towards various Solanaceous plants, the physiological facts may be represented as follows:



A Solanaceous plant which is resistant to the attack of the Colorado beetle may possess several characters which can produce adverse effects in the larval life. The value of such a plant depends essentially upon the combined action of these characters which can progressively weaken the larva as development proceeds.

Though the chemical identity of many of the substances concerned is still obscure, the writer hopes that the present studies have thrown some light upon how the selection of food plants is effected by the Colorado beetle and upon the nature of the factors that may be responsible for the resistance of plants to the ravages of this insect.

SUMMARY

1. As an oligophagous insect the larva of *Leptinotarsa decemlineata* Say has a restricted food range which is confined to some plants belonging to the family Solanaceae. Species of this family show a continuous gradation from those preferred to those rejected.
2. The rate of feeding of the larva increases with rising temperature and reaches a maximum about 32 °C, above which it decreases. Although the food range of the larva below the optimal temperature for development is stable, it seems to be extended at superoptimal temperatures.
3. The effective distance at which the larva can perceive the food plants is about 2 mm. The perception is effected essentially through its short-ranged olfactory sense. The antennae play a major role in olfaction. The maxillary and labial palpi are less important in this respect though it has been proved that they also take part in olfactory perception. The lateral ocelli are concerned with the perception of light intensity and guide the animal in its phototactic movement.
4. The odours of the plants are the most important factor for eliciting the biting response. The water vapour tension and the texture of the substratum play a minor part. The physiological conditions of the larva generally influence the threshold of the stimuli.
5. The gustatory properties of the leaf seem to be the decisive element in continuing or discontinuing the feeding action. It is very probable that quantitative and qualitative differences in the "taste" substances in different plants are the principal cause of the feeding preference which is manifested in the frequency and total duration of the feeding process and the extent of food consumption. The gustatory sense is not connected with the antennae and the palpi but seems to be located in the mouth cavity.
6. Under laboratory conditions, the relative length of the periods of feeding, resting, and wandering which can be used as a criterion for food preference are determined by sensory reactions.
7. The antennae and palpi contain sensilla basiconica and sensilla styloconica in definite numbers and positions. They are regarded as the olfactory receptors. On the inner surface of the labrum and on the labium, there are gustatory receptors, the sensilla placodea and the sensilla trichodea. The distribution of the tangoreceptors is rather diffuse throughout the body surface.
8. *Petunia hybrida* is definitely toxic to the larva; the third and fourth instars with the greatest tolerance can survive on this plant only for four days.
9. Under our experimental conditions, about 20 % of the newly hatched larvae could grow and mature on a resistant "line" of *Solanum demissum*. The mortality rate of the larvae feeding on the flowering plant of *Atropa belladonna* is about 50 %. The adverse effect of this species is most conspicuous in the first larval instar.

10. A large percentage of the larvae feeding alternatively on *Solanum tuberosum* and *S. demissum* can reach maturity, but none can do so when *Petunia hybrida* is used instead of *Solanum demissum.*
11. In the thermal range suitable for development the adverse effect of *Solanum demissum* is enhanced by lower temperatures.
12. With the increase in body weight as the criterion, the larva feeding on *Solanum tuberosum* possesses an S-shaped growth curve. Very rapid growth takes place in the third and the early part of the fourth instars where the process of fat synthesis prevails. The growth curve of the larva feeding on *Atropa belladonna* is similar to that of the larva on *Solanum tuberosum*, except in the first instar, where the growth is rather slow. The larva feeding on *Solanum demissum* may also show the S-shaped growth curve, but this is usually marked by an inconsistent rate of growth.
13. In my experiments, the larvae uniformly had four instars; irregular and additional moults did not appear even if the diet was unsatisfactory.
14. The larval instars on unsuitable plants were generally prolonged. On *Atropa belladonna*, only the first instar was lengthened, the others being normal. On *Solanum demissum* the instars were progressively prolonged, indicating that the adverse effect of this plant is different in nature from that of *Atropa belladonna.*
15. On a suitable food plant, the linear growth of the head capsule and mandibles are in close accordance with Dyar's rule. The later instars of the larvae feeding on *Solanum demissum* showed a defective linear growth of the parts mentioned.
16. The rates of passage of food through the empty digestive tract of the larva newly induced to feed on different plants were nearly the same. The shortest time was found to be three hours. Larvae having fed on *Solanum tuberosum* and *S. demissum* for several days seemed to have a different rate of passage of food: it was about two hours on *Solanum tuberosum* and more than three hours on *S. demissum.* The well-fed larva newly induced to feed on *Petunia hybrida* has the same rate of food passage as that on *Solanum tuberosum.*
17. On *Solanum tuberosum*, larvae of successive instars showed an increase in the percentage of food retention: it was 71.4 ± 2.3 in the second instar, 75.1 ± 1.3 in the third, and 82.9 ± 0.6 in the fourth. Larvae feeding on *Solanum demissum* had a lower percentage of food retention: it was 58.4 ± 5.5 in the third instar and 61.8 ± 5.2 in the fourth.
18. In the excrement, the contents of the cells in the inner part of the leaf fragments eaten were intact: only the cells cut open at the margins appeared to be empty. No essential difference in the defecated fragments from different plants could be detected in this respect. The sizes of the leaf fragments ingested by the larvae at different ages were different; they became greater as the larvae grew older.
19. The epithelial cells of the ventriculus or mid-gut of the fourth instar larvae living on a restistant „line" of *Solanum demissum* and when starved underwent hastened disintegration and replacement. These were shown by the appearance of vacuoles in the digestive cells and the increased mitoses of the regenerative cells.
20. The rate of heart beat of the larva at a constant temperature is conditioned by the metabolic rate. A direct and conspicuous influence of food plants on the heart rate in general could not be detected.

21. The CO_2 output per unit body weight of the fourth instar larvae feeding on potato decreases progressively as the animals grow older. In the larvae feeding on unsuitable plants or suffering inanition, the CO_2 output per unit body weight also decreases, and when compared with their growth curves, this decline in the metabolic rate is seen to be pathological.
22. During growth, carbohydrates seems to be the essential substances utilized in the body of the larva. Under conditions of malnutrition and starvation, fats and proteins are utilized for energy production, as shown in the decrease in the R. Q.
23. The resistant "lines" of *Solanum demissum* are characterized by being attractive to the olfactory senses, repellent to the gustatory senses and deficient in nutritive properties for the feeding of the larva. An acute toxic effect could not be found though a slow chronic effect may not be excluded. The adverse effect of these plants on the larva is very similar to that due to inanition.

SAMENVATTING

Inleiding. Men pleegt de phytophage insecten op grond van de voedselkeuze in te delen in drie groepen: monophage (insecten die hun ontwikkeling slechts op één enkele plantensoort kunnen doormaken), oligo- of stenophage (insecten met een beperkt aantal voedselplanten, dikwijls behorende tot één plantenfamilie) en poly- of euryphage (insecten die van zeer vele plantensoorten kunnen leven). Mono- en oligophage insecten leveren problemen op van verschillende aard; evolutionistische, oekologische, physiologische en, wanneer specialisatie plaats vindt op één onzer cultuurgewassen, toegepast-entomologische.

De coloradokever (*Leptinotarsa decemlineata* SAY) heeft b.v. enkele soorten van de familie der Solanaceae, en onder deze vooral de cultuuraardappel als voedselplant, terwijl andere soorten van dezelfde familie, soms zeer nauw met de aardappel verwant, niet worden geaccepteerd of zelfs giftig zijn. Dit artikel vormt het verslag van onderzoekingen, uitgevoerd in 1948 en 1949 met het doel na te gaan welke physiologische factoren bij deze eenzijdige voedselkeuze een rol spelen.

De proeven werden uitgevoerd met larven; uit vroegere gegevens was bekend dat deze nog iets meer beperkt zijn in hun voedingsmogelijkheden dan de imagines. Zij vormen in dit opzicht de beperkende factor in de ontwikkelingscyclus. Het plantenmateriaal bestond uit een groot aantal Solanaceeën, afkomstig uit de Hortus Botanicus te Amsterdam, in het bizonder *Solanum tuberosum*, *Petunia hybrida* en *Solanum demissum*. Laatstgenoemde soort is een der weinige die „resistent" zijn tegen de coloradokever en tevens voldoende verwantschap met de aardappel vertonen om hiermee te kunnen worden gekruist. Zulke kruisingen worden thans in verschillende landen uitgevoerd met het doel, resistente aardappelrassen te verkrijgen. Over de aard van de resistentiefactoren was tot dusver nog weinig bekend.

Hoofdstuk III. Aangetoond wordt dat er binnen de familie der Solanaceeën geen scherpe grens te trekken is tussen soorten die wél en zulke die niet als voedsel worden geaccepteerd; eerder is er sprake van een zeer geleidelijke overgang. Ook wanneer een plant eenmaal door de larve als voedsel is geaccepteerd kan het resultaat echter verschillend zijn. Sommige soorten worden na korte tijd weer verlaten, andere worden blijvend geaccepteerd, nog andere zijn giftig. In totaal bleek slechts op een zevental van de onderzochte 56 Nachtschaden de ontwikkeling te kunnen worden doorgemaakt (kweek in vitro, bij kamertemperatuur).

De vraag deed zich voor of de temperatuur invloed uitoefent op de voedselkeuze. In het positieve geval zou dit de verklaring kunnen vormen voor het feit, dat de coloradokever in Zuidelijke landen enkele soorten onder zijn voedselplanten telt, die in Nederland niet als zodanig worden geaccepteerd, b.v. de tomaat. Proeven in een seriethermostaat wezen uit dat inderdaad bij temperaturen boven het optimum voor de voedselopname (± 32 °C) een relatief grotere hoeveelheid wordt gegeten van minder geprefereerde voedselplanten, mits er keuze mogelijk is. Bij suboptimale temperaturen is de voedselkeuze stabiel.

Hoofdstuk IV. De maximale afstand waarop de larve de voedselplant kan waarnemen is ongeveer 2 mm. De perceptie heeft in hoofdzaak plaats door middel van de reukzin. Deze zetelt zowel in de antennen als in de maxillaire en labiale palpen; de antennen zijn echter het belangrijkst. De stemmata schijnen vooral te dienen voor het waarnemen van lichtintensiteiten en spelen bij de voedselkeuze een ondergeschikte rol.

Het uitvoeren van de eerste beet in het blad (bijtreactie) kan belangrijk worden bespoedigd door de geur van de plant. De luchtvochtigheid en de structuur van het bladoppervlak zijn minder belangrijk. De physiologische toestand van de larve, in het bizonder de voedingstoestand oefent een belangrijke invloed uit op de drempelwaarde van de benodigde prikkels.

Of de voedselopname zal worden voortgezet, hangt nu in de eerste plaats af van de gustatorische eigenschappen van het blad. De qualitatieve en quantitatieve verschillen, die in dit opzicht tussen verschillende plantensoorten bestaan zijn de belangrijkste factor bij de voedselkeuze. Een maatstaf voor de preferentie is de duur van de voedselopname en de hoeveelheid opgenomen blad, maar ook de relatieve duur van de perioden van voedselopname, rust en rondlopen gedurende het verblijf van de larven op het blad. De smaakzin zetelt niet in de antennen en palpen, maar zeer waarschijnlijk in de mondholte.

Bij microscopisch onderzoek blijken op de antennen en palpen sensilla basiconica en sensilla styloconica aanwezig te zijn. Door amputatieproeven werd aangetoond dat dit reukzintuigen zijn. Aan de binnenzijde van het labrum en het labium bevinden zich sensilla placodea en trichodea, die beschouwd kunnen worden als smaakzintuigen. Trichoïde tastsensillen zijn diffuus over het gehele lichaamsoppervlak verspreid.

Hoofdstuk V. Petunia hybrida heeft toxische eigenschappen; larven van het derde en vierde stadium, waarbij de tolerantie het grootst is, kunnen maximaal 4 dagen op *Petunia* leven. Desondanks is *Petunia* voor deze stadia olfactorisch even aantrekkelijk als de aardappel; dit werd in hoofdstuk IV met behulp van een olfactometrische methode aangetoond.

Bij kweken in vitro onder zeer gunstige omstandigheden ontwikkelt 20 % van de larven, ab ovo op een resistente stam van *Solanum demissum* gekweekt, zich tot imago. Blad van volgroeide *Atropa belladonna* gaf een mortaliteit van 50 %, in hoofdzaak in het eerste stadium. Bij afwisselende voeding (telkens 24 u. *Sol. tuberosum* en *Sol. demissum*) ontwikkelde het merendeel van de larven zich tot imago. Werd daarentegen *Sol. tuberosum* met *Petunia* afgewisseld, dan was totale mortaliteit het gevolg.

Het ongunstig effect van *Solanum demissum* wordt verhoogd door lage temperaturen binnen het traject dat ontwikkeling toelaat.

Hoofdstuk VI. De groeicurve van de larven (groei uitgedrukt als de toename van het lichaamsgewicht) is S-vormig. Bij voeding met verschillende Solanaceeën verloopt de curve niet gelijk. Voeding met *Atropa belladonna* vertraagt de ontwikkeling in het eerste stadium; de overige stadia ontwikkelen zich even snel als op aardappel. De groeicurve op *Solanum demissum* is onregelmatig en blijft lager dan die op aardappel.

Het aantal larvale stadia was onveranderlijk vier; invloed van de voedselplant werd in dit opzicht niet waargenomen.

Voeding met ongunstige voedselplanten verlengde in het algemeen de duur van de larvale stadia. Op *Atropa belladonna* werd alleen de duur van het eerste stadium verlengd, op *Solanum demissum* die van alle stadia, en wel in toenemende mate.

Bij kweken op een gunstig voedselgewas werd gevonden dat de lineaire groei van kopkapsel en mandibulae in overeenstemming was met de regel van Dyar. Bij het derde en vierde stadium van de larven, gevoed met *Solanum demissum*, waren de genoemde delen van het chitineskelet belangrijk kleiner dan normaal; de larven blijven in groei achter.

Hoofdstuk VII. Vervolgens werd de vertering van de verschillende voedselplanten nagegaan. In de eerste plaats werd de snelheid bepaald, waarmee het voedsel de darmtractus passeert. Wanneer larven, die geruime tijd hebben gehongerd, weer opnieuw worden gevoederd, is de passagetijd het langst (bij 20–23°C voor het vierde stadium 3–5 u.) en zijn bij verschillende voedselplanten geen verschillen waarneembaar. Kweekt men de dieren echter enige dagen op de verschillende voedselplanten, dan blijkt de passagetijd verschillend te zijn (*Sol. tuberosum* 2 u., *Sol. demissum* 3 u.).

Ook de *utilisatie* van het voedsel vertoont verschillen. Bij voeding met aardappelblad was de retentie van de droge substantie bij de larven van het tweede stadium 71,4 ± 2,3 %, van het derde stadium 75,1 ± 1,3 %, van het vierde 82,9 ± 0,6 %. Bij voeding met *Solanum demissum* was de retentie in stadium 2 : 58,4 ± 5,5 %, in stad. 3 : 61,8 ± 5,2 %. De voedingswaarde van het blad van *Solanum demissum* is dus geringer dan dat van de aardappel.

Van de afgebeten bladfragmenten werden alleen de randcellen verteerd.

Hoofdstuk VIII. Het epitheel van de middendarm ondergaat bij voedselgebrek belangrijke veranderingen: de naar het lumen gekeerde, resorberende cellen worden sterk gevacuoliseerd, de regeneratiecellen delen zich in versneld tempo. Bij voeding met *Petunia* (van welke plant maar weinig wordt gegeten) vertoont de darmwand hetzelfde microscopische beeld als bij hongerdieren, doch in iets mindere mate. Bij voedering met *Solanum demissum* wordt dit beeld eerst na veel langere tijd verkregen en zijn de verschijnselen minder duidelijk.

Hoofdstuk IX. Het hartrhythme van de larven bij constante temperatuur vertoont samenhang met de intensiteit van de stofwisselingsprocessen; het wordt langzamer naarmate de larve een hoger stadium bereikt (de stofwisseling verloopt dan trager, zie Hoofdstuk X) en naarmate de activiteit vermindert. Een specifieke invloed van de voedselplant op het hartrhythme kon niet worden waargenomen.

Hoofdstuk X. De CO_2 productie per eenheid lichaamsgewicht neemt bij de larve van het vierde stadium af naarmate de leeftijd vordert. Bij inanitie of bij voeding met een ongeschikte plant wordt eveneens afnemende CO_2 productie waargenomen; omgerekend per eenheid lichaamsgewicht is de daling echter veel sterker dan bij het normaal gevoede dier. Een specifieke invloed op de CO_2 productie kon noch bij voedering met *Petunia*, noch met *Solanum demissum* worden vastgesteld. De proeven werden echter alleen met larven van het vierde stadium genomen.

Gedurende de groei, bij normale voeding, is het respiratiequotient van de larve van het vierde stadium ongeveer 1, hetgeen wijst op de verbranding van koolhydraten. In het praepupale stadium is het R.Q. sterk gedaald, de vetvoorraad is dan de voornaamste energiebron. Ook bij inanitie daalt het R.Q. Een specifieke invloed van de voedselplant, die niet door verschillen in de opname van voedingsstoffen kan worden verklaard, werd niet waargenomen.

Conclusie. Uit de beschreven proeven blijkt, dat de voorkeur die dit oligophage insect voor bepaalde voedselplanten vertoont, uitsluitend door een beperkt aantal zin-

tuiglijke prikkels wordt bepaald. Samenhang met de voedingswaarde of toxiciteit van de plant werd niet waargenomen. De prikkels die de voedselkeuze bepalen zijn overwegend van chemische aard; hierbij is zowel de aanwezigheid van aantrekkelijke als de afwezigheid van afwerende stoffen van belang. De geur van de plant bepaalt de reactietijd bij het begin van de vreterij; de smaak bepaalt of deze zal worden voortgezet. *De combinatie van olfactorische, gustatorische, nutritieve en toxische eigenschappen bepaalt de geschiktheid van een gewas als voedselplant.*

De resistente lijnen van *Solanum demissum* vertegenwoordigen in de eerste plaats de groep met olfactorisch gunstige, gustatorisch ongunstige eigenschappen; daarnaast echter is de voedingswaarde laag. Acute vergiftiging van de larven op deze plant werd niet waargenomen.

LITERATURE

Acqua, C.	1921	Ricerche sperimentali sui processi digestion della larva del filugello. Boll. Lab. Portici., 11: 3–44.
Anonymous	1937	Rapports sommaires sur les travaux accomplis dans les laboratoires en 1936. Ann. Epiphyt. Phytogen., N. S. 3 (2): 275–90.
Barber, G. W.	1933	Insects attacking *Solanum sisymbriifolium* in eastern Georgia. J. Econ. Ent., 26: 1174–5.
Biederman, W.	1919	Die Verdauung pflanzlichen Zellinhalts im Darm einiger Insekten. Arch. ges. Physiol., 174: 392–425.
Brues, C. T.	1940	Food preferences of the Colorado potato beetle (*Leptinotarsa decemlineata*). Psyche, 47: 38–43.
Brues, C. T.	1946	Insect dietary, an account of the food habits of insects. Harvard University Press.
Buchmann, W. W.	1928	Changes in midgut cells during secretion and absorption: *Pyrausta* larva. Zool. Anz. 79: 223–43.
Busnel, R. G.	1939	Etude physiologiques sur le *Leptinotarsa decemlineata* Say. Librairie E. le Francois, Paris.
Busnel, R. G. and M. Chevalier	1938	Note sur la microphysiologie de *Leptinotarsa decemlineata* Say à l'état larvaire. Etude du comportement des larves pendant leur croissance sur *Solanum demissum* et sur les hybrides de cette race. Rev. de Zool. agr. et appl., 37 (12): 180.
Crowell, H. H.	1943	Feeding habits of the southern Army-worm and rate of passage of food through the gut. Ann. Ent. Soc. Amer., 36: 243–9.
Crozier, W. J. and H. Federighi	1925	Effect of temperature on heart beat in *Bombyx mori*. J. gen. Physiol., 7: 565–70.
Davenport, D.	1949	Studies in the pharmacology of the heart of the orthopteron *Stenopelmatus*. Physiol. Zool., 22: 35–44.
Deegener, P.	1909	Changes in midgut cells during secretion: *Deilephila*. Arch. Naturgesch., 75: 71–110.
Dethier, V. G.	1937	Gustation and olfaction in lepidopterous larvae. Biol. Bull., 72: 7–23.
Dethier, V. G.	1948	Chemical insect attractants and repellents. The Blakiston Co., Philadelphia.
Dethier, V. G. and L. E. Chadwick	1948	Chemoreception in insects. Physiol. Rev., 28: 220–54.
Feytaud, J.	1931	Comment progresse l'invasion doryphorique. Rev. zool. agric., 30 (5): 69–82.
Feytaud, J.	1932	Rapports sommaires sur les travaux accomplis dans les laboratoires en 1932. Ann. Epiphyt. Phytogen., 19 (1–2): 1–46.

Feytaud. J. 1934 La question du Petunia envisagée comme remède contre le doryphore. C. R. Acad. agric. Fr., 17 janvier, 1934.

Fink, D. E. 1925*a* Physiological studies on hibernation in the potato beetle. Biol. Bull., 49 (5): 301–406.

Fink, D. E. 1925*b* Metabolism during embryonic and metamorphic development of insects. J. gen. Physiol., 7: 527–43.

Frenzel, J. 1885 Einiges über den Mitteldarm der Insekten, sowie Epithelregeneration. Arch. mikrosk. Anat., 26: 229–306.

Gilman, H. 1938 Organic chemistry, an advanced treatise. John Willey & Sons, New York.

Glaser, R. W. 1927 Evidence in support of the olfactory function of the antennae in insects. Psyche, 34 (6): 209–15.

Götz, B. 1936 Beiträge zur Analyse des Verhaltens von Schmetterlingsraupen beim Aufsuchen des Futters und des Verpuppungsplatzes. Z. verg. Physiol., 23: 429–503.

Gourévitch, A. 1928 Le quotient respiratoire des Blattes en fonction de la nourriture. C. R. Soc. Biol., 98: 26–27.

Grison, P. and R. Roehrich 1947 Bilans nutritifs chez le doryphore adulte. Bull. Soc. ent. Fr., 51: 133–37.

Guillaume, A. 1934 Le *Petunia* et le doryphore de la pomme de terre. Rev. Bot. appl., 14 (156) : 659–63.

Hodge, C. 1933 Growth and nutrition of *Melanoplus differentialis* I. Growth on a satisfactory mixed diet and on diets of single food plants. Physiol. Zool., 6: 306–28.

Hodge, C. 1944 Some effects of the diet on the gastric epithelial cells of the grasshopper *Melanoplus differentialis* Thomas. Biol Bull., 72 (2): 203–211.

Kozlovsky, S. 1936 Le cycle biologique du *Leptinotarsa decemlineata* sur différentes variétés de tomates. Rev. zool. agric., No 7.

Kozlovsky, S. 1937 Sur le décalage des générations du *Leptinotarsa* decemlineata au milieu artificiel. Ann. Epiphyt. Phytogen., N. S. 3 (1): 99–111.

Kremer, J. 1932 Die fortlaufenden Veränderungen der Amphibienleber im Hungerzustande. Zeitschr. Mikrosk. Anat., 28: 81.

Von Kuhn, R. and Gauhe 1947 Über die Bedeutung des Demissins für die Resistenz von *Solanum demissum* gegen die Larven des Kartoffelkäfers. Z. für Nat., 2b (11/12): 407–9.

Von Kuhn, R. and Löw 1947 Über Demissin, ein Alkaloidglykosid aus den Blättern von *Solanum demissum.* Chem. Ber., (5/1947): 406–10.

De Lepiney, J. 1929 Note préliminaire sur le rôle de la vision ocellaire dans le comportement des chenilles de *Lymantria dispar* L. Bull. Soc. Zool. Fr., 53: 479–90.

Maas, J. A. 1938 Stoffwechseluntersuchungen am Fuss von *Helix pomatia* L. mittels des thermischen Diaferometers. Auszug Dissertation, Utrecht, 1938.

Mayer, A. G. and C. G. Soule 1906 Some reactions of caterpillars and moths. J. exp. Zool., 3: 15–33.

Mayer, J. 1949 Problems of organic growth. Nature, 164 (4158): 65.

McIndoo, N. E. 1935 The relative attractiveness of certain Solanaceous plants to the Colorado potato beetle. Proc. ent. Soc. Wash., 37 (2): 36–42.

Newport, G. 1837 Temperature and circulation in insects. Phil. Trans. Roy. Soc., 127.

Noyons, A. K. M. 1938 On a polyfilar diaferometer for micrometabolism. Acta brev. neerl., 8: 108.

Painter, R. H. 1943 Insect resistance of plants in relation to insect physiology and habits. J. Amer. Soc. Agronomy, 35 (8): 725–32.

Pilewiczowna, M. 1926 Sur le métabolisme azoté des insectes. Trav. Inst. Neucki, Warsaw, 3 (4): 1–25.

Plateau, F. 1874 Recherches sur le digestion chez les insectes. Mém. Acad. Roy. Belg., 41: 1–124.

Przibram, H. 1927 Diskontinuität des Wachstums als eine Ursache diskontinuierlicher Variation bei *Forficula.* Zeitschr. Wiss. Biol. Abt. D. Wilh. Roux's Arch. Entwicklungsmech. Organ., 112 (1): 142–8.

Przibram, H. and Megusar 1912 Growth measurement: Sphodromantis. Arch. Ent. Mech. 34: 680–741.

Punt, A. 1943 De gaswisseling van enkele bloedzuigende parasieten van warmbloedige dieren. Onderz. Physiol. Lab. Utrecht, 8e R. III.

Raucourt, M. and B. Trouvelot 1936 Les principes constituants de la pomme de terre et le doryphore. Ann. Epiphyt. Phytogen., N. S. 2 (1): 51–98.

Romeis, B. 1948 Mikroskopische Technik. Leibniz verlag, München.

Sayle, M. H. 1928 Factors influencing the rate of metabolism of *Aeschna umbrosa* nymphs. Biol. Bull. 54: 212–30.

Schaper, P. 1939 Arbeiten und Probleme zur züchterischen Bekämpfung des Kartoffelkäfers. Zeitschr. Zücht. Reihe A. Pflanzenzücht., 23 (2): 239–322.

Schlottke, E. 1937 Untersuchungen über die Verdauungsfermente der Insekten II. Die Fermente der Land- und Feldheuschrecken und ihre Abhängigkeit von der Lebensweise. Z. verg. Physiol., 24: 422–50.

Schwartz, M. 1948 Kartoffelkäferresistez. Nachrichtenb. f. d. Deuts. Planzenschutzd., 2-5/6, -5/6 - '48, 65-69.

Shinoda, O. 1927 Contributions to the knowledge of intestinal secretion in insects. II. A comparative histo-cytology of the mid-intestine in various orders of insects. Z. Zellforsch. und Mikrosk. Anat., 5 (3): 278–92.

Simpson, G. G. and A. Roe 1939 Quantitative zoology. McGraw-Hill Book Co., N.Y.

Snodgrass, R. E. 1935 Principles of insect morphology. McGraw-Hill Book Co., N. Y.

Steiner, G. 1925 The problem of host selection and host specialization of certain plant-infecting Nemas and its application in the study of Nemic pests. Phytopath., 15: 499–534.

Stober, W. K. 1927 Ernährungsphysiologische Untersuchungen an Lepidopteren. Z. verg. Physiol., 6: 530–65.

Swingle, M. C., J. B. Gahan, and A. M. Phillips 1941 Laboratory rearing of certain leaf-eating insects. J. econ. Ent., 34: 90–95.

Teissier, E. 1931 Recherches morphologiques et physiologiques sur la croissance des insectes. Trav. Stat. Biol. Roscoff., 9: 29–238.

Teissier, E. 1936 Dyar's law. Livre Jubilaire E. L. Bouvier, 1936, 334–42.

Titshack, E. 1926 Untersuchungen uber das Wachstum, den Nahrungsverbrauch und die Eierzeugung. II. *Tineola bisselliella* Hum. Z. wiss. Zool., 128: 509–69.

Toxopeus, H. J. 1949 The significance of resistance in tuber-bearing wild *Solanum* species for the breeding of a commercial potato resistant to the Colorado beetle. Progress report for the meeting of the C. E. Z. A. at Wageningen, 12th, Sept. 1949.

Tower, W. L. 1906 An investigation of evolution in Chrysomelid beetle of the genus *Leptinotarsa.* Publication of by the Carnegie Institution of Washington.

Trager, W. 1947 Insect nutrition. Biol. Rev., 22 (2): 148.

Treviranus, G. R. 1831 Versuche über das Atemholen der nieder Tiere. Z. Physiol. von Tiedmann und Treviranus, 4: 1–39.

Trouvelot, B. and Thenard 1931 Remarques sur les éléments des végétaux contributant à limiter ou à empecher la pullulation du *Leptinotarsa decemlineata* sur de nombreuses espèces ou races végétales. Rev. Path. vég. Ent. agric., 18: 277–85.

Trouvelot, B. Lacotte, Dussy, and Thenard 1933*a* Observations sur les affinités trophiques existant entre les larves de *Leptinotarsa decemlineata* et les plantes de la famille des Solanées. C. R. Acad. Sci. Paris, 197: 273–75.

Trouvelot, B. Lacotte, Dussy, and Thenard 1933*b* Les qualités élémentaires des plantes nourricières du *Leptinotarsa decemlineata* et leur influence sur le comportement de l'insecte. C. R. Acad. Sci. Paris, 197: 335.

Trouvelot, B., Dixmeras, and Grison 1935 Variabilité de l'attaque du doryphore sur diverses Solanées tubérifères. C. R. Acad. agric. Fr., 11 décembre, 1935.

TROUVELOT, B. and BUSNEL	1937	Modification du rythme des battements cardiaques chez les larves de doryphore suivant les *Solanum* dont elles se nourrissent. C. R. Acad. Sci. Fr., 205 (23): 1171–73.
TROUVELOT, B. and MÜLLER-BÖHME	1938	Étude sur la valeur alimentaire pour les larves du doryphore d'hybrides *Solanum demissum* et *S. tuberosum.* C. R. VIIe Congr. internat. d'Ent. Berlin.
TROUVELOT, B., MÜLLER-BÖHME et LACOTTE	1938	Remarque sur le comportement du doryphore sur les hybrides de *Solanum tuberosum* et *S. demissum.* Rev. Path. vég. et Ent. agryc., 24 (4): 273–76.
TROUVELOT, B. et BRÉJOUX	1939	Effects d'engourdissement causés aux larves du doryphore par l'ingestion du *Solanum demissum.* Rev. Path. vég. et Ent. agric., 26 (2): 142–47.
TRUSZKOWSKI, R.	1927	Studies in purine metabolism. IV. The nuclear-plasmic ratio in dogs in carbohydrate and protein feeding and in starvation. Biochem. Jour. 21: 1047.
TRUSZKOWSKI, R.	1928	Studies in Purine metabolism. V. The nuclear-plasmic ratio of frogs. Biochem. Jour. 22: 198.
TSCHANG, Y. T.	1928	Bull. Soc. zool. Fr. 53: 56–60.
TSCHANG, Y. T.	1929	L'histogenèse et l'histophysiologie de l'epithélium de l'intestin moyen chez un lépidoptere (*Galleria mellonella*). Supplement 12, Bull. Biol. Fr. et Belg.
UVAROV, B. P.	1928	Nutrition and metabolism. Trans. Ent. Soc. London, 255–343.
VERSCHAFFELT, E.	1910	The cause determining the selection of food in some herbivorous insects. Proc. Sec. Sci. K. Akad. Wetensch., Amsterdam, 13: 536–42.
WILDE, J. DE	1946	Verslag Entomologisch Laboratorium Tuinbouw. (unpublished).
WILDE, J. DE	1948*a*	Over de oorzaken der „resistentie" van *Solanum demissum* tegen aantasting door de Coloradokever (*Leptinotarsa decemlineata* Say). Tijdschr. Plantenz. 54 (5): 90–94.
WILDE, J. DE	1948*b*	Développement embryonnaire et postembryonnaire du doryphore (*Leptinotarsa decemlineata* Say) en fonction de la température. C. R. VIIIe internat. Congr. Ent. Stockholm.
WIGGLESWORTH, V. B.	1939	The principles of insect physiology. Methuen Co., London.
WOJTUSIAK, R. J.	1932	Sense of smell in the orientation of larvae of *Pieris*, Lep. Biol. Abstr., 6 (1932), *24933*.
YAGI, N.	1926	Analysis of the growth curves of the insect larvae. Kyoto Imp. Coll. Agric. Mem., 1: 1–35.